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Cooperative Marine Technology Program for the Middle East (Phase IV)

M15-062

FINAL REPORT

PART II

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PN-ACF 301

DEPARTMENT OF ENVIRONMENTAL & INDUSTRIAL HEALTH
SCHOOL OF PUBLIC HEALTH

SEAFOOD SAFETY AND FISH DECONTAMINATION TECHNOLOGICAL AND HEALTH IMPLICATIONS

Project Final Report (1993 - 1997)
Submitted to

United States Agency for International Development
Middle East Regional Cooperation Program

VOLUME I SURVEYS OF CONTAMINANTS
CHEMICAL DEPURATION
HEALTH RISK ASSESSMENT
REGULATORY APPROACHES

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B

FOREWORD

A primary goal of this study was the promotion of peace through scientific cooperation between Israel and Egypt This was an attempt to normalize and enhance relationships and demonstrate the utility and the benefits of the peace treaty between Egypt and Israel This study succeeded in engaging a number of highly qualified scientists from Egypt and Israel and provide them with the appropriate milieu and mechanism for scientific cooperation in a mutually beneficial developmental project

The topic selected for this study is Seafood Safety, which is a major concern in rich and poor countries alike Research findings will certainly enhance the awareness of policy and decision makers with seafood safety problems and provide them with options of regulatory approaches Needless to say, this study has helped to establish durable linkages between Egyptian and Israeli institutions and scientists and promoted hopes for continued future cooperation

This study was supported by the Middle East Regional Cooperation Program, The US Agency for International Development Additional support was provided by the National Institute of Oceanography and Fisheries in Egypt, The Hebrew University of Jerusalem in Israel, and The University of Michigan in the USA

It is important to acknowledge the leadership and guidance received throughout the study from Professor Hillel Shuval, Dr Badri Fattal, Dr Hussein K Badawi, Mr Ahmad El-Ibiary, and Dr Sayed Z El-Sayed

Finally, our Egyptian-Israeli team wishes to dedicate this final project report to the memory of the Co-Principal Investigator, Dr Mohyi Easa, who passed away on January 17, 1998 at the age of 48 Dr Easa has been a most distinguished scientist who is well recognized for his studies on fish parasites

Submitted By

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School of Public Health
The University of Michigan

May 29, 1998

C

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EXECUTIVE SUMMARY

(This report is dedicated to the memory of Co-Principal Investigator Dr Mohyi Easa who died on January 17, 1998 at the age of 48)

RATIONALE

Due to increasing awareness of the relation between eating habits and good health, consumers have begun to alter their diets incorporating foods rich in nutrients, vitamins and protein This trend has led to increased consumption of seafood, perceived as an excellent source of protein with properties which may reduce the risk of cardiovascular disease

Seafood includes an array of products encompassing literally hundreds of edible species that have little in common other than an aquatic origin The US Food & Drug Administration defines the term "fish" to include all fresh and salt water finfish, molluscan, shellfish, and crustaceans This study is concerned with finfish only, both wild-caught and farm-grown varieties

In recent years, worldwide concern has evolved regarding the safety of seafood Humans have found themselves at increased risk of developing seafood-borne illnesses due to several factors, including polluted waters, improper handling and preparation of seafood and higher consumption rates of fish and shellfish

Contamination may result from microbial pathogens, toxins, parasites and toxic chemicals including pesticides and heavy metals In addition, improper storage, shipping, handling and preparation of seafood play a significant role in spreading fish contaminants via cross-contamination The issue of seafood safety is particularly relevant in the Middle East, where seafood not only constitutes a significant proportion of the population's diet, but also contributes substantially to the financial resources of the region Economic considerations, coupled with human health concerns, warrant a need to increase awareness of health risks associated with seafood contaminants, as well as reverse situations which have fostered the proliferation of contamination

In response to these concerns, prominent institutions in Egypt, Israel and the United States have joined forces to promote health and safety awareness and the prevention of seafood contamination This includes guidelines which will serve as the basis for the development of regulations which will assure consumers that the seafood products they purchase meet accepted minimum standards for safety

GOALS AND OBJECTIVES

The overall goal of this study is to promote seafood safety practices in Egypt and Israel This includes the awareness of the public health risks of seafood contamination and the strengthening of prevention and control practices

The specific objectives of the study include the following

1 Conduct a comprehensive review of the literature to compile existing public health criteria on seafood contamination and develop a data bank for

contaminants and bioaccumulation-elimination rates in fishes and what influences them

2 Screen representative samples of fish from different aquatic sources for organic and inorganic hazardous chemical residue and pathogen levels, so as to identify problem contaminants

3 Assessment of the rates of elimination of certain infectious microorganisms (viruses, including Hepatitis A virus and bacteria) from contaminated fish This includes the determination of the effect of environmental and stress factors on the rates of uptake and release of microorganisms and storage sites

4 Determine the survival of Hepatitis A virus in fish culture water and the influence of environmental factors on die-off rate

5 Assessment of the rates of elimination (depuration) of problem chemical pollutants from contaminated fish

6 Develop mathematical models for predicting the rates of elimination of chemical contaminants during depuration in "clean water" and/or dilution of contaminants by growth in "clean water" and using "clean feed" following the wastewater growth

7 Assess public health guidelines and regulatory options

8 Communicate project findings to policy and decision makers, the public and the scientific community

RESEARCH APPROACH

The basic framework of this study was based on engaging a number of highly qualified scientists and engineers from Egypt and Israel to investigate seafood safety issues This included the provision of an appropriate milieu for collaboration, (b) specialized training, e g in the areas of computer applications and health risk assessment, and (c) mechanism for disseminating the generated information to policy and decision makers as well as citizen groups, e g NGOs

The institutional framework of the study is illustrated in Figure 1 The program committee, which included the coprincipal investigators and research leaders met twice per year Project principal investigators were also members of the Cooperative Marine Technology (CMT) Steering Committee, which met once per year

Specialized training was offered to project scientists by the collaborating institutions This included methods for health risk assessment and the application of advanced computer techniques for data processing and modeling purposes

The distribution of tasks among the different collaborating institutions is shown in Figure 1 In general, research activities at the Hebrew University dealt with viral and bacterial contaminants in fish and rates of depuration Professors Hillel Shuval and Badri Fattal were primarily responsible for research activities in Israel In addition, they participated in the planning and review of research activities in Egypt

Project activities in Egypt were under the supervision of Dr Hussein K Badawi and Mr Ahmad El-Ibiary from NIOF Research investigations in Egypt were largely directed towards the (a) assessment of the type and levels of contaminants in fish harvested from the Nile and its tributaries and the coastal zones of Alexandria, Port Said and Suez, (b)

epidemiology of fish parasites in the Lake Manzala Region, (c) effectiveness of fish preparation practices for the inactivation of infectious parasites, (d) determination of depuration rates of certain chemicals and pathogenic bacteria from contaminated fishes, and (e) evaluation of seafood regulations in Egypt

The overall coordination of research activities in both Egypt and Israel was the responsibility of Professor Khalil H Mancy from The University of Michigan This included the planning and implementation of training courses, workshops and annual meetings

OVERVIEW OF RESEARCH FINDINGS

Review of Literature and Data Banks

A comprehensive review of the literature on "Seafood Safety" was conducted This included natural and anthropogenic contaminants in seafood Emphasis was given to contaminants in finfish (in contrast to shellfish and crustacea), harvested from surface waters and aquaculture The review also included public health implications of fish preparations, marketing and depuration and decontamination practices The review emphasized conditions in Egypt and Israel

All research groups in the USA, Egypt, and Israel have participated in the literature review with the aim of establishing a data base This included seafood safety regulations, standards, and guidelines in the USA & Canada, Europe, FAO, Egypt, and Israel This information is currently stored in Data Banks at NIOF-Suez and the University of Ain Shams in Cairo

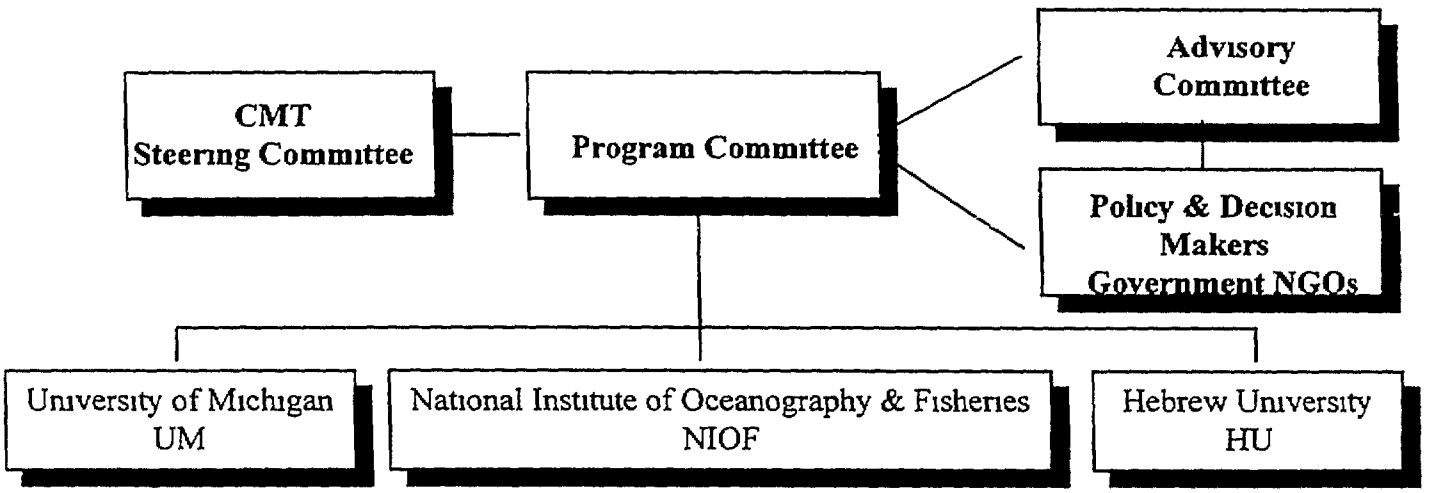
Survey of Fish Contaminants

Survey of fish contaminants in Egypt were conducted by Mohyi Easa (microbial pathogens including bacteria and parasites), Dr Mohamad Sherif (pesticides), and Mohamad El-Samra (toxic metals and PAHs) Fish samples from Lake Nasser and the southern reaches of the Nile had the lowest levels of contaminants Samples collected from the Delta, Northern Lakes and coastal waters at Alexandria, Port Said and Suez had the highest levels of contaminants This pattern followed closely the increasing pollution burden from south to north, caused by the discharge of municipal, industrial and agricultural waste It is interesting to note that fish reared in treated sewage effluents in the Suez Experimental Station was found to be of higher quality than fish harvested from certain locations in Lake Manzala and the Delta region

Health Risk Assessment

Assessments of health risk resulting from consumption of toxic chemicals in fish in Egypt and Israel were conducted The chemical contaminants detected were largely pesticide residues and certain toxic metals, commonly found in agricultural drainage water and industrial waste effluents Except for certain "hot spots" the concentrations detected were within international guidelines The health risk models and the health advisory guidelines used in this study were based on procedures used in the Great Lakes region in the USA Dr Mohyi Easa and Dr Mohamad Sherif supervised this work in Egypt, while Dr Badri Fattal was responsible for the work in Israel

Seafood Safety and Fish Decontamination
(Institutional Framework)



Personnel
K H Mancy
Graduate Student

Tasks
*Literature
*Health Risk
Assessment
*Plan Coordinate
Review
*Program Reports

Personnel
Hussein K Badawi
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Mohyi Easa

Tasks
*Microbial Pathogens
*Epidemiology of fish
parasites
*Fish preparation &
safety
*Health Risk Analysis

Ain Shams University
Mohamad Sherif
*Literature
*Pesticides in fish
*Pesticides in depuration
*Health Risk Analysis

NIOF - Suez
Mohamad El-Samra
*Literature
*Toxic Metals &
Organics in Fish
*Toxic Chemicals
Depuration

Personnel
Hillel Shuval
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Abdel Fatah Nasser

Tasks
*Literature
*Microbial Contaminants
*Microbial Depuration
*Health Risk Analysis

Results of these assessments were communicated to Egyptian authorities and summaries were reported in the news media The main public health concerns with seafood safety continues to be microbial infectious agents, including virus, bacteria, and parasites The health risk from consumption of chemically contaminated fish is insignificant in comparison to the acute infectious diseases caused by fish pathogens

Decontamination/Depuration Studies

Dr Badri Fattal at the Hebrew University studied the rates of elimination of different types of microorganisms (E Coli, MS2 coliphage and poliovirus 1)from various fish tissues Dr El-Samra in Suez, Egypt studied the depuration of metals and PAHs from fish Dr Sherif was responsible for the investigations and the depuration of pesticide residues Research results were reported in project workshops and some of the findings were published

Fish farming practices frequently entail a depuration or a decontamination step where the harvested fish is held in a clean water tank for a period of time Our research findings indicate that the rate of depuration is largely dependent on the type and concentration of the contaminant, the type of fish, water quality and environmental factors, e g temperature For a given type and size of fish and controlled environmental conditions, rates of depuration vary widely for viruses and bacteria, as well as for toxic chemicals, e g pesticide residues and toxic metals Depuration rates are faster from the fish digestive tract and the gills, than from other deep organs

By simply changing the clean water, the time required for active depuration of E Coli and MS2 Phage, from the digestive tracts of Tilapia and Mugil species was shortened from 21 days to 6 days The addition of disinfectants to the water (e g 30 mg/l H_2O_2 and 84 ppb Ag^+) increased the die-away of certain pathogens, with no observed effects on the fish

Similarly, the active depuration of copper, cadmium, and fenitrothion (a common organophosphorus pesticide) varied from 7 - 24 days

Empirical rate equations were applied to the modeling of the depuration process, based on laboratory data These models will be helpful in assessing the feasibility of depuration practices A preliminary conclusion could be that effective depuration will take weeks and not one or two days as frequently practiced

Regulatory Approaches

Effective seafood safety programs, applicable to both Israel and Egypt, should include (a) domestic inspections of seafood processors, (b) sampling and analyses of local and imported fish and fisheries products, (c) cooperative programs of training and technical support sponsored by the government and industry, (d) research in support of the regulatory mission, (e) public education (e g advising certain at-risk populations about consuming raw or partially cooked fish), and (f) adoption of international public health and safety guidelines

In Egypt, there are significant concerns based on the following

1 Lack of effective legislation and enforceable regulations for seafood safety (including production, processing and marketing)

2 Increasing levels of contaminants in the canals, Delta Lakes, and coastal areas which are the sources of the majority of fish harvested in Egypt
3 Fish farming practices are largely unregulated
4 Government policy restricts the water available to fish farming, to irrigation drainage and coastal and inland lakes which are mostly polluted Use of ground water is permitted mainly for fish hatcheries
5 In the Lake Manzala region, fish parasites are causing significant health problems

For both Israel and Egypt, it is proposed to adopt a preventive approach for the control of seafood contamination This is known as HACCP or "Hazard Analysis Critical Control Point," where the aim is to keep health hazards from occurring or keep the likelihood of their occurrence to a minimum This is not a government operated system, but rather it is the responsibility of the seafood industry It is science-based because it relies on a knowledge of what the reasonable likelihood of food safety hazards are for a given product and a process and what are the controls for those hazards The government, through inspections, verifies the adoption of HACCP by industry

RESEARCH SUMMARIES

I Microbial Contaminants in Fish

Dr Mohyi Easa, Cairo University, Egypt

Foodborne parasitic disease

This study addressed microbial agents in fish, including bacteria and parasites An extensive survey of fish harvested at different sites in Egypt showed that the degree of contamination increased from south to north and it is highest in the Delta, northern lakes and coastal waters Levels of chemical contaminants, such as pesticides and toxic metals were highest in samples collected from polluted sites in Lake Manzala

In depth investigations were conducted on foodborne parasites transmitted to humans from eating fish The most common fishborne parasite in Egypt are the flukes (trematodes), e g Heterophyes heterophes The parasite is maintained in the aquatic environment as a result of the presence of susceptible snails, in which larvae taken in by the ingestion of eggs, will multiply to release cercariae, infective to fish The cercariae penetrate the fish skin, enter the flesh to encyst, become a matacercariae, which is the infective form to humans The dietary habit of eating raw, partially cooked, or partially pickled fish is the primary mode of transmission

These investigations found high rates of infection in some of the most common fish in Egypt, e g Tilapia species and Nile-catfish, which varies with season and location Fisherman communities and villages in the northern delta exhibited high rates of infection In such communities eating raw-salted fish or whole partially cooked fish (Bisaria) is common Disease complications were most evident in children

Laboratory experiments provided quantitative data on methods of fish preparations and their effectiveness in the destruction or inactivation of the metacercarie These practices include frying, grilling, smoking, pickling, etc Procedural guidelines were developed, e g required temperature and time for inactivation of infectious agents

Publications

Ph D Thesis

1 Pathological Changes in Some Fish Eating Mammals as a Result of Consumption of Fish Infested with Encysted Matacercariae
Thesis presented by Iman Bakr Mohamed to Cairo University, Faculty of Veterinary Medicine, Department of Pathology (1996)

2 Diseases Due to Infestation of Fish with Metacercariae
Thesis presented by Amany Abdel-Rahman Abbas to Zagazig University Benha Branch, Faculty of Veterinary Medicine, Moshtohor, Department of Poultry and Fish Diseases (1997)

Master's Thesis

1 The Role of Fish Raised in Sewage in the Transmission of Food Poisoning Organisms to Man
Thesis presented by Gehan Mohamed El-Moghazi, Cairo University, Faculty of Veterinary Medicine, Department of Hygiene and Zoonoses (1993)

Published Papers

1 Mahdy , Olfat, A , Manal, A A and Easa M El-S (1995)
Parasitological and Pathological Studies on Heterophyid Infection in Tilapia spp From Lake Manzala, Egypt
Egypt Journal of Comparative Pathology and Clinical Pathology 8(2) 131-154

2 Shaaban, A I , Tamos, A I , El-Shater, M A and Easa, M El-S (1995)
Bacteriological Examination of Fish and Water at Nasser Lake
Egypt Journal of Comparative Pathology & Clinical Pathology Vol 9(1) 195-199

II Seafood Contaminants in Israel & Microbial Decontamination
Dr Badri Fattal, Hebrew University, Israel

Microbes and Heavy Metals Detected in Fresh Water Aquaculture Fish Marketed in Israel

This study was conducted because of the increased use of aquaculture in Israel as well as other countries The organism Aeromonas (opportunistic pathogen) was present at the highest level in both species of fish Tilapia and Muril Overall, microbes (Aeromonas, fecal coliforms and F+ coliphages) and heavy metal concentrations (mercury, cadmium, copper and zinc) in the fish were within Israeli and International limits However, prevention of cross-contamination of edible tissue by the digestive tract and skin is recommended

Fish Grown in Fecally Contaminated Fish Pond

Tilapia fish from commercial fish ponds in Israel were examined for E Coli and enterococci The digestive tract contained a higher level of these two organisms than the water In addition, when these organisms were at sufficiently high levels in the water, the liver and tissue of the fish became contaminated Elimination of the organisms was tested in starvation conditions in a static tank The results indicated no apparent decrease in the level of organisms for the fish reared in fish ponds, yet there was an increase for artificially contaminated fish This study shows that uptake and elimination for artificially contaminated fish is different than fish reared in fish ponds over long periods of time The primary public health concern is for the individuals who handle and process the fish that are at risk for Aeromonas wounds infections

Impact of Water Quality on Fish Production based on Egyptian Studies in Israeli Practices

Studies were conducted in Egypt and Israel on Tilapia fish species The studies were focused on the quality of fish production from those reared in fish ponds supplied with wastewater vs Fish ponds without wastewater The results indicated that the production of fish was greater in fish ponds supplied with wastewater However, the wastewater should be diluted with freshwater in order to evade negative effects on fish production Microbial assays indicated that there was no difference between wastewater-enriched and non-wastewater aquacultures for both E coli and Aeromonas Fish obtained from both sites reflected the amount of Aeromonas that were in the water In addition, the digestive tract contained a higher concentration of bacteria than the water in which they were grown

Active Decontamination and Detection of Microbes and Heavy Metals in Fish Marketed in Israel

This study was conducted in two parts in order 1) to examine the efficiency of changing the water, in the holding tanks, to reduce the level of microorganisms in various fish, tissues, 2) to test for the presence of microbes and heavy metal in fish marketed in Israel Fish contamination was accomplished by seeding 10^9, 10^{11}, 10^{10} cfu or pfu ml^{-1} of E coli, MS2 coliphage and poliovirus 1, respectively, into 100 l of water These microorganisms were tested in the skin, muscle, liver, spleen and the digestive tract (DT) A single inoculation of the test microorganisms was followed by 3-24 water change (tank volumes) and compared with self purification (control) experiments without changing the water In the decontamination experiments, no microorganisms were detected in the DT 5-6 days after inoculation, as compared to 11 - 21 days in the control experiments The results of this study indicate that faster decontamination of fish is achieved by repeated changing of the water in the holding tanks and thus may lower the risk of disease transmission by fish handling or consumption

In the second part, two species fish *Tilapia sp* and *Mugil sp*, grown in fresh water aquaculture and marketed in Israel were tested for the presence of microbes and heavy metals Samples of digestive tract (DT), liver, spleen, skin and muscle were assayed for *Aeromonas*, *Fecal coliforms*, and F+ coliphages DT and muscle were assayed also for enteroviruses The most frequent microorganism found was *Aeromonas*, followed by *Fecal coliforms*, and F+ coliphage In both fish species, the highest concentration of Aeromonas was present in the DT, followed by skin, liver, spleen and muscle *Fecal coliforms* were not found in any of the muscles sampled Enteroviruses were not detected in either DT or muscle Even though our results show that both microbial and heavy metal concentrations were within the acceptable Israeli ranges, we recommend that measures be taken in processing to prevent cross-contamination of edible tissue by DT and skin Model for estimating health risk associated with eating fish cultivated in polluted fish ponds to be developed within 3 months

Publications

1 Fattal, B , Dotan, A , Tchorsh, Y , Parpari, L And Shuval, H I Penetration of E Coli and F2 bacteriophage into fish tissues In Viren und Plasmide in de Umwelt, eds J M

Lopez Pila, E Seeber, K Jander Stuttgart/New York, Gustav Fischer Verlag, pp 27-38, 1988

2 Iger, Y , Abraham, M , Dotan, A , Fattal, B And Rahamim, E Cellular responses in the skin of carp maintained in organically fertilized water J Fish Biol, 33 711-720, 1988

3 Dota, A , Parpari, L , Fattal, B And Shuval, H I Microbial studies of water and fish at Ein-Boquq wastewater reservoir In Environmental Quality and Ecosystem Stability, 4th International Conference of the Israel Society for Ecology and Environmental Quality Sciences, Jerusalem, 1989, eds M Luria, Y Steinberger, and E Spainer Vol IV-A, pp 529 -538

4 Fattal, B , Eisawy, A M , Dotan, A , Shuval, H I and Mancy, K H Impact of water quality on fish production based on Egyptian and Israeli practices Wat Sci Tech , 21(3) 27 - 33, 1989

5 Mancy, K H , Eisawy, A M and Fattal, B Comparative analysis of yields of fishes grown in brackish and wastewaters in Egypt and Israel In Environmental Quality and Ecosystem Stability, 4th International conference of the Israel Society for Ecology and Environmental Quality Sciences, Jerusalem, 1989, eds M Luria, Y Steinberger, and E Spanier Vol IV-A, pp 493-494

6 Fattal, B , Dotan, A And Tchorsh, Y Rates of experimental microbiological contamination of fish exposed to polluted water Water Research 26(12) 1621-1627, 1992

7 Nasser, A M , Tchorsh, Y and Fattal, b Detection of Aeromonas and microbiological indicators in fish marketed in Israel International Journal of Environmental health Research, 2 99-105, 1992

8 Fattal, B , Dotan, A , Parpari, L , Tchorsh, Y and Cabelli, V J Microbiological purification of fish grown in fecally-contaminated commercial fish pond Wat Sci Tech 27 7-8, 303-311, 1993

9 Nasser, A M , Tchorch, Y And Fattal, B Comparative Survival of E Coli, F+ bacteriophages, HAV, and Poliovirus 1 in wastewaters and groundwaters Wat Sci Tech 27 3-4, 401-407, 1993

10 Zuaretz-Peled, S , Tchorsh, Y , Nasser, A M , and Fattal, B Active microbial decontamination of telapia fish Int J Environ Health Res 6 63-66, 1996

11 Chaouat, A , Tchorsh, Y And Fattal, B Microbes and heavy metals detected in fresh water aquacultured fish marketed in Israel ISEEQS Pub Jerusalem, Israel, Ed Y Steinberger, Vol VIB 644-647, 1996

III Chemical Contaminants & Fish Decontamination

Dr Mohamed Shereif, Ain Shams University, Cairo, Egypt

Chemical Residues in Fish (Published in Wat Sci Tech Vol 32, No 11 pp 155 - 161 1995)

Chemical residues were analyzed from fish taken from two different sites in Egypt One was from lake Manzala which receives agricultural drainage and untreated sewage effluents and from the SES (Suez Experimental Stations) where fish is grown in treated sewage effluents Results indicated that there were a higher concentration of organochlorine pesticides and heavy metals (lead, copper, zinc, cadmium) present in Lake Manzala than in fish from the SES In addition, lead and cadmium levels from fish of Lake Manzala surpass international contaminant levels for human consumption

Uptake and Depuration of Contaminants by Fish (Menofiya J Agric Res, vol 20 (1995) No 2 657-673)

The uptake and elimination of the insecticide fenitrothion was examined in Tilapia fish from the river Nile Uptake was higher in larger fish compared to smaller fish Elimination of the insecticide in clean water occurred at the end of 15 days

Effect of Environmental Factors on the Uptake and Depuration of Chemicals

The effect of environmental factors such as pH and salinity on toxicity, uptake and depuration of the insecticide fenitrothion on Tilapia fish from the river Nile was studied The results indicated that at low pH values there is

- an increase in uptake of the insecticide
- an increase in accumulation of the insecticide
- an increase in the toxicity of the insecticide

Fish Surveys

Studies on Fish in Egypt indicate that in certain location, the organic chemicals chlordane, endrin and PCB exceed the most conservative international standards However, the metals Cadmium, lead and chromium levels were below international maximum limits Mercury and selenium concentrations exceeded the limit but this was only found in one study and therefore data from other regions is needed to give more conclusive results

Uptake and Depuration of herbicides by fish grown in the rice-fish farms

This study gives some guidance on the accumulation of the applied herbicides in rice fields and in fish

Modeling of Chemical Uptake and Depuration Rates

Mathematical models were developed based on laboratory data These models could be used to estimate the rate and effectiveness of fish depuration practices

Publications

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Theses

M Sc
Studies on the uptake and depuration of some pesticides by fish of economic importance

M Sc
Environment factors affecting the elimination of pesticides by fishes from polluted areas

Ph D
Environmental, Nutritional, and toxicological studies on some fish species from fish farms in Fayoum Governorate, Egypt

Lectures

- Lecture entitled "Estimating the hazards and elimination of pollutants in the aquatic Environment" 2nd Conference On Animal & Pollution Organized by Cairo University, Faculty of Veterinary Medicine, March, 1993
- Lecture entitled "Low cost technologies to reduce the toxic pollutants in fishes of Lake Mazala", 3rd Conference on food and toxins Organized by Banha University, Faculty of Agriculture, May, 1994
- Lecture entitled "Environmental pollution by pesticides How to protect our aquatic resources", 3rd Conference on Environmental Protection is a must Organized by Ain Shams University, October, 1994
- Lecture entitled "Seafood Safety and Decontamination", 5th Conference on Agricultural Development Organized by Ain Shams University, Faculty of Agriculture, December, 1995

Articles in Newspapers

- Article entitled "Paddy fields and contamination by toxic herbicides' Al-Ahram newspaper, May 1994
- Article entitled "Pollution in Egyptian lakes and ways of curing" Al-Ahram newspaper, Jan, 1996

IV Microbial and Chemical Contaminants in Fish and Decontamination Rates

Dr Mohamed El-Samra, NIOF, Suez, Egypt

Accumulation and Elimination of Pathogenic Bacteria by Fish Tilapia Sp

Tilapia nilotica fish species were exposed to raw sewage and then analyzed for total aerobic bacteria, total coliforms and fecala coliforms Results indicated an increase in the level of organisms over time and when put in elimination tanks the level of organisms were reduced to their initial values or lower

Metals, Protein, Lipid & PAHs in Fish from different Egyptian sites I-Levels, II-Uptake & Elimination of Cadmium

Muscles of the fish showed the lowest levels of cadmium, lead, copper and zinc and were within the safe concentration levels recommended by the FAO Cadmium and lead levels were highest in the gills of the fish while copper and zinc levels were highest in the liver The results indicate organ and metal specificity

Fish Surveys

Nile river fish and fish samples from the Mediterranean Sea indicate high levels of polycyclic aromatic hydrocarbons This is found in polluted areas with heavy ship traffic and industrial/urban waste effluents

V Health Risk Assessment

Dr Khalil H Mancy The University of Michigan

The health risk, given in terms of the probability of incidence of disease was estimated utilizing the data from surveys of chemical contaminants in fish in Egypt and Israel This included the following steps

Hazard Identification The determination of whether a particular chemical contaminant poses a health risk to consumers

Exposure-Response Assessment Evaluation of the relationship between the magnitude of exposure and the probability that the potential health effect will occur

Exposure Assessment Determination of the extent of exposure of consumers to the chemical hazard, based on the contaminant levels, consumption rates, duration of exposure

Risk Characterization Determination of the probability of incidence of disease, including uncertainty analysis

Research findings revealed that the health risk from the consumption of fish in Egypt is generally within limits acceptable elsewhere The exception is with fish harvested from highly polluted areas (hot spots) where the calculated risk was higher than acceptable levels in the USA and elsewhere (e g one in a million over a lifetime exposure)

Risk models and their applications for the assessment of health risk from consumption of contaminated fish were presented in a workshop in Egypt This included demonstrations of how to estimate advisories for consumers and the development of standards and regulations

VI Science-Policy Linkages & Regulatory Approaches

Khalil H Mancy, The University of Michigan
Ahmad I El-Ibiary, NIOF

Effective Management of Seafood Safety Should Incorporate HACCP-based Prevention Programs

The estimated 1995 fish production in Egypt was 407,141 metric tons (mt), of which 70 6% (287,456 mt) derived from marine and brackish waters (coastal areas and delta lakes), 14 2% (57,872 mt) from freshwater (the Aswan High Dam, the Nile, and irrigation and drainage canals), and 15 2% (61,815 mt) from fish farming (1, 2, 3) The total fish supply in 1995 was 547,952 mt (of which 26% was imported) This amounts to an average consumption of 9 kg/cap/yr The Government of Egypt (GOE) aims to increase consumption to 10 kg/cap/yr by the year 2000

Fish harvested from the northern coastal areas and the delta lakes exhibits high levels of contaminants, in comparison to fish caught in the Aswan High Dam, the Nile river, and irrigation canals The main reason is water pollution with sewage effluents, agricultural drainage and industrial waste Some of the hot spots are the coastal areas off Alexandria and Lake Manzala

Aquacultural development has a high priority in the GOE plans, which includes the increase of fish farming in various governorates However, the GOE policy prohibits the use of fresh water from the Nile and from irrigation canals for fish farming In addition, the use of groundwater is permitted mainly for fish hatcheries Water use for fish farming is currently restricted to irrigation drainage water (which contains variable levels of contaminants e g pesticides, fertilizers, and domestic and industrial waste) and coastal and delta lakes

We have provided the relevant authorities in Egypt with research results indicating that the reuse of treated domestic wastewater is highly preferred over the reuse of agricultural drainage water in fish farming These research findings were based on a full scale demonstration of the public health and economic advantages of using treated sewage effluents for aquaculure-agriculture applications The treatment of domestic wastewater in Egypt should include the option of reusing these treated effluents in fish

farming applications The effluents from the fish farms can be further used in irrigation applications

Fish farming practices in Egypt are largely unregulated In 1995, 17% of the fish farms were run by the government, and only 20% of the remaining private farms were licensed Sustainable development of fish farming in Egypt will largely depend on the close coordination of the policies of the Ministry of Water Resources & Public Works and the Ministry of Agriculture Fresh water resources allocations should be based on integrated aquaculture-agriculture systems, and cost-benefit analysis This should include considerations for water quality management and pollution prevention

An effective seafood safety program must include both the public and private sectors as well as citizen groups (the consumers) Efforts should be made towards the gradual adoption of the Joint FAO/WHO Food Standards Program, developed by the Codex Alimentarius Committee on Fish and Fish Products In addition, the GOE should start adopting HACCP-based Seafood Safety program HACCP is a "Hazard Analysis Critical Control Point' nongovernmental management system It defines specific hazards and preventive measures for their control rather than relying on end-product testing, which has been proven to be inadequate elsewhere, e g the USA

Finally, there exists a pressing need for public education programs to advise consumers on how to minimize the health risks from eating contaminated seafood This includes (a) advisories on eating salted raw fish or improperly prepared fish, (b) guidelines for purchasing, storing and cooking and (c) information on the nutritional values of seafood, economic fraud and other matters related to the industry

INTRODUCTION

Seafood includes an array of products encompassing literally hundreds of edible species that have little in common other than an aquatic origin The US Food & Drug Administration defines the term "fish" to include all fresh and salt water finfish, molluscan, shellfish, and crustaceans This study is concerned with finfish only, both wild-caught and farm-grown varieties

Seafood is nutritionally recognized for their high protein content and it includes a higher percentage of amino acids than any other animal meat In addition, seafood has a low carbohydrate content and is a rich food source for many of the important minerals The protein content in seafood is easily digestible and presents a healthy alternative for meat consumption The edible portions of fish vary according to species, size, sex, and the fishing season It ranges usually between 40% to 60% of the total fish size

Seafood is marketed either as a fresh or processed food Since seafood is a highly perishable food product, humans have discovered different means of preservation to prolong the shelf-life of fish and maintain its nutritional value as an important food source The first methods used for preservations were refrigeration and freezing in cold countries and salting and drying in tropical warm countries The ancient Egyptians were considered pioneers in preserving fish by salting and more recently many processing techniques were introduced such as smoking

In recent years, worldwide concern has evolved regarding the safety of seafood Humans have found themselves at increased risk of developing seafood-borne illnesses due to several factors, including polluted waters, improper handling and preparation of seafood and higher consumption rates of fish and shellfish

Contamination may result from microbial pathogens, toxins, parasites and toxic chemicals including pesticides and heavy metals In addition, improper storage, shipping, handling and preparation of seafood play a significant role in spreading fish contaminants via cross-contamination

There is increasing concern about the safety of seafood in poor and rich countries alike In view of the widespread pollution of surface waters, seafoods including finfish, crustaceans and mullusks are frequently contaminated with microbial pathogens, toxins, parasites, and toxic chemicals In developing countries, the health risk attributed to seafood contamination is great Recent examples include the 1992 cholera epidemic in Peru and the 1991 seafood poisoning cases in Cairo, Egypt

Furthermore, fish farming is growing at an unprecedented rate throughout the world In Egypt and Israel, it is the source of a good portion of the nationally marketed fish and it provides significant economic returns from the exporting of certain types of fish Fish farming practices in Egypt were unregulated and decontamination (depuration) practices, when applied, could be ineffective

When evaluating the safety of seafood, it is important to assess the various biological as well as chemical contaminants in the fish and which inhabit the same waters in which they are bred Through ingestion, absorption or penetration, fish may become contaminated or infected with these microbial agents While many of these agents may pose a direct risk to the fish, their effect on humans may be minimal The reverse,

however, may also be true Some pathogens do not appear to adversely affect fish, but the fish may pass these pathogens on to humans, producing disease or infection

To determine actual human exposure, various factors must be considered These include the quality of the water in which the fish were bred, the uptake or ingestion rates of the fish, the depuration capacity of the fish in relation to the contaminant, the organs of the fish which will be consumed by humans and methods used in the handling and preparation of the seafood

The issue of seafood safety is particularly relevant in the Middle East, where seafood not only constitutes a significant proportion of the population's diet, but also contributes substantially to the financial resources of the region Economic considerations, coupled with human health concerns, warrant a need to increase awareness of health risks associated with seafood contaminants, as well as reverse situations which have fostered the proliferation of contamination

In response to these concerns, prominent institutions in Egypt, Israel and the United States have joined forces to promote health and safety awareness and the prevention of seafood contamination This includes guidelines which will serve as the basis for the development of regulations which will assure consumers that the seafood products they purchase meet accepted minimum standards for safety

GOALS AND OBJECTIVES

The overall goal of this study is to promote seafood safety practices in Egypt and Israel This includes the awareness of the public health risks of seafood contamination and the strengthening of prevention and control practices

The specific objectives of the study include the following

1. Conduct a comprehensive review of the literature to compile existing public health criteria on seafood contamination and develop a data bank for contaminants and bioaccumulation-elimination rates in fishes and what influences them
2. Screen representative samples of fish from different aquatic sources for organic and inorganic hazardous chemical residue and pathogen levels, so as to identify problem contaminants
3. Assessment of the rates of elimination of certain infectious microorganisms (viruses, including Hepatitis A virus and bacteria) from contaminated fish This includes the determination of the effect of environmental and stress factors on the rates of uptake and release of microorganisms and storage sites
4. Determine the survival of Hepatitis A virus in fish culture water and the influence of environmental factors on die-off rate
5. Assessment of the rates of elimination (depuration) of problem chemical pollutants from contaminated fish
6. Develop mathematical models for predicting the rates of elimination of chemical contaminants during depuration in "clean water" and/or dilution of contaminants by growth in "clean water" and using "clean feed" following the wastewater growth

7 Assess public health guidelines and regulatory options
8 Communicate project findings to policy and decision makers, the public and the scientific community

RESEARCH APPROACH

The basic framework of this study was based on engaging a number of highly qualified scientists and engineers from Egypt and Israel to investigate seafood safety issues This included the provision of an appropriate milieu for collaboration, (b)specialized training, e g in the areas of computer applications and health risk assessment, and (c) mechanism for disseminating the generated information to policy and decision makers as well as citizen groups, e g NGOs

The institutional framework of study is illustrated in Figure 1 The program committee, which included the coprincipal investigators and research leaders met twice per year Project principal investigators were also members of the Cooperative Marine Technology (CMT) Steering Committee, which met once per year

Specialized training was offered to project scientists by the collaborating institutions This included methods for health risk assessment and the application of advanced computer techniques for data processing and modeling purposes

The distribution of tasks among the different collaborating institutions is shown in Figure 1 In general, research activities at the Hebrew University dealt with viral and bacterial contaminants in fish and rates of depuration Professors Hillel Shuval and Badri Fattal were primarily responsible for research activities in Israel In addition, they participated in the planning and review of research activities in Egypt

Project activities in Egypt were under the supervision of Dr Hussein K Badawi and Mr Ahmad El-Ibiary from NIOF Research investigations in Egypt were largely directed towards the (a) assessment of the type and levels of contaminants in fish harvested from the Nile and its tributaries and the coastal zones of Alexandria, Port Said and Suez, (b)epidemiology of fish parasites in the Lake Manzala Region, (c) effectiveness of fish preparation practices for the inactivation of infectious parasites, (d) determination of depuration rates of certain chemicals and pathogenic bacteria from contaminated fishes, and (e) evaluation of seafood regulations in Egypt

The overall coordination of the research activities in both Egypt and Israel was the responsibility of Professor Khalil H Mancy from The University of Michigan This included the planning and implementation of training courses, workshops and annual meetings

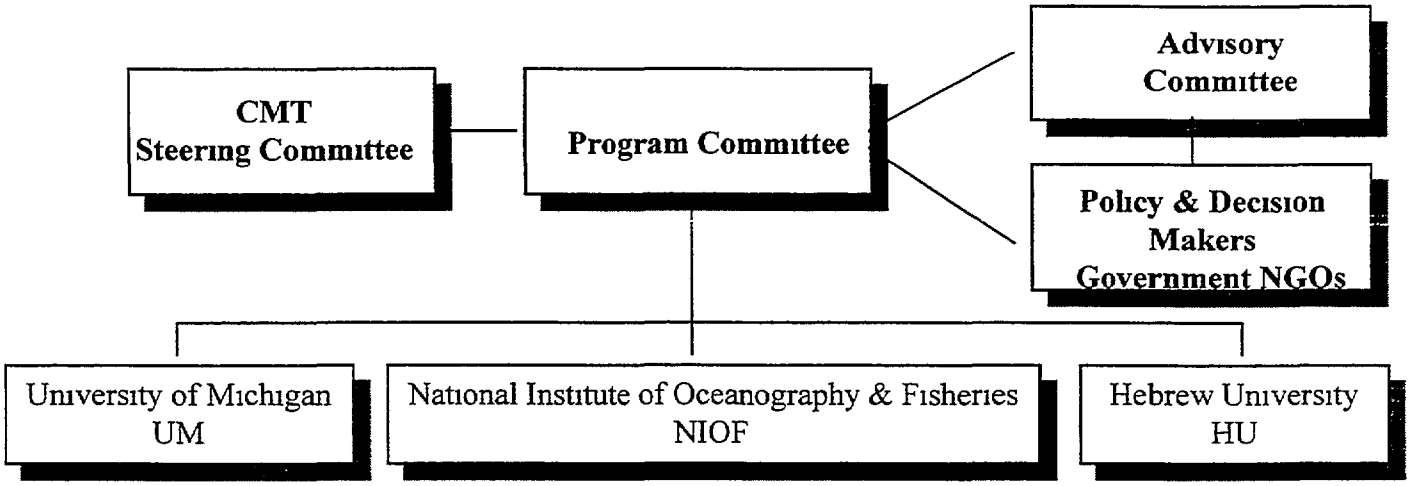
A comprehensive account of the study is presented in this four volume report This includes the following contributions

Volume I

Project Goals & Research Approach
Surveys of Fish Contaminants
Chemical Depuration Models
Health Risk Assessment
Seafood Regulatory Approaches

Figure 1

Seafood Safety and Fish Decontamination (Institutional Framework)



Personnel
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Tasks
*Literature
*Health Risk Assessment
*Plan, Coordinate, Review
*Program Reports

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*Epidemiology of fish parasites
*Fish preparation & safety
*Health Risk Analysis

Ain Shams University
Mohamad Sherif
*Literature
*Pesticides in fish
*Pesticides in depuration
*Health Risk Analysis

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Volume II
Depuration of Bacterial & Viral Contaminants
Disinfection using Hydrogen Peroxide & Silver
Survival of Hepatitis A Virus & Seafood Safety
Survey of Heavy Metals and Microbial Pathogens in Fresh Fish in Israel

Prepared by
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Volume III
Toxic Metals in Fish from Egypt Coastal Waters
Uptake & Depuration of Metals from Fish
Metal Bioassay Investigations
Prepared by Mohamad El-Samra and Khalid I El-Moselhy, National Institute of Oceanography and Fisheries, Red Sea Branch, Suez, Egypt

Volume IV
Fish Parasites in Egypt
Fish Viral and Bacterial Contaminants in Egypt
Prepared by Mohyi Easa, Faculty of Veterinary Medicine, Cairo University, Cairo, Egypt

Seafood Safety and Fish Decontamination Technical and Health Implications

Pathogens Associated with Seafood

With Emphasis on Egypt and Israel

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EXECUTIVE SUMMARY

Due to an increasing awareness of the relation between eating habits and good health, consumers have begun to alter their diets, incorporating foods rich in nutrients, vitamins and protein This trend has led to increased consumption of seafood, perceived as an excellent source of protein and having properties which may reduce the risk of cardiovascular disease With this growth in consumption has come increasing concern regarding the potential for seafood contamination and disease outbreaks Some of the primary agents responsible for seafood-related illnesses include naturally-occuring toxins, bacteria, viruses and metazoan helminthic parasites

While seafood-borne intoxicants vary according to species and region, there are essentially seven major toxins, ciguatera and scombroid poisoning being the most common in the United States Most toxins are produced by various species of dinoflagellates and accumulate in shellfish or fish after the consumption of these organisms Through a process called bio-accumulation, toxin concentrations increase as they move up the food chain with the result that the largest, most frequently consumed species may be the most poisonous

Bacteria represent a primary source of contamination for fish and shellfish Adulteration of these foods may result from various factors such as fecal contamination of the aquatic environment, consumption of indigenous bacterial contaminants and cross-contamination of food during processing or preparation Discharge of human wastes into surface waters results in fecal contamination of harvest areas, while poor sanitation and contact with infected foods can also lead to seafood contamination Because similar types of bacteria may be involved in both processes it is often difficult to determine the origin of adulteration Fortunately, many of these outbreaks can be controlled by treating human wastes and utilizing better sanitation and processing techniques These practices are well-established in the industrialized world, but many developing countries, including Egypt, lack the infrastructure to ensure that stringent standards are met

Naturally-occurring pathogens of the genera *Vibrio* are among the most common causes of seafood-borne bacterial disease outbreaks These organisms, endemic to most aquatic environments, reach peak populations during the warm summer months They are often linked to

the consumption of raw shellfish Illnesses caused by *Vibrio* spp are usually self-limiting and not life-threatening in healthy individuals Raw shellfish consumption, however, is highly discouraged among people with a chronic medical condition, as these populations may experience much more severe infections, especially when *V vulnificus* is involved

Viruses may also cause many seafood-related illnesses, most of which are associated with the consumption of raw shellfish The Hepatitis A virus and Norwalk viruses have often been implicated in seafood-borne disease outbreaks and may be much more widespread than once thought Indicators for viral contamination, however, have proven inadequate, due to the persistent nature of viruses Moreover, simple accurate diagnostic procedures are not widely available for identifying the types of viruses involved in outbreaks Consequently, viral outbreaks are very difficult to identify and control

Infections with parasitic helminths, while fairly rare in the United States and many other countries, are widespread in several Asian regions Raw fish consumption is often responsible for human infection with cestodes, trematodes and nematodes It has also been linked to gastroenteritis caused by the protozoa, *Giardia lamblia* In a parasitic infection, humans act as a host in the parasite life cycle, passing the parasite eggs or larvae in their feces and consequently contaminating water bodies used for sewage disposal Humans, however, are not always the definitive hosts, and, as a result, parasite eradication efforts may be more difficult

Control measures to limit seafood-related intoxications include

a) proper processing and handling to reduce the incidence of scombroid poisoning

b) monitoring harvest waters for toxigenic dinoflagellates

c) closing of harvest areas when necessary

d) rapid and accurate methods of detecting toxins in seafood products and

e) restricting the importation/consumption of puffer fish

Recommendations for limiting seafood-borne disease outbreaks include

a) thorough cooking of all seafood products

b) better indicators for *Vibrio* spp and viruses

c) adequate treatment and disposal of human wastes

d) education of consumers

e) improved processing and sanitation, and

f) decontamination/depuration of fish before marketing

Another important alternative to controlling seafood-related illnesses is the use of aquaculture for the rearing of fish and shellfish Fish farmed in controlled environments are easier to monitor In addition, pathogenic organisms can often be eliminated from the waters in which the fish are raised, yielding larger, healthier and safer seafood products

INTRODUCTION

Seafood can serve as a vehicle for the transmission of various disease-inducing organisms Finfish or shellfish contaminated with naturally-occuring toxins, bacteria, viruses or paraistes have caused numerous disease outbreaks Contamination of seafood with infectious agents may be due to several factors, such as organisms of fecal origin in polluted water, naturally occurring aquatic microorganisms, and improper handling or preparation practices (Ahmed ed , 1991) The last method of contamination is generally the most common Seafood-borne outbreaks are often directly related to behavioral practices, such as the consumption of raw shellfish or raw fish Due to the rise in consumption of seafood, especially such delicacies as sashimi, ceviche and green herring, related outbreaks are taking on a new importance (Higashi, 1985)

Assessing the health risks from seafood consumption is difficult due to the regional disparities between levels of environmental contamination, cultural practices, varying degrees of sanitation and completely different methods of handling and preparing seafood In the United States for example, 10 5% of all food-borne disease outbreaks between 1978 and 1987 were attributed to fish and shellfish consumption By comparison, however, chicken is 200 times more likely to cause disease than contaminated fish and 100 times more likely to result in the death of the consumer Significantly, the consumption of raw or partially cooked shellfish is 100 times more likely to result in illness and there is a 250 fold increase in the risk of death (Nightingale, 1990)

Likewise there is regional variation in the types of pathogens of concern, due to local species or cultural methods of food consumption For example, infection with the liver fluke (*Clonorchis*) is related to the practice of eating raw, partially cooked, or inadequately preserved freshwater fish While the incidence in the population may exceed 40% in certain areas of China, the disease does not exist in Europe or the United States (Higashi, 1985) Similarly, in Egypt, the primary infectious agent transmitted from freshwater fish is the intestinal trematode parasite, *Heterophyes heterophyes*, which poses a threat when the infected fish has not been adequately

cooked or cured The incidence of infection is especially high during the period surrounding the "Sham El Nessim" celebration (Higashi, University of Michigan)

Many feasible options exist to minimize the risks associated with the consumption of seafood Reducing pathogen levels in the environment by treating domestic wastes adequately is an important step, but may be impossible to implement on a worldwide basis, especially in those countries without adequate financial resources Moreover, seafood is often harvested in polluted waters and exported to other areas, thus spreading contamination to those regions In addition, there is no generic pollutant indicator that can predict levels of all pathogens found in the harvesting region For example, the measurement of fecal coliforms present in the water does not correlate with naturally occurring *Vibrio* species, viruses or helminthic parasites that might be present in the seafood (Elliot and Colwell,1985) Similarly, improving sanitation by introducing necessary changes in handling and processing procedures in all regions would be extremely difficult due to economic disparities and a lack of infrastructure in many areas

Depuration offers a potential alternative for cleansing fish and shellfish of bacterial and viral pathogens prior to consumption Studies have shown that transferring living fish and shellfish to clean water before processing increases the rate at which harmful bacteria and viruses may be removed from the harvested species An obvious limitation, however, is that depuration may be more effective for select pathogens and is completely ineffective for helminthic parasites and some viruses In addition, studies in Israel have shown that when fish were heavily pollluted, depuration was not very effective (Buras et al , 1987) Thus, this procedure would necessitate the use of additional techniques to ensure that the safety of the food was adequate

FISH AND SHELLFISH TOXINS

Naturally-occuring seafood-borne toxins have affected consumers globally While these toxins are found in various parts of the world, most cases of poisoning seem to be limited to North America, Asia and Europe Consequently, seafood-borne toxins have not been a major factor in seafood-related illnesses in Egypt, Israel and other Middle-Eastern countries

Seven types have been recognized as the most important ciguatera, scombroid, paralytic shellfish poisoning, neurotoxic shellfish poisoning, diarrhetic shellfish poisoning, puffer fish poisoning, and amnesic shellfish poisoning (Ahmed ed , 1991) These toxins are particularily insidious because they cannot be detected by sensory inspection and are not inactivated by heat Natural intoxications, with the exception of scombroid poisoning, are region-specific and often species-specific (See Table 1 for a complete summary of toxin characteristics)

All of the toxins mentioned above, with the exception of that responsible for scombroid poisoning, bioaccumulate in fish and shellfish as a result of food chain magnification and are, consequently, present in the organisms at harvest Scombroid poisoning,which has a wide geographic distribution and is present in various fish species, results from improper handling procedures These procedures allow bacteria to proliferate on the fish surface and subsequently convert free histidine to histamine in the fish tissues

Table 1 Information on Diseases Caused by Natural Toxins and Poisons

(Adopted from Ahmed ed 1991)

	Paralytic Shellfish Poisoning (PSP)	Puffer Fish Poisoning (PFP)	Ciguatera	Diarrhetic Shellfish Poisoning (NSP)	Neurotoxic Shellfish Poisoning (NSP)	Scombr Poisonin
Seafood Involved	Mussels clams some fish poison in digestive gland siphon	Puffer or globefish (tetrodon) poison in liver gonads and roe	Most common in barracuda kahala snapper and grouper	Mussels clams scallops toxin in digestive gland	Bivalves and most plankton feeders	Mahimal bluefish skipjack
Source of poison or toxin	Toxic dinoflagellates *Gonyaulax catenella* (Pacific) *G tamarensis* (Atlantic)	May be produced by fish some evidence from food chain	Not definitely known possible *Gambierdiscus toxicus*	Dinoflagellates *Dinophysis fortii D acuminata*	Dinoflagellate *Gymnodinium breve*	Bacteria with hig histidine
Areas commonly found	NW and NE North America, south Chile North Sea area, Japan	Areas of Pacific around China and Japan rare in U S	Tropical areas around world in U S mainly around Florida	Heaviest around Japan and Europe no cases in U S	Mostly west coast of Florida Caribbean	Worldw
Method of assay	Mouse unit is minimum intraperitoneal dose to kill in 15 minutes HPLC ELISA (specify toxicity 7 g/kg mouse)	Use mouse essay for PSP HPLC (specific toxicity 7 g/kg mouse)	Mouse test (poor) RIA ELISA immunological stick test under development	Mouse unit minimum intraperitoneal dose to kill in 24 hours (specific toxicity in mouse 500 g/kg)	Mouse test	Chemica histamin
Type of poison or toxin	Neurotoxin purine base very water soluble	Neurotoxin slightly water soluble	Lipid soluble polyether multicomponent	Okadaic acid dinophysis toxin	Brevetoxin lipid soluble polyether	H stem.r histamin substanc
Extent of U S and worldwide problem	Local areas worldwide few cases now	Important seafood in Japan 100 cases 50 deaths per year rare in U S	Largest seafood problem 50 000 cases per year <0 1 mortality	High morbidity rate potentially worldwide problem none in U S	Massive fish kills environmental problems	Japan 10 some in
Stability	Stable to cooking most stable at pH 7 and below	Stable to cooking stable between pH 4 and 9	Stable to cooking	Stable to cooking	Heat stable	Heat stat
Symptoms of poisoning in humans	Numbness paralysis after eating death 2 12 hours prognosis good after 24 hours	Very similar to PSP	Abdominal pain diarrhea vomiting neurological symptoms rarely fatal	Abdominal pain nausea vomiting severe diarrhea within 4 hours after eating rarely fatal	Feeling of nausea from red tide spray symptoms like ciguatera from eating bivalves	Itching sympton dizzines peppery
Dangerous and lethal human dose per 100 g of edible meat	1 mg (sickness) 2 mg or more (death)	Same as for PSP	Actual dose not known any amount is dangerous	Actual dose not known	Actual dose not known	Dose va individu.
Treatment	No antidote artificial respiration rest	No antidote artificial respiration rest	No effective antidote mannitol may be effective in acute cases	No specific treatment	No specific treatment	Unneces duration
Control measures	FDA limit 80 g/100 g of meat minimum cases due to good management	Education on identification of toxic species	No harvesting where toxic fish are found	None in U S close shellfish harvest	No established controls close shellfish harvest	Educatc freshly c below 1(

Remarks In most cases toxic shellfish are not detectable by organoleptic means It is therefore important that practical chemical or biological tests specific for the detection of the toxins be developed Although not all the listed diseases are problems in the United States seafood inspectors and processors should always be aware that toxigenic dinoflagellates or other microorganisms producing toxins that get into fish and shellfish may become established in areas where fish and shellfish are harvested for U S consumption An example diarrhetic shellfish poisoning is not a problem in the United States but the dinoflagellate that produces the toxin may become established in shellfish areas that supply U S markets

Ciguatera

Ciguatera poisoning is endemic to tropical islands and areas with extensive warm water reefs The toxin, ciguatoxin, is thought to be produced by the dinoflagellate*Gambierdiscus toxicus*, which is associated with reefs and other benthic algae (Bagnis et al , 1980) The predator fish ingests the toxin, which then concentrates up the food chain as smaller fish are preyed upon by larger fish The largest, often most commercially-desirable, fish are also usually the most poisonous Over 400 species of fish have been associated with ciguatera poisoning, but the most common species involved include amberjack, snapper, grouper, barracuda, goatfish and other reef fish belonging to the *Carrangidae* (Ahmed ed , 1991) In Egypt, various species of fish have been identified with the poison These include *Tilapia* spp , *Mugil* spp , *Chrysophrys* auratus, *Parupeneus* spp , *Solea bleekeri Boul*, *Sacina aquibla*, *Argyropus* spp , *Latis niloticus*, *Gerresoyena* spp and *Aconthurus* spp (Naguib et al , 1990)

Ciguatera poisoning is often accompanied by both gastrointestinal and neurological symptoms Nausea, vomiting, diarrhea, or abdominal pains may occur within 3-5 hours of toxin ingestion, while neurological symptoms appear 12-18 hours after consumption and may last from 1 to 82 days and, in some extreme cases, have lasted years Currently, no antidote is available though several remedies have been utilized to reduce the intensity of the symptoms (Ahmed ed , 1991)

Scombroid Poisoning

Scombroid poisoning occurs worldwide and is not limited to fish from tropical areas Fish with high levels of free histidine in their tissues are the usual sources of this illness Examples of such fish include tuna, mackerel, bonito, mahimahi, bluefish, jack, herring, sardine and anchovy (Ahmed ed , 1991) Harvested fish may sometimes be stored at improper temperatures, facilitating the growth of bacteria on the surface Histidine, which is naturally present at high levels in the above-mentioned fish, is converted into histamine by an enzyme secreted by the bacteria.

The ingestion of histamine is reponsible for the symptoms associated with scombroid poisoning The illness is characterized by a rapid onset of symptoms which include nausea,

vomiting, diarrhea, cramping, headache, palpitations, flushing, tingling, burning, itching, rash, edema and localized inflammation Fortunately, the disease is usually self-limiting and of short duration Though treatment is generally not required, anti-histamine drugs can provide relief (Ahmed ed , 1991)

Paralytic Shellfish Poisoning

Paralytic Shellfish Poisoning results from the consumption of bivalve molluscs (clams, mussels, oysters and scallops) that have fed upon toxigenic dinoflagellates (Halstead and Schantz, 1984) Toxins are incorporated into the flesh and stored by shellfish Blooms of the toxigenic dinoflagellates may occur often during the warmer months These blooms appear in many geographical regions and may result from the upwelling of nutrients associated with the formation of the thermocline in the spring or its destruction in the fall (Ahmed ed , 1991) During these episodes shellfish become toxic and may remain so for several weeks after the dinoflagellates have perished Some shellfish may remain hazardous indefinitely, necessitating the restriction of harvesting of some species

Paralytic shellfish poisoning is potentially fatal, as the toxins involved are exceptionally poisonous Symptoms, neurological in nature, often appear within an hour of consumption Severe cases may cause respiratory paralysis and possibly death within 24 hours In milder cases, symptoms may last a few days, but patients usually recover completely The only treatment used is respiratory support, no antidote is currently available (Ahmed ed , 1991)

Neurotoxic Shellfish Poisoning

Neurotoxic shellfish poisoning results when shellfish consume another type of toxigenic dinoflagellate which causes the red tide Red tides occur sporadically in the Gulf of Mexico and the southern Atlantic Blooms of the organism *Gymnodinium breve* turn the water red, producing massive fish kills Molluscs feed upon the toxigenic dinoflagellate and retain the toxin for lengthy periods

Symptoms resulting from the consumption of toxic shellfish appear rapidly and include tingling and numbness of the lips, tongue and throat, muscular aches, gastrointestinal upset and

dizzness (Ahmed ed , 1991) Intoxication is generally not fatal, and symptoms are usually resolved within hours or days of onset Currently, no treatment is available

Diarrhetic Shellfish Poisoning

Diarrhetic shellfish poisoning results when people consume bivalve molluscs which have previously fed on *Dinophysis fortii* or *D acuminata* (Edler and Hageltorn, 1990), as well as other dinoflagellates This illness is common in Japan and becoming increasingly common in Europe, although it is not yet an important cause of disease in the United States

Symptoms of this disease appear rapidly , sometimes within 30 minutes of consumption They include diarrhea, nausea, vomiting, and abdominal pain Diarrhetic shellfish poisoning is self-limiting, and symptoms usually subside within a few days in severe cases Presently, there is no antidote

Puffer Fish Poisoning

Tetrodotoxin, the causative agent of puffer fish poisoning, was originally thought to be produced by species of fish belonging to *Tetraodontidae* (puffer fish) There is now some evidence, however, that the toxin is produced by indigenous bacteria and stored by the fish in its tissues and organs (Ahmed ed , 1991) Puffer fish are a delicacy in Japan and, consequently, most of the poisoning incidents have been reported there

Symptoms associated with this illness are nearly identical to those of paralytic shellfish poisoning nausea and vomiting, subsequently followed by neurological symptoms and, in severe cases, respiratory paralysis and death Tetrodotoxin may lead to death with the ingestion of just 1-4 mg Treatment is supportive, as there is no antidote

Amnesic Shellfish Poisoning

Amnesic shellfish poisoning was first classified in 1989 by Todd, following an outbreak in Canada The disease results from the consumption of shellfish containing a toxin known as domoic acid This toxin is produced by several varieties of the diatom *Nitzschia pungens,* which are consumed by mussels and clams during periods of blooms

Amnesic shellfish poisoning is characterized by vomiting, abdominal cramps, diarrhea, disorientation and memory loss (Perl et al , 1988) Short-term memory loss has lasted for longer than a year in some cases, and degeneration of the hippocampus in three fatalities was evident Manifestations of the disease appear more severe in older victoms, some of whom died in the Canadian outbreak (Ahmed ed , 1991)

INFECTIOUS AGENTS

BACTERIA

Bacterial contamination of seafood may originate from fecal contamination of harvesting areas, indigenous bacteria found in the waters, and cross contamination due to poor handling or processing practices Different species of bacteria can serve as indicators of each source of contamination For example pathogenic bacteria of the *Vibrio* genera are normally resident in most aquatic ecosystems and, thus, do not necessarily indicate fecal contamination The presence of other species of bacteria from the genera *Salmonella*, *Shigella* and *Campylobacter* indicate either cross contamination due to improper processing or the presence of fecal material in the waterbody In many cases it is difficult to assess whether adulteration of the food occurred as a result of fecal contamination in the environment or poor sanitation during handling and/or preparation

Naturally Occurring Bacterial Pathogens

Organisms belonging to the genera *Vibrio*, *Aeromonas* and *Plesiomonas* may inhabit freshwater or estuarine environments These bacteria are generally not associated with fecal contamination but may act as pathogens under certain conditions Bacterial populations reach a maximum during the summer, when water temperatures are highest Reported cases of illness due to consumption of seafood containing these organisms also peak in this season (Ahmed ed , 1991) Species typically associated with seafood borne disease outbreaks are *Vibrio cholerae*, *Vibrio parahaemolyticus*, *Vibrio vulnificus*, other *Vibrio*, *Aeromonas*, and *Plesiomonas*

Vibrio cholerae

Vibrio cholerae organisms are classified according to the type of O antigen present Members of O group 1 are responsible for cholera outbreaks while strains from other O groups (non-O1) usually cause less severe illnesses O group 1 strains were originally thought to be of fecal origin Though this is often the case in large outbreaks, there is evidence that free living strains of these bacteria do inhabit marine environments (Morris and Black, 1985)

Virulent strains of *V cholerae* produce an enterotoxin which, when released into the intestinal tract, causes a severe debilitating watery diarrhea which can lead to dehydration and sometimes death when proper treatment is not received The most insidious of all types of cholera, cholera gravis, is fairly rare in developed countries, but outbreaks in developing countries are still common Most seafood-borne cholera outbreaks are mild with 4 to 100 asymptomatic cases for every documented case (Bart et al , 1970)

In January 1991, a cholera epidemic spread to Peru, eventually extending to other areas of South and Central America By July of 1992, over 553,000 cases and 4,700 deaths had been reported from 19 countries in the hemisphere (Swerdlow et al , 1992) Though contaminated water was the main vehicle of this outbreak, there is clear evidence that the epidemic was exacerbated by the consumption of a marinated raw fish dish called ceviche Seawater from a fishing port registered a count of 9300 mpn *Vibrio*/100ml This same water was also used for processing and cleaning harvested fish prior to their consumption in ceviche (WHO, 1991) The Peruvian President even appeared on television, eating ceviche to reassure citizens that the consumption of raw fish was safe How many cases of cholera this led to is not clear As previously mentioned, cholera poses a grave threat to developing countries due to both inadquate waste treatment facilities and lack of stringent sanitary practices While cholera is not a significant factor in seafood-borne illnesses in Egypt and Israel, both countries, as well as other Middle-Eastern nations, are at risk due to their proximity to the current pandemic Moreover, it is probable that Egypt and lesser-developed countries are at a higher risk due to their lack of infrastructure to support monitoring and sanitation activities

Vibrio parahaemolyticus

Various strains of *V parahaemolyticus* can be isolated from virtually any marine environment The ability to induce gastroenteritis in humans is correlated to the organism's ability to produce a heat-stable hemolysin (Miyamoto et al , 1969) As most strains do not produce this toxin, their overall disease incidence is quite low, although not insignificant *V parahaemolyticus* can reproduce very quickly at temperatures exceeding 20°C and has been found to reach infectious levels (>10^5 colony forming units) in shrimp and crabs within 2-3 hours when stored at these temperatures (Liston, 1973)

Vibrio vulnificus

V vulnificus is also ubiquitous in marine environments and is commonly isolated from oysters harvested in warm waters These organisms are usually not associated with gastroenteritis but may cause septicemia, especially in individuals with other underlying conditions such as cirrhosis hemochromatosis or in immunocompromised people (Klontz et al , 1988) Infections, though uncommon, may result in mortality rates which exceed 50% for people in the aforementioned high risk categories (Morris, 1988)

Other *Vibrio* Species

Several other *Vibrio* species have also led to human illness as a result of the consumption of contaminated seafood *V mimicus*, *V hollisae*, *V fluvialis*, and *V furnissii* have all been associated with disease outbreaks Infections with *V mimicus* and *V hollisae* have almost always been associated with the consumption of raw shellfish, especially oysters Cases involving these organisms however are rare and, thus, do not pose as great a health risk as the other *Vibrio* species previously discussed

Aeromonas

Aeromonas species, present in virtually all estuarine areas, are commonly isolated from seafood, as well as other types of foods (Palumbo et al , 1989, Kaper et al , 1979) Evidence that *Aeromonas* species cause disease in humans, however, is limited and circumstantial at best (Ahmed ed , 1991) Nevertheless, it is important to note that levels of *Aeromonas* were found to

be comparatively high in a study conducted by the Division of Environmental Studies at Hewbrew University in Israel ******* Thus, the potential for illness due to *Aeromonas* should not be ignored when formulating a program of seafood safety in the Middle East

Plesiomonas

Plesiomonas shigelloides is common in freshwater but may also inhabit estuarine environments (Van Damme and Vandepitte, 1980) There is evidence that there are few virulent strains of this organism which may cause gastroenteritis in humans *Plesiomonas shigelloides* infections can usually be traced back to the consumption of raw shellfish, especially oysters (Kain and Kelly 1989)

Recent studies show that certain strains of this organism are able to grow at temperatures as low as 0°C and, consequently, survive refrigeration in shellstock oysters (Ahmed ed , 1991) As the organism is sensitive to a salt concentration higher than 5%, it is improbable that it would be responsible for seafood-borne outbreaks in the Middle East In general, illnesses caused by this bacterium are rare, from 1978-1987, it accounted for less than 5% of all reported cases in the United States associated with molluscan shellfish (Ahmed ed , 1991)

Pathogens Transmitted via Fecal or Cross-Contamination

In many seafood-borne disease outbreaks, it is difficult to determine the source of the responsible pathogen Dumping of untreated or partially treated wastes into bodies of water is commonplace throughout the world and is often done in areas where fish or shellfish are harvested This increases danger that fish catches will be contaminated with organisms of fecal origin Moreover, seafood may not be processed or cooked under sanitary conditions, further increasing the probability of contamination

Certain bacteria which adulterate other foods such as chicken can also contaminate seafood For example,*Salmonella, Campylobacter, Escherichia, Yersinia, Shigella, Listeria, Clostridium* and *Staphylococcus* have all been identified as causal organisms in seafood-borne disease outbreaks

Salmonella

Though there are several pathogenic species of *Salmonella* , the most virulent is *S typhi*, the cause of typhoid fever Seafood-borne outbreaks of this disease are increasingly rare in industrialized nations but still occur in less developed countries (Ahmed ed , 1991) In Egypt, widespread distribution of *S typhi, S paratyphi* and other serotypes important in gastroenteritis implies that these bacteria may play an important role in food and seafood-borne illnesses (Higashi, University of Michigan) A study by Tawfik and Hegazi found that 11 sero-types of *Salmonella* sp were present in isolates from 14 specis of freshwater and saltwater fish (Tawfik and Hegazi, 1990) This disease appears sporadically in many regions and may be endemic in others

The pathogen is spread via the fecal-oral route, posing potential danger in fish harvesting areas where domestic wastes are dumped The contaminant may also be spread by poor hygiene during processing This route of contamination is especially troublesome to control, as infections may occur asymptomatically and, consequently, would be difficult to identify in food handlers

Salmonella species which are not responsible for typhoid fever have been identified in many seafood-borne disease outbreaks A study in Florida found *Salmonella* species present in nearly 20% of all clams, crabs and oysters in the marketplace, regardless of the season (Fraiser and Koburger, 1984) As *Salmonella* outbreaks are linked to many different types of food, cross-contamination from other contaminated foods during processing or preparation is a distinct possibility

Campylobacter

Campylobacter jejuni, another organism of predominately fecal origin, has been isolated from both marine and freshwater environments and identified as the causative agent in illnesses stemming from the consumption of raw shellfish (Arumugaswamy and Proudford, 1987) This species does not appear to survive for long periods of time in harsh environments (Franco, 1988), but the infectious dose required to cause disease appears to be low, approximately 500-800 organisms (Black et al , 1983) Like *Plesiomonas shigelloides*, *C jejuni* appears to be sensitive

to sodim chloride, limiting its importance in marine species, especially in particularly saline areas such as Israel and Egypt

Escherichia

Pathogenic *E coli* infect many people in less developed areas and virulent strains are often ubiquitous (Ahmed ed , 1991) Because these organisms are transmitted by the fecal oral route, it is suspected they have been involved in seafood-related outbreaks, but presently there is no data to confirm this A study conducted at the Hebrew University of Jerusalem found *E Coli* numbers to be very high in at the Ein Boqeq Wastewater Reservoir in Israel While the numbers were at least 100 times greater than those in stabilization ponds in the US, they were considerably lower than those found in similar ponds in Israel and Peru (Dotan et al , 1988) Nevertheless, these numbers stress the need to monitor *E coli* counts in attempts to regulate seafood safety in Israel, as well as other Middle Eastern countries

Yersinia

Yersinia enterocolitica has often been implicated in food-borne disease outbreaks, but there are few documented cases of this illness being caused by consumption of contaminated seafood Studies of finfish and shellfish sold in markets, however, have found these bacteria to be present in a significant number of samples A study in Texas identified this species in 21% of all blue crab samples (Peixotto et al , 1979) Due to its worldwide distribution, it is possible that these bacteria could pose a threat to the safety of seafood harvested in the Middle East More research regarding seafood contamination with *Y enterocolitica* is needed

Shigella

Shigella species normally inhabit the intestinal tracts of humans, primates and, to some extent, other animals (Ahmed ed , 1991) This indicates that fecal pollution of the aquatic environment or poor sanitation during food handling are the main routes of seafood contamination involving this organism (Blake and Feldman, 1986) *Shigella* outbreaks associated with the consumption of contaminated seafood are well-documented in the United States

The infectivity of Shigella is exacerbated by the long survival periods of many strains in the aquatic environment and the low infectious doses required to cause disease (Flowers, 1988) Researchers have found that it may take as few as 100 organisms to initiate an infection in a human host (Dupont et al , 1969)

Listeria

Listeria monocytogenes has been consistently isolated from the feces of man and other organisms (Ahmed ed , 1991) This species can cause a wide range of human diseases, including meningitis and perinatal disease (Lamont et al , 1988) This pathogen usually causes infections in people with other underlying illnesses, but may also cause disease in healthy individuals (Ahmed ed , 1991)

There is little data linking this organism to seafood-borne disease outbreaks, but it has frequently been isolated from various types of seafood and, thus, may be an under-reported cause of illness (Ahmed ed , 1991)

Staphylococcus

Staphylococcus aureus is a common cause of food poisoning in the United States, as well as other countries It grows quickly in foods held at improper temperatures, secreting an enterotoxin into the food The ingestion of the toxin is responsible for the symptoms, which usually include nausea and vomiting

Because this bacteria is closely associated with human skin and 25-30% of all people carry enterotoxin-producing strains, *S aureus* contamination of seafood occurs almost exclusively from poor hygiene/sanitation practices during handling or preparation (Ahmed ed , 1991) Thus, in less-industrialized countries such as Egypt, where hygiene and sanitation methods are not sufficient, this organism is most likely a common cause of food poisoning

Clostridium

Clostridium botulinum , an anaerobic spore producer, is the cause of botulism The spores of *C botulinum* are exceptionally persistent, withstanding temperatures that would inactivate living cells and other toxins The spores are ubiquitous and, thus, readily contaminate seafood

during processing The spores germinate under anaerobic conditions, producing the botulin toxin It is the ingestion of the toxin which is responsible for the symptoms

Most botulism outbreaks occur as a result of improper canning techniques, often performed at home, which do not incorporate the proper time/temperature regimen to inactivate the spores (Eklund, 1982) All seafood-related botulism cases reported in the United States have stemmed from inadequate processing or preservation techniques These incidents, fortunately, are rare, as botulism is characterized by mortality rates as high as 70% in developed countries and is probably greater in less developed regions (Ahmed ed , 1991)

Clostridium perfringens, another anaerobic sporeformer, has also often been implicated in food-borne disease outbreaks, usually as a result of contamination from an infected handler This may also be the main route by which these organisms are introduced into seafood *C perfringens*, however, has also been identified in freshly harvested seafood A study conducted in marine waters surrounding Alexandria, Egypt found that the organism was present in as many as 40% of the shellfish samples taken from highly polluted areas Data directly implicating these organisms in seafood-related outbreaks is lacking, but it is possible that it is an under-reported cause of infection in consumers of seafood (El-Sharkawi et al , 1982)

VIRUSES

At least 110 known enteric viruses have been isolated and identified in human feces (Melnick and Gerba, 1980) These viruses enter marine environments when sewage is dumped directly into the ocean or when it is released into waterways which connect to the ocean (Goyal, 1984) Of these enteric viruses, only Hepatitis A, Non-A, Non-B Hepatitis, Norwalk, Astroviruses, Calicviruses and Small Round Viruses have been epidemiologically linked to seafood-related disease outbreaks Other viruses may also act as disease-causing agents in seafood, but both the current methods of viral identification and the inherent difficulty of diagnosing viral disease outbreaks which occur months after exposure, limit this list (De Leon and Gerba, 1990) Viral concentrations can reach very high levels , particularly in water-saving

countries like Israel and developing countries, where estimated ranges of viruses in raw sewage are 6,000 to 490,000 per liter (De Leon and Gerba, 1990)

Seafood-borne viral outbreaks are almost exclusively associated with the consumption of shellfish (De Leon and Gerba, 1990) Very few viral outbreaks have been attributed to the contamination of finfish Shellfish are bottom dwellers and obtain their food by filtering the water, ingesting viruses either directly or through particles of food to which the virus are attached These viruses may remain viable for long periods of time in the gut or mucus membranes of the shellfish Shellfish thus act as passive carriers for both viruses and bacterial pathogens (Gerba, 1988)

Hepatitis A Virus (HAV)

The first case of shellfish-related HAV infection was documented in Sweden in the 1950's (Cliver, 1988) Since, HAV has been routinely isolated from waters contaminated with human feces and from the shellfish grown in these areas (Ahmed ed , 1991) HAV is spread by fecal-oral contact and is endemic in many countries, including Egypt, where sanitation is poor and people live in crowded conditions

HAV infections are often considered the most serious type of viral disease related to the consumption of seafood Seafood-related HAV outbreaks account for a large proportion of all HAV cases in developed countries One study conducted in the southeast of England indicated that the consumption of contaminated shellfish accounted for 25% of all HAV infections in that part of the country (O'Mahoney et al , 1983)

HAV is of particular importance because it is more resistant to chlorine disinfection than bacteria and can withstand higher temperatures for longer periods of time than most viruses (White and Fenner, 1986, Grabow et al , 1983, Keswick et al , 1985) HAV outbreaks can occur due to the consumption of raw or partially cooked seafood or through cross-contamination

Non-A, Non-B, Hepatitis (NANB)

Little information is available regarding this type of virus It is however known to be spread by fecal-oral contact and occasionally by person-to-person contact (Overby et al , 1983) Several studies have linked NANB Hepatitis to the consumption of raw shellfish Overby et al

(1983) reported that enteral NANB appears to be endemic in the Middle East and Africa Similarly, Alter et al , (1983) found that 12 5% of all NANB Hepatitis cases in Baltimore, Maryland could be attributed to the consumption of raw shellfish Eating raw shellfish was in fact the second highest attributable risk factor in contracting NANB Hepatitis behind parenteral drug use

Norwalk Virus

Norwalk viruses are responsible for up to 42% of all nonbacterial gastroenteritis outbreaks in the United States (Kaplan et al , 1982) These viruses are fecally-orally transmitted and have been isolated in marine waters and in samples of clams and oysters Norwalk viruses were first attributed to a shellfish-related gastroenteritis outbreak in 1979, in Australia, where over 2000 people were infected nationwide (Gerba, 1988) Subsequent outbreaks involving shellfish in Florida, and New York have also been linked to this virus (Gunn et al , 1982, Morse et al , 1986) A review by Guzewich and Morse (1986) illustrated that shellfish-related gastroenteritis outbreaks due to the Norwalk virus continue to be a problem in the United States In most developing countries, anitbodies to the Norwalk agent are acquired early in life (Benenson, ed , 1991) Consequently, in many Middle Eastern countries, seafood-borne outbreaks due to the Norwalk virus would most likely be minimal

Astroviruses

Astroviruses have been isolated from the feces of children suffering from gastroenteritis (Appleton and Higgins, 1975) They were also associated with a shellfish-related gastroenteritis outbreak at a naval base, which took place in two stages The first stage, caused by a Small Round Virus, caused symptoms which subsided within four days Shortly afterwards, diarrhea appeared again with the simultaneous shedding of massive quantities of Astroviruses in the feces (Caul, 1987a) It appears that more studies regarding seafood contamination and disease outbreaks caused by astroviruses are warranted

Calciviruses

Human Caliciviruses have been recovered from human feces and have been shown to cause diarrhea and vomiting in children and flu-like symptoms in adults (Cubitt, 1987) A study by Cubitt (1987) showed that Caliciviruses can be transmitted by shellfish In this study, illness was caused by the consumption of contaminated raw oysters and was characterized by a high attack rate among those who ate them

Small Round Viruses (SRV)

While little is known about the characteristics of the Small Round Virus group, it is believed that the group may encompass several different types of viruses Identification of the viruses requires an electron microscope These agents have been given several names by researchers, including the Snow Mountain Agent, Hawaii Agent, etc (De Leon and Gerba, 1990) SRV have been identified as the causative agents in several shellfish -related gastroenteritis outbreaks In one outbreak involving oysters, there was a 79% attack rate among consumers, with nearly 69% of the people that ate just one oyster also affected (Gill et al , 1983) Other outbreaks have been associated with the consumption of clams There is some evidence that dual infections with Norwalk viruses can take place (Caul, 1987b)

PARASITES

Giardia lamblia

Giardiasis is a protozoan infection principally of the upper small intestine *Giardia lamblia*, the causative agent, is associated with freshwater-borne epidemics of gastroenteritis in the United States and worldwide While the fecal-oral route of transmission is well-established, debate exists about its potential to infect seafood and be passed on to humans Symptoms include cramps, bloating, low-grade fever, weight loss and chronic diarrhea At least one outbreak of giardiasis, however, has been traced to contaminated salmon (Eastaugh and Shepherd, 1989) If the protozoa can indeed be transmitted to humans via seafood, this could be particularly problematic in Israel, where raw salmon, in the form of lox, is a popular dish among the Jewish population In countries with adequate wastewater filtration processes, giardiasis does not pose a significant risk,

as the cysts are generally filtered out during treatment It should be noted, however, that concentrations of chlorine used in routine wastewater treatment do not kill *Giardia* cysts, especially when the water is cold (Benenson ed , 1990)

Helminths

Helminths are often not considered an important source of human infection in industrialized countries Worldwide, however, metazoan helminthic parasites represent the most commonly acquired infectious agents from the consumption of fish or shellfish In Egypt, the primary infectious agent transmitted from freshwater fish is the intestinal trematode parasite (Higashi, University of Michigan) Moreover, in some countries helminthic infestation may reach levels as high as 40% of the population (Rim, 1982)

Virtually all infections involving these organisms are associated with the consumption of raw, partially-cooked or improperly-preserved fish or shellfish For many, consuming seafood in this manner is an intrinsic element of their culture, making it extremely difficult to promote modification of this behavior Furthermore, in many countries, including the United States, the consumption of raw seafood products may actually be increasing as delicacies such as sashimi, ceviche and green herring become popular (Higashi, 1985)

Helminths contracted from seafood of concern to man can be broadly classified into three groups Cestodes (tapeworms), Trematodes (flukes), and Nematodes (roundworms)

Cestodes (Tapeworms)

Two fish tapeworms commonly infect man, *Diphyllobothrium latum* and *Diphyllobothrium pacificum* *D latum* is commonly found in fish in North America, Northern Europe and Japan, while *D pacificum* infects marine fish, with most cases occurring in Japan, Peru and Chile (Higashi, 1985)

The life cycle of both tapeworms is similar and requires two intermediate hosts prior to human infection Adult worms residing in the human intestine release eggs which are passed with the human feces Once in the environment, the eggs hatch into larvae which then infect a snail or crustacean Within the first intermediate host, larvae develop into procercoids which, when

released, can then infect fish Humans or other mammals then consume raw or partially cooked fish, completing the circle Adult worms have reached sizes of up to 10 feet in the host intestine Infection often leads to anemia because of the tapeworms preferential absorption of vitamin B-12 (Higashi, 1985)

Species of fish commonly infected with *D latum* include eel, perch, salmon, and lake trout, in Europe, salmon in Japan, and northern pike, wall-eyed pike, burbot and salmon in North America (Higashi, 1985)

Trematodes (Flukes)

Four types of trematodes infect human hosts *Clonorchis sinensis*, *Opisthorchis viverrini* *Heterophyes heterophyes*, and *Metagonimus yokogawai* (Higashi, 1985) Fluke infections related to seafood consumption do not pose a significant health threat in most industrialized nations In less developed regions, especially Asia, these parasites represent the most prevalent disease due to the consumption of raw, partially cooked, or improperly preserved freshwater fish In some areas of southern China human *Clonorchis* infection rates are as high as 40%, while rural areas of South Korea have shown infection rates of at least 11% in school-age children (Rim, 1982)

The fluke life cycle is similar to that of the tapeworm Adult worms live in the bile ducts and release eggs with the feces Eggs are consumed by susceptible snails (*Parafossarulus* spp), larvae develop and multiply, forming cercariae which are then released by the snails Cercariae attach to fish, burrow into the flesh and become encysted metacercariae Metacercariae complete the cycle, infecting humans, as well as other hosts such as cats, dogs and other mammals Over 80 species of fish are susceptible to infection with the metacercariae (Higashi, 1985) One study in Egypt by Tawfik and Hassanien found that of 14 species of freshwater, brackish water and salt water fish, five had encysted metacercariae (Tawfik and Hassanien, 1990)

Adult worms inhabit the bile ducts for many years, potentially leading to cirrhosis of the liver or cancer of the biliary duct *H heterophyes* and *M yokogawai* infections are usually well tolerated, but invasions of the visceral cavity do occur, endangering the life of the host (Africa et al , 1935) *H heterophyes* is transmitted by eating fresh or brackish-water fish, such as mullet,

in a raw, salted or dried state This trematode is a common parasite in the lower Nile valley near the Mediterranean coastline and has also been isolated in the Orient and western India (Ahmed ed , 1991) In Egypt, infection with *H heterophyes* appears to follow a seasonal distribution, peaking during the spring period surrounding the "Sham El Nessim" celebration, although the infection is present year-round (Higashi, University of Michigan) *M yokogawai* closely resembles *H heterophyes* and may be present in freshwater trout in the Orient, the former Soviet Union, northern Siberia and the Balkans (Ahmed ed , 1991)

Nematodes (Roundworms)

There are four species of nematodes commonly spread from fish to humans *Gnathostoma spinigerum*, *Capillaria philippinensis*, *Anisakis simplex* and *Phocanema* spp (Higashi, 1985) All are transmitted to humans through the consumption of raw or partially cooked or preserved fish

From a public health point of view,*Gnathostoma spinigerum* is the most important The organism is acquired through the ingestion of raw or undercooked freshwater fish Infections with this organism have been reported in eastern and Southeast Asia, as well as India and Israel (Ahmed ed , 1991) It is possible that *G spinigerum* is responsible for illness in other parts of the Middle East, but may not be reported due to lack of sufficient data or incorrect diagnosis

The life cycle of *G spinigerum* is similar to that of other fish-borne parasites Eggs are released into the water through the feces of the host (human, dog, cat, etc) Larvae hatch from the eggs and are ingested by the *Cyclops* crustacean, the first intermediate host This crustacean is then consumed by various species of fish Once inside the fish, the larvae migrate to the muscles and become infective larvae Human or other animals are infected when they consume the muscle (Higashi, 1985) Infections in humans are characterized by the migration of the worms from the stomach wall to various parts of the body including subcutaneous locations on the thorax, arms, head and neck The infection induces a creeping eruption and traveling edema in the effected regions (Miyazaki, 1960) Various fish species as well as chickens and ducks may also serve as a source of infection for humans (Daengsvang et al , 1966)

Capillaria philippinensis was first recognized as a human pathogen in 1962 in the Philippines 1,700 cases were documented within a span of six years (Cross and Bhaibulaya, 1983) This parasite is transmitted to humans through the consumption of small freshwater fish which have ingested the embryonated eggs The worm inhabits the host's intestinal tract where it causes severe diarrhea, sometimes leading to death by dehydration Humans, however, are thought to be an incidental host (Cross and Bhaibulaya, 1983) Since the initial burst of cases, the incidence of this disease has declined rapidly (Higashi, 1985), although it is not known why so many infections appeared in one short period of time Single cases have also been reported from Iran and Egypt (Benenson ed , 1990)

Infections due to the parasites *Anisakis simplex* and *Phocanema* spp have also been associated with the consumption of raw fish Hundreds of cases of anisakiasis have been reported in Japan and the Netherlands (Asaishi et al , 1980, Van Thiel, 1976)

The definitive hosts of *A simplex* and *Phocanema* spp are usually marine mammals (seals, dolphins, etc) Adult parasites inhabit the small intestines of the host and discharge eggs with the animals feces The larvae hatch and are preyed upon by various crustaceans which are, in turn, eaten by fish Larvae develop into infective forms in the intestinal tract, organs and muscle tissues of the fish Fish are then consumed by humans or sea mammals to complete the cycle (Smith and Wootten, 1975, 1978) Symptoms appear in humans when ingested worms burrow into the walls of the gastrointestinal tract and cause severe abdominal pains, often mimicking other critical intestinal problems (Oshima, 1972)

In all, over 160 different species of marine fish can be infected by *A simplex* and *Phocanema* spp , including such commonly harvested fish as tuna, salmon, herring and mackerel (Oshima, 1972)

HEALTH IMPACTS

Fish and Shellfish Toxins

Naturally occuring toxins in seafoods cause many outbreaks each year and may be under-reported as a source of seafood-related illness In the United States, seafood-related intoxications were responsible for 17 8% of all food-borne disease outbreaks recorded by the Center for Disease Control between the years 1978-1987 Most of these illnesses were due to either ciguatera or scombroid poisoning

In ciguatera endemic regions of the United States (Hawaii, Puerto Rico, Guam, The Virgin Islands and Florida) 15-20 outbreaks involving 50-100 cases per year are reported Studies conducted in the Virgin Islands, however, indicate that the incidence rate may be closer to 73 cases/10,000 population per year (Morris et al , 1982) There is evidence that a significant number of cases are not reported, implying that the latter number may be more realistic Ciguatera poisoning accounted for approximately half of the recorded intoxication cases in the United States between 1978 and 1987

Scombroid poisoning appears to have a worldwide distribution While it is associated with warm temperatures, it is not limited to tropical or subtropical regions In the United States cases of this intoxication were reported in 45 of the 50 states during the period from 1978 to 1987 The United States Center for Disease Control reports between 12 and 20 outbreaks per year resulting in less than a total of 100 cases per year These numbers are most likely too low, as the disease is generally mild, passes quickly and, consequently, is not reported (Ahmed ed , 1991)

Paralytic shellfish poisoning appears to be highly regional Cases observed in the United States, for example, have been limited to New England in the east and California, Oregon, Washington and Alaska on the west coast Over a 10 year period from 1978-1987 a total of 282 cases were reported by the Northeast Technical Support Unit of the United States Food and Drug Administration Though this disease can be quite severe and possibly fatal, it is possible that mild cases contracted by recreationl clam diggers go unreported or are misdiagnosed

Neurotoxic shellfish poisoning is intimately tied to the red tide phenomena Monitoring areas where episodes occur and closing affected harvest areas when the titer of organisms becomes too high has proven very effective at limiting disease outbreaks in the United States The last reported cases in the U S occured during a red tide incident in North Carolina from 1987 to 1988, in which 48 people were affected due to consumption of contaminated shellfish (Ahmed ed , 1991)

Diarrhetic shellfish poisoning has long been a problem in Japan and is increasingly so in Europe This disease has not yet been identified in the U S , but one documented case occured in Canada in 1990 (Todd, 1989) The toxin responsible for the disease has been found in waters of many different regions, indicating the potential for more widespread outbreaks

Puffer fish poisoning is not a common cause of illness in fish consumers, especially as most people who eat puffer fish are aware of the toxic potential of their meal Occasionally, cases arise in the United States, seven were reported in Florida from 1951 to 1974 with three fatalities, while at least another seven deaths were reported in Hawaii (Ahmed ed , 1991) In Japan however, where fugu is an exquisite delicacy, 20-100 fatalities arise per year, despite stringent regulations controlling marketing and preparation of the dish (Ogura, 1971)

A series of outbreaks in Canada of amnesic shellfish poisoning occured between November and December of 1988 and involved 103 cases with three fatalities This disease has not been reported outside of Canada, but the organism which produces the toxin consumed by shellfish is present in U S waters

Infectious Agents

Infectious agents found in seafood can be classified in three ways (1) pathogens which routinely cause disease in healthy individuals, (2) opportunistic agents which do not normally cause illness in healthy adults but which may affect certain susceptible subgroups (i e children, immunocompromised individuals) and (3) those lacking conclusive evidence of pathogenicity (Ahmed ed , 1991) As detailed in the previous section, *V cholerae, V parahaemolyticus,*

Salmonella, HAV, *Anisakis simplex*, etc , can all cause disease in healthy individuals *V vulnificus* rarely infects healthy adults and is more often associated with illness in patients with other underlying conditions such as cirrhosis of the liver *Aeromonas* and *Plesiomonas* are often found in seafood, but there is insufficient data to implicate these organisms in seafood-related disease outbreaks

Illnesses caused by organisms in the first two categories may exhibit different severities Infections in healthy individuals are usually less invasive and self-limiting, while those in immunocompromised individuals may be more severe and even life-threatening Thus, it is apparent there is a need for different strategies to prevent disease outbreaks in the different population subgroups

The infectious doses required by various pathogens to initiate disease are also quite variable For example some bacteria such as *S aureus* must achieve levels in the food of at least 100,000 organisms/gram before enough toxin exists to cause illness (Ahmed ed , 1991) Other bacteria, such as *Shigella,* may only require as few as 100 organisms to initiate disease (Dupont et al , 1969) Viral and Helminthic pathogens theoretically can cause an infection with just one viable agent Bacteria, on the other hand, can grow in the contaminated food, with time and temperature abuse potentially leading to higher levels of bacterial pathogens over longer periods of time For example, the *S aureus* population can, under proper conditions, double every 10-15 minutes, resulting in levels of organisms greater than 100,00/gram (Potter, 1986) Nevertheless, the higher the dose of the infectious agent, the more likely it is to result in illness

Exposure to seafood containing infectious agents is a function of many different variables The type of seafood consumed, the harvest area , the water quality and the handling and processing of the seafood all determine the level of contamination to which the consumer is subjected For example, in Egypt, infection with *Heterophyes heterophyes* is high during the "Sham El Nessim" celebration This disease is associated with inadequately-cooked or cured fish On the other hand, illnesses related to inadequately cooked Boulti fish (*Tilapia notica*) are not common, as these fish are not eaten raw (El-Zanfaly and Ibrahim, 1982) Because factors surrounding the level of

contamination to which the consumer is exposed vary tremendously and the risks associated with each type of pathogen are distinct, it is difficult to determine the level of risk associated with the consumption of seafood Similarly, it also becomes difficult to mandate comprehensive water quality or product safety standards (Ahmed ed , 1991)

As *Vibrio* populations are highest during the warmer seasons, disease outbreaks due to naturally occurring *Vibrio* spp often take place in the summer months Other factors which may limit or enhance the growth of these species include salinity and the presence of competing organisms (fecal coliforms may compete with indigenous bacteria) (Ahmed ed , 1991) Most *Vibrio* outbreaks are related to the consumption of raw shellfish

Exposure to viruses in seafood is usually directly related to the consumption of raw or improperly-cooked shellfish Those cases not involving shellfish are primarily caused by cross contamination during processing, with the Hepatitis A virus being the most common Viruses are species specific, and those that cause illness in humans will not infect finfish Shellfish, on the other hand, act as passive carriers of the viruses, which may be trapped in their gut or mucus membranes Survival of viruses in marine environments is dependent upon several factors including, temperature, salinity, ultraviolet inactivation and the presence of suspended organic materials or sediments to which viruses can adhere (Kapuscinski and Mitchel, 1980) Low temperatures and the presence of high quantities of organic material in the water help protect viruses from inactivation (Block, 1983) As previously mentioned, the high sodium chloride content in the Middle Eastern waters is a factor which limits the survival of many potentially-harmful viruses

Exposure to metazoan helminthic parasites depends upon several factors including the presence or absence of intermediate hosts, human waste disposal practices and the presence of other non-human definitive hosts Most helminthic parasites rely on several hosts to complete their life cycles, if any of these hosts can be eliminated, the cycle can be broken This, however, is quite difficult as there may be several substitute hosts Humans, for example, are an incidental host of the trematode *Anisakis simplex*, and thus, are not crucial to the survival of this species

Likewise, dogs and cats are often infected with the cestode *Diphyllobothrium latum* and can perpetuate the life cycle even when humans are not infected Treating human wastes adequately before dumping them into water bodies could significantly reduce the prevalence of these infections Similarly, utilization of molluscicides to kill the snail intermediate hosts for a similar parasite, *Schistosoma mansoni*, has resulted in disease reductions in affected areas

In characterizing the health risks associated with the consumption of seafood, it is clear that the practice of eating raw, partially-cooked or improperly-preserved fish and, particularly shellfish, poses the greatest threat to human health This is true in the United States where raw molluscan shellfish account for less than 0 1% of the seafood consumed but cause a far higher proportion of reported outbreaks (Rippey and Verber, 1988) In areas of Asia and the Middle East, however, raw or under-cooked fish is responsible for the higher incidence of parasitic helminth infections, but shellfish also may cause other disease outbreaks

Though the type of seafood associated with illness is generally recognized, the etiological agent may not always be clearly identified In the United States, the infectious agent for the majority of seafood-related disease outbreaks is never clearly established (Rippey and Verber, 1988, CDC, 1989) A small percentage of these illnesses may be non-microbiological in nature, but many are probably due to infections with Norwalk or related viruses (Morse et al , 1986) A great deal of past outbreaks reported in the United States have been characterized by similar incubation periods and symptoms consistent with these agents Difficulty in identifying these viruses, coupled with the fact that only a handful of labs in the United States are capable of diagnosing these infections, leads to a paucity of reported cases by Norwalk and related viruses Research by Morse et al , (1986) suggests that Norwalk and related agents may be the most common pathogens associated with shellfish and cold water clams

Pathogens contracted from shellfish consumption constituting the greatest threat to public health are Hepatitis viruses of unknown etiology and *Vibrio* species including, *V parahaemolyticus*, *V vulnificus*, and Non-O1 *V cholerae* (CDC 1989)

Overall, the risk of infection from bacterial pathogens seems to be lower than that for viruses Studies on the Gulf Coast in the United States, where consumption of shellfish would seem to be the highest, indicate that *Vibrio* species cause approximately 5-1 case/100,000 population/year (Hoge et al , 1989) The number of *Shigella* and *Salmonella* outbreaks related to seafood is difficult to ascertain, because it is often impossible to determine the vehicle of infection in the vast majority of these outbreaks

Assessing the risk from viral contaminated shellfish is difficult due to techinal limitations in isolation and identification of viral strains It is also important to realize that more cases of Hepatitis A will be cited as seafood-related in developed countries because the disease is not common in these areas, facilitating identification of the source of the outbreak In regions where Hepatitis infections (A, Non-A, Non-B) are endemic it is much more difficult to assess from where the disease originated Moreover, lifetime immunity is conferred upon people previously infected with HAV, hence outbreaks may appear more limited than expected

Parasitic infections resulting from seafood consumption in developed countries are even more rare than bacterial or viral-related outbreaks (Ahmed ed , 1991) Risk factors associated with the contraction of Helminthic parasites, such as consuming raw or partially cooked fish, are, however, rising in some areas This may lead to an increasing number of outbreaks in these countries (Higashi, 1985) Parasitic infections in developing areas such as the Middle East, however, are still commonplace

In many regions of Asia, where most of the cases of parasitic infections are found, reducing the numbers of individuals infected will be challenging In these locations, the parasite life cycles are well-established and traditional practices are often ingrained Consequently, there is is a higher probability of helminthic infection when raw fish are consumed here, as compared to viral or bacterial infections The low infective dose also contributes to the high prevalence of parasitic infections

MITIGATION OF HEALTH IMPACTS

Fish and Shellfish Toxins

There are many effective measures that can be taken to limit disease outbreaks due to toxin-laden seafood It is advisable to monitor harvest areas for the presence of the toxigenic dinoflagellates responsible for producing the toxins When levels in the water exceed a certain limit, harvest areas should be closed until the threat has dissipated This approach has worked very well in the United States in the prevention of paralytic shellfish poisoning and neurotoxic shellfish poisoning, but is not highly effective in prevention of ciguatera or scombroid poisoning

In addition to better monitoring of harvest areas, simple and accurate tests to identify toxins in shellfish and fish need to be developed and implemented on a wide scale Domestic and imported seafood should be regularly tested for the presence of toxins Furthermore, information and rapid detection methods should be made available to recreational fishermen and shellfish gatherers to prevent outbreaks in these groups

Puffer fish poisoning could be virtually eliminated through bans on imports of fugu and restricting public consumption of this delicacy People, however, are generally aware that these fish are poisonous, yet continue to eat them Consequently, forbidding consumption of this potentially lethal food may not be feasible

As scombroid poisoning is a result of improper handling procedures which lead to the formation of histamine in the tissues of the fish, prevention methods should focus on better sanitation Cooling fish immediately after capture would generally be enough to prevent growth of the bacteria responsible for the formation of histamine

Infectious Agents

Many strategies have been designed to reduce the potential for contracting infectious diseases from the consumption of seafood These range from simple measures, such as cooking all seafood thoroughly, to complex methods, such as irradiating raw shellfish prior to consumption Clearly, more appropriate methods of monitoring harvesting areas need to be

developed and utilized Techniques for limiting disease outbreaks will be different for each region according to its unique seafood-related pathogens Moreover, the means available to a country to prevent and diagnose outbreaks will cover an enormous spectrum Rich countries, which serve as a market for seafood harvested in poorer regions, may find it worthwhile to work with these governments in developing comprehensive monitoring networks and universal water quality standards to reduce disease outbreaks in all regions

The single most important step in reducing the probability of contracting an infectious disease from seafood is to ensure thorough cooking of all fish and shellfish Virtually all seafood-borne pathogens can be killed or inactivated after a relatively short exposure to a high temperature The cestode *Diphyllobothrium latum*, for example can be inactivated by heating the fish to a temperature of 56°C for just five minutes (Higashi, 1985) HAV inactivation however, requires a higher temperature (White and Fenner, 1986) Almost all pathogens can be eliminated from seafood, given the right combination of time and temperature The *S aureus* enterotoxin, however, is quite heat resistant, and may not be inactivated even under high temperatures Though this is the simplest and most practical preventative measure to implement, it is difficult for people to accept, as various types of raw seafood are considered delicacies in many regions of the world Raw seafood will, therefore, continue to be consumed despite the higher risk of infection

Educating consumers about the risks involved in consuming raw seafood may also prove an effective strategy Information campaigns should focus on alerting people of the various pathogens associated with seafood, proper handling procedures, and seasons in which raw shellfish should not be consumed Health workers need to be especially diligent in warning members of high risk categories, such as those with chronic diseases or elderly and very young consumers, to avoid raw shellfish entirely, as the consequences of an infection may be much more severe in these groups (Ahmed ed , 1991)

Attention also needs to be directed towards proper wastewater treatment methods, emphasizing the hazards involved in the dumping of human wastes in harvest areas Many of the pathogens found in seafood are of fecal origin and could be reduced or eliminated by proper

wastewater treatment The life cycle of the cestode *D latum*, for example, is largely dependent upon the human definitive host Adequate processing of human wastes, however, could lead to a significant reduction in infections attibutable to this organism In Finland, for example, the prevalence of this disease declined from 20-25% in 1952 to 1 8% in 1972, almost exclusively as a result of better sewage treatment (Von Bonsdorff and Bylund, 1982) There is a similar potential to reduce outbreaks caused by HAV, *Salmonella*, *Shigella*, and other organisms as well Some parasites and naturally occurring *Vibrio* species, however, will not be significantly affected by better sewage treatment practices

Traditional water quality indicators such as fecal coliform counts do not adequately predict levels of indigenous *Vibrio* spp , nor are they indicative of virus concentrations or survival potential in marine water or shellfish Moreover, fecal coliform counts in shellfish may be elevated by the presence of indigenous species such as *Klebsiella pneumoniae*, which pose no threat to humans and may outnumber *E coli* by as much as 1000 to 1 (Paille et al , 1987) The fecal coliform count, in this case, would be of marginal relevance Clearly, new indices for measuring *Vibrio* spp must be developed and implemented When indicators show high *Vibrio* populations, as in the warm summer months, harvesting areas should be closed and illegal fishing curtailed Similarly, measures of viral contamination need to be developed which closely mimic the survival of HAV and Norwalk viruses in the marine environment and shellfish Various indicators of viral contamination have been proposed, but all have proven less than ideal Sobsey (1990), for example, found that HAV persists for significantly longer periods of time in clams and oysters than either *E coli* or poliovirus Thus, there is a need for more research in this area

In addition to better indicators of seafood-borne pathogens, new diagnostic methods to detect *Vibrio* spp , HAV and Norwalk viruses must be developed Routine sampling of seafood in markets may help to prevent some disease outbreaks More importantly, however, better diagnosis of seafood-related diseases could help track the sources of the outbreak, thus preventing future occurrences If rapid diagnostic tests were available, it would also help clarify the nature of disease outbreaks and provide more insight into their etiology

Reducing disease outbreaks by utilizing better processing technologies is another possibility Simple measures, such as proper refrigeration, may be enough to keep *Vibrio* and other bacterial pathogens from multiplying in recently-harvested shellfish Studies conducted by Cook and Ruple (1989), showed that bacterial counts in shellfish grew rapidly when they were left unrefrigerated on the decks of boats on warm days, even for short periods of time Rapid cooling procedures used immediately after harvest have also shown promise in reducing the growth of bacterial pathogens (Ahmed ed , 1991)

Low temperatures are also fairly effective at reducing the probability of contracting some helminthic parasites Infective larvae of the cestode *D latum* were killed by freezing fish at -18°C for 24-48 hours (Bylund, 1982) Similarly, other parasites may be inactivated by storing fish at low temperatures for specific periods of time

Bacterial standards for fish have been proposed by Buras et al , (1987) Muscle tissue containing 0-10 bacteria/ml is considered to be very good quality, while those with counts of 10-30 bacteria/ml would be medium quality Fish with muscle tissue showing counts above 50 bacteria/ml should be rejected These guidelines could be used to evaluate the quality of both native and farmed fish Widespread inspection of fish muscle samples could help reduce outbreaks Toxic components, such as histamine, could also be evaluated and action taken to minimize the potential for human ingestion of these contaminants

As natural environments become more polluted, and indigenous fish stocks become depleted, aquaculture will be increasingly considered as a viable alternative for raising seafood Aquaculture offers the opportunity to control the environment and the pathogens to which the fish are exposed This type of farming could help limit seafood-related disease outbreaks and produce healthier, larger fish for consumption

Aquaculture, however, has to be performed in a scientific manner in order to ensure control of fish pathogens which also can infect humans Measures must also be adopted to prevent over-crowding and stress of the farmed fish, as this is the most important factor in bacterial and viral penetration of fish tissues (Dotan et al , 1988)

When wastewater is used in farming activities, analysis of the waste constituents should be performed to limit fish exposure to excessive bacterial concentrations or harmful chemicals present in the water Studies by Dotan et al (1988) conducted in wastewater reservoirs in Israel showed that fish grown under these circumstances may harbor bacteria in the digestive tract at levels 1 to 3 orders of magnitude higher than in the surrounding waters Exposure to chemicals, especially detergents in the waters, is believed to be the cause of these high levels, as these contaminants exacerbate microbial uptake by the fish

Another approach for reducing pathogen populations in seafood is the use of irradiation Gamma irradiation of live shellfish and fresh or frozen seafood is very effective at reducing *Vibrio* populations to safer levels Oysters irradiated with 1-1 5kGy (10^5 rads) reduced initial *Vibrio* counts of 10^6 to undetectable levels (Giddings, 1984, Kilgen et al , 1988) At this level of exposure, there was no significant organoleptic deterioration (Kilgen et al , 1988) Similarly, parasitic helminths will also be inactivated at these levels, but some viruses may be more resistant to radiation than either bacteria or infective parasite larvae (Potter, 1986) A study by Digirolamo et al , (1972) found that a 90% reduction of poliovirus 1 was achieved in raw oysters only after the oysters had been exposed to 4 kGy (10^5 rads) This level of exposure, however, led to an unacceptable level of organoleptic deterioration of the oyster

Methods for limiting the outbreak potential of seafood need not be so complicated or expensive as those mentioned above Less cost-intensive practices, such as immediate cleaning of the seafood, may also help Much of the increase in the incidence of anisakiasis, for example, can be attributed to new processing procedures Before refrigeration, fish caught at sea were cleaned immediately, removing most of the worms which normally live in the intestinal wall and other abdominal viscera Presently, however, fish are cleaned and processed onshore, allowing the larvae to migrate into the muscle tissue and subsequently infect consumers of the raw product, 'green herring' (Oshima, 1972, Smith and Wooten, 1975, Higashi, 1985)

Similarly, many of the infective larvae of various nematodes can be inactivated by marinating the raw fish in 4% acetic acid for 5 5 hours (Daengsvang, 1949) Other chemical treatments or methods of preservation need to be investigated as well

Depuration, a technique used to reduce seafood-borne outbreaks, involves placing live fish and shellfish in a controlled clean water environment to permit them to cleanse themselves of impurities, including bacteria and viruses Depuration rates vary according to species, environmental conditions, specific types of pathogens involved and differential uptake rates (Richards, 1988) Depuration of tilapia, a popular fish among Egyptian consumers, has been shown to be effective, especially under conditions of low bacteria concentration (Buras et al , 1985) This same experiment also concluded that tilapia was an unusually strong species, capable of surviving higher concentrations of both chemical and bacterial pollutants

Evidence demonstrates depuration is a useful technique for removing bacterial pathogens of fecal origin Organisms such as *E coli*, *Salmonella* spp , and others can be depurated fairly rapidly and efficiently under appropriate conditions For other species, including most of the naturally occurring *Vibrio* species, there is little evidence that depuration allows elimination of these organisms and may even facilitate multiplication in shellfish (Richards, 1988)

Furthermore, depuration rates are not the same for viruses as for bacteria Sobsey (1990), reported that HAV persists far longer in oysters and clams than either *E coli* or Poliovirus Richards (1988), reported that improperly-depurated shellfish have been implicated in several seafood-borne viral outbreaks, suggesting it is not reliable to assume depurated shellfish are safe to eat

Generally, data involving depuration rates for HAV, Norwalk viruses, *Vibrio* spp and some other enteric bacterial pathogens is lacking Moreover, laboratory studies may not accurately reflect naturally-occurring phenomena There is some evidence to suggest that uptake rates may be higher in natural environments, where organic particles absorb viruses, and depuration rates occur more slowly (Richards, 1988) Other complications include the fact that depuration may not occur at a uniform rate, rates of cleansing decrease with time, and pathogens may only be reduced to low

levels rather than being completely eliminated from the organism (Richards, 1988) This low level of contamination may be acceptable for most bacterial pathogens, but would be unacceptable for viruses due to the low infectious doses required to cause disease

Depuration may also be useful for removing bacterial pathogens from fish Fish living in waters where bacterial counts exceed certain levels may have bacterial penetration of tissues Placing fish in waters with lower bacterial counts has been shown to reduce bacterial concentrations in the muscle tissues (Bartone et al , 1990) Helminthic parasites, however, are not affected by depuration

For depuration to be used effectively, well-designed flow-through systems are required Detailed information regarding the species of shellfish and specific pathogens that might be present could then be used to determine whether or not this procedure was valid and the time intervals required for adequate cleansing Moreover, bacterial concentration thresholds should be established above which depuration would not be attempted Clearly, more studies are needed to better explore the usefulness of this option

CONCLUSION

The risks involved with the consumption of seafood vary greatly according to where the seafood is harvested, how it is prepared and how it is eaten In the United States and other industrialized countries, the main threats from seafood are due to the presence of toxins, especially ciguatera and scombroid, and viruses followed by naturally occurring *Vibrio* spp in raw shellfish In regions of Asia and the Middle East, helminthic parasites pose the greatest threat to human health due to the widespread consumption of raw or inadequately-preserved fish

Strategies for reducing disease outbreaks in all regions, however can be implemented Through the regulation of harvesting in areas characterized by high numbers of toxic dinoflagellates, the prevention of fecal contamination of harvesting areas and the establishment of representative indicators of both viruses and natural *Vibrio* spp , many disease outbreaks could be avoided While altering people's customs and traditions may not be feasible, the consumption of raw seafood could be made safer with the establishment of comprehensive monitoring and inspection networks

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Seafood Safety and Fish Decontamination: Technical and Health Implications

Chemical Contamination of Fish in Egypt

EXECUTIVE SUMMARY

ORGANIC CHEMICALS

HEAVY METALS

CONCLUSIONS

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Executive Summary

Evaluating chemical and heavy metal concentrations in fish harvested from Egyptian waters is not a clear cut process Data concerning chemical concentrations in fish is lacking for many substances and is inconsistent across regions Additionally, when data is available it is difficult to compare these levels to accepted international standards because there is generally no consensus as to what levels in the fish are safe For example cadmium standards range from 0 05 - 5 5 mg Cd/kg wet weight of fish depending on which country's standard one chooses Nevertheless for some chemicals there has been enough data collected from most regions to determine if a threat to public health exists

Of the organic chemicals investigated only chlordane, endrin and PCB levels exceed the most conservative international standards and then only infrequently Heavy metal concentrations however pose more of a problem Cadmium, lead and chromium levels in many fish samples were higher than the minimum standards and in some cases higher even than the maximum limits Mercury and selenium concentrations also exceeded the most conservative international standards in some cases but this information comes from one study of the Wadi El-Kaiyan Lakes and thus more data from other regions is required before any conclusions can be drawn

ORGANIC CHEMICALS

Table 1 shows maximum levels of organochlorine contaminants in fish from Egyptian fish harvesting areas and table 2 illustrates legal limits for organic compounds from various countries

Table 1

Maximum Levels (ug/kg wet weight) of Organochlorine Contaminants in Fish From Egyptian Fish Harvesting Areas (1981-1987)

Location	Reference	Number of Samples	Aldrin	Dieldrin	Chlor-dane	Sum DDT	Endrin	Hepta-chlor	HCB	Lindane	Mirex	PCI
Mediterranean Sea												
Alexandria	(1)	2	N.D.		N.D.	37		N.D.		N.D.	N.D.	
Abu Qir Bay	(2)	10	20	11		157	12	5.3	7.8	29	0.96	9
Port Said	(1)	17	17		N.D.	119		9.0		N.D.	N.D.	
	(3)	24	69		5.6	298	70		18	28		120
Suez Canal												
Ismalia	(1)	16	5 6		36	399		N.D.		N.D	N.D.	
Suez	(1)	17	71		N.D.	223		8.1		9.2	N.D	
Red Sea												
Hurghada	(1)	21	34		N.D.	964		N.D.		N.D	N.D	
Lakes												
Nasser	(1)	3	4.0		N.D.	19		7.0		N.D.	N.D.	
Maryut	(2)	6	13	10		40	7.7	2.8	9.1	34	0 41	2
	(4)	7				135*				76*		
Idku	(2)	3	3.4	1.3		41	3.8	1.5	3.2	19	0.17	1
Burullus												
Manzala	(1)	11	24		N D.	192		N.D.		59	N.D.	
Nile River												
Aswan	(5)*	10	N.D.	N D.	N.D.	65*	16	N.D.		12		
Assiut	(5)	10	N.D.	N.D.	N.D.	139*	43	N.D.		16		
Cario	(5)	10	N.D.	N.D.	N.D.	87*	28	N.D.		12		
El-Mansora	(5)	10	N.D.	N.D.	N.D.	162*	45	N.D.		13		
Faraskour	(5)	10	N.D.	N.D.	N.D.	113*	35	N D		16		
Edfina	(5)	10	N.D.	N.D.	N.D.	98*	28	N.D.		15		
Canals												
El-Mahmodia	(5)	10	N.D.	N.D.	N D.	199*	86	N D.		16		
Abo El-Gheit	(5)	10	N D	N.D.	N.D.	180*	52	N D		19		

80 samples in study - assumed 10/station

* Mean value

(1) Naguib (1988)
(2) Nabawi et al (1987)
(3) El-Dib and Badawy (1985)
(4) Sadd et al (1981)
(5) Aly and Badawy (1984)

Table 2

Legal Limits For Hazardous Organic Chemicals in Fish and Fishery Products

(mg/kg wet weight).

try	Aldrin/ Dieldrin	Chlor- dane	DDT	DDTs	TCDD[1] 2,3,7,8 Dioxin	Endrin	Hepta- chlor[2]	Hexa- chloro- benzene	Kepone	HCH Lindane	Mala- thion	Mirex	Para- thion	PCBs	Toxa- phene	Ch
da	0.1	0.1	5.0	5.0	20	0.1	0.1		0.1	0.1	0.1	0.1	0.1	2.0	0.1	
ark			2.0-5.0													
any (FRG)[4]	0.5-1.0	0.01		2.0-5.0		0.01	0.01	0.5		2.0		0.01				
and										5.0						
herlands														5.0		
ien	0.1			5.0				0.2		0.5				2.0-5.0		
tzerland														1.0		
land	0.1, 0.3			5.0		0.3	0.3			0.5	0.6		0.2			
ed States	0.3	0.3	5.0	5.0	25	0.3	0.3	0.3	0.3-0.4			0.1		2.0	5.0	
e																
imum	0.1	0.01	2.0	2.0	20	0.01	0.01	0.2	0.1	0.1	0.1	0.01	0.1	1.0	0.1	
imum	1.0	0.3	5.0	5.0	25	0.3	0.3	0.5	0.4	2.0	0.6	0.1	0.2	5.0	5.0	0

ng/kg

& Heptachlor epoxide

Legal limits exist for other organic chemicals

erences; Nauen (1983), U.S. FDA (1982,1984)

Aldrin

With the exception of the Nile River Aldrin has been detected in fish from all the major water sources in Egypt including the Mediterranean Sea (Alexandria, Abu Qir Bay, Port Said), the Suez Canal (Ismalia, Suez), the Red Sea (Hurghada), and various lakes (Nasser, Maryut, Idku, Burullus, Manzala) Fish from the Mediterranean Sea have shown levels ranging from 17 - 69 ug/kg wet weight of fish The highest concentration recorded in harvested fish was reported in the Suez canal where a value of 71 ug/kg wet weight of fish was found The mean concentration of Aldrin found in fish harvested in Egyptian waters was 26 1 +/- 25 0 ug/kg wet weight of fish Standards for Aldrin concentrations in fish from nine different countries range from 100 - 1000 ug/kg wet weight of fish Therefore the values detected in fish harvested from Egyptian waters are generally safe for human consumption

Dieldrin

Data for Dieldrin levels in fish harvested in Egypt is generally lacking, however values of 11, 10, and 1 3 ug/kg wet weight of fish have been recorded at Abu Qir Bay, Lake Maryut, and Lake Idku respectively These levels are significantly below international standards which range from 100 to 1000 ug/kg wet weight of fish and thus there does not seem to be any imminent danger from consuming fish containing these levels of dieldrin

Chlordane

Chlordane was detected in fish at only two locations in Egypt Values of 5 6 and 36 ug/kg wet weight of fish were detected at Port Said and in the Suez Canal at Ismalia respectively

Though chlordane contamination of fish does not appear to be a widespread problem in Egypt the concentration detected in fish at Ismalia exceeds the chlordane standard set by Germany of 10 ug/kg wet weight of fish but is safely within the limits set by the United States of 300 ug/kg wet weight of fish More research is required to better determine an acceptable standard for chlordane contamination of fish

DDT(sum)

DDT or its derivatives was found in fish from every location tested in Egypt i e , the Mediterranean Sea (Alexandria, Abu Qir Bay, Port Said), the Suez Canal (Ismalia, Suez), the Red Sea (Hurghada), various lakes (Nasser Maryut, Idku, Burullus, Manzala), the Nile River (Aswan, Assiut Cairo, El-Mansora, Faraskour, Edfina) and in Canals El-Mahmodia, Abo El-Gheit) The range of concentrations spread from 19 to 964 ug/kg wet weight of fish with a mean value of 183 5 +/- 205 9 ug/kg wet weight of fish The highest concentration was reported in fish caught in the Red Sea at Hurghada Though DDT or its derivatives is ubiquitous in the Egyptian environment the concentrations do not exceed the lowest legal limit as set by Germany or Denmark of 2000 ug/kg wet weight of fish

Endrin

Endrin concentrations in fish have been studied extensively in the Nile basin, in the two canal locations (El-Mahmodia, Abo El-Gheit), but data is lacking from the Suez Canal, the Red Sea, Alexandria and the following lakes, Nasser, Burullus and Manzala Endrin concentrations ranged from 3 8-86 ug/kg wet weight of fish The highest value was reported from the El-Mahmodia Canal and the mean value for all the sites tested in Egypt was 35 5 +/- 25 2 ug

endrin/kg wet weight of fish According to German standards for endrin concentrations in fish every level above 10 ug endrin/kg wet weight of fish would be unacceptable If this standard was used in Egypt every fish sampled except for fish caught at Lake Maryut or Lake Idku would be unacceptable for human consumption If the U S standard of 300 ug endrin/kg wet weight of fish was applied all samples would be considered safe

Heptachlor

In most areas of Egypt where fish was tested for heptachlor it was not detected In areas where fish did show this chemical the levels were low ranging from 1 5 to 9 0 ug heptachlor/kg wet weight of fish The highest value was found in fish harvested from the Mediterranean Sea at Port Said These values however all fall below the conservative German standard requiring less than 10 ug heptachlor/kg wet weight of fish and thus the samples pose relatively little risk to consumers

Hexachlorobenzene

Fish were not tested for this compound in the Nile basin, Lakes, Nasser, Burullus and Manzala, the drainage canals, the Red Sea, nor the Suez Canal Existing data shows a range of 3 2 - 18 ug hexachlorobenzene/kg wet weight of fish These values however should not be reason for concern as they are all significantly below the lowest legal limit of 200 ug hexachlorobenzene/kg wet weight of fish set by Sweden More data may be useful in determining if this is true for all areas of Egypt

Lindane

Lindane was found in Fish caught from most regions except for the Red Sea, Alexandria, Ismalia, and Lake Nasser The range of concentrations was from 9 2-76 ug lindane/kg wet weight of fish, the highest value was reported from Lake Maryut The average value was 24 88 +/- 18 9 ug lindane/kg wet weight of fish Lindane standards of less than 100 ug lindane/kg wet weight of fish were the most stringent as set by Canada All samples taken from Egyptian harvest areas easily conform to these restrictions and thus there is little threat to public health

Mirex

Concentrations of mirex in fish have not been evaluated in the Nile river basin or adjacent canals Mirex levels of 0 96, 0 41, and 0 17 ug/kg wet weight of fish have been found in fish harvested from Abu Qir Bay, Lake Maryut, and Lake Idku respectively Standards for mirex concentrations in fish range from 10 to 100 ug mirex/kg wet weight of fish Clearly the levels detected in fish harvested in Egypt are well below even the most conservative international standards and there is thus little cause for concern in theseareas

PCBs

PCB concentrations of fish have only been investigated at four locations in Egypt The four sites, Abu Qir Bay, Port Said, Lake Maryut, and Lake Idku yielded fish with concentrations of 90, 1200, 22, and 18 ug PCB/kg wet weight of fish respectively Though these levels are safely under the most conservative standard of 2000 ug PCB/kg wet weight of fish it would be

useful to determine PCB concentrations in fish from the Nile river and adjacent canals as well as from the Red Sea to ensure that all fish taken from these waters comply with this standard

HEAVY METALS

Table 3 summarizes heavy metal concentrations in fish taken from the Wadi El-Raiyan Lakes, table 4 illustrates legal limits of heavy metals in fish and fishery products from selected countries

Table 3
Concentration of Heavy Metals in Fish *(Tilapia zilli)* of Wadi El-Raiyan Lakes (ug/dry g)

Organ and tissue	Year	Pb	Hg	Se	Cd	Cu	Zn
Whole body	1	3 7-4 4	3 8-4 9	3 6-4 1	0 7-1 3	2 2-0 2	5 2-0 2
	2	5 0-7 8	3 5-5 0	5 6-6 0	0 8-1 2	3 8-4 7	6 4-9 1
	3	6 9-8 9	3 0-4 1	2 9-4 1	0 3-1 3	4 7-5 3	2 5-8 8
Muscle	1	3 1-5 0	2 9-3 7	3 3-3 8	0 5-1 2	1 3-2 0	4 1-8 5
	2	4 3-8 8	2 4-3 8	5 7-6 3	0 5-1 2	2 9-4 0	5 2-9 2
	3	5 1 -7 2	2 3- 3 0	1 4-5 0	0 1-1 1	3 4- 5 3	1 8-14 1
Skin	1	15 8-25 5	18 2-20 0	14 6-16 8	1 0-2 4	12 8-14 3	16 8-19 1
	2	13 5-14 8	21 1-25 4	13 9-21 2	0 7-1 3	14 5-16 1	18 9-22 4
	3	14 6-18 1	25 2-27 1	12 1-18 9	0 3-1 5	16 8-17 2	13 4-15 9
Head,tail and fins	1	22 4-25 6	9 5-10 1	5 8-6 4	1 6-2 2	15 0-18 1	5 6-6 8
	2	27 7-3 1 1	7 3-8 2	7 2-7 9	1 9-2 4	14 1 - 16 3	7 8-9 2
	3	27 9-30 8	5 0-6 0	4 5-6 1	1 8- 3 8	7 5-10 0	6 5-9 8
Internal organs	1	I 1 9-25 9	6 5-15 1	9 7-10 1	1 8-4 8	11 0-14 4	5 2- 15 9
	2	16 4-30 4	7 2-10 2	6 0-8 2	1 3-2 3	16 1 - 18 2	6 3-21 3
	3	14 3-28 3	3 5-4 4	3 5-4 1	1 7-2 8	14 2-20 1	5 6- 18 4
Skeleton	1	5 8-6 0	11 1 - 12 2	3 9-4 5	0 1-0 9	4 5-6 1	8 9-9 4
	3	4 6-5 5	15 2-16 9	2 I -3 0	0 1-0 7	6 8-8 0	3 4-4 1

Taken from Saleh et al (1988)

Table 4
Legal Limits for Hazardous Elements in Fish and Fishery Products
(mg/kg wet weight)

Country	Sb	As	Cd	Cr	Cu	Pb	Hg	Se	Zn
Australia	1 5	1,1 5[2]	0 2-5 5		10-70	1 5-5 5	0 5,1	1,2	40-1000
Brazil							5[3]		
Canada		3 5				5	5[3]		
Chile		0 12,1	05		10	2		05, 3	100
Denmark							5		
Ecuador		1			10	5	1		
Finland		5				2	1[3]		
France							5, 7		
Germany			5			5	1		
Greece							7[4]		
Hong Kong	1	1 4-10	2	1			6	5	
India		1			10	5	5[3]		50
Israel							5		
Italy						2	7[3]		
Japan							3, 4[3]		
Netherlands			05-1			5,2	1 0[3]		
New Zealand	1	1	1		30	2	5[3]	2	40
Philippines		3				5	5[4]		
Poland		4			10-30	1-2		30-50	
Spain								5	
Sweden						1-2	1[3]		
Switzerland			1			1	5		
Thailand		2			20	1	5		
United Kingdom		1		20-10					
United States							1[3]		
U S S R.							2-1		
Venezuela		1	<LOD- 1		10	2	1- 5		
Zambia		3 5-5			100	5-10	2- 3[4]		100

1 Limit varies aong states
2 Inorganic
3 Total
4 Methyl
References Nauen (1993), U S FDA (1982, 1984)

Lead

Figure 1 shows the frequency distribution of lead levels in fish flesh from Egyptian fish harvesting areas Lead is a common contaminant found in fish harvested in Egyptian waters Fish taken from the Red Sea area have lead concentrations ranging from 1 1 -32 3 mg Pb/kg wet weight of fish The mean level is 6 73 mg Pb/kg wet weight fish Fish from the Mediterranean Sea showed a range from 1 9 - 5 6 mg Pb/kg wet weight fish with a mean value of 3 07 mg Pb/kg wet weight fish Waters of the Nile and adjacent canals yielded fish with concentrations from 0 92 - 19 6 mg Pb/kg wet weight fish International standards for lead levels in fish range from 0 5 - 10 mg Pb/kg wet weight fish An appropriate standard range might be from 0 5 - 5 0 mg Pb/kg wet weight fish (Nriagu, 1993) If a protective standard of 2 mg Pb/kg wet weight fish is used then data from a study by Naguib (1988) showed that 93, 78, and 84 percent of all samples from the Red Sea, the Mediterranean Sea and all freshwater sources respectively, exceeded this limit This study indicates that lead contamination of Seafood may be a serious problem in Egypt

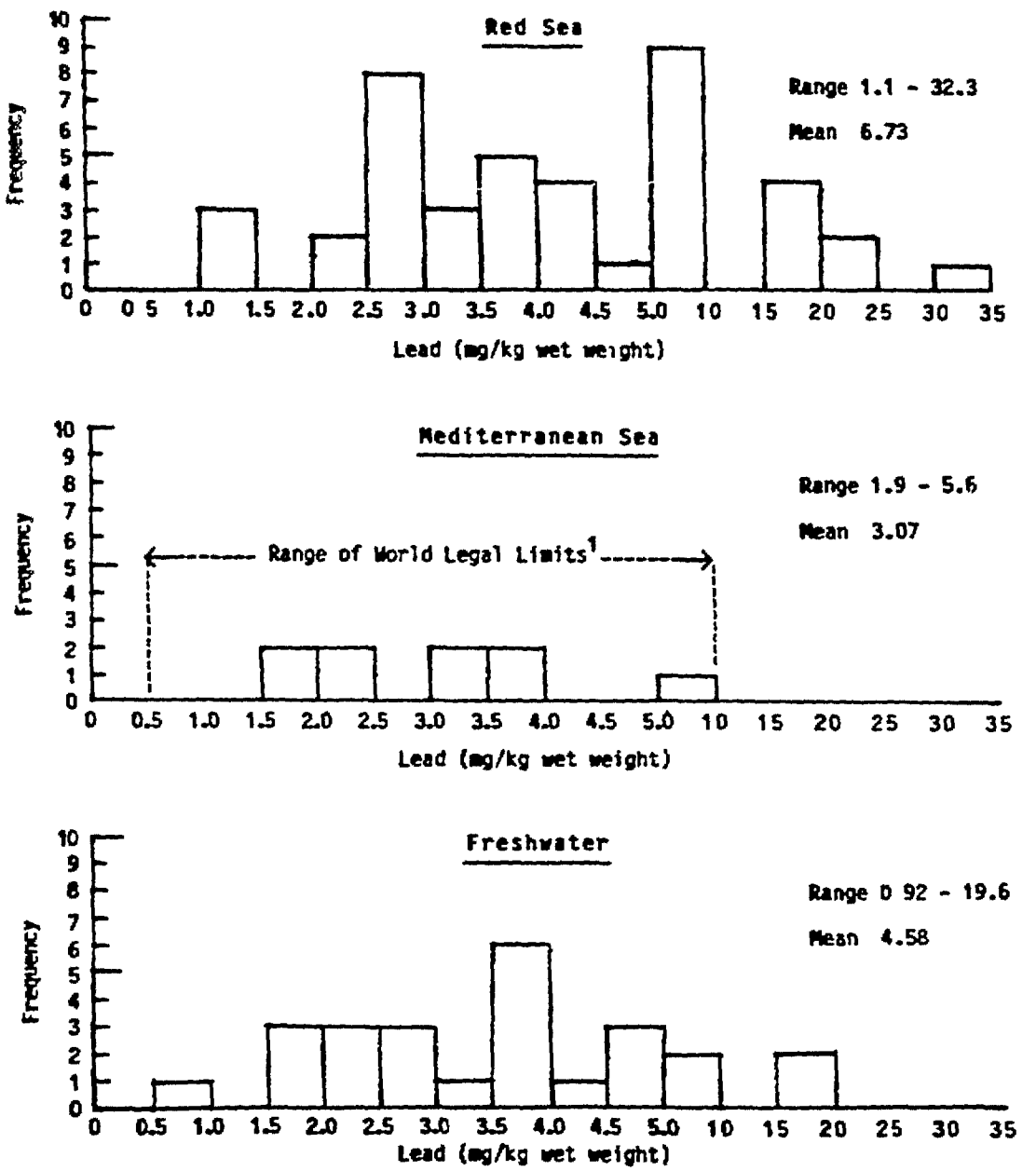
Another study by Saleh et al , (1988) illustrated that older (and usually larger fish) had higher lead body burdens and that lead was not distributed evenly throughout the fish The highest concentrations were detected in the head, tail and fin regions, followed by the skin and internal organs The muscle tissues of a fish showed the lowest concentrations and thus eating only this portion of the fish may reduce the exposure to lead

Mercury

Figure 1

Frequency Distribution of Lead Levels in Fish Flesh

From Egyptian Fish Harvesting Areas (1986-87)



Data from Naguib (1988)

1) Nauen (1983)

Data regarding mercury contamination of fish harvested from Egyptian waters is fairly limited Saleh et at , (1988) found mercury concentrations in fish from the Wadi El Raiyan lakes to range approximately from 3 - 5 mg Hg/kg wet weight fish (the original data was for mg Hg/kg dry weight) International standards regulating mercury in fish range from 1 - 1 0 mg Hg/kg wet weight fish There is evidence that mercury is absorbed by the body more readily than lead because mercury often exists in a methylated form in the fish (Nriagu, 1993) The appropriate standard depends upon whom consumes the fish and how often it is consumed A standard of 1 0 mg Hg/kg wet weight fish implies that 227 g of such fish can be safely consumed by a typical 70 kg male in one week If conditions vary from this ideal then the standard should be revised accordingly Although the values given fall in between the range of standards, the paucity of data regarding concentrations of mercury in fish makes it difficult to determine the degree of hazard to which consumers are subjected to from other fish taken from Egyptian waters

As with lead, data from Saleh et at , (1988) suggests mercury is not stored uniformly in all the tissues of the fish The highest levels of mercury were detected in the skin and skeleton Removing the skin before consumption may reduce this source of exposure

Selenium

Selenium contamination of fish harvested from Egyptian waters has only been studied by Saleh at al , (1988) for the Wadi El Raiyan lakes Levels were found from 0 29 - 0 60 ug Se/kg wet weight of fish International standards for selenium range from 50 -2000 ug Se/kg wet weight of fish Selenium levels reported from these lakes are well below either of these standards but more data from other areas is required to ensure that there are no hot spots

Selenium also appears to be predominantly stored in the fish skin and thus removal of this component may reduce exposure to this compound

Cadmium

Figure 2 illustrates the frequency distribution of cadmium levels in fish flesh from Egyptian harvesting areas Cadmium levels in fish were reported from the Red Sea, the Mediterranean Sea and freshwater sources In the Red Sea values ranged from 0 13 to 1 0 mg Cd/kg wet weight of fish with a mean of 0 35 mg Cd/kg wet weight of fish Levels in the Mediterranean Sea ranged from 0 14 - 0 56 mg Cd/kg wet weight of fish with a mean of 0 30 mg Cd/kg wet weight of fish Freshwater fish concentrations were slightly higher ranging from 0 0 - 1 7 mg Cd/kg wet weight of fish with a mean of 0 41 mg Cd/kg wet weight of fish There is no consensus in the international limits of cadmium in fish, the values show an extreme range from 0 05 - 5 5 mg Cd/kg wet weight of fish A conservative limit may be preferable to protect human health If a standard of 1 mg Cd/kg wet weight of fish was adopted most of the fish samples would be acceptable for human consumption but advisories nay still be necessary because approximately 2 -5% of all samples could exceed this limit

Cadmium distribution in fish appears to be more uniform than for the other metals previously mentioned There is thus no practical way to reduce cadmium exposure from fish consumption but since levels are relatively low this is not a major concern

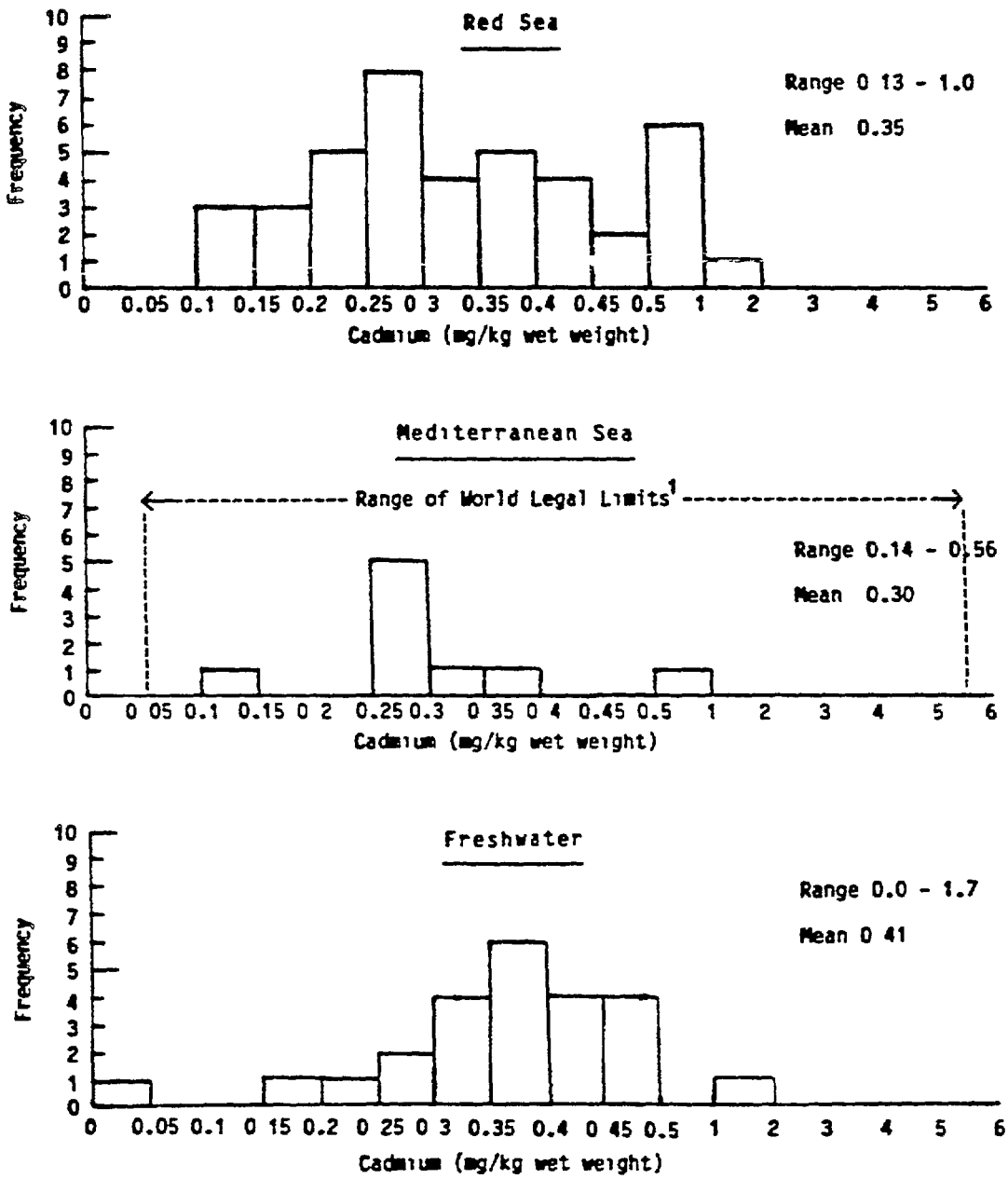
Chromium

Figure 3 shows the frequency distribution of chromium levels in fish 'flesh taken from Egyptian fish harvesting areas Chromium contamination of fish in the Red Sea, Mediterranean

Figure 2

Frequency Distribution of Cadmium Levels in Fish Flesh

From Egyptian Fish Harvesting Areas (1986-87)



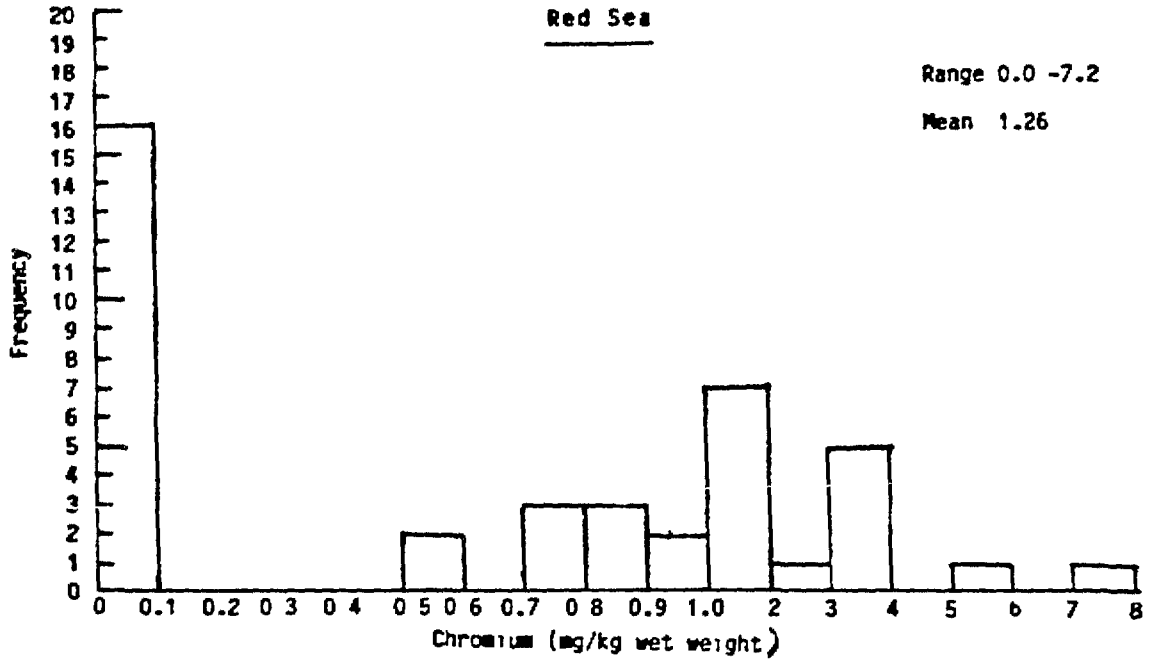
Data from Naguib (1988)

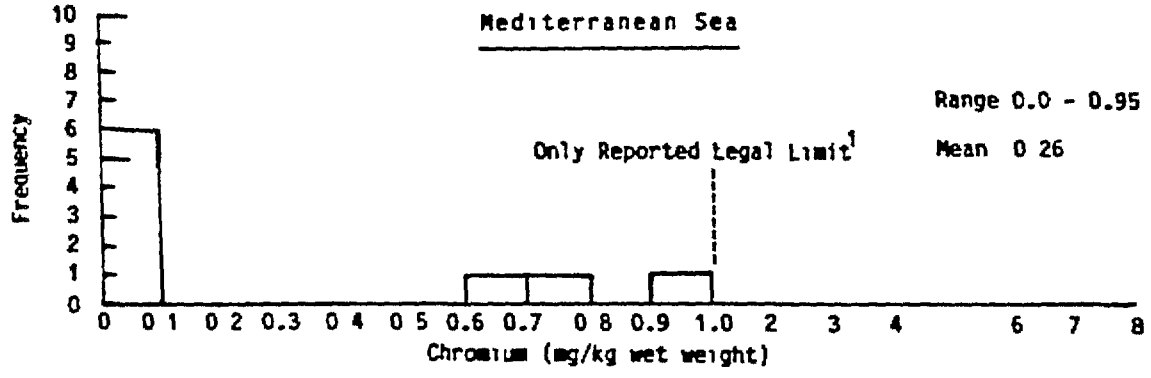
1) Nauen (1983)

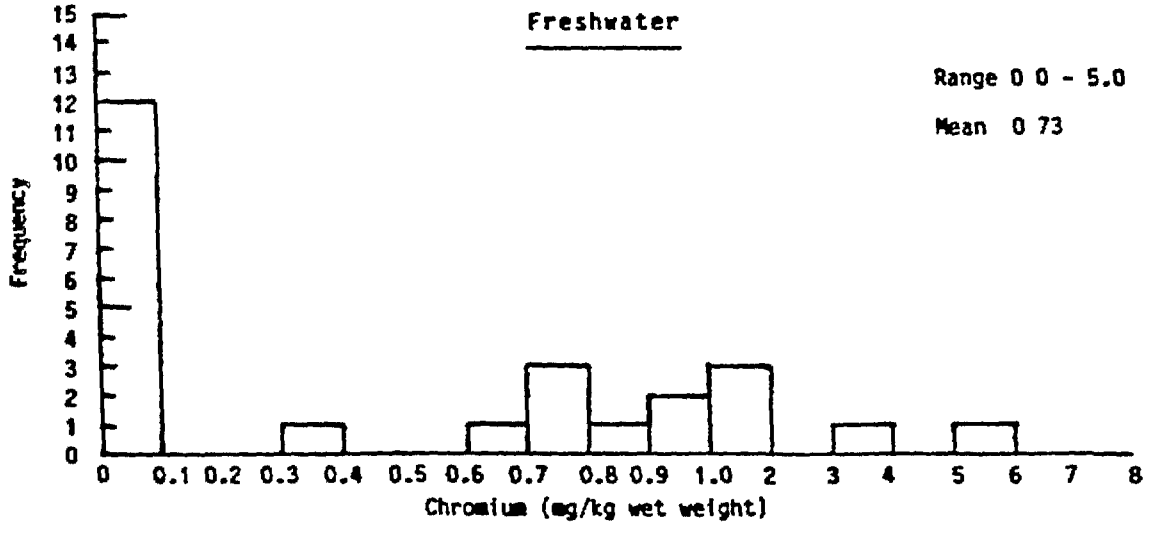
Figure 3

Frequency Distribution of Chromium Levels in Fish Flesh

From Egyptian Fish Harvesting Areas (1986-87)







Data from Naguib (1988)

1) Nauen (1983)

Sea and in fresh water fish has been reported by Naguib (1988) Red Sea levels from 0 0 - 7 2 mg Cr/kg wet weight of fish were reported with a mean of 1 26 mg Cr/kg wet weight of fish Concentrations found in Mediterranean fish were generally lower with a maximum value of 0 95 mg Cr/kg wet weight of fish, the mean was 0 26 mg Cr/kg wet weight of fish Freshwater levels of 0 - 5 0 mg Cr/kg wet weight of fish were recorded with a mean of 0 73 mg Cr/kg wet weight of fish The only reported standard for this metal is 1 0 mg Cr/kg wet weight of fish from Hong Kong If this number is an acceptable standard then approximately 37, and 20 percent of all fish samples taken from the Red Sea and freshwater sources respectively exceeded this limit Chromium contamination in Egypt may therefore be a problem, but more research may be needed to determine an adequate standard

Copper

Data for concentrations of this element in harvested fish is lacking but a study conducted by Saleh et al , (1988) found levels of copper from 0 02 - 0 53 mg Cu/kg wet weight of fish in the Wadi El Kaiyan lakes Because international standards for copper start at 10 mg Cu/kg wet weight of fish, these copper levels are not currently a concern in these lakes but other areas should also be investigated

Zinc

Zinc levels in the Wadi El Raiyan lakes as reported by Saleh et al , (1988) ranged from 0 02 - 0 91 mg Zn/kg wet weight of fish Standards from other countries consider zinc levels above 30 mg Zn/kg wet weight of fish to be hazardous and thus these levels are of no concern However, levels in fish from other areas must also be investigated to ensure that locally contaminated sites are not causing problems

CONCLUSION

Continued investigation of contaminant levels is warranted especially with regard to heavy metal concentrations in fish harvested from various waters Additionally Egypt needs to evaluate chemical standards and adopt levels that protect human health and at the same time take into account the special conditions present in Egypt Lead concentrations seem to be quite high in some regions especially pans of the Red Sea and various freshwater sources Areas with high contaminant levels should be restricted to fishing Remedial efforts and pollution prevention measures also should be incorporated to reduce contaminant levels

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Chemical Decontamination Models

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Abstract

There are three general approaches for reducing the levels of chemical contaminants in aquatic organisms that are centered around the pharmacokinetics of uptake and clearance of a chemical and biodilution of toxic residues by growth These include, 1) reduction of the concentration of the chemical in water, 2) allowing contaminated organisms to depurate in clean water and providing them with uncontaminated food and 3) increasing the growth rate during the uptake and /or depuration phases to dilute the contaminant concentration in the organism In this section of the report research effects to model the depuration and biological processes is presented

The overall depuration rate is taken to be a first order rate process This is combined with a zero order intake rate to provide a simplified model which could be used to start the chemical depuration experiments

A detailed discussion of the kinetics of the rates of bioaccumulation and depuration of chemicals in fish is given in the appendix

Potential Public Health Problems From Chemical Contaminants

The Hidden Threat

Throughout the world chemical contamination of food is an issue of growing concern This is more evident in the case of seafood where organisms are continuously exposed to highly complex mixtures of hydrocarbons in fuel oils, pesticides herbicides heavy metals and other exotic organic and inorganic compounds With worldwide industry using about 65,000 chemical compounds and around 1,000 new compounds being introduced each year, there is a myriad of possible combinations of chemicals that may directly or indirectly find their way into wastewater (U S GAO 1988) Currently one would be hard-pressed to find surface water, including that used in any type of aquaculture, that is free from pollution Because of numerous reports on the contamination of aquatic environments and the importance of seafood in the human diet, aquatic organisms require special attention

Metallic and organic toxicants entering a pond in wastewater or leached from pond fixtures and equipment (Boydon and Romerial 1975) may affect fish and shellfish survival, or at sub-lethal concentrations they may impair their growth It is important to note in this discussion that water pollution will exhibit a more harmful effect on aquaculture than natural aquatic systems because the cultured species have a limited capacity to adapt, avoid or escape pollutants It is possible that very low levels of contaminants may not visibly affect fish and shellfish growth and survival yet via bioaccumulation these low concentrations could pose a public health to consumers and thereby affect the viability of the sewage aquaculture industry (Little and Muir 1987)

The potential health hazard from consumption of organisms which have been harvested from chemically contaminated sewage could most likely no be human toxicosis from large doses of toxicants over a short period of time (Brock, 1986) Rather it is quite likely that the consumer would be exposed to low levels of an indeterminable number of hazardous substances over an extended period of time with no observed effects for years However, their lifetime risk of disease such as cancer would be increased (Cordle, 1981)

The magnitude of the public health threat may be substantial Scientists do not know the human health risks from exposures to 90 percent or more of all chemicals that get into the environment since it is difficult for them to develop direct relationships between levels of many chemical contaminants and human illness Because of these uncertainties, the potential hazard of these chemicals remains unclear (U S GAO, 1988)

Hypothetical Approaches to Show Potential Problems

Some examples of xenobiotics which may not threaten fish and shellfish growth, survival, or reproduction, and yet bioaccumulate to levels which could pose a public health problem, can be provided by using two similar hypothetical approaches

The first approach involves comparing the maximum acceptable toxicant concentration (MATC) or hypothetical toxic threshold concentration for aquatic organisms to a criteria level in water which is set to protect humans from undue risks form seafood consumption (U S EPA, 1986) for example, the MATC for PCBs using fathead minnows has been reported to be between 1 1 to 15 ug/l (McKim, 1985) this is substantially higher that 0 079 ng/l for water which is the U S EPA criteria concentration that may result, via bioconcentration, in 10^{-6} lifetime risk of increased cancer incidence from consumption (Table 1)

Table 1 General Information on Health Aspects, Fish Survival, and Legal Limits For Hazardous Compounds in Fish and Fishery Products

Compounds	Priority Pollutant	Known Carcinogen	MATC[2] ug/l	U S Criteria Conc [1 4] Fish Consumption, ng/l	U S mg/kg	World mg/kg
Aldrin	Y	Y		079	3	1 1 0
Dieldrin	Y	Y		076	3	1-1 0
Chlordane	Y	Y		48	3	01- 3
DDT	Y	Y	36 1 5	024	5 0	2 0-5 0
DDTs	Y	Y				
Dioxin	Y	Y		000014	25 (ng/kg)	20 -25
Endrin	Y	N	14 - 30	1000[o]	3	01- 3
Heptachlor & Heptachloro-epoxide	Y	Y	086 1 84	.29	3	01 - 3
Hexachloro-benzene	Y	N		74	3	.2 5
Kepone					3 4	1 - 4
Lidane	Y	Y	9 1 - 23 5	N E	N E	1 2
Malathion	N	N		N E		1 5
Mirex	N	N		N E		01 - 1

	Priority Pollutant	Known Carcinogen	MATC[2] ug/l	U S Criteria Conc [1 4] Fish Consumption ng/l	U S mg/kg	World mg/kg
Parathion	N	N		N E	N E	1 2
PCBs	Y	Y	1 1 15	079	2 0	1 0 5 0
Toxaphene	Y	Y	025 299	73	5 0	1 5 0
Vinyl chloride	Y	Y		525 000	N E	01
Element						
Antimony	Y	N		45 mg	N E	1 0 1 5
Arsenic	Y	Y		17 5 ng		1 10
Arsenic III	Y	Y		N E		
Arsenic V	Y	Y		N E		
Cadmium	Y	N	1 1 80 [H]	10 [O] ug	N E	0 5 5
Chromium III	N	N		3433 mg	N E	1 0
Chromium VI	Y	N				
Copper	Y	N	9 5 104 4 [H]	N E	N E	10 100
Lead	Y	N	31 3 253 [H]	50 [O] ug	N E	5 10
Mercury (methyl)	Y	N	07 93	146 ug	1 0	1 1 0
Selenium	Y	N		10 0 [O] ug	N E	05 2 0
Zinc	Y	N	26 0 1368 [H]	N E	N E	30 1000

1 U S EPA (1986)
2 McKim (1985)
3 Nauen (1983) U S FDA (1982) U S FDA (1986)
4 Risk level for carcinogens = 10^{-6}

[O] Value for water and fish ingestion
H = Hardness dependent
Y = Yes
N = No
N E = Not established

The second approach is more direct and involves looking at the levels accumulated in organisms during chronic bioassays where no observed effect is reported For example, McKim, et al (1976) observed that brook trout could accumulate methyl mercury to levels which exceeded legal limits without affecting the fish's survival, growth, or reproduction Similar results have been reported for mirex (Skea et al, 1981) these examples show that chemical contamination of organism is not always apparent and that there is a need foe chemical analysis of tissues to determine if there is potential public health threat from their consumption

What are Acceptable Levels for Contaminants in Seafoods?

The acceptable levels for contaminants in seafood depends upon what levels of risk an agency group society or person is willing to tolerate These levels are not permanent They may change due to public pressure, increased knowledge about the toxic effects of

may change due to public pressure increased knowledge about the toxic effects of contaminants, or improved techniques in determining risk The following discussion is constructed to illustrate different degrees of concern over the consumption of contaminated seafood and how these concerns relate to what are considered acceptable levels The purpose of this discussion is to show that in certain cases sewage-aquaculture systems may need to be modified to meet different ideals

Compliance with legal limits is probably at the top of the list of concerns with management personal and regulators However, these limits will vary from place to place because people are different and are willing to accept different risks, and also because the database and method of calculating legal limits varies Levels of contaminants in wastewater-cultured organisms would not meet legal limits if these products were sold in other countries Hence there would be a need to modify these wastewater aquaculture systems if the products were sold somewhere else

There may be potential public health hazards, even though a nation's legal limits are met, or if one place adopts the legal limits of another (e g , the Marianna Islands use U S FDA Standards) For example when the U S FDA sets action levels or tolerances, they make generalizations about consumers and their consumption patterns, e g , 6 5 g of seafood are consumed by a 70 kg make per day (U S EPA 1980) Average consumption rates for portions of the US population may vary from 6 to 100 g seafood/day and hence the risks from seafood consumption are not evenly distributed throughout the population (Suta 1978, SRI 1980, Puffer et al 1982) Also, legal limits may not take into account sensitive subgroups in a population Eating contaminated seafood before and during pregnancy, even in small amounts, may result in substantial developmental problems in the exposed fetus and in nursing children Also, young children who are not yet sexually mature may be especially sensitive to the effects of chemical contaminants (National Wildlife Federation 1989) Therefore it is often difficult to determine, based on legal limits that a sewage-aquaculture system will produce a product that is acceptable

for human consumption by an entire population This further illustrates the need for options to reduce contaminant levels in wastewater grown organisms

Even if local standards are set for contaminants in seafood less assumptions are made about the consumer and sensitive subgroups are considered there still may be a need for further reduction in contaminant levels before the seafood may be considered unquestionably safe and acceptable for human consumption

Seafood can be simultaneously contaminated by many chemicals Little is known about the toxic effects of some of these chemicals and toxic interactions between chemicals Thus, the magnitude of the risk due to consumption of the seafood is unknown The total risk from all the chemicals present in seafood may be higher than the risks that calculated, usually individually, for the known toxicants

For carcinogens the levels in any medium should theoretically be zero for maximum protection of human health However, this may not be feasible and thus residue levels are chosen that may result in a small (e g 10^{-6}) increased risk of cancer over a lifetime Since most of the models used to determine these risks are linear at low dose (U S OTA 1979) any significant decline in contaminant levels should translate proportionally into reduced risks

Given the aforementioned problems it would be responsible product stewardship to keep toxic residue levels as low as possible Again, this shows the need for options to reduce contaminant levels in wastewater-grown organisms

Ways to Reduce Residue Levels in Cultured Aquatic Organisms

In order to emphasize the importance of reducing contaminant levels in cultured organisms as a means of reducing risks from their consumption, it is of value to consider other possibilities for lowering these risks

One way to reduce risks from consumption of contaminated cultured aquatic organisms is to eat less of them However, this might increase risks from other disease like malnutrition from insufficient protein intake or it might increase risk of heart problems Another way to

reduce risks from consumption of these organisms is to use food preparation and cooking techniques which lower the contaminant levels This could be an effective approach but it would be difficult to get universal compliance because is some places there would be problems related to information dissemination and the changing of personal habits and local customs The most effective approach to reduce risks from consumption of contaminated cultured aquatic organisms would be to reduce residue levels in these organisms

The ways in which residues levels in cultured aquatic organisms can be reduced are centered around the pharmacokinetics of uptake an depuration of a chemical Prior to describing the pharmacokinetics it is necessary to define what uptake routes are going to be considered An organism will obtain its pollutant from eating contaminated food and also from breathing contaminated water The potential for residue accumulation by fish and shellfish through the food chain is usually assumed to be relatively insignificant, i e , < 10% (Macek, 1979) However, in certain cases exposure through the food chain is likely to occur only for chemicals which are very lipophilic, i e , $P_{ow}>10^5$ (Bruggeman et al 1988, Thomann, 1989) Hence, for many chemicals and aquatic organisms bioconcentration from water is adequate to describe accumulation of chemical residues

Bioconcentration is often modeled by considering it to be a balance between two kinetic processes, uptake across the gill membrane from water and depuration from the organism (also referred to as clearance) Both processes generally can be satisfactorily described using first order kinetics with the uptake rate dependent upon the contaminant concentration in water, and the uptake rate constant and the depuration rate a function of the residue level in the organism and the depuration rate constant This can be mathematically described by the following

$$C_f = (k1/k2)\ C_w\ (1-e^{k_2 T})$$

where C_f C_w = contaminant concentration in fish and water respectively

k_1, k_2 = uptake and depuration (d^{-1}) rate constants, respectively

t = time

At steady state the ratio C_f/C_w is called the bioconcentration factor (BCF) and equals k_1/k_2 (Neely 1979)

The residue level is an organism at any given time will depend upon the contaminant concentration in water, duration of exposure uptake and depuration rates, as well as the growth rate which facilitates a biodilution of accumulated residues (Spacie and Hamelink, 1985) Extensive environmental factors such as biomass and organic content of the water and sediments may have and influence on residue levels in organisms by reducing the availability of a toxicant for uptake (Vanderford and Hamelink, 1977 Ellgehausen et al 1980) Also, intensive environmental factors like temperature can affect accumulated residue levels (MacLeod and Pessah 1973 Reinert et al 1974)

There are at least three approaches for reducing the levels of contaminants in aquatic organisms 1) reduce the concentration of the toxic constituent in water by decreasing direct inputs implementing pretreatment or maintaining a high organic content and/or biomass in pond water or sediments 2) depurate the organism in "clean" water and provide "uncontaminated" feed, and 3) increase the growth rate during uptake and/or depuration phases

Depuration of Residue Levels

Biological Half-lives

The feasibility of depuration to significantly reduce contaminant levels in fish heavily depends upon the clearance rate constant or the biological half-life In this study, the depuration of toxicants following the introduction of fish into clean water will be described predominantly by a first-order single compartment model with the understanding that this is a mathematical construct of a multicompartment system which may at times follow a non-first order clearance (Spacie and Hamelink 1982) Depending on the exposure level and the mode of elimination, models with biphasic second order or Michaelis-Menten kinetics may at times prove to be better choices (Spacie and Hamelink 1982) If elimination occurs through passive diffusion at the gills or other membranes first-order kinetics are generally appropriate The clearance rate of a

foreign chemical from an organism changes with time and this can be mathematically represented by the following differential equation

$$\frac{dC}{dt} = -k_2C \qquad (2)$$

When integrated this becomes

$$C_t = C_0e\text{-}k_2t \qquad (3)$$

Where (Ct) is the concentration of the chemical in the organism at time (t) of depuration and (C0) is the initial concentration at the beginning of depuration (t=0) The elimination rate constraint (k_2) in units of time-1 is determined from the slope of the line

$$\ln C_t = \ln C_0\text{-}k_2t \qquad (4)$$

The first order elimination rate constant (k_2) is a proportionality constant that relates rate of elimination and amount in the organism For first order elimination the biological half-life, $t_{1/2}$, is determined by k_2 as follows

$$t_{1/2} = \ln 2/k_2 = 0\ 693/k_2 \qquad (5)$$

The chemical's biological half-life will often be used to describe the elimination of the toxicant from fish because it is an easily understood parameter by laymen as well as scientists Furthermore, for first order elimination $t_{1/2}$ can be directly converted to the depuration rate constant (equation 5) Also either of these descriptions, $t_{1/2}$ or k_2, can be used to determine the time required to reach a percentage of steady state accumulation of BCF (Zitko, 1977, Spacie and Hamelink, 1985),

i e ,

$$\%BCF = 100\ (C_f/Cw)/(k_1/k_2) = 100\ (1\text{-}e^{k_2t}) \qquad (6)$$

The k_2 can be used to calculate the time required by a fish to achieve the BCF level, but not the magnitude of this level Knowing the time to reach steady state may be of value for some chemicals (especially those with long $t_{1/2}$) when determining the trade off between the growing time of fish in contaminated water verses the degree of tissue contamination Although the biological half-life of a substance is very useful for comparing the retention of chemicals and time to steady state, the feasibility of using depuration in order to lower residual levels to acceptable values will also highly depend on the extent to which a substance exceeds the desired level since the elimination rate is concentration dependent (equation 2)

Ways to Predict Biological Half-lives

Approaches for predicting biological half-lives may be divided into inter-chemical predictions (predicting k_2s for unknowns using one of their physical properties and relationships between that property and k_2s of known compounds) or intra-chemical predictions (predicting k_2s for a given compound using relationships between that compound and experimental variables)

There exists no simple formula for predicting, for all chemicals, their biological half-lives For some non-metabolized non-polar organic contaminants, negative relationships have been established between these chemicals n-octanol/water partitioning coefficient and their depuration rate constants (Ellgehausen et al 1980 Konemann and Van Leeuwen, 1980, Spacie and Hamelink 1982) This concept was expanded upon by Gobas et al (1986) and Barber et al (1988) to include in their models, along with the physiochemical property of the chemical, biological attributes of the fish These relationships are useful for making predictions within a homologous series of compounds and generalizations about this relationship for other chemicals (e g elimination rate constants are extremely low for "super lipophilic" compounds) They cannot be applied to other hazardous substances where metabolism plays a decisive role in excretion (Southworth et al 1980 Southworth et al 1981) or when the chemical does not have a partitioning coefficient (e g metals) While these models are useful for a rough estimation of the depuration rate constant for some untested chemicals, they often contain large errors of a

magnitude or more, and thereby they are not much use for accurately predicting depuration rates in the "real world For this purpose intra-chemical models using tested chemicals are needed rather than inter-chemical models

Ideally in order to accurately estimate the biological half-life of hazardous substances in the "real world " laboratory experiments should include many experimental variables (e g , fish species, fish weight water temperature, etc) Unfortunately this is not economically feasible Uptake and/or depuration experiments are usually conducted independently by different researchers to answer specific questions related to a chemical (e g , K_1 k_2, BCF) using convenient fish and environmental conditions which are conducive to survival This lack of standardized test conditions has often resulted in discrepancies in the literature on biological half-lives and other pharmacokinetic descriptors (e g BCF) This fact, along with at times the quality of research has created a literature database that, at first glance, one would be hard-pressed to use to make certain predictions from This is especially so when a statement might influence decision makers For example, the half-life of hexachlorobenzene has been reported to be 5-12 days (Neely et al 1974, Sanborn et al 1977, Konemann and Van Leeuwen 1980) and around 200 days (Niimi and Cho 1981, Niimi and Palazzo 1985) One could rather safely say that the half-life for hexachlorobenzene is between 1 week and 8 months This degree of accuracy may not be adequate for certain types of decisions, especially when money and time constraints are considered, like in the case of depurating chemically contaminated cultured fish

Scheme for Determining Which Model to Use

When making a decision on what model and kinetics is most appropriate to describe the elimination of a foreign substance from the fish, the following scheme, using information from Spacie and Hamelink (1982) shall be followed For persistent, apolar, lipophilic compounds, which are eliminated by passive diffusion, first order kinetics shall be chosen to describe the elimination If a chemical is eliminated predominantly by a metabolic pathway that involves enzyme systems, then the clearance may follow Michaelis-Menten kinetics This can be identified by the half-life increasing with residue concentrations and the kinetics shifting form

zero order to first order as the residue level declines and the enzyme system changes from a saturated to an unsaturated states In this case the kinetics used to describe the data will depend upon which relationship is significant (P<0 05) and best fits the data The exception to this rule for choice of kinetics occurs when the experiment is conducted for a time period less than the half-life In this case it is difficult to distinguish first order from zero order clearance If evidence from other experiments suggest that the kinetics follows first order clearance then this will be selected over zero order elimination even thought the latter may fit the data

If only two data points are reported for the elimination phase, the selected kinetics model will be one that is consistent with similar studies on the chemical

If two or more compartments exist which clear at different rates, this can be identified by the half-life increasing with decreasing concentrations Some researchers have tried to describe this phenomena by use of concentration dependent second order kinetics (Ellgehausen et al 1980) Since this model implies that residues become more persistent as they decline, which is mechanistically difficult to account for (Spacie and Hamilton 1982), a two compartment model employing first order kinetics will be used to describe the biphasic data

Variables Which Affect Depuration

Variables which affect depuration are tabulated and discussed in Appendix A Variables include such factors as (1) chemical and fish species (2) fish size (initial weight before uptake and average weight during depuration) (3) exposure and elimination duration and temperature, (4) media analyzed (whole fish muscle or elimination of water), (5) the initial level of the toxicant in the organism or its muscle tissue at the beginning of depuration and (6) lipid content A comprehensive list of factors which may affect elimination rates is given in Table 2 below Not all of these factors are included in the compiled studies because some of the factors are only rarely looked at

Table Two List of factors that may affect laboratory derived elimination

Experimental Methodology	Biological
Exposure duration	Species
Elimination duration	Tissue
Water renewal time	Lipid Content
Dose level	Egg and sperm maturation & spawning and sex
Model selection	
Growth compensation	
Preexposure to chemical	
Exposure Route	**Related to Metabolism**
Food	Weight
Water	Temperature
Other (injection, intragastrically, etc)	Ration (starved or fed)
	Ventilation volume
	Oxygen concentration
	Fish behavior (activity)
Chemical	
Species	
Properties	
Interaction of accumulated contaminants	
Calcium concentration in the water	

Since growth can proportionally reduce reported half-lives based on concentration it is of value to elaborate on the implications of increasing body size Historically it has been assumed that the change in fish weight during a laboratory study is small and has insignificant effect on the chemical half-life, but this is not always the case When the depuration phase of an experiment is quite long, as may be the case for very lipophilic compounds, substantial growth dilution may be occurring along with elimination Both of these processes will facilitate a reduction in the contaminant level in the fish If they occur simultaneously this will result in a higher calculation of K_2 or lower estimate of $t_{1/2}$ than that produced by elimination along unless one of the following procedures is followed

1) Fish are depurated under a maintenance diet that does not result in growth A 4% ration is considered a maintenance ration for many fish (Hastings 1969) This may be an appropriate approximation (Fisher et al 1986) or may not be appropriate (Argyle et al 1975) In either case the level of the toxicant can significantly affect the growth rate (Argyle et al 1975) So it may be difficult to determine if there is growth based on ration,

2) Growth is measured and the half-life determination is corrected for the influence of growth dilution (e g , Niimi and Cho 1981 Branson et al 1985),

3) Body burden is measured by either monitoring a radioactive, non-metabolizable substance in a live fish or by monitoring the decline of the amount of substance in whole fish or tissues by multiplying the contaminant concentration by the weight of the fish or tissue

The clearance of xenobiotics from biological systems is a complex dynamic process resulting from the interplay of several parameters which not only include properties of the compound involved (such as solubility and structure) but also characteristics of the biological system (lipid content weight species age sex etc) environmental influences (temperature dissolved oxygen, etc), and behavioral patterns (e g activity)

In spite of the complexity of reactions and processes involved in elimination of a substance and the confounding influence of internal and external factors which affect the elimination rate it is important to determine if simple relationships can be established between experimental variables and a chemical's ability to be eliminated These relationships could aid in extrapolating laboratory date to different field conditions

Statistics and Relationships

According to Norstrom et al (1976) the clearance of a chemical from the whole organism should be directly related to turnover of the tissue components, which, in turn should be related to metabolic rate or some other species-specific time-dependent, and definable fraction, such as body size and/or adiposity Relationships between the depuration rate constant and such variables as ration, temperature and weight all of which can influence metabolic weight, may or may not show a metabolic dependence of chemical depuration

Excellent examples of these relationships are provided for methyl mercury (Fagerstrom et al 1974 Sharpe et al 1977 Rodgers and Beamish 1982) Collectively these studies suggest that the depuration of methyl mercury is not strictly dependent on metabolic rate and may be species specific Regardless of the mechanism a number of these studies show a strong

dependency of depuration on weight (DeFreitas et al 1975, Sharp et al 1977) There is little evidence in the literature to either contradict or confirm this type of dependency for most other chemicals Norstrom et al (1977) and Thomann and Connolly (1984) showed that the dependency of PCB elimination rate constant on body weight is consistent with their findings From a very preliminary investigation using some of the data that has been compiled for this proposal, relationships have been established between body weight and depuration rate constants This indicates that this approach shows promise in revealing some very useful information However, the extent to which other experimental variables could influence elimination may be difficult to assess at the present time because of limited information available on a contaminant with different half-lives over a range of experimental variables

Beyond the field application of relationships between experimental variables and clearance rates the relationships could help to better delineate to what degree k_2 relationships may aid in refining inter-chemical comparisons based on physicals properties (e g , log P-log k_2) by normalizing the data (e g , k_2 for 100g fish) Also, relating the clearance function to fish weight, or some other parameter would provide valuable information for clearance models used in this study as well as models concerned with predicting pollution accumulation in the environment (e g Norstrom et al 1976)

Biodilution of Residue Levels

The biodilution of contaminant levels in fish tissue by growth in "clean" water using "clean" food is modeled by incorporating a growth function into the previously mentioned single compartment models (Branson et al 1985, Spacie and Hamelink 1985) A mathematical description of incorporating growth into both linear and exponential short term (<1 year) growth models is given in Appendix 1

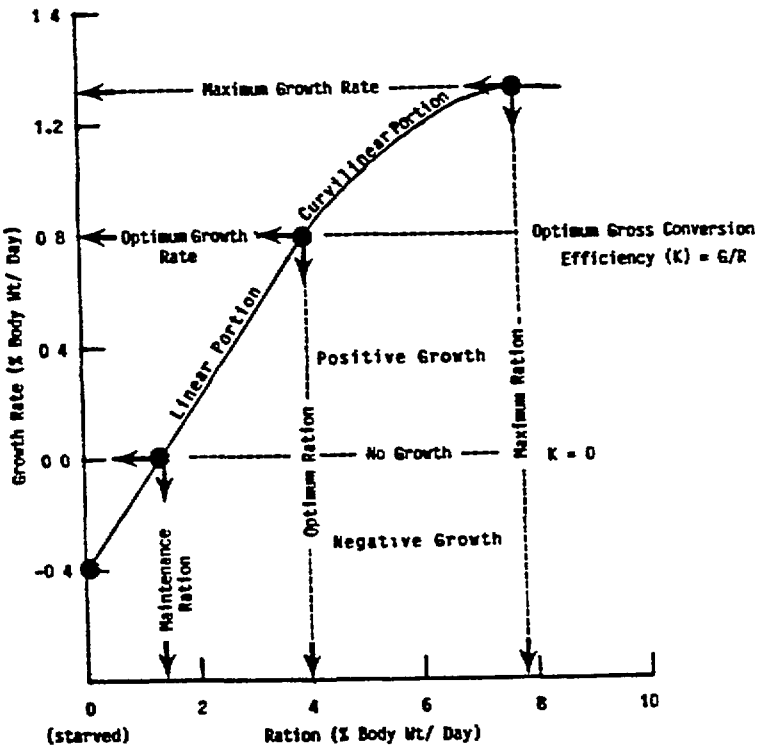
Those industrial and agricultural compounds that have half-lives which may range into years, such as mirex PCBs, and methyl mercury (Skaar et al 1981, Niimi and Oliver 1983, Ruohtula and Miettinen 1975) can only undergo significant reduction in tissue levels in a reasonable period of time by growth dilution The prior choice of an appropriate growth model to

study this effect is critical The time required for growth dilution of a chemical contaminant to half its original concentration could vary depending upon the model (linear or exponential) by a factor of as much as 1 44 if only the initial growth rate (day 0 - day 1) is considered or if a percentage/day figure is converted to a growth rate and inserted into these models

Another important consideration when studying the effect of biodilution on residue levels is the selection of fish growth rates and setting the conditions for those factors which influence growth rates Fish growth is the difference between metabolizable energy (calories consumed x assimilation efficiency) and respiration requirements (standards metabolism + specific dynamic action + active metabolism) Many of the aforementioned variables that determine growth are themselves affected by other factors such as temperature, salinity, food availability, fish size etc Because growth rates are controlled by various biotic and abiotic factors which often are interrelated it is of value to assume a set of conditions so as to simplify things Making assumptions when dealing with aquacultures can be done with more certainty that in nature because these systems can be manipulated by feeding and not allowing the fish to reach maturation For example growth rate can be related to and to a large degree, controlled by the feeding rate and food conversion efficiency (100 x weight gain/amount of food) Thereby, this reduces the influence of a number of physiological and environmental variables on growth

The maximum physiological growth rate is a condition where food availability (quality and quantity) will not limit growth (Hepher 1978) This occurs when fish consume the maximum ration (see Figure 1 at the top of the next page) The maximum growth rate can be achieved by a fish as long as food a particular nutrient or unfavorable environmental conditions (e g low D O) do not limit growth (Hepher 1978) This growth rate can be maintained for the fish in a pond up to the critical standing crop, the biomass at which limiting factors come into play This level can be extended by satisfying the limiting factors Temperature can also greatly influence the growth rate (Brett 1979)

Figure 1 Maximum Growth Rate



Modified from Brett (1979)

Feasibility of Depuration and Biodilution to Decontaminate Fish as Determined by Size, Time, and Cost Constraints

In order to determine the feasibility of depuration-growth to lower fish residue levels, market size, time, and cost constraints must be considered The main factors (output variables) that need to be determined are (1) The time for a set percentage of the fish population to reach specific chemical concentration level, (2) the average weight of the fish at the end of the process, and (3) the cost of producing the fish The values for the three factors (time, weight, and cost) will need to be compared to "criteria" values to determine the feasibility of the process Based on this comparison, and the initial contaminant levels and risk information, one of three decisions can be made First, a decision can be made on whether to go ahead and depurate the fish prior to marketing for human consumption Secondly, a decision can be made not to depurate the fish in order to market them for some other use (e g , animal feed, fertilizer, etc) Or, a decision can be made to simply discard the fish

Desired Level

The desired level will either be a legal limit or a lower value that has been determined by risk analysis The risk analysis-derived values will be estimated using realistic exposure information (e g consumption of y) and for noncarcinogens, Reference Doses (RfD's) which are conceptually similar to Acceptable Daily Intakes (ADI's) The desired level for carcinogens will be determined using a linear dose response model, an acceptable level of risk (e g , 10^{-6} excess lifetime risk of cancer) exposure information and carcinogen potency factors (Q *'s) Integrated Risk Information System (IRIS) will be used as the primary source of information for RFD's and Q*'s Some RFD's and Q*'s are listed in Table 3 and examples of risk models can be found in Appendix 2 Based on the aforementioned factors, management may also pick a value between the legal limit and the risk analysis value because of economic considerations

Table 3 Information on Risk from Consumption of Hazardous Organic Compounds and Elements That Have Legal Limits in Fish and Fishery Products

		Human Threshold Criteria		Human Cancer Criteria	
Compound/Element	Known Carcinogen	RfD[1] mg/kg/day	Uncertainty[2] Factor	TMRC mg chemical/kg body wt/day	Q [4] Potency Factor
Compounds					
Aldrin	Y			8 73 E 8	11 45
Dieldrin	Y			3.29 E 8	30 37
Chlordane	Y			7 69 E 7	1 3
DDT	Y			1 19 E 7	8 422
DDTs	Y				
Dioxin (2 3 7 8 TCDD)	Y				
Endrin	N	000045	1 000		
Heptachlor	Y			2.22 E 7	4 5
Heptachloro-epoxide					
Hexacholorbenzene	Y			5 88 E 7	1 7
Kepone					
Lindane	Y			7 54 E 7	1 326
Malathion	N				
Mirex	N				
Parathion	N				
PCBs	Y			2 30 E 7	4 3396
Toxaphene	Y			8 84 E -7	1 131
Vinyl chloride	Y			4 35 E 7	2 3

Compound/Element	Known Carcinogen	Human Threshold Criteria: RfD[1] mg/kg/day	Human Threshold Criteria: Uncertainty[2] Factor	Human Cancer Criteria: TMRC mg chemical/kg body wt/day	Human Cancer Criteria: Q* [4] Potency Factor
Elements					
Antimony	N	0042	100		
Arsenic	Y				
Arsenic III	Y				
Arsenic V	Y				
Cadmium	N	0005	10		
Chromium III	N	5 1	1 000		
Chromium VI	N	00482	500		
Copper	N				
Lead	N				
Mercury	N	000158	1 000		
Selenium	N	001	10		
Zinc	N				

1 RfD (Reference Dose) or ADI (Acceptable Daily Intake) = NOAEL or LOEAL/uncertainty factor

2 Uncertainty factor- A safety factor for exptrapolating from animals to man etc

3 TMRC (Theoretical Maximum REsidue Contribution) or RAI (Risk Associated Intake) calculated for excess tumor incidences = 10^{-6}

4 Q (Cancer Potency Factor) TMRC (mg chemical/ kg body wt/day) x Q* (excess tumor incidence/mg chemical/ kg body wt/day) = Excess tumor incidence

Reference State of Wisconson (1989)

Size

The size criteria is determined by the market size of the fish and/or fish maturation This is a somewhat flexible value and could be a range (e g , 150-250g)

Time

The time criteria will depend upon the aquaculture system It must be at least as long as the duration required for depuration of pathogens and no longer than the time required for the next crop to grow out or complete a phase of growth In other words, the ongoing production should not be interrupted by this phase

Cost

The cost associated with depuration and growth will be compared to the market price of fish to determine if the process is economically feasible The cost requirements for feed will depend upon such variables as depuration time, fish size, growth rate, food conversion ratio, and unit feed price Formulas that may be used to calculate feed-related costs and feeding rates are

provided in Appendix I It should be noted that the feed costs for the extensive sewage aquaculture phase will be very low because natural flora and fauna provide the feed, so virtually all the feed costs will derive from the depuration phase On the other hand labor requirements and associated costs will be relatively high for the sewage growth phase but probably not for the depuration phase if it is assumed that the added tasks do not put an excessive burden on personnel

Problems With Extrapolation of Laboratory Data to Field Data

Extrapolation of laboratory data to field data include problems such as species variability and temperature differences Less evident field problems, such as multiple chemical exposure interactions can influence depuration rates (Macek et al 1970, Mayer et al 1970 Denison et al 1985, Cuvin and Furness 1988 Wicklund et al 1988)

The Importance of Field Studies

The importance of performing field studies to practically test clearance rates and the feasibility of depuration to reduce risk from chemically contaminated fish cannot be overemphasized Specific populations dependent on a seafood diet will continue to be at risk from chemical contamination until practical knowledge is gained about seafood chemical contamination This report provides state of the science information about the practical application of modeled depuration and biological dilution processes data and represents an important step towards protecting the public health

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APPENDIX I

Appendix I

Simple Ways to Estimate Feeding Rates and Feed Related Costs for Depurating and Feeding Sewage-Grown Fish

---

Using the Exponential Growth Model

$$W_t = W_o e^{gt}$$

Assumptions 1) Food Conversion Ratio (FCR) = $\frac{\text{Feed used by fish}}{\text{Weight gained by fish}}$ is constant

2) Temperature and salinity are constant

3) Maximum growth rate and critical standing crop are constant

Per fish estimates

Daily feeding rate as a fraction of fish rate = g x FRC

Weight gain in time(t) = $W_t - W_o = W_o (e^{gt}-1)$

Amount of feed/fish = $FRC(W_t - W_o) = FCR\ W_o(e^{gt}-1)$

Cost of feed/fish = amount of feed/fish x unit price of feed

= $FCR\ (W_t - W_o)$ unit price of feed

= $FCR\ W_o\ (e^{gt} - 1)$ unit price of feed

Depuration cost/kg fish = FCR price/kg feed

Growth in sewage followed by depuration cost/kg fish (assume cost of sewage = 0) = $\frac{FRC(W_t - W_o)}{W_t}$ price/kg feed

Area estimates

= per fish estimates x fish/area e g fish/ha

Yield

= weight gain/fish/time x fish/area e g kg/ha/day

Appendix I

Setting Desired Levels for Carcinogens in Fish

---

1) Set Risk
2) Calculate RAI, Total lifetime dose, Fish lifetime dose, and Fish concentration

RAI x Q* = Risk	Risk Associated Intake (RAI)	mg chem /kg body wt/ day
	Q* (Cancer Potency Factor)	excess tumor incidence/ mg chem./kg body wt/day
	Risk	excess tumor incidence (10^{-5} or 10^{-6} or 10^{-7})

$$RAI = \frac{\text{Total Lifetime Dose (mg chem)}}{\text{Body wt (kg) x Lifetime (days)}}$$

Body weight	avg consumer body wt
Lifetime	avg consumer life expect

Total Lifetime Dose	=	Fish Lifetime Dose	=	Chemical Concentration (mg/kg fish)	x	Contact Rate (kg fish/day)	x	Exposure Duration (days)	x	Absorption Efficiency (ratio)
		+ Food Lifetime Dose	=	' (mg/kg food)	x	(kg food/day)	x	(days)	x	(ratio)
		+ Water Lifetime Dose	=	' (mg/ml water)	x	' (l water/day)	x	' (days)	x	' (ratio)
		+ Air Lifetime Dose	=	' ' (mg/m^3 air)	x	(m^3 air/day)	x	' (days)	x	(ratio)

Fish Lifetime Dose =	Total Lifetime Dose	-	Food Lifetime Dose	-	Water Lifetime Dose	-	Air Lifetime Dose

Fish Concentration = Fish Lifetime Dose / [Contact Rate x Exposure Duration x Absorption Efficiency]

Appendix I

Setting Desired Levels for NonCarcinogens in Fish

1) Calculate Maximum Permissible Intake Intake from Fish and Fish Concentration

Maximum Permissible Intake (mg chem /day)	=	ADI (mg chem /[kg body wt x day])	x	Body Weight (avg consumer-kg)

Intake from fish (mg/day) = Max Permissible intake Intake form other sources

Fish concentration (mg chem /kg fish) = Intake from fish (mg/day)/Consumption (kg fish/day)

Depuration Models that Include a Growth Dilution Component

First Order Single Compartment Depuration Models

With Exponential Growth Incorporated[1]	With Linear Growth Incorporated[2]
$dC/dt = -(k_2 + g)\ C$	$WdC/Dt + CdW/dt = -k_2CW$
Following Integration	Substitution and Integration
$C_t = C_oe^{-k_2t}$	$C_t = C_oe^{-k_2t}/(1 + gt)$
In Linear Form	
$LnC_t = lnC_o - (k_2 + g)t$	$LnC_t = lnC_o - k_2t - ln(1 + gt)$
Biological Half-Life	
$T_{1/2} = ln2/(k_2 + g)$	$Ln2 = k_2t + ln(1 + gt)$ (solve for t)
Exponential Growth Model	Linear Growth Model
$dW/dt = Wg$	$dW/dt = W_og$
Following Integration	
$W_t = W_oe^{gt}$	$W_t = W_o(1 + gt)$
In Linear Form	
$lnW_t = lnW_o + gt$	$W_t = W_ogt + W_o$

C_o = Concentration of chemical in fish at the end of uptake
C_t = Concentration of chemical in fish at time (t)
k_2 = Depuration rate constant (d^{-1})
Wo = Weight of fish at end of uptake
Wt = Weight of fish at time = t
g = Growth rate constant (d^{-1})

1 Spacie and Hamelink (1985)
2 Branson et al (1985)

APPENDIX 2

APPENDIX 2

Bioaccumulation models

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APPENDIX 2

BIOACCUMULATION MODELS

The overall model for bioaccumulation is in the form of a differential equation which when solved should give either a concentration of the contaminant in the fish or a whole body burden Sources have also transfered the concentration to a lipid basis in the fish to more accurately isolate the long-term storage compartment for contaminants The terms in the differential equation as summerized from the literature are

1) uptake via gills

2) uptake by adsorption to the body

3) uptake by consumption (sometimes called food-chain accumulation)

4) elimination by excretion from the gills

5) elimination by defecation

6) loss from "growth dilution'

7) loss from biotransformation

8) reproductive loss

The approach for modeling each of these terms is not always consistant and some models have not included every one

UPTAKE

The uptake of persistant contaminants from a fish's environment to its tissues is governed by either absorption onto the gills or by ingestion with the possible addition of adsorption to the body In the first case the contaminant diffuses from the water across the gills into the body Following consumption of contaminated food the chemical transfers from the gut into the body Adsorption occurs at the skin passing through to the tissues beneath Each of these processes has its own term in the bioaccumulation model Due to their high lipid solubility contaminants are then concentrated in tissues with large lipid contents The processes of intake from food and water are assumed to be additive in the model but competition is possible [1] (although this latter point has not been pursued in the literature) The rate of

uptake is influenced by a chain of other parameters starting with the water temperature which affects the respiratory rate and caloric intake each of which influence the metabolic rate which in turn factors into the rate of uptake [1]

One of the questions that a bioaccumulation model may answer is what is the relative importance of food and water in the accumulation process This question was not answered adequately by lab and microcosm studies which gave inconsistant results relative to one another Factors affecting the relative importance of the two main exposure routes include species locale food web and the physico-chemical properties of the contaminant [12] Several studies have determined the relative contributions of food and water in the bioaccumulation process for different fish food sources and contaminants

A plateau test which exposes the fish with a constant concentration until steady state is reached can determine this relativity by including three groups of organisms One group of fish would have only contaminated water as a chemical source while another would also have a contaminated food source A third group without any contaminated source would act as a control [1] "Linear additivity" is expected for the combined intake of contaminated food and water [4]

The two basic methods available for fitting intake rate parameters are regression analysis and estimation from experimentation Data is scarce for long-term rate constants These constants are difficult to calculate in the lab are poorly understood and have not usually been determined [2] Long term kinetics in general have not been adequately described although two-compartment kinetics have been shown to be better than one compartment [2]

UPTAKE FROM WATER–UPTAKE BY GILL

The mechanism of uptake operating at the gill membrane is passive diffusion across a concentration gradient The non polar contaminant molecules must cross through a lipid bilayer Proteinaceous pores will allow compounds through with a molecular weight up to about 100 [1] The overall process can be described by Fickian diffusion

$$\frac{\Delta C \times \text{area} \times \text{temperature}}{\text{distance}} \qquad \Delta C = \text{change in concentration}$$

The countercurrent blood flow at the gill site creates a large concentration gradient The uptake of a contaminant at the gill membrane is directly affected by the availability and persistance of the contaminant in water This bioavailability of contaminants will be discussed later Respiration rate and other factors are also important for determining the rate of uptake for a contaminant at the gills It appears that the uptake rate constant remains relatively constant over a wide range of bioconcentration factors (BCFs) held by contaminants [1]

In order to determine the uptake rate constant for transport across the gills an accelerated bioconcentration test is prescribed After four days the rate constant is calculated by a non-linear least squares method from concentration data. If it may be assumed that the uptake rate is constant for all contaminants than the value calculated by this method should be universally applicable However this test fails to account for non-ideal processes including a model based one more than one compartment. Thus there is a problem with accuracy for long-term exposures [1] A rough determination of the uptake rate constant (k_1) can be made by experiment when the concentration in the water (C_w) and the depuration rate (k_2) are known From a uptake experiment a plot of the fish concentration (C_f) versus $1 - \exp(-k_2 t)$ should result in a straight line with a slope equal to $k_1 C_w / k_2$ Since the concentration in water and the depuration constant are known, the uptake rate can be estimated [13]

Given enough data about the fish and its environment and using typical literature values an accurate representation of uptake at the gill can be constructed for the model The most crucial parameters are the respiration rate of the fish the oxygen concentration in water and the dissolved chemical concentration in the water Some additional parameters, for which the treatment varies in the literature must also be supplied to complete the uptake term The basic outline of the term is

Uptake = $k_u C$
where k_u = the uptake rate constant
and C = the chemical concentration in water

Depending on the differential variable the term s units may be something like [µg/g(w)•day g(w) = wet weight[of the fish] which could be multiplied by the weight of the fish in order to determine a total body burden A lipid-normalized uptake (for a chemical concentration on a lipid basis in the fish) may be

described by the relation $k_u = k'_u/p$ where k'u is the original uptake rate and p is the fraction of lipid in the fish by weight [11]

The uptake rate constant can be predicted more accurately for natural settings by basing it on physiology and chemical characteristics Connolly used the ratio of mass transfer coefficients for oxygen and the chemical of concern to develop an uptake rate constant in addition to the respiration rate and oxygen concentration in water [6] The representation is as follows

$K_{ui} = (K_{Li}/K_{LiO2})(r'_i/c_{O2})$ where
K_{ui}=transport across gill to blood [L/day g(w)],
c_{O2}=oxygen conc in water [g/L]
r'_i=respiration rate [g of O_2/g(w) day]
and K_{Li}/K_{LiO2}=the ratio of mass transfer coefficients for the chemical/oxygen

The ratio of mass transfer coefficients may be derived from literature values (if available) developed from the characteristics of the chemical and the physiology of the animal Otherwise estimates could be obtained from measurements of the chemical and oxygen uptake efficiencies when the initial body burden is zero Studies measuring these coefficients have shown them to be equal for $\log K_{ow}$ values between 3 and 6 [6] Data from various sources has indicated that the maximum uptake efficiency is reached at a $\log K_{ow}$ of about 6-7 [11]

The respiration rate as a function of the weight of the fish (W) and the temperature of the water (T) can be represented as follows

$R = \beta W^{\gamma} \exp(\rho T)$ [6]

This relationship can be determined by the regression of data from laboratory respiration studies The oxygen concentration in water and the dissolved chemical concentration in water may be measured directly to complete the data need for the uptake rate

Thomann has presented a slightly different version of the uptake rate coefficient which includes a transfer efficiency parameter and an oxygen-based respiration rate [11] With the assumption that

with r as the respiration rate on an oxygen basis [g of O_2/daykg(lp)]
then $k_u = r'E/c_o$
and relating r to r (day^{-1}) thru $r = (a_{oc}a_c/a_{wd}p)r$

The uptake rate may be calculated from the equation

$k_u = (a_{oc}a_c rE/a_{wd}pc_o)$ where

ku = uptake rate
a_{oc} =the oxygen to carbon ratio,
a_c = the carbon/dry wt ratio
r = the standard respiration rate
a_{wd} = the wet/dry wt ratio
E=efficiency of transfer of the chemical
p=fraction lipid weight in the fish
c_o as the oxygen conc (kg/L)

The respiration rate as with the previous model is dependent on the fish weight and was formulated in the present model as

$r = \phi w^{-\gamma}$, where for w [g(w)]

This relationship may be given specific value ranges of ϕ and γ for routine metabolism The transfer efficiency E, is related to K_{ow} and is somewhat analagous to a bioconcentration factor The value of E which has and can be determined by studies varies for different ranges of K_{ow} The route described by this efficiency is balanced between transport through an aqueous diffusion layer (where water solubility is a factor) and through a lipid membrane A higher ventilation volume (translated as respiration) reduces the efficiency E, due to increased diffusion dead space and a shunting away of inspired water from respiratory surfaces [11] Given typical values for each of these parameters including the remainder which should be available to the modeler k_u should be on the order of $10^3(w^{-\gamma}/p)E$ [11]

A method which models biological processes of respiration a little more in depth to determine gill sorption was presented as part of a lake ecosystem model by Leung [16] In addition to the water contaminant concentration this model included the volume of water passing through gills the volume of blood passing through the gills, the concentration of the chemical in blood and a partitioning coefficient between blood and water This last parameter could be derived experimentally or, if the chemicals are in the same class its value is assumed to be constant in relation to other partitioning coefficients in the body tissues such as between water and lipid The volume of water passing through the gills is dependent on factors previously seen namely the concentration of oxygen in water and the respiration rate In addition a coefficient of correlation between oxygen uptake and respiration (g O_2/g respired) and the withholding efficiency of oxygen by the gills fill in the rest of the data needed for a determination of the volume of

water transfered [16] Depending on fish size on the order of 10-1000 L/day of water is processed by the gills [13]

The respiration rate in this formulation as with the previous models is dependent on the temperature and biomass but also assumes a relationship with a consumption rate a starvation rate and a daily consumption rate For more in depth modeling studies these parameters could also be included For brevity s sake this model is not shown here

UPTAKE BY ADSORPTION TO BODY

Little has been done to study addition of chemicals to the body burden of fish by this route but there is a possibility that there may be some significance in some cases [16] This process is important for microorganisms due to their high surface area-to-volume ratio but generally this is not an efficient route for reaching critically toxic areas in other organisms The appropriate modeling method is to employ the Freundlich equation where

$x/m = KC^n$

x=conc of chemical
m=mass of adsorbent
K=measure of extent of adsorption (α solubility^{-1})
C=equilib conc
n=coeff which deals w/the nature of the adsorbent

This relation has also been used determining pesticide fate on soil surfaces [1] [16]

UPTAKE FROM CONSUMPTION

Biomagnification accounts for the high ratio of contaminant in the fish compared to the water due to the increase of fish contaminant level via consumpive uptake It is an important part of the overall bioaccumulation process One explaination for the cause of biomagnification is the that food digestion causes a fugacity increase in the gastrointestinal tract [4] The generation of this hypothesized chemical gradient in the gut of the animal is caused by preferential lipid breakdown [11] Biomagnification is most important for very hydrophobic slowly clearing nonmetabolizing chemicals The physiological process of food uptake relies on variable chemistry The concepts of both diffusion and special transport apply to

gastrointestinal tract absorption There is a lipoprotein membrane transport mechanism across the gut wall of the organism and into the blood stream A simple representation of consumption which does not include food-chain effects may look like

EF_tC_{fd} where
E=the absorption efficiency
C_{fd}=the concentration of chemical in food
$F_t=fW_t$ = food consumed by and organism in a week
W_t=fish weight
f=feeding rate

The rate of food consumption can also be estimated based on the rate of energy usage as follows

rate of food consumption $C_{ij} = (\lambda_i/\lambda_j)(R_i + G_i)/a$, (or F_t as above)
a=fraction of energy assimilated or food assimilation efficiency
λ_i=caloric density of animal's tissue
λ_j=caloric density of prey
R=metabolic rate or respiration rate
G=growth rate

This method applies to pelagic feeding animals whereas the food consumption for deposit feeding animals should be based on carbon instead [6] The absorption efficiency or assimilation efficiency as it is sometimes called can be determined by studies which measure the chemical concentration at the end of an exposure period (with the only source of the contaminant being the food) The assimilation efficiency may either be constant or for more hydrophobic chemicals will vary as a function of K_{ow} in much the same relationship as in gill transfer The trend shown in studies is to increase to $\log K_{ow}$ ~5 5 and then to decrease [11] The particular food affects the amount taken up during consumption. The amount assimilated is the difference between consumption and defecation

Respiration rate can be calculated as previously described in the water uptake section In a controlled environment the influence of the sampling and feeding scenerio may be seen in the results Discontinuous feeding rates coupled with periodic sampling will result in a sawtooth pattern in a graph comparing the concentration of the chemical in the organism with time [2] The feeding rate is expected to fall with increased fish volume [4]

A more detailed model of consumption indicates differential uptake based on a variable feeding rate and on food preferences

$$SUM1 = \sum_{j=1}^{n} W(i,j)*B_j$$

$$SUM2 = \sum_{j=1}^{n} W(i,j)*C_j$$

$$CONSUM1_i = CMAX(i)*B_i*SUM2*[1 - BMIN_i/\sum_{j=1}^{n} B_j]/Q_i + SUM1 \quad \text{where}$$

C_j=conc of pest in j
$CMAX_i$=max consump rate of predator (g/g/day)
B_i=biomass of predator i in lake (g/m^3)
W(i,j)=preference of predator for j prey (unitless)
BMIN=min food level before feeding occurs (g/m^3)
Q=half-saturation coefficient for feeding (g/m^3)

ELIMINATION (DEPURATION)

The overall rate constant in tandem with the uptake efficiency comprise the key parameters for modeling bioaccumulation Contaminants are released from the fish by exchange back out through the gills from defecation and primarily from the female gender through gonadal loss The contaminant concentration in organisms may also be lowered by metabolism into daughter products and by an increase in lipid content causing "growth dilution" Many bioaccumulation models incorporate terms for growth sex reproduction and biotransformation but some contradictory results have been to date indicating the need for further investigation of these processes [2] The routes of elimination of chemical contaminants in aquatic organsims are affected by such factors as temperature
water chemistry
tissue damage
preexposure to toxicants
and the presence of competing chemicals

These are additional factors to the ones presented in the models seen here and must also be taken into consideration to determine changes in the chemical s pathway which may affect a model s ability to predict the degree of bioaccumulation The rates of elimination as with uptake are affected by parameters specific to the fish including the metabolic rate which in turn is determined by the respiration rate and caloric intake by the fish and by the size and lipid content of the fish The overall

rate of elimination is highly dependent on $\log K_{ow}$ and more chemical is removed by metabolism than the gills with increased lipid partitioning [4] A variety of empirical equations have been suggested relating the overall depuration rate to K_{ow} A simplified relation is found under the assumptions of zero growth steady state and a lipid-normalized BCF equal to the octanol-water coefficient Given these assumptions the excretion rate should equal the ratio of uptake rate over the K_{ow} for the contaminant This also assumes that the same mechanisms occur through the lipoprotein membranes in excretion as with uptake [11] Sijm et al found that the elimination rate constants are independent of the life stage of the organism [2]

Experiments may be conducted to determine the overall depuration rate constant One method is to dose the fish at a constant exposure concentration (either water or food) until a steady state is obtained and then transport the fish to a clean environment to initiate a depuration phase Assuming a simplified model of depuration

$$\ln C = \ln C_o - k_d t$$

then $t_{1/2} = \ln 0.5/k_d$

where C = the concentration in the fish or the whole body burden
C_o = concentration/body burden at the start of the depuration phase
k_d = the overall depuration rate constant
t = time in the experiment
$t_{1/2}$ = depuration half-life

Using the tangents/slopes to the uptake curve $\Delta C/\Delta t$ k_d may be estimated This depuration rate constant may represent the additive processes of elimination and biotransformation To also include a growth dilution term into the depuration rate constant it is neccesary to use the logarithm of the concentration rather than the whole body burden

A non-linear least squares regression procedure can also be used to estimate k_d assuming the relationship

$$C/C_w = BCF[1-\exp(-k_d t)]$$

C=concentration of contaminant in fish
C_w=concentration of contaminant in water
BCF=the predetermined bioaccumulation factor

then for points of data $(C_1\ t_1)$ and $(C_2\ t_2)$

k_d may be solved numerically using the derived equation

$$C_1/C_2 = 1 - \exp(-k_d t_1)/[1-\exp(-k_d t_2)]$$

However these are not unique solutions and the broad assumption is made that the food and water elimination rates are first-order and identical An accelerated bioconcentration test over four days is used for the above determinations and as mentioned earlier in the uptake section thus fails to account for non-ideal processes which conceptually, have more than one compartment [1] Long-term depuration rate constants have yet to be developed satisfactorily The plateau test for bioconcentration provides an estimate of the depuration rate constant but is expensive lasting one month or more and thus may need to be corrected for growth in the fish [1]

EXCRETION

In a more focused modeling procedure the net process of depuration would be divided into several terms describing the individual processes separately Excretion may be isolated as a separate term or more accurately it would be subdivided by route into loss via the gills and loss via defecation The excretion rate is analagous to the simplified relationship involving the depuration constant as mentioned above

$K_1 = K_{u1}/N_1$
for K_{u1} = rate of uptake
K_1 = excretion rate
$N_1 = f_{L1}K_{ow}$ = BCF
and f_{L1}=lipid fraction of the animal

The physiology behind excretion is as follows Contaminant material that was taken in but not accumulated in tissues is excreted Also excreted are accumulated chemicals in tissues during routine metabolic processes growth and starvation These processes have been modeled using biological parameters specific to the fish under study An assumption is made that the breakdown of fatty tissue results from endogenous respiration under starvation conditions Otherwise excretion is related to standard metabolism and specific dynamic action [16] Study results show that one would expect the

excretion rate to be lower for larger fish due to decreased respiration and for the most part a greater ratio of volume to membrane area [11]

LOSS FROM GILLS

Removal of the contaminant from the organism s body burden at the site of the gills is by passive elimination Studies have shown this site to be most important for compounds which are slowly transformed and non-polar

LOSS FROM DEFECATION

The other organs of importance for eliminating toxic agents from the organism include the liver gallbladder and the kidney The first two organs process polar chemicals and metabolites and sometimes hold the chemical through enterohepatic cycling Analysis of bile is important for determining the fate of the contaminant in the tissues Contaminants above a molecular weight of around 400 are eliminated in bile while the rest exit with the urine A good deal of variation is found amongst species Physico-chemical properties of the contaminant are important for determining the rate of excretion into the urine via the kidneys [1] Fecal elimination is partially responsible for the limitation on BCFs with increasing K_{ow} such that an increasing fecal elimination lowers the slope of the relationship [6]

Defecation may be represented as the loss associated with a particular prey that is not absorbed in the organism s gut. It can be determined by the amount of pesticide consumed in the prey multiplied by the inverse of the assimilation efficiency of consumption (proportion not absorbed in gut) A formulation by Leung follows

$$DEF_{li} = \sum_{j=1}^{n} CONSUM_{ij} * E(i,j)$$

DEF_{li}=rate of pest loss thru defecation (g/m^3-day)
$CONSUM_{ij}$=rate of consumption of pesticide assoc w/ prey, j (g pest/g fish/day)
E(i,j)=proportion of pest not absorbed in gut (g pest defected/g pest consumed)

GROWTH RATE AND LOSS FROM GROWTH DILUTION

Growth is usually assumed to be a first-order elimination process but it can change physiological properties which influence biomagnification kinetics A "growth dilution" of the chemical concentration occurs due to an increase in lipid weight of the organism Conceptually growth dilution is similar to

metabolism The growth rate effect is to dominate the "loss" of chemical at higher K_{ow} values due to the increased mass of the lipid pool

The growth dilution affects tests by reducing the time to steady state and reducing the depuration half-life However studies show that steady state is not always reached for growing fish [2] The growth dilution term is used for testing in the field but lab BCF studies may use a maintenance diet for zero growth [11] As mentioned before a growth correction term may be neccessary for the plateau method of testing bioconcentration

Growth is a major control on the biomagnification of slowly eliminating hydrophobic compounds The lower the elimination rate constant ($k_d + k_m$) the greater is the contribution of growth dilution to overall elimination ($k_d + k_m + g$) This effect is important when comparing different life stages [2] Sijm et al found growth dilution to be the only important process in modeling the bioaccumulation of higher chlorinated biphenyls [2] A study of northern pike in Scandinavia partly attributed lower pollutant levels in fish inhabiting lakes with higher productivity to the higher growth rate of the pike in these lakes [5] One study showed a reduction in simple lipid-based partioning by a factor of 2-5 due to the effect of the growth rate [11]

The term for growth dilution may appear as a rate constant multiplied by the concentration or body burden of the contaminant in the organism, gC It may also be incorporated into the overall depuration rate constant or the elimination rate constant ($k = k_d + k_m + g$) as above The growth rate constant can be calculated from a growth equation

$$dW/dt = W_0 \exp(gt)$$

dW/dt = growth rate
W = weight
W_0 = weight at the start of the growth period or test
g = growth rate constant
t = time

Growth rates are determined from field analysis Across a general food chain the growth rate is approximated by

$$G = \delta w^{-\beta}$$

where for w [g(w)] δ is ~0 002 at 10°C and β varies from 0 2-0 3 according to a study by Thomann [11]

The ranges in coefficients are functions of the specific organism and ecosystem conditions When modeling the chemical conc on a lipid basis in predator organism

G= net growth rate (day^{-1}) of the lipid wt (w_{lp}) of the organism
$= (dw_{lp}/dt)/w_{lp} = (dw_p/dt)/w_p$
w_p = the total weight of the organism

For a constant lipid fraction over time the growth rate can be related to the respiration rate and the food assimilation efficiency by

$G = aC - r$
r=organism respiration rate(day^{-1})
a=food assimilation efficiency

LOSS FROM BIOTRANSFORMATION

Biotransformation is a function of a toxic substance s aqueous solubility with degradation occuring in an aqueous state [16] Detoxification may be important in decreasing storage and accumulation of toxic chemicals in the food chain This process may be very complex involving reactions such as

oxidation (abphatic and aromatic)		dechlorinations
hydrolysis (ester and amide bonds)		reduction
dealkylation (C-R or N-R bonds)	and	aromatic ring cleavage

Solubility in lipids stores contaminants away from detoxification sites A biodegradability index (B I) is a measure of the ratio of polar to nonpolar pesticide fractions in a model ecosystem and is given here for some typical values

$\log(B\ I) = 1\ 41 + 0\ 87 \log (H_20 \text{ solubility})$ [16]

Another informative index is the

%parent compound = 41 1086 + 40 7901σ [16]
σ=Hammet constant

In general not enough is known about the temporal aspects of toxicant elimination in organisms to be represented accurately

Polar metabolites are eliminated with greater ease and in a different manner than other daughter compounds Radiolabeling is used to determine the rate of metabolite elimination. Metabolites eliminate

at a different rate than their parent compounds The case when the rate constant for metabolite elimination is much less than the overall depuration rate constant is a signal for persistant metabolized residues A study on PCB elimination showed that biotransformation was the most important elimination process for some congeners [2] In general biotransformation may be dominant in the elimination of very hydrophobic, slow eliminating chemicals Rate constants for biotransformation can t always be quantified but those compounds with a higher degree of elimination than expected with a certain hydrophobicity may indicate a degree of biotransformation

Biotransformation is generally assumed to be first order and independent of the concentration and the age of the fish Possible exceptions and other factors affecting the rate are concentration dependency dependence on environmental parameters variation by species and a higher rate in adults than in young fish [2] However biotransformation can be estimated in individual studies Correlations between bioconcentration and physical properties are poor for chemicals that are readily metabolized by fish [13] A generalized term for biotransformation follows

BTRANS = CON * TOXD

BTRANS=pest transformed thru biological processes ($g/m^3/day$)
CON=pest conc (g/m^3)
TOXD=% pest cleared by detox (day^{-1})

REPRODUCTIVE LOSS

Lower half-lives of chemicals have been seen in females relative to males This may be because of the greater mass of lipid-intensive material lost with the female gonadal excretions or from transfer to the offspring before exit from the fish Reproduction is a frequently recurring process in females with a zero-order elimination rate constant Due to its high lipid content the change in gonadal mass changes the fish weight and the pesticide storage Because of elimination via the offspring bioaccumulation data cannot be transfered between males and females Questions about important characteristics which may affect this process are

Is the fish viparous?
What is the gestation time?
How many eggs are produced?
What is the amount of vitellogenin produced?
and How many hatchlings per year?

Part of the discrepancy between genders arises from the amount and nature of the gonadal products For males the geminal tissue is ~2% of the body weight, whereas for females it may consist of up to 15% of the body weight and has a higher fat content than male gonadal products [5] The process of gonadal enlargement and sudden loss with contained contaminants is cyclic and specific for the type of fish [16] Some species show only slight differences in gonadal product shedding between the sexes at least for the period of shedding The temperature, photo period and water level are all environmental cues for the onset of spawning The basic term may be represented as

$Rk_r n$ where
R = a trigger variable
k_r = zero order reproduction
n = concentration or body burden of chemical in the organism

IMPORTANT FACTORS INFLUENCING TERMS IN MODEL

FOOD CHAIN EFFECTS

A typically assumed food chain route in an ecosystem for transfer of chemicals to edible fish is

phytoplankton ⇒ zooplankton ⇒ small fish ⇒ large fish ⇒ human consumption

In fugacity terms the fugacity increases with the trophic level The concentration of the predator due to food intake would be expected to be greater than that of the prey under conditions of low excretion and high food assimilation efficiency This elevated concentration is a function of the energetics of the organism (food conversion efficiency respiration and growth) and the characteristics of the chemical (assimilation efficiency and excretion rate) In terms of the BAF

$$N_2 = N_{w2} + f_{21}N_{w1}$$
$$N_3 = N_{w3} + f_{32}N_{w2} + f_{32}f_{21}N_{w1}$$
$$N_4 = N_{w4} + f_{43}N_{w3} + f_{43}f_{32}N_{w2} + f_{43}f_{32}f_{21}N_{w1}$$

where $f_{i\,i-1} = \alpha_{i\,i-1}[C_{i\,i-1}/(K_i + G_i)]$
N_i = the BAF at trophic level i
N_{wi} = the BCF (water only) for trophic level i
$\alpha_{i\,i-1}$ = the chemical assimilation efficency
$C_{i\,i-1}$ = consumptionrate of predator i on prey $i - 1$
K_i = the overall depuration rate constant
G_i = the growth rate of the organism

Thus the parameters mentioned above are applied to each animal in the food chain The basic effect of a food chain is to increase the BAF at each trophic level During steady state, $N/N_w>1$ indicates that food chain accumulation has occurred Models have shown that above log P ~5-6, food is the dominant source of chemical to the food chain Assimilation efficiency is then a controlling model parameter [6]

The bioconcentration of chemicals from water by algae zooplankton, and the like can lead to food chain accumulation in fish at the top of the pelagic food chain The uptake of chemicals by macroinvertebrates such as oligochaete worms from contaminated sediments and detritus can lead to food chain accumulation in fish at the top of the benthic food chain [13] The latter fish classification tends to have a greater chemical concentration than the former due to the much higher concentrations found in sediments For log P of 5-7 calculated and observed field concentration factors in top predators indicated significant (1-2 orders of magnitude) observations above calculated field BCF values in Thomann s study [11] Also above log P = 7 food chain effects are sensitive to the chemical assimilation efficiency and phytoplankton BCF

As with uptake from water the chemical concentration can be represented on a lipid basis in the pred

BCF DETERMINATION

Estimation of the bioconcentration factor (BCF) from properties of the chemical can be essential in determining the uptake and depuration constants The BCF is equal to the ratio of the concentration at steady-state to the concentration in water This ratio includes all of the rate constants in the model Several values have been given in the literature relating log BCF to the logarithm of the octanol/water partitioning coeffient log P The influence of other factors (protein non-lipid organic matter) is presumed to be small [11] The reasons for the use of a lipid based chemical concentration are that lipid partitioning is often favorable in organisms such that the concentration tends to vary with lipid content Estimates of log P have been measured directly or taken from established relationships with the water solubility S of the compound Some literature values are taken from Spacie and Hamelink

$$\log BCF = 0.76 \log P - 0.23 \qquad \text{and} \qquad \log P = 5.00 - 0.670 \log S$$

The overall uptake rate is found to be relatively constant over a wide range of P values while the overall depuration rate is highly dependent on log P [1] During a plateau test the steady state BCF can be

measured directly [1] The BCF can be estimated by measuring the chemical concentration in fish at steady state after exposing the fish for the appropriate length of time to a constant chemical concentration in the water [13] In addition to the steady-state approach, BCFs can also be calculated by a kinetic approach for chemicals with a known half-life in the organism [13] The two approaches give similar results for short half-lived compounds but significantly different results for compounds with longer half-lives Predictions based on lab BCFs for long half-life chemicals may be too low where contaminated food is the major chemical source [13]

The log-log relationship between bioconcentration and hydrophobicity breaks down for medium to high molecular weight solutes which bioconcentrate slightly or not at all [3] Specifically the standard equation for the relationship between BCF and P given above breaks down for log P greater than 6 giving a maximum log BCF of ~5 5 [3] This can be a problem for compounds such as most PCB congeners which have log P > 6 [12 5] The reason for this disruption is partially attributed to a lack of lipid solubility Unfortuneatly lipid solubility data is generally unavailable In another perspective the assumption that octanol can be used to approximate lipids may be incorrect because the thermodynamics for equilibrium partitioning between water and fish lipid and between water and octanol may not be similar [12] An explaination for this effect is that hydrophobes tend to be large which gives them difficulty in cavity formation in lipids Their size may also give them trouble passing through the gill membrane [3] Thus size may be more important than hydrophobicity For an increasing molecular weight γ_{lipid} increases more rapidly than $\gamma_{octanol}$ and the BCF ($\gamma_{water}/\gamma_{lipid}$) decreases when γ_{lipid} increases more rapidly than water which is essentially the case Banerjee and Baughman showed that the inclusion of an octanol solubility term is able to help the relationship (r=0 95 for tested compounds) [3] This is a more practical alternative to finding a better lipid substitute The term is a solute activity coefficient which stimulates lipid behavior by combining more than one octanol-related property The resulting formulation is

$$\log(BCF) = c3 + c4\log(Kow) + c5\log(Soctanol) + c6(mp - 25) \quad mp=\text{melting point}$$

The melting point term allows for both liquids and solid by using a melting point=25 for liquids This formulation is more practical than using some other material such as membrane vessicle data Since the

Soctanol term is evolved only through correlation it should be confirmed independently by experiment and included among other factors affecting bioconcentration such as growth dilution metabolism and differences in solute bioavailability An assumption is made for this solution that the structure of the lipid matrix leads to strong solute nonideality [3]

Other explainations for limits on BCF with increasing P are increasing fecal elimination as mentioned earlier and a decrease in freely dissolved chemical due to complexation with dissolved and colloidal organic matter [6] This latter effect is dependent on the concentration of organic matter in water and will be expanded more in the section on bioavailability Other proposed correlations include added quadratic terms to account for steric factors as well as terms of metabolism [12 5] Compounds metabolized by the fish also do not correlate well with P

Lipid solubility is a subset of the types of partioning that can be modeled in fish The affinity for any tissue in fish can be determined by the phycico-chemical characteristics of toxic chemicals The amount accumulating can be determined from the partition coefficient between the predominant tissue of accumulation and blood [16]

BIOAVAILABILITY

In order to be taken up by the fish directly via the gill or indirectly through the food chain a xenobiotic must be in dissolved form in the water Factors which may reduce the dissolved fraction include

1)adsorption to suspended solids
2)adsoprtion to sediments
3)adsorption to humic acids & other macromolecules
4)formation of colloidal suspensions
5)chelation
6)complexation
7) ionization [1]

The lipophillic nature of compounds with a high BCF also facilitates their partitioning into the organic fraction of sediments or suspended solids reducing their bioavailability This may be modeled as

$K=C_a/C_w$ K_{oc} = K/fraction of org content
K = concentration factor for adsorption to seds or soil
C_a = concentration in sediments or soil
C_w = concentration in water

K_{oc} = conc factor normalized for the org content of soil

The humic acid level helps determine the bioavailable fraction in the water column Another formulation determines the dissolved concentration of contaminant in the water directly

$C_w = C_t/(1 + K_{pw}X)$ where
C_w=dissolved concentration,
C_t=total concentration
K_{pw}=partition coeff,
X=sorbent concentration
where K_{pw} is related to K_{ow} and K_{oc}

The latter relation uses Y such that $K_{pw}X = K_{ow}Y$ Y = conc of suspended organic carbon [4] The dissolved fraction can be an important determination in bioaccumulation Not included in this determination is the possible dependence of resistances on diffusivity of the chemical in water and organic media [4] The study of pike in Scandinavia mentioned before also attributed reduced contaminant levels in fish to productivity due to a higher turnover time and sedimentation of particles namely phytoplankton to which the pollutants are adsorbed A greater amount of humic substances in lakes also lowers the concentration since humus adsobs persistant compounds rendering them less available for uptake [5] For utility's sake the substrate should by characterized according to %organic matter and particle size [1]

The dissolved chemical in the water column is the source for both gill sorption and for the base of the pelagic food chain (phytoplankton) The amount of chemical dissolved in the sediment is the major source for the base of the benthic food chain [6] The section on bioaccumulation showed the importance of the concentration of organic matter in the water in decreasing the amount of the freely dissolved chemical A depressed phytoplankton BCF affecting the food chain model may be due to the use of total dissolved rather than bioavailable dissolved chemical in the BCF calculation [6]

AGE AND FAT CONTENT

Due to important physiological differences separate life stages of each upper level species should be modeled separately The proportion of fat increases with the age of the fish which has a large impact on the bioaccumulation rates of organic compounds However it is assumed that adult fish are of the

most concern for human consumption The fish age affects uptake rates due to variations in metabolic rates consumption rates food preferences, and several other parameters [16]

There is a general trend for a lesser degree of biomagnification in earlier stages compared to older fish as determined by the changing uptake and elimination in each life stage [2]

The protein content of fish tends to stay fairly constant, while the fat content fluctuates widely during the year and is also determined by the growth stanza of the fish Variation in fat content occurs due to food availability and quality, seasonal and hormonal cycle age and general physiological condition This variation may cause a change in the accumulation rate Depending on the species and reproductive status there may be some differences in fat content between the sexes as seen earlier [16]

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CHEMICAL UPTAKE & DEPURATION BY FISH

By

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Uptake, Accumulation and Elimination of the Organophosphate Insecticide Fenitrothion (Sumithion) by the Fish Tilapia (Oreochromis niloticus)

Pesticides and chemical compounds contaminate aquatic environments through both direct and indirect methods Point source contamination, originating from factories, sewage discharge areas and other industrial activities, are relatively easy to identify and control Nonpoint sources, including agricultural run-off and atmospheric deposition, also contribute substantially to water pollution but are more difficult to identify and control

Excess use of various pesticides in agriculture leads to high levels of contamination due to run-off of these substances Water sources, including fish ponds, which are located closer to agricultural areas typically display higher levels of residue from these chemicals Pesticides and other chemical compounds pose acute and chronic hazards to the aquatic organisms, producing deleterious effects for indigenous fish and invertebrate populations

The following sections highlight findings regarding chemical contamination of fish examined in Egypt and their rates of depuration The contaminants examined include the organophosphate insecticide fenitrothion (sumithion), polycyclic aromatic hydrocarbons, and various heavy metals

FENITROTHION

Fenitrothion (sumithion) is one of the organophosphate insecticides used extensively to control agricultural and woodland pests Sources of contamination of the aquatic environment with this pesticide include aircraft, atmospheric drift, erosion and surface runoff Fenitrothion inhibits the activity of acetylcholinesterase (AchE) and is lethal to various fish species at concentrations ranging from 1 to 5 mg/L (Johnson 1980)

In this study, researchers attempted to evaluate, under laboratory conditions, the processes of uptake, bioaccumulation and elimination of fenitrothion by the fish *Tilapia* (Oreochromis niloticus) In addition, the scientists involved in the study attempted to determine the relationship between these processes and fish body size

The Insecticide

Fenitrothion (Sumithion) o,o-Dimethyl o-(4-nitro-m-tolyl) phosphorothioate (purity liquid concentrate 50%)

The Fish

The fish, Oreochromis niloticus, were obtained from one of the recommended hatcheries near Cairo The study examined two size of fish 30-40 mm and 60-70 mm in length These fish were acclimated for two weeks to the laboratory environment During this period, the fish were fed a commercial dry pellet food, the same food used in all experiments involving fenitrothion The fish were then acclimated for another week to the glass aquaria (50 x 35 x 35 cm) used in the experiments, at a constant temperature of 27°C

The Experiments

Acute Toxicity Test

The median lethal concentration (LC50) of fenitrothion was measured with a static test (USEPA, 1982) using a series of concentrations with three replicates Separate water controls were used Fingerlings of O. niloticus were placed in the aquaria and received no food for the duration of the test (96 hours) Investigators recorded mortalities at four hour intervals for the first day and at 24 hour intervals for each day thereafter Fish were considered dead when there was no movement of the operculum, at which point the dead fish were removed The value of the LC50 was computed using the probit analysis method of Finney (1971)

Uptake and Elimination Test

This test was carried out in glass aquaria filled with aqueous solutions containing concentrations equivalent to 1/100 of the LC50 of fenitrothion The renewal exposure system was used to conduct the uptake and elimination experiments Test solutions and control water were renewed periodically (usually 24 hour intervals) by transferring the test organism to different chambers with freshly-prepared material or by removing and replacing the material in the original containers Each of the three aquaria contained 15-20 fish, where they were raised for 15 days and then transferred to clean water for another 15 days Commercial dry feed was given once a day (4% of fish body weight) throughout the experiment

At each sampling time (1, 3, 7, 10 and 15 days), two fish were washed with running tap water, weighed and analyzed for chemical residue At the same time, water samples were taken and subjected to residue analysis

Residue Analysis in Fish - Extraction

For each fish sample, 10-40 g of fish were blended twice in a sorvall omni mixer, after adding 10 g of sodium sulfate anhydrous and 50 ml of acetonitrile This mixture was filtered under a vacuum using Whatman glass filter paper The extract was then concentrated by rotary evaporation

Residue Analysis in Fish - Clean-up

The concentrate (residues) were dissolved in hexane, then transferred to a 125 ml separatory funnel and extracted three times with 25 ml portions of acetonitrile The acetonitrile extracts (lower layer) were combined and concentrated again in the same manner as earlier mentioned The residues were dissolved in 2 ml of acetone for gas-liquid chromatography injections Extraction and clean-up procedures followed those developed by Kanazawa (1982)

A Varian 3700 GLC equipped with flame ionization detector (FID) was used with following operation conditions column - glass column (4 mm ID and 2 m length) packed with 3% OV-17 on 80/100 chromosorb W , oven temperature - 200°C, detector temperature - 300°C, injection port temperature - 300°C, carrier gas - nitrogen at a flow rate of 40 ml/min

Residues in Water (USEPA 1974)

A 50 ml water sample was extracted three times with 25 ml portions of methylene chloride in a 25 ml separatory funnel The mixture was then shaken vigorously for one minute, and the two layers were completely separated The methylene chloride extracts (lower portion) were collected and combined in a round bottom flask The solvent phase was then poured through a sodium sulfate anhydrous column, and the extract was concentrated by rotary evaporation The dried extract was dissolved in 2 0 ml of acetonitrile for GLC injections A Varian GLC, equipped with flame ionization detector, was used under the same operating conditions as outlined earlier

Results

The static 96 hour LC50 of fenitrothion to O. niloticus was calculated to be 4 0 mg/l

Uptake Phase

The uptake phase is the period during which fish are exposed to the chemical Figure 1 and table 1 portray uptake and elimination of sumithion by the two sizes of fish exposed to 40 ug/l (1/100 of LC50) of fenitrothion Insecticide concentrations in fish increased gradually until day 10 and nearly reached equilibrium at day 15 of exposure Fish tissue showed residues of 2 41 mg/kg in small fish and 3 05 mg/kg in larger fish Thus accumulation (residue

concentration in the fish tissue divided by that in the water) was 60 for the small fish and 76 for the larger ones

Depuration (Elimination) Phase

Depuration of chemicals is a process whereby a chemical contaminant is eliminated from the fish As displayed in Figure 2, when the fish were transferred to a clean water environment, fenitrothion concentrations in the fish declined gradually At the end of the 15 day exposure period, the small fish contained 0 03 mg/kg fenitrothion, while the large fish contained 0 04 mg/kg Differences were not significant between large and small fish at the end of the 15 day elimination period (see table 2)

Review of Other Studies

Sundaram (1973, 1974a) found that Sumithion concentrations decrease rapidly in natural water and disappear within hours to days Lockhart et al (1973), in their studies on stream-caged rainbow trout exposed to 275 g/ha Sumithion spray, reported that residues in rainbow trout averaged 0 5 mg/kg in the 24 hour period after spraying Peak residue values in the fish were as high as 1 84 mg/kg, but these declined to less than 0 02 mg/kg in four days At a level of 10 mg/kg-food, brook trout growth and food conversion efficiency are not affected (Wildish and Lister 1973) Green et al (1984) found that the uptake of the organophosphate acephate by rainbow trout from water was rapid and reached equilibrium concentrations in 5 to 8 days Elimination of 50% of acephate from the fish required 1 37 to 2 43 days

Korn (1973) reported that channel catfish exposed to 2 8 mg/kg per week by diet eliminated residues of carbaryl rapidly after they were placed on a carbaryl-free diet for 28 days Residues remained constant for 28 days in fish previously exposed to 0 25 mg of carbaryl per liter in their bath for 56 days

The insecticide diflubenzuron accumulated from the water into the tissues of the bluegill sunfish at levels up to 80 fold within 24 hours when the fish were exposed to concentrations of 10 µg/l (Schaefer et al 1979) The fish, however, degrade and eliminate diflubenzuron 24 to 48 hours after exposure Ohkawa et al (1980) reported that fenvalerate was taken up into carp fish more slowly than DDT Residues were readily excreted while DDT residues persisted

When Kanazawa and Tomizawa (1978) exposed the freshwater fish topmouth gudgeon to 20 µg/l of CNP (2,4,6-trichlorophenyl-4'-nitrophenyl ether), CNP concentration in the fish increased and reached equilibrium after 7 days After transferring it to clean water, the fish slowly excreted the CNP, decreasing levels to 0 115 mg/kg after 7 days

Duangsawasdi and Klaverkamp (1979) concluded that the LC50 value for fenitrothion was approximately 600 to 1000 times less than that for acephate in rainbow trout This difference in toxicity is probably due to the difference in solubility of the chemicals in water and

fat Fenitrothion is lipid-soluble, while acephate is water-soluble Sasaki et al (1982) reported a bioconcentration factor (concentration of the chemical in the fish/concentration of the chemical in water) of 50 for fenitrothion in killifish exposed to 800 µg of fenitrothion/l for 10 days The bioconcentration factor for coho salmon exposed to 560 µg of fenitrothion/l for one day was 16 (Bull 1974)

Giam et al (1974) observed a relationship between the size of the fish and residues, especially in highly contaminated areas Boileau et al (1979) noted a similar relationship between age and residue concentrations in pike from the Richelieu River in Canada Shannon (1977) found that large catfish accumulated greater concentrations of dieldrin in muscles than did small catfish after 28 days of exposure to the insecticide

Metals, Protein, Lipid & PAHs in Fish From Different Egyptian Sites
Levels, Uptake & Elimination of Cadium

METALS, PROTEIN, LIPID AND PAHs IN FISH

In order to identify problems associated with chemical contaminants, researchers in the Seafood Safety and Fish Decontamination Project screened representative samples of fish from different sites for chemical residues The sites selected represent the full spectrum of degrees of environmental contamination and exposure in Egypt Sampling took place at the following sites

1) Lake Manzala I - mixed lake and Mediterranean Sea water,
2) Lake Manzala II - southern part of the lake, which is most contaminated,
3) Lake Mariut in the Alexandria region - complex, heavily-polluted lake, receiving many different types of industrial, agricultural and sewage wastes,
4) Lake Timsah at the middle portion of the Suez Canal - lake ecosystem affected by sewage water and other pollutants from ships crossing the canal,
5) The Nile River - relatively high concentration of contaminants,
6) Fish ponds in the Suez Experimental Station - sewage wastes treated via stabilization ponds,
7) Mediterranean Sea at Port Said - heavily contaminated sea site in the immediate vicinity of the waste sources

Tilapia sp were collected from the first six sites, while *Mugil sp* samples were collected from the Port Said site Both species are bottom feeding fish

Based upon the work plan for the project, researchers chose to measure levels of metals (Cd, Pb, Cu and Zn) in organs of the fish samples collected from the above sites In addition, utilizing the GC/MS technique, they analyzed polycyclic aromatic hydrocarbons (PAHs) and total hydrocarbons in the muscle of *Scomber japonicus* of the Gulf of Suez Scientists also measured the protein and lipid content of fish muscle from the sites

Metals

Analysis Technique

Fish samples were weighed, measured and dissected in order to separate the organs (muscle, gills and liver) According to methods recommended by the FAO (1976), subsamples were digested in concentrated HNO_3, diluted to a specified volume and then measured using an atomic absorption spectrophotometer (Buck Science, model no 200A) Data obtained were expressed in ppm net weight

Results

Figures 3-6 show levels of metals in organs of fish collected from the various sites Cadmium and lead concentrations exhibit their highest levels in the gills of fish, with one exception in which Cd concentration is highest in the liver of *Mugil sp* collected from the Mediterranean Copper and zinc levels, on the other hand, are highest in the liver of the fish This illustrates organ and metal-specific accumulation which is in agreement with recent literature (Fujise 1988) Muscle tissue shows the lowest values for all metals

Lead in the muscles of fish collected from the Mediterranean and the northern region of Manzala Lake shows values less than 0 1 ppm, while other sites reveal levels ranging from 0 125 to 0 275 ppm Cadmium levels, however, show no variation (^{2}0 03 ppm), with the exception of levels observed in *Tilapia* from the Suez fish ponds, where levels were 0 085 ppm High levels of copper were found in the muscles of fish collected from the Mediterranean, reflecting the effect of heavy water traffic upon pollutant levels Concentrations of each metal seem to correlate with the pollution sources and environmental conditions of the area in which they are found

Absolute maxima of lead and zinc were found in the muscle of *Tilapia sp* collected from Mariut, while the absolute maximum value of cadmium was found in the muscle of *Tilapia* from the Suez fish ponds

Calculating the ration c/w (where c is the concentration of Cd in the muscle of *Tilapia sp*, and w is the fish weight) produces considerable changes in the pattern of metal variation between different areas (see figure 7) Maxima of the ratio c/w were observed in the Mariut ecosystem, highlighting the heavy pollutant levels of that lake

Levels of the four metals in the muscle are within the safe concentrations recommended by the FAO (1991) Table 3 shows the level of metals in the muscle of fish from the various Egyptian sites The levels reported in this table are within the range of levels reported in other studies

<u>Total Hydrocarbons and Polycyclic Aromatic Hydrocarbons (PAHs)</u>

Analysis Technique

50 grams of fresh samples of fish muscle were digested by KOH in CH_3OH and refluxed for one hour with 15 ml of n-pentane (Farrington and Mederos 1975) The combination of high-resolution capillary gas chromatography and high-performance mass-spectrometric identification of compounds was used under the conditions illustrated in the accompanying figures

Results

Identification of individual hydrocarbons was carried out by Com-GC/MS using a Hewlett-Packard Model MS-5988 Series of PAHs were measured and quantified using the mass fragmentographic profiles (see figures 8-11)

Due to its severe oil contamination, the Gulf of Suez was chosen as a site from which to collect fish Researchers collected *Scomber japonicus* from 3 locations Ain El Sukhna, the immediate vicinity of the oil exploration area of Petro Bel Company, and the southern region of the Gulf of Suez The figures illustrate that the muscle tissue of fish collected from the oil exploration area have accumulated many varieties of PAHs of high molecular weight The most abundant PAHs found in fish muscle at this location are Dibenzo cronene, Benz[a] anthracene/crysene and 1-methyl dibenzo [a,h] anthracene The other two locations showed less variety and lower concentration of PAHs in the fish muscle tissue The present data are similar to that present in other work (Grimalt et al 1984) in the coastal zone of Spain

Figure 12 shows that fish samples from the Mediterranean Sea at Port Said contain the highest levels (19 42 ppm) of total hydrocarbons El Samra et al (1982) reported that heavy ship traffic had adversely impacted the area Moreover, industrialization of Cairo and Giza has rendered the Nile River an environment heavily contaminated with oil and its derivatives This would explain the high levels of hydrocarbons found in the tissue of Nile River fish

Carcinogenicty of various PAHs has been demonstrated in rodent skin tests Analysis of PAHs in tissues generally indicates that levels should be quite low in the most commonly-consumed fish products (GESAMP 1993)

Total Protein and Lipid Content

Analysis Technique

The total protein content in the fish muscle was determined according to the method developed by Peters (1968) Total lipid content was carried out according to the method specified by Knight et al (1972)

Results

Figure 13 shows that total protein content of fish muscle exhibits non-significant variation at the different sites The values of total protein ranged from 5 9 to 8 2 gm%, while those of total lipid varied from 0 38 to 1 29 gm%

UPTAKE AND ELIMINATION OF CADMIUM

The work in task 3 of the Seafood Safety and Fish Decontamination Project is devoted to assessing the rate of elimination of chemical pollutants from contaminated fish In order to determine these rates, scientists undertook long-term experiments to study the uptake and elimination of cadmium The experiments took place under controlled conditions in the bioassay laboratory in the Suez branch of the National Institute of Oceanography and Fisheries

Cadmium was selected due to its ubiquity, high toxicity and ability to accumulate in aquatic organisms Cadmium uptake from water can take place via either passive diffusion through the gills or through the body (Del Ramo et al 1993)

Experiment

Live fish of *Tilapia sp* were caught and acclimated to laboratory conditions for one week, after which time the experiments began Groups of active and healthy fish of 7 different sizes were placed in glass tanks containing 1 ppm of cadmium Initial concentrations of cadmium were measured in the entire body for small fish and in different organs in the larger fish Researchers calculated the mean value of concentrations and found that the standard deviation was within the accepted limits

Fish were removed from the containers at different time periods to measure Cd concentrations in the muscle, liver, gills and whole body Using the static-renewal procedure, actual concentrations of Cd in pooled water samples were determined at the beginning and after 24 hours of exposure before water removal

The elimination experiment began after 30 days of accumulation, when the alive and active fish were transferred to tanks containing clean seawater The same procedures were followed as those implemented in the uptake experiments

Results

Figures 14-15 show cadmium uptake in different organs, as well as uptake and elimination for the entire fish Cadmium accumulated most rapidly in the gills Accumulation during the first 48 hours showed a 12 fold increase over the initial concentration, although the other organs (muscle and liver) showed no response to cadmium uptake during the same period After a 30 day period, cadmium levels in the gills had increased 65 fold over the initial concentration, while that in the liver had increased to levels as high as 8400 fold No saturation was recorded in the gills or liver tissues after 30 days

No response was recorded in the muscle during the first 10 days of the experiment After this period, a rapid increase (55 fold) was observed up to day 20 The uptake rate slightly

decreased to 21 fold over the initial concentration after 30 days It appears that these results were due to detoxification processes

In the whole fish, cadmium increased 37 fold in the first 24 hours over the initial concentration Accumulation, however, reached a plateau between the first and fifth day and was then followed by a rapid rise to day 30 Figure 15 shows that, after a rapid cadmium uptake in the whole fish, the elimination from the tissue was slow Whole body concentrations reached 4 38 ppm after a 24 hour period and then decreased at a slow pace to 1 82 ppm after 20 days of depuration

Table 1

Mean residues (mg/kg) and uptake of fenitrothion by O. niloticus exposed to 40 µg fenitrothion/l for 15 days

Fish size	Time (days) from beginning of exposure				
	1	3	7	10	15
35-40 mm	0 75	1 02	1.90	2.40	2 41
60-70 mm	1 05	1 80	2.45	3 04	3 05

Table 2.

Mean residues (mg/kg) and elimination of fenitrothion by O. niloticus in clean water following 15 days of withdrawal from exposure to 0 04 mg fenitrothion/l

Fish size	Time (days) from end of exposure period				
	1	3	7	10	15
35-40 mm	2 08	1 60	0.58	0.19	0 03
60-70 mm	2 42	1.75	0.85	0 28	0.04

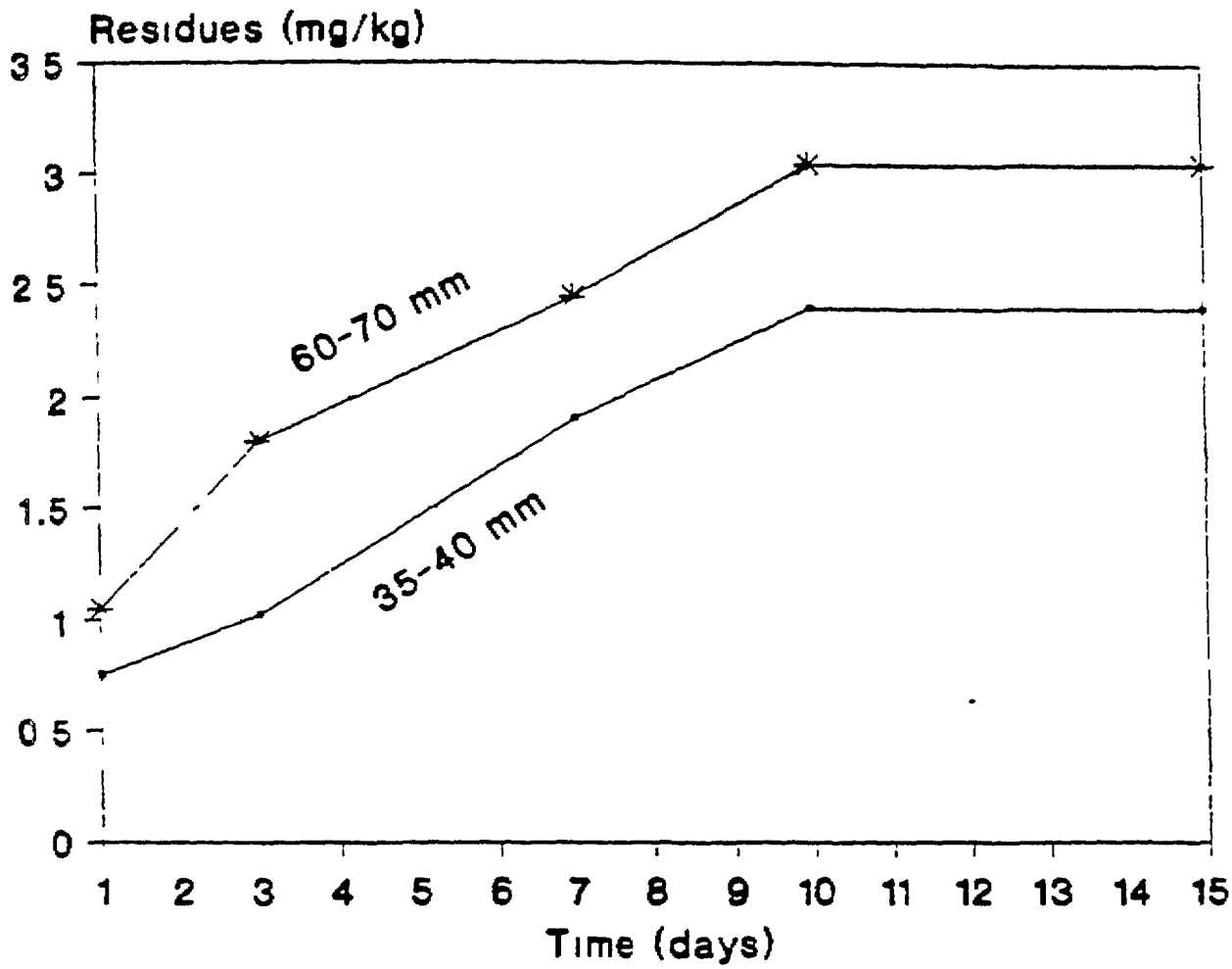


Figure 1.

Uptake of fenitrothion by Oreochromis niloticus exposed to 0 04 mg fenitrothion/l for 15 days

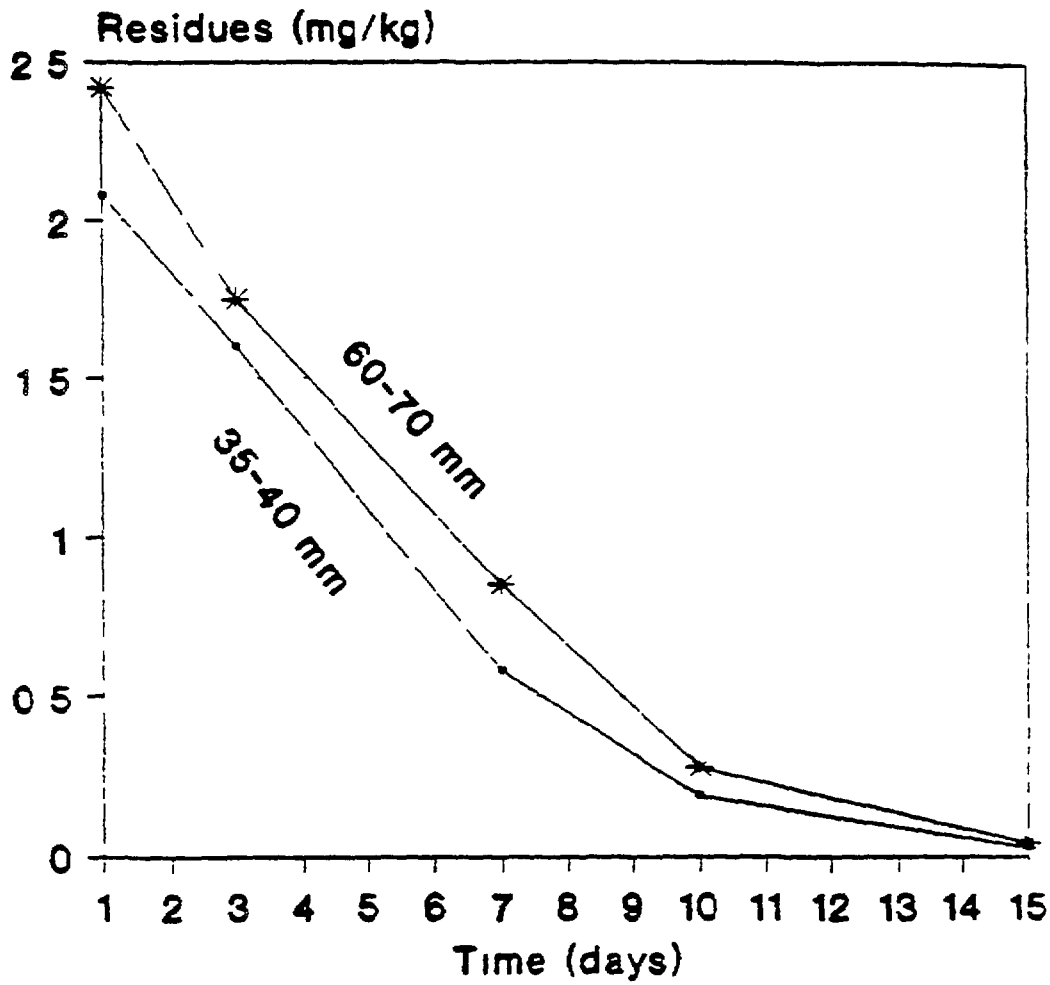


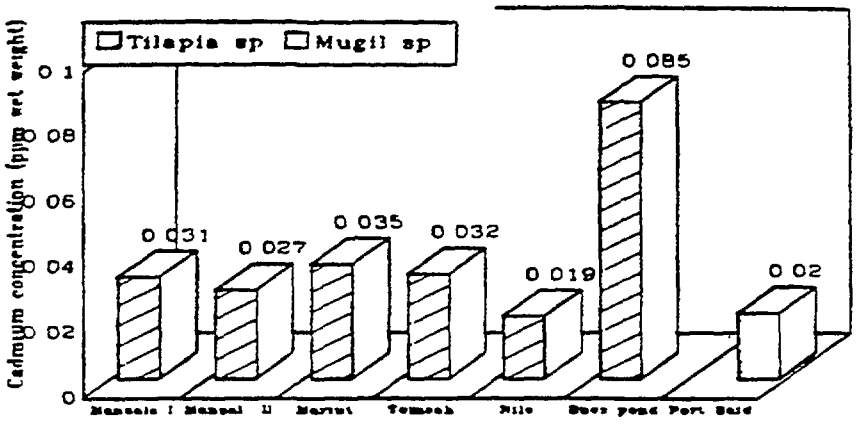
Figure 2

Elimination of fenitrothion by Oreochromis niloticus in clean water for 15 days after exposure to 0 04 mg fenitrothion/l for 15 days

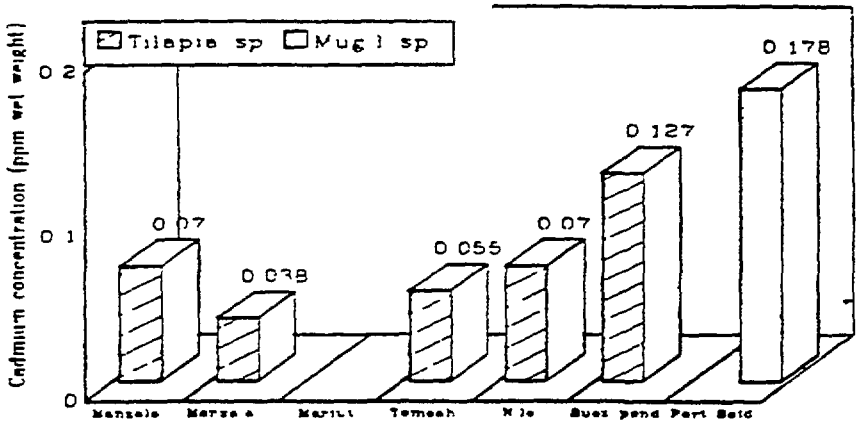
Table 3

Level of metals in the muscle of fish from various sites in Egypt

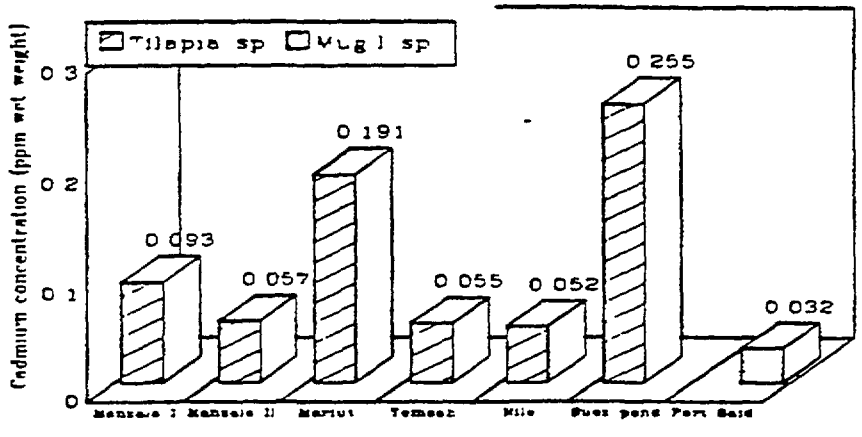
Species	Location	Cd	Pb	Cu	Zn	References
Tilapia Sp	Manzala	0 015- 0 055	0 225- 0 700	1.013-1 475	5 225-7.29	Abou-Donia (1990)
Tilapia Sp	Mariut	0 06-0 093	0 9 - 2 425	0.88 - 1 56	6 143-6 973	Abou-Donia (1990)
Mugil Sp	Red Sea	-	7.14	16 75	15 56	Abdel-Salam (1981)
Mugil Sp	Ismailia	-	3 54	-	13.04	El- Deak (1990)
Mugil cephalus	Suez Bay	0 13	0 54	0 590	15 28	El- moselhy (1993)
Tilapia Sp	Manzala I	0 031	0 086	0 523	4.038	Present study
Tilapia Sp	Manzala II	0 027	0 176	0 446	6.48	Present study
Tilapia Sp	Mariut	0 075	0 275	0 550	8 271	Present study
Tilapia Sp	Temsah	0 052	0 234	0 620	3.185	Present study
Tilapia Sp	Nile	0 019	0 125	0 373	3.537	Present study
Tilapia Sp	Suez pond	0 085	0 234	0 298	4.610	Present study
Mugil Sp	Port Said	0 02	0 088	1 362	5.584	The present study



Muscle



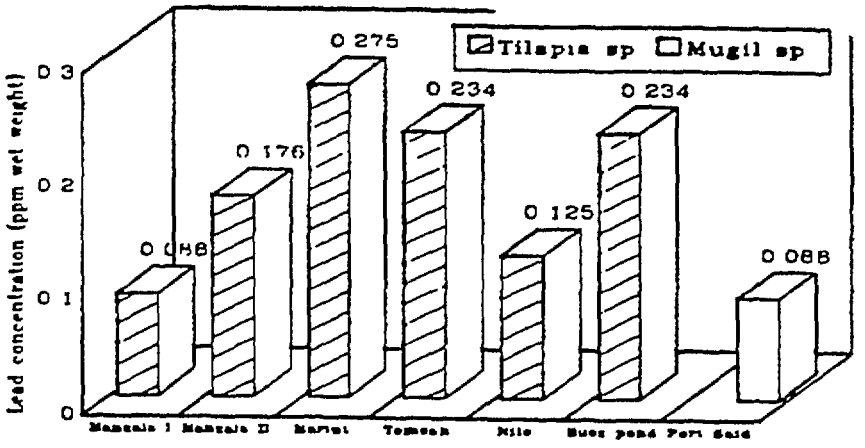
Liver



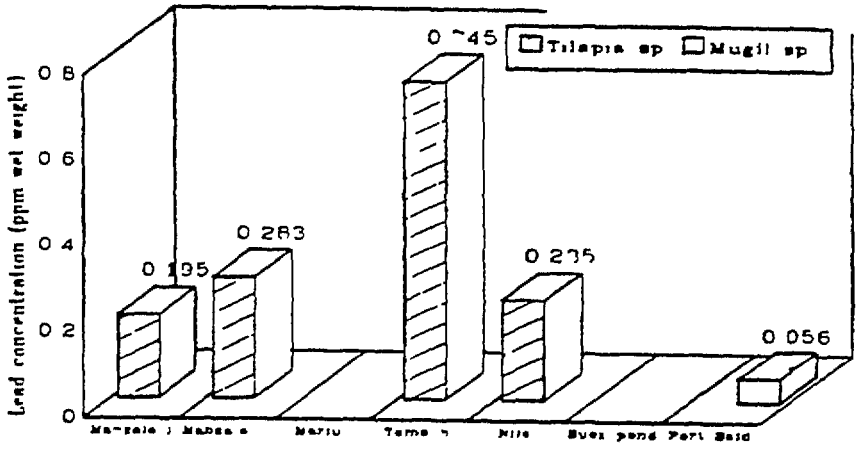
Gills

Figure 3

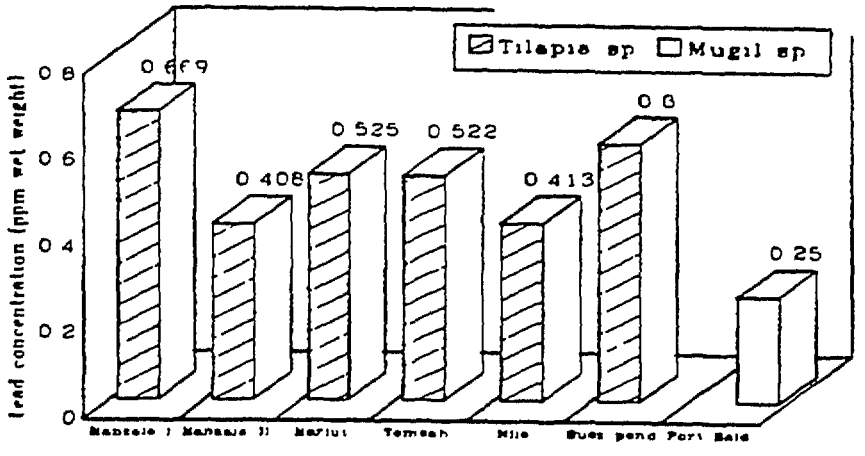
Cadmium levels in fish collected from various sites



Muscle



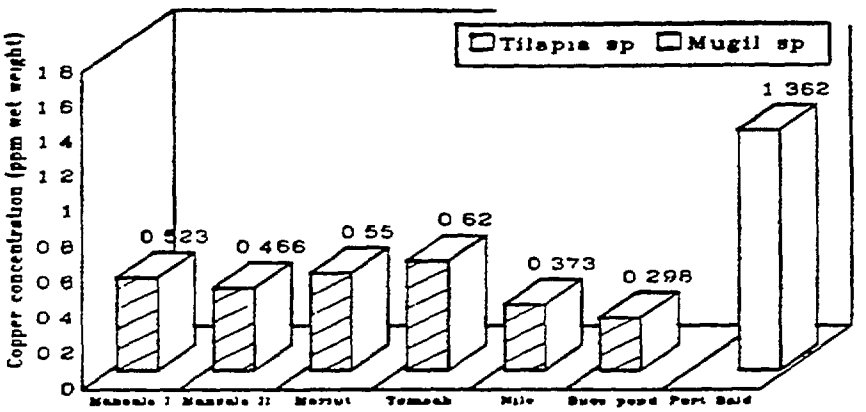
Liver



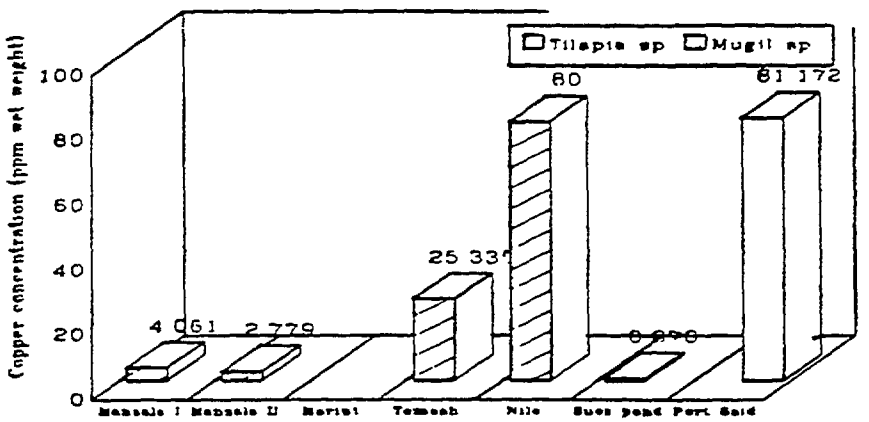
Gills

Figure 4

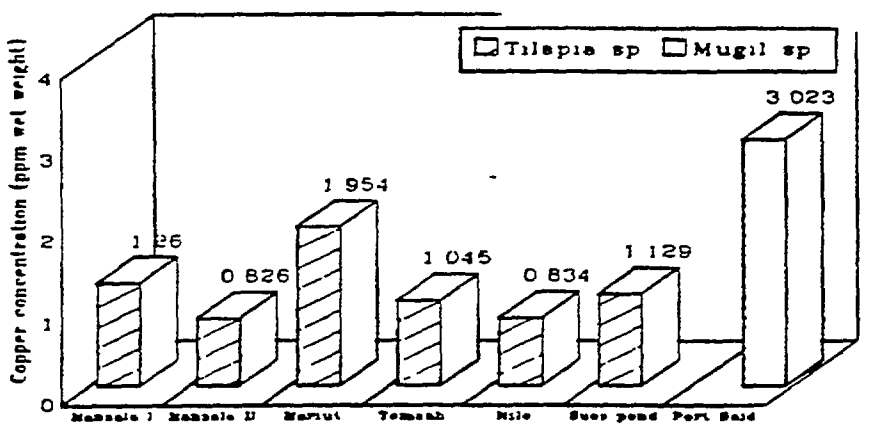
Lead levels in fish collected from various sites



Muscle



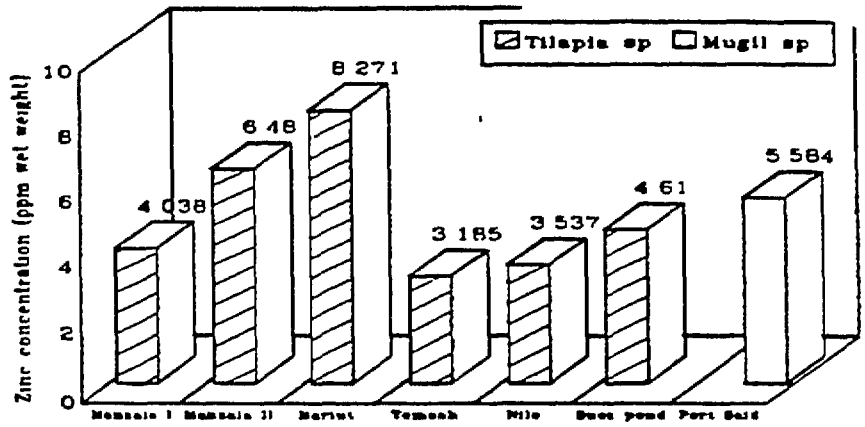
Liver



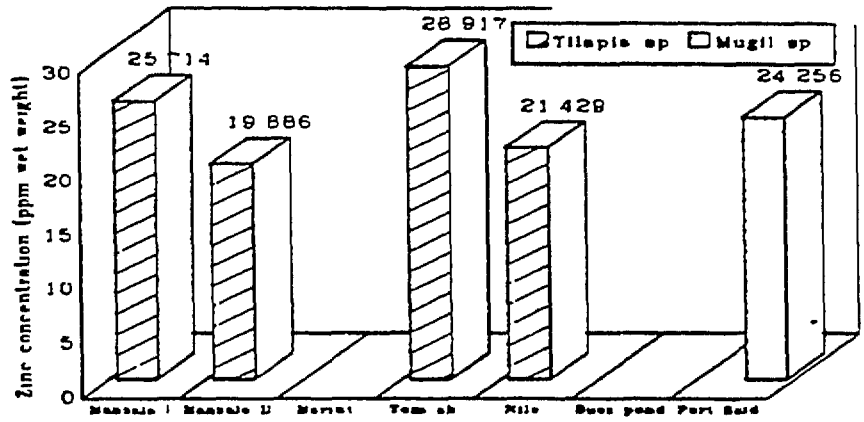
Gills

Figure 5

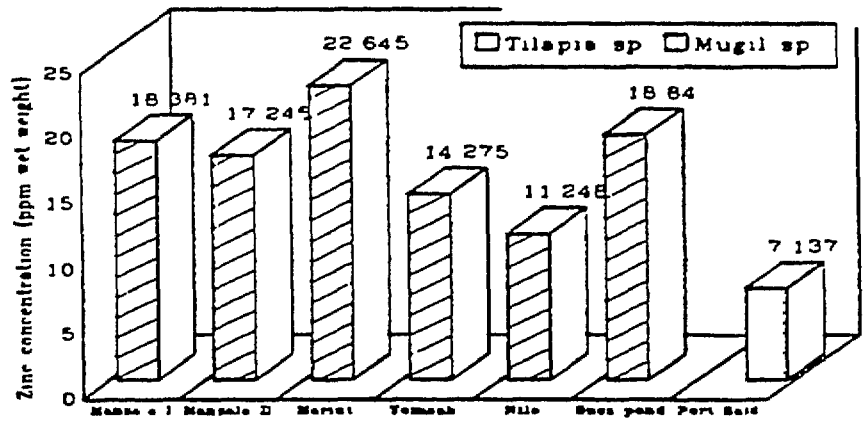
Copper levels in fish collected from various sites



Muscle



Liver



Gills

Figure 6

Zinc levels in fish collected from various sites

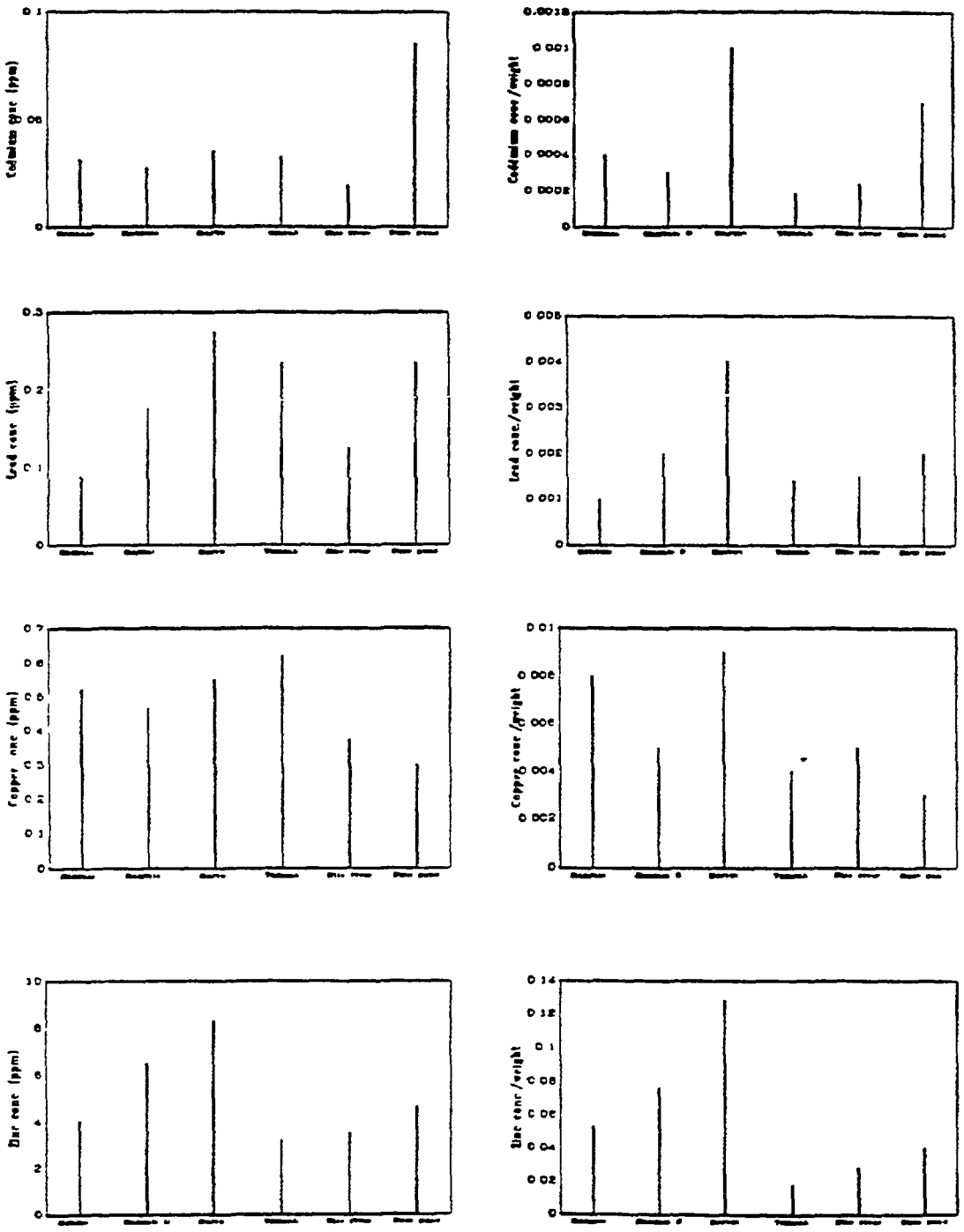
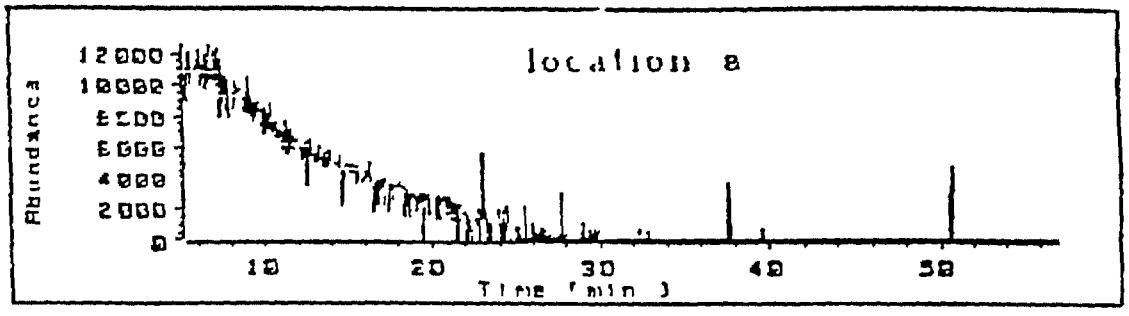


Figure 7.

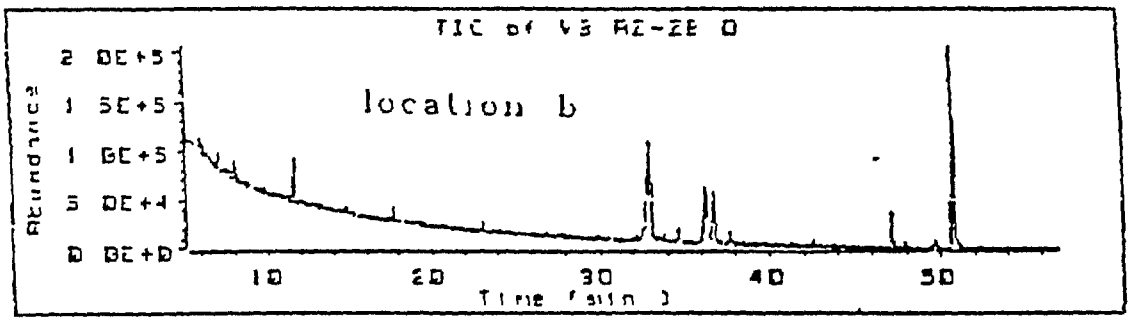
Metals concentration and metal/weight of *Tilapia sp* from various sites



T Scan 39 676 min of V3 ZOH

Y Scan 39 825 min of V3 ZOH

X Scan 50 676 min of V3 ZOH



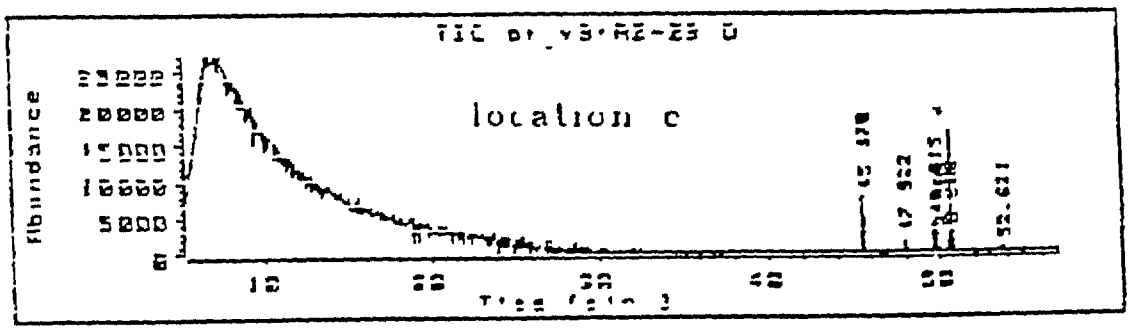
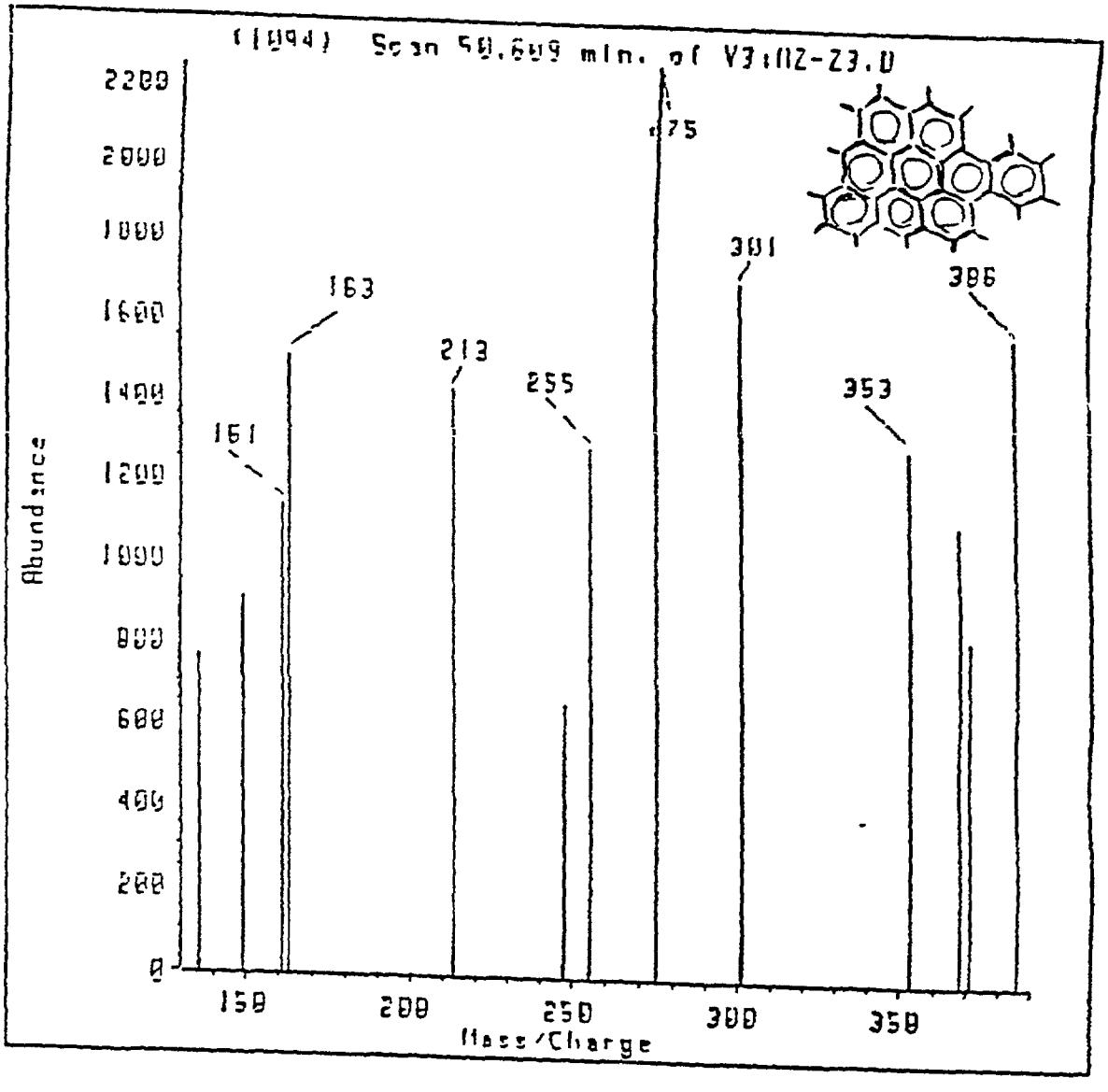


Figure 8

GC fractionation of hydrocarbon residues in muscle of *Scomber japonicus* of the Suez Gulf



FILE NAME
HP MODEL MS 5988

SAMPLE INFORMATION NO 22

ELECTRON ENERGY 15eV
SERIAL NO. 25949

6 Sep 93 3 20 pm
INLET TYPE :GC
FLOW RATE
COLUMN TYPE :HP-1
TEMP RATE :5 C/MIN
FINAL TEMP :280 C

Figure 9

MS fragmentation of dibenzo cronene detected in muscle of *Scomber japonicus* of the Suez Gulf (location b)

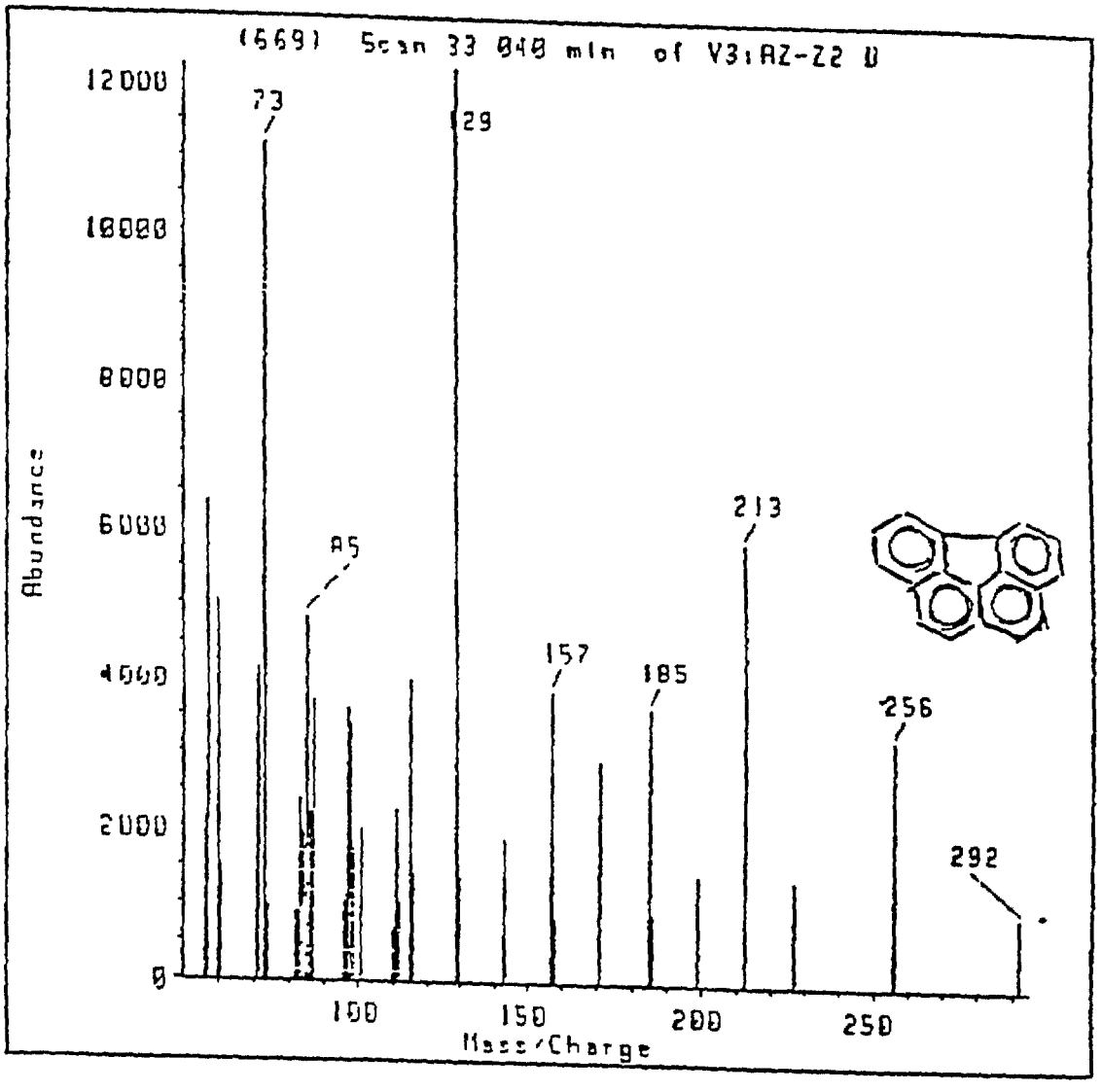


Figure 10

MS framentation of benz[A]anthracene/crysene detected in muscle of *Scomber japonicus* of the Suez Gulf (location b)

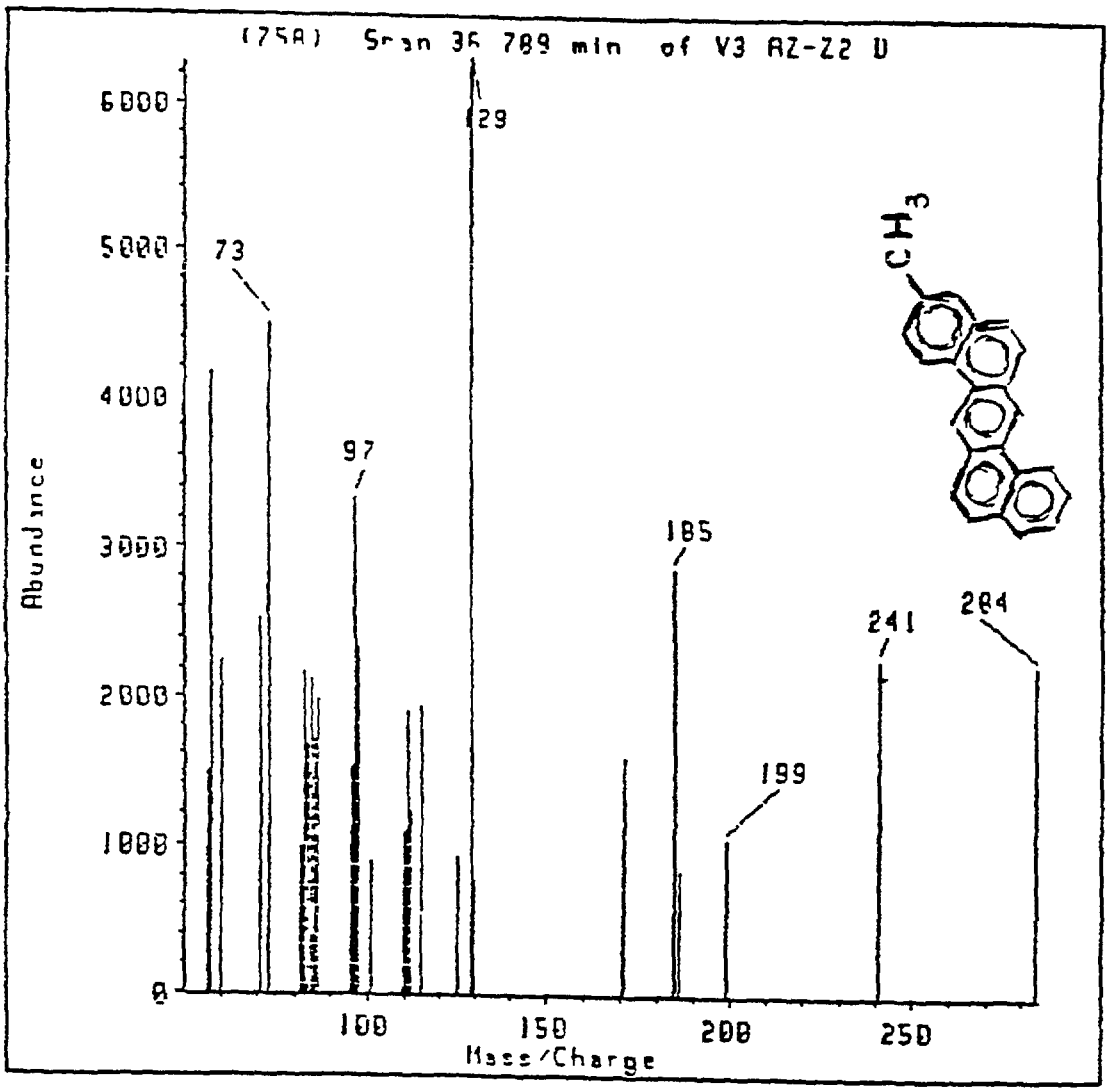


Figure 11

MS framentation of 1-methyl dibenzo [a,h] antrhacene detected in muscle of *Scomber japonicus* of the Suez Gulf (location b)

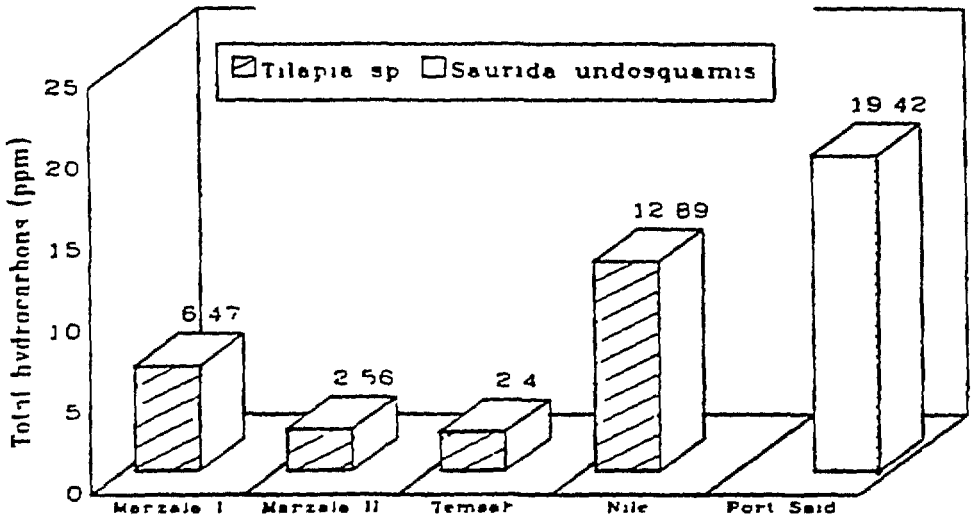
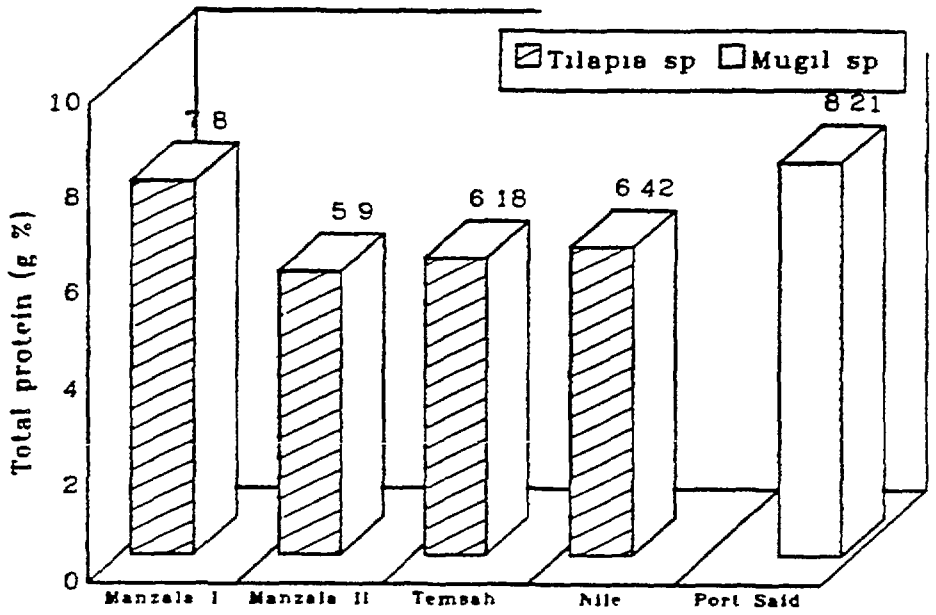


Figure 12

Total content of hydrocarbons in fish from different sites



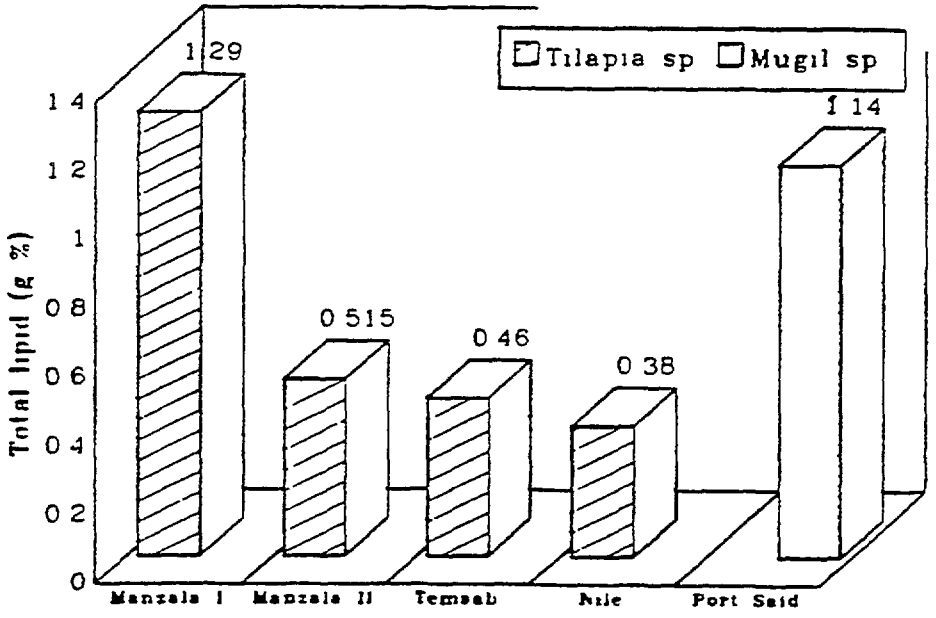


Figure 13.

Total protein and lipid content in fish from different sites

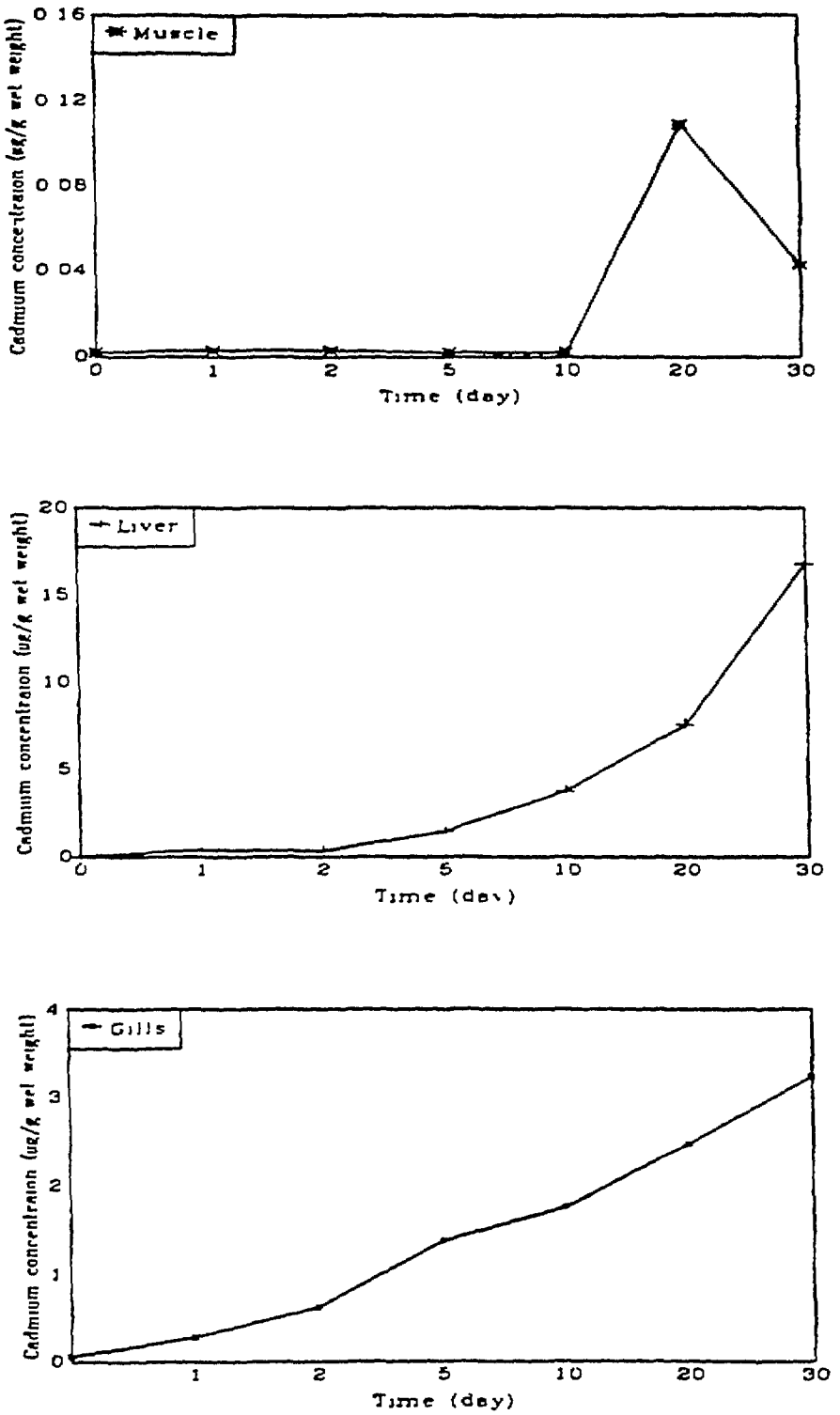


Figure 14

Uptake of cadmium in organs of *Tilapia sp*

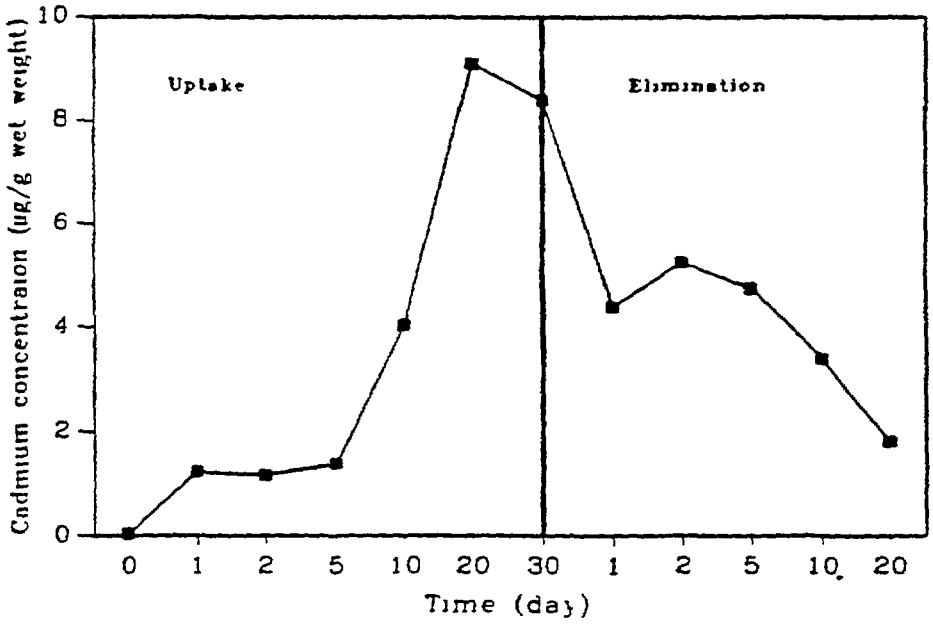


Figure 15

Uptake and elimination of cadmium in whole fish of *Tilapia sp*

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EFFECT OF WATER PH AND SALINITY ON THE TOXICITY, UPTAKE AND DEPURATION OF THE ORGANOPHOSPHORUS INSECTICIDE FENITROTHION (SUMITHION) BY THE NILE TILAPIA *OREOCHROMIS NILOTICUS*

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INTRODUCTION

The effect of pesticides on fish is influenced by a number of environmental factors which may increase or decreas the toxic effect of these chemicals to fish As a consequence, the extent of uptake and depuration of pesticides by fish could be altered Environmental factors that may affect the toxicity, uptake and depuration of pesticides by fish include temperature, pH, dissolved gases, dissolved salts, turbidity, alkalinity, organic compounds, salinity and hardness

Literature reveals some conflicting data concerning the effect of such environmental conditions on the toxicity, uptake and depuration of pesticides by fish In a previous study (Shereif, 1995), a positive relationship was found between the uptake of the organophosphorous insecticide fenitrothion and Nile tilapia (*Oreochromis niloticus*) from a solution containing 40 ug fenitrothion/l This study is an attempt to evaluate, under lab conditions, the effect of hydrogen ion concentration (pH) and salinity on the toxicity, uptake and depuration of the organophosphorus insecticide fenitrothion by Nile tilapia *O niloticus*

MATERIAL AND METHODS

I. The Fish .

The Nile tilapia *Oreochromis niloticus* was selected as a very common and popular fish in Nile and its tributaries and in Egyptian fish farms Fingerlings (mean weight 5 5 g, mean length 3 5 cm) were obtained from one of the recommended hatcharies near Cairo They were acclimated for two weeks in the lab During this period, fish were fed a commercial dry pellet food at 4% of their body weight The fish were acclimated for another week in glass aquaria (50 x 35 x 35 cm), filled with 50 l of water before exposing them to fenitrothion

II. The Pesticide Fenitrothion

Chemical Name	O,O-Dimethyl O-(4-nitro-m-tolyl) phosphorothioate
Alternate Names	Accothion, Agrothion, Bay 41831, CAS 122-14-5, ENT-25715, Folithion, MEP, Methyl-nitrophos, Novathion, Nuvanol, Sumithion, Cytel, S-5660
Principal Use	Insecticide
Purity	40% (EC)

III Acute toxicity Bioassays·

The values of static 96h-LC_{50} were determined for fenitrothion (Sumithion) according to procedures developed by Standard Methods (1986) A series of concentrations of fenitrothion with the adjusted values of pH and salinity (three replicates and controls) were used Pesticide was added to the aerated test medium 45 minutes before the fish tilapia were placed No food was provided during the 96 hrs of the test

Mortalities were recorded at four hours intervals for the first day and at 24 hour intervals for each day thereafter Fish were considered dead when there was no movement of the operculum, and dead fish were removed immediately after death

IV Chronic Toxicity Bioassays

Chronic toxicity bioassays were performed in glass aquaria (50 x 35 x 35cm) filled with 50 l of water and 40 ug fenitrothion/l with the adjusted values of pH and salinity This concentration is $^1/_{10}$ of the acute toxic value (400 ug/l) previously determined by the author (Shereif, 1995) The renewal exposure system was followed where the test solutions and control water were renewed periodically (usually at 24-hr intervals) 15-20 fingerlings of Nile tilapia were placed in each aquarium, with three replicates for 15 days, then transferred into clean water for another 15 days Commercial dry pellet food was provided to fish at 4% of their wet body weight

V. pH and Salinity:

The pH was adjusted to 5 (acidic range), and 9 (alkaline range) by addition of concentrated hydrochloric acid (HCl) or saturated solution of sodium hydroxide (NaOH), respectively The pH was usually measured 45 minutes after NaOH or HCl is added pH values were measured and

monitored by a pH meter Preliminary experiments showed that these pH values were not toxic within 96 hrs to tilapia After acclimation (96 hrs), fish were transferred to aquaria filled with water of the identical pH values but containing 40 ug fenitrothion/l

Salinities in test aquaria were adjusted to 8% and 35% by addition of pure sodium chloride (NaCl) Salinity values were measured using EC meter The fingerlings of tilapia were slowly accliminated to salinity values before exposure, then transferred to aquaria containing the same salinity values and 40 ug/l of fenitrothion

VI. Residues of fenitrothion

At each sampling time (1, 3, 7, 10 and 15 days), two fish were taken, washed with running tap water, weighed, and analyzed for pesticide residues in muscles (the edible part) Water samples were taken at the same sampling time and subjected to residue analysis

The extraction and clean-up procedures of fenitrothion in fish and water samples followed those developed by Kanazawa (1975) and USEPA (1974), respectively A varian 3700 GLC equipped with flame ionization detector (FID) was used for the detection of fenitrothion in fish and water samples

RESULTS

I pH

Data in Table (1) and Figure (1) show that the toxicity of fenitrothion to Nile tilapia increased with the decrease of pH values The 96-h LC50 of fenitrothion for Nile tilapia was previously recorded as 400 ug/l using Nile water (Shereif, 1995) This value increased at pH = 5 2 (280 ug/l), while it decreased at pH = 9 1 (480 ug/l)

The uptake of fenitrothion by the fish was more pronounced at the lower values of pH (pH = 5 2) than the other two values of pH (Table 2) It is noticed that concentrations of fenitrothion in fish muscles increased gradually until day 10 and nearly reached equilibrium at day 15 of exposure However, the muscles of the fish exposed to fenitrothion at the lower pH accumulated greater concentrations than did muscles at higher pH (Table 2 and Figure 2) Accordingly, the accumulation factor (residues of fenitrothion in fish muscles divided by that in water) for fish exposed at pH = 5 2 was greater (71) than those fish at pH = 6 8 (60) and pH = 9 1 (57) (Table 2)

When the fish transferred to clean water, fenitrothion concentrations in fish declined gradually at the different pH values (Table 3 and Figure 3) Differences in residues after depuration of fenitrothion from fish were insignificant ($P < 0\ 05$) between fish exposed to fenitrothion at the different pH values

II Salinity:

The toxicity of fenitrothion to Nile tilapia was most higher at the highest salinity value (35%) than the other two values (Table 4 and Figure 4) Thus, the accumulation factor was comparatively higher for the fish exposed to the pesticide at the higher water salinity than those at the lower salinity (8%) or Nile water (Table 5 and Figure 5)

On the other hand, there was no general trend in the effect of water salinity on the depuration of fenitrothion from fish (Table 6 and Figure 6)

Table (1) Acute Toxicity data of fenitrothion to *O niloticus* in different pH values

pH value	96-h LC_{50}
5 2	280 ug/l
6 8 (Shereif, 1995)	400 ug/l
9 1	480 ug/l

Acute Toxicity Data
At pH Values

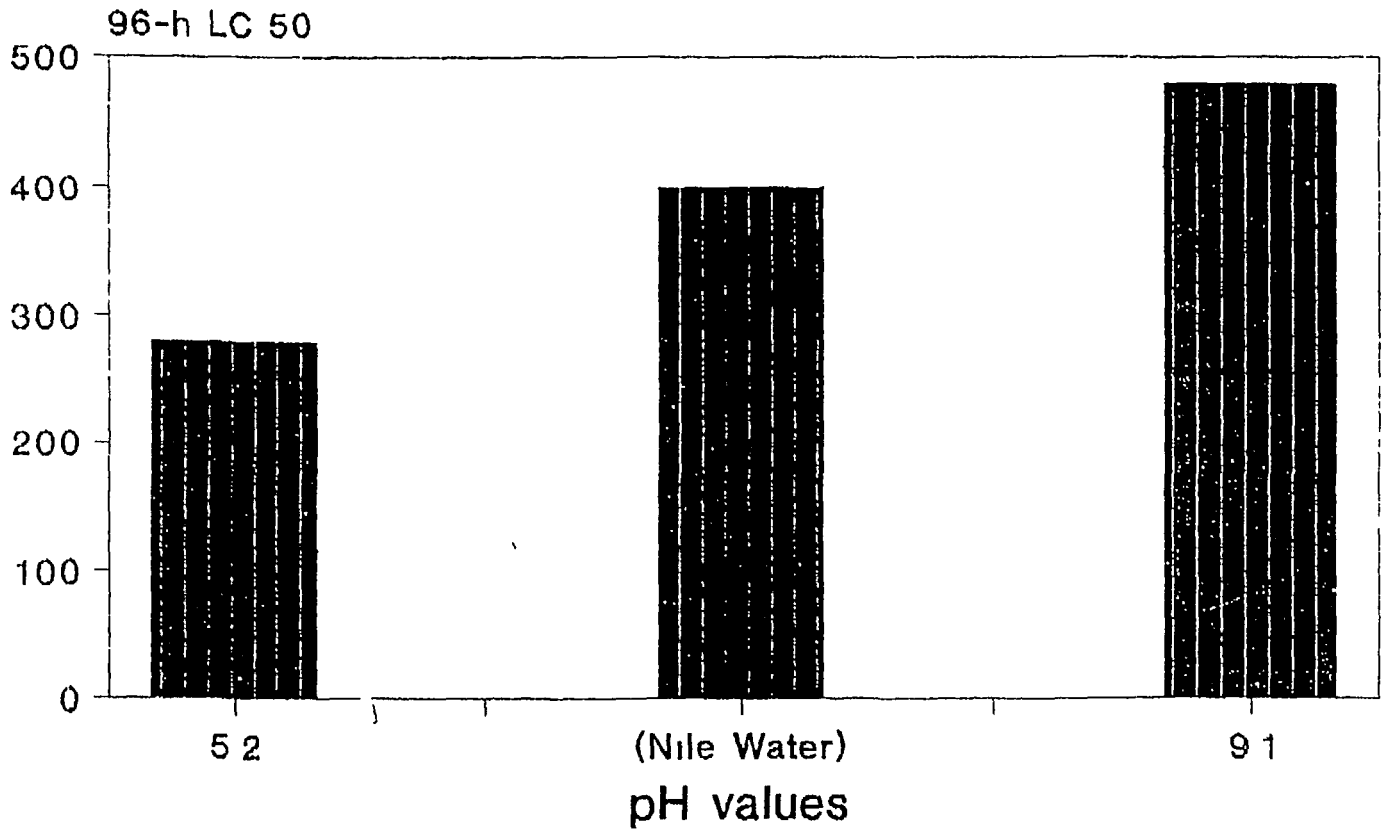

Fig. (1) : Acute Toxicity Data at pH Values

Table (2) Mean residues (mg fenitrothion/kg fish) and uptake of fenitrothion by *O niloticus* exposed to 40 ug/l at different pH values

pH values	Time (days) From the beginning of exposure period					Accumulation Factor
	1	3	7	10	15	
5 2	1 00	1 47	2 35	2 85	2 85	71
6 8 (Shereif,1995)	0 75	1 02	1 90	2 40	2 41	60
9 1	0 70	0 98	1 65	2 25	2 26	57

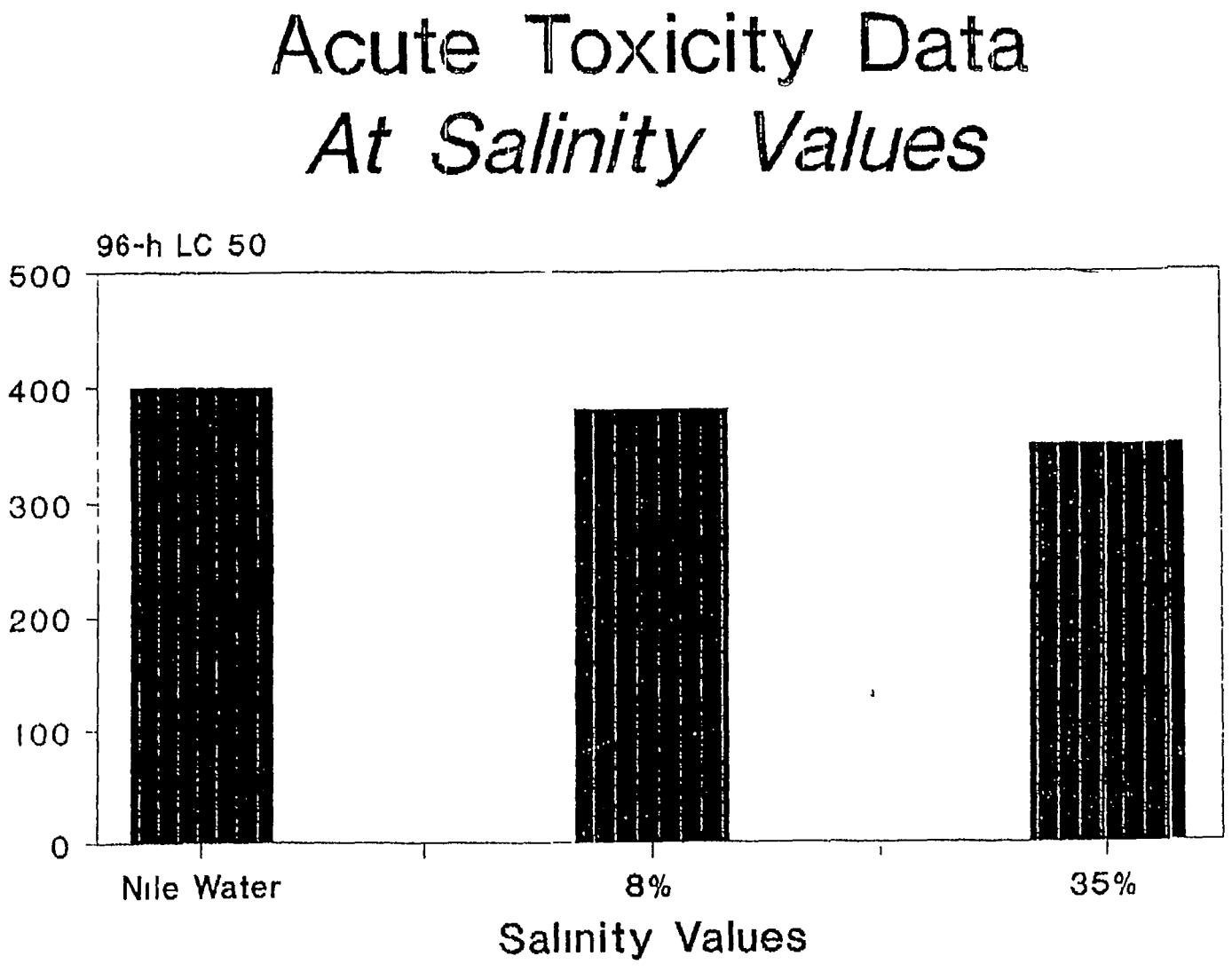

Fig. (2) : Acute Toxicity Data at Salinity Values

Table (3) Mean residues (mg fenitrothion/kg fish) and depuration of fenitrothion by *O niloticus* in clean water folllowing 15 days of withdrawal from exposur to 40 ug/l at different pH values

pH values	Time (days) from end of exposure period				
	1	3	7	10	15
5 2	2 25	1 75	0 90	0 59	0 29
6 8 (Shereif, 1995)	2 08	1 06	0 58	0 19	0 03
9 1	2 01	1 62	0 79	0 32	0 06

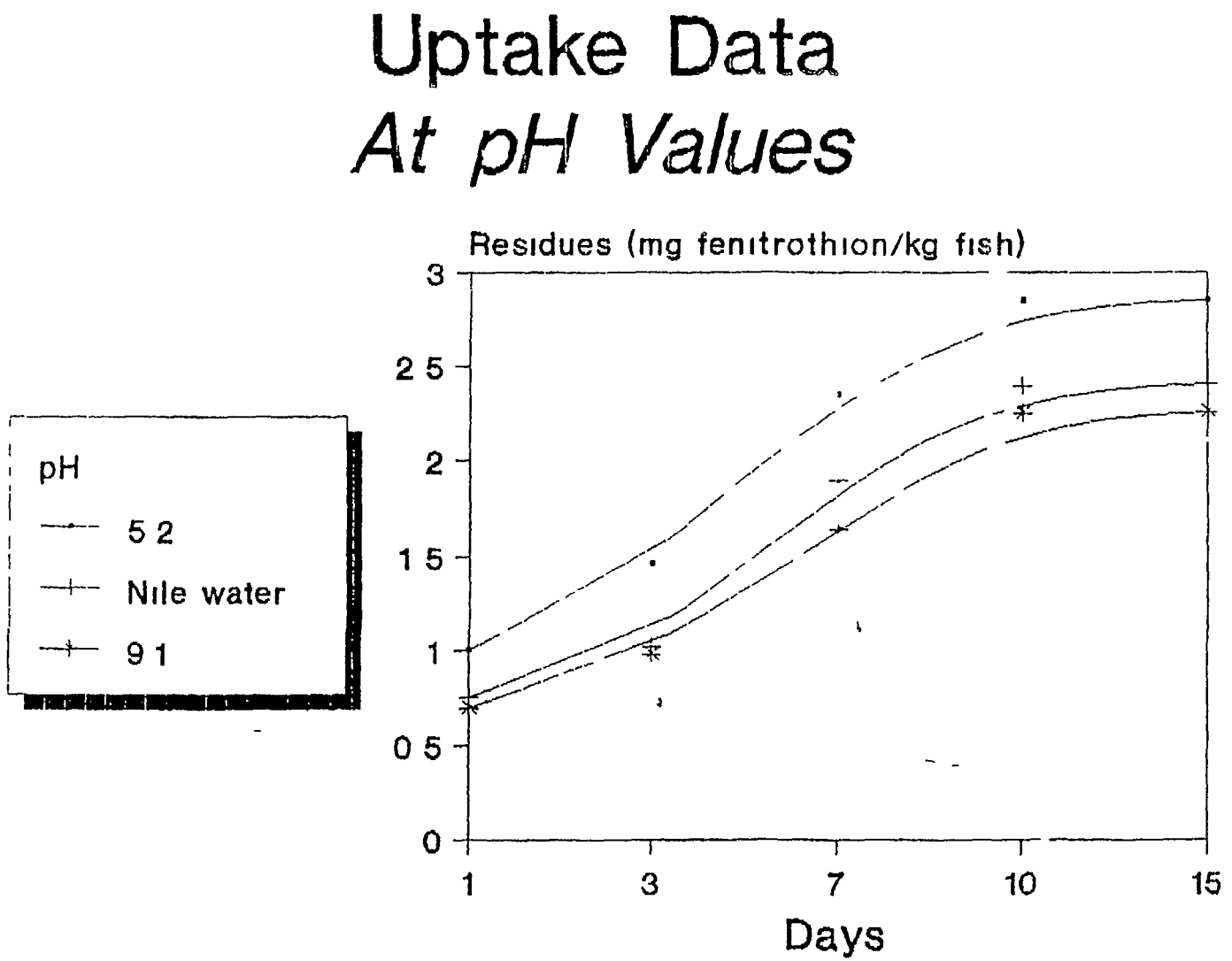

Fig (3) . Uptake Data at pH Values

Table (4) Acute toxicity data of fenitrothion to *O niloticus* in different water salinities.

Salinity Value	96-h LC_{50}
Nile water (Shereif, 1995)	400 ug/l
8%	380 ug/l
35%	350 ug/l

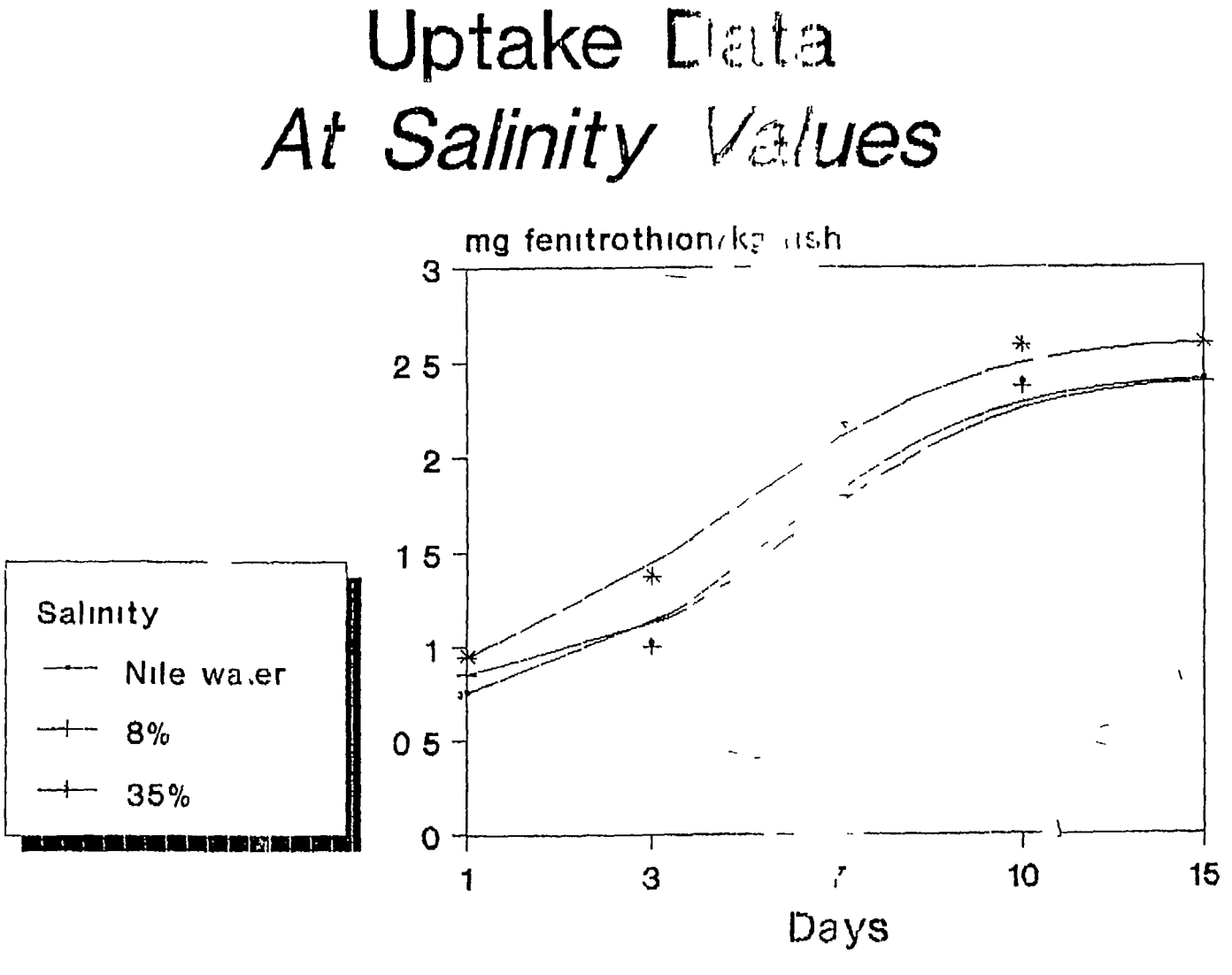

Fig (4) . Uptake Data at Salinity Values

Table (5) Mean residues (mg fenitrothion/kg fish) and uptake of fenitrothion by *O niloticus* exposed to 40 ug/l at different water salinities

Water Salinity	Time (days) From the beginning of exposure period					Accumulation Factor
	1	3	7	10	15	
Nile Water *	0 75	1 02	1 90	2 40	2 41	60
8%	0 85	1 00	1 80	2 38	2 40	60
35%	0 95	1 38	2 18	2 59	2 60	65

* (Shereif, 1995)

Depuration Data
At pH Values

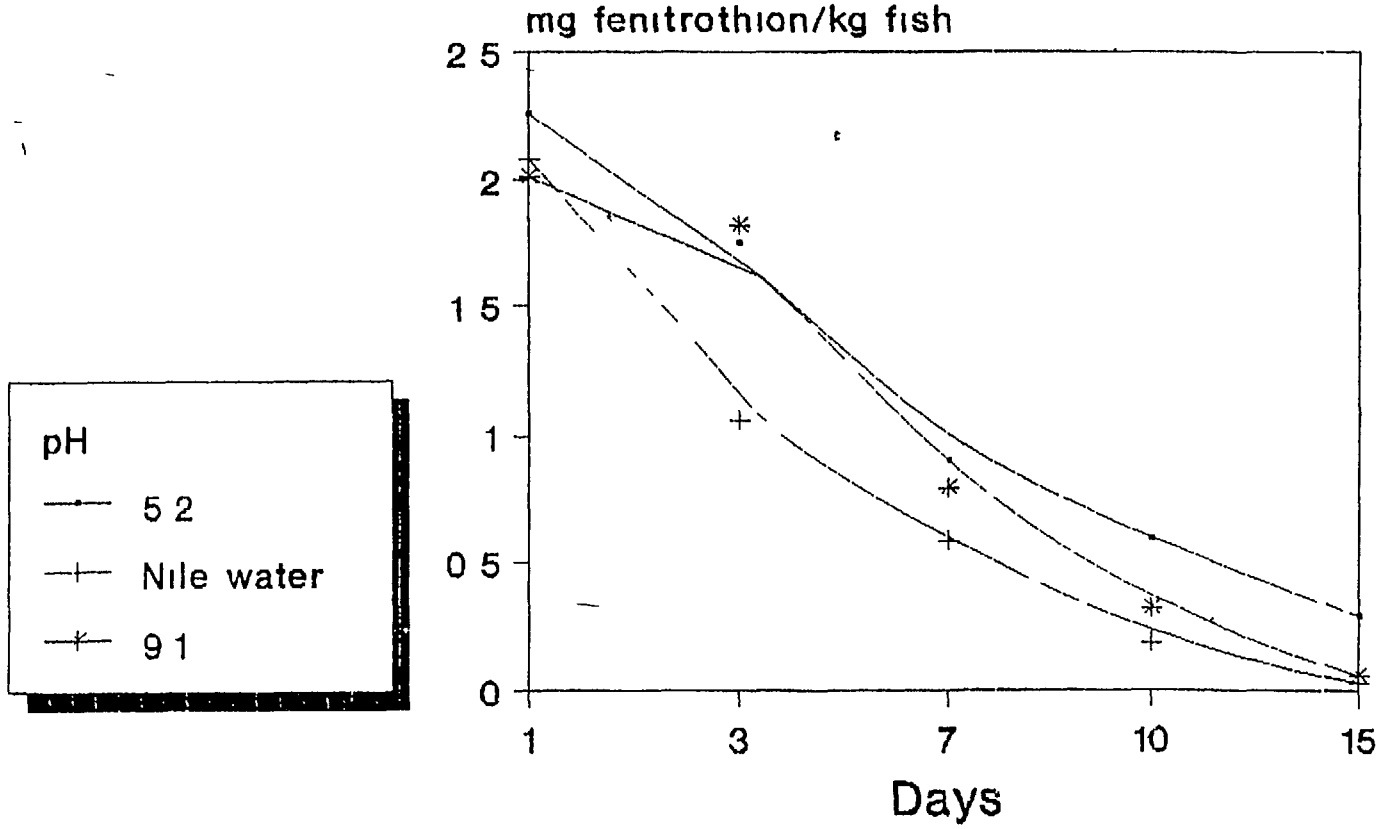

Fig (5) · Depuration Data at pH Values

Table (6): Mean residues (mg fenitrothion/kg fish) and depuration of fenitrothion by *O niloticus* in clean water folllowing 15 days of withdrawal from exposur to 40 ug/l at different water salinities

Water salinity	Time (days) from end of exposure period				
	1	3	7	10	15
Nile water *	2 08	1 06	0 58	0 19	0 03
8%	2 15	1 63	0 72	0 28	0 05
35%	2 25	1 80	0 95	0 53	0 17

* (Shereif, 1995)

Depuration Data
At Salinity Values

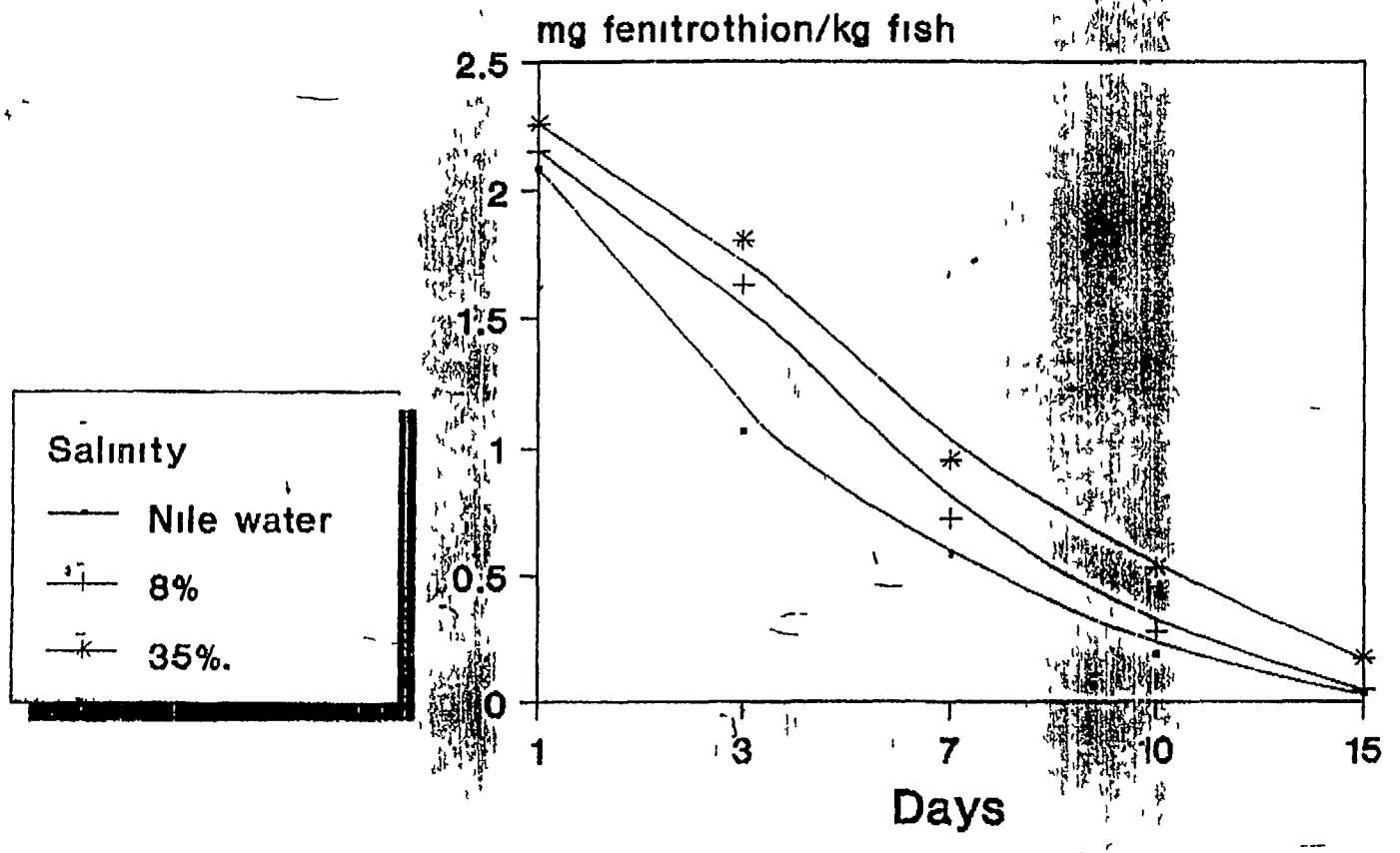

Fig. (6) : Depuration Data at Salinity Values

Modelling Uptake And Depuration Of Contaminants

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DIFFERENT TYPES OF CONTAMINANTS

Microbial Agents (Pathogens)

Pathogens can be classified as micro or macroparasites (Dobson & May, 1987) The former include viruses, bacteria, fungi and protozoa They are characterized by their small size, short generation times and their ability to multiply within an infected host On the other hand, macroparasites of fish include helminthes, acanthocephalans, crustaceans and agnathans (Wootton, 1990)

While they filter many liters of water per hour to satisfy their nutritional needs, fish retain suspended particles to which are attached microorganisms, some of which may be pathogenic The concentration rate between the water and the fish differs according to many factors including the particular microbial agent and the species of fish In fact, the major factors of significance for fish contamination and disease are the susceptibility of species to a particular pathogen present, the virulence of the pathogenic agent, and also the environmental conditions which may trigger epizootics Physical and environmetal stress have an important impact in the maintenance of healthy conditions for fish (for the water hardness, pH, temperature, among other parameters) On an other hand, the susceptibility (or the resistance) of the fish species to the action of the disease agent is governed by its physical barriers, its exposure conditions, and its age Among the physical barriers are the skin, scales, and mucous membranes which all limit the entry of infectuous and parasitic agents Finally, adequate nutrition is vital for the rapid growth and the vigor needed to cope with a variety of pathogens

Bacteria

These microorganisms may not cause disease in the fish themselves Rather, the fish may serve only as an host subsequently transmitting the disease to humans who consume the contaminated fish The role of the fish as the vector of transmission is particularly important, due to the pathogen's ability to survive and multiply within the gut, mucous, and tissues of the fish (Issa, 1993) According to the possible sources of contamination, we may distinguish between

- naturally occurring bacterial pathogens, generally not associated with fecal contamination Vibrio cholerae, Vibrio parahaemolyticus, Vibrio vulnificus, other Vibrio, Aeromonas, and Plesimonas
- pathogens transmitted via fecal or cross-contamination Salmonella, Campylobacter, Escherichia, Yersinia, Shigella, Listeria, Clostridium, and Staphylococcus

Viruses

Of the enteric viruses, only Hepatitis A, non-A and non-B Hepatitis, Norwalk, Astroviruses, Calciviruses, and Small Round Viruses have been presented to be epidemiologically linked to seafood-related disease outbreaks This list is limited by the difficulty to identify the agents and to make an accurate diagnosis -the disease generally occurs months after exposure-

Shellfish are more implicated than finfish

Macroparasites

Some macroparasites may have complex life histories in which the fish is only an intermediate host, and the parasite only becomes sexually mature when the infected fish is eaten by another predator which is then the definitive host While most parasites are found in a range of sites within the internal organs of fish, some are attaching themselves to the external surface of the fish, such as the gill filaments or fins

Fish may be host to an impressive number of protozoans and macroparasites Any individual fish carries only a sub-set of the parasites that infest that particular species Another important feature of many macroparasite species is their uneven distribution within the fish population Most fish may carry none or only a few parasites, but a few fish are heavily infested (Wootton, 1990) In such case, the effects of the parasite are not spread approximately equally across all members of the fish population, but only a small proportion of individuals in the population are exposed to heavy infestations

The following parasites have been especially documented during the performed studies in Israel and Egypt Giardia lamblia, and three groups of Helminths (Cestodes or tapeworms, Trematodes or flukes, and Nematodes or roundworms)

Chemical Contaminants

Sherif *& al* proceeded to a "Comprehensive review of literature on chemical pollutant residues, uptake, accumulation and elimination by seafood and fish" (Progress Report of Nov '83) Different sorts of water contaminants may have physiological effects on fish

Inorganic toxicants

Metal bioavailability is influenced by physical, chemical, and biological factors in the aquatic environment Physical factors include temperature, phase association (solid, or disolved), physical adsorption, sequestration by occlusion within a solid phase, or deposition Chemical factors comprise those influencing the metal speciation at thermodynamic equilibrium, presence of ligands and complexation kinetics, lipid solubility, and phase transitions such as those associated with precipitation, coprecipitatiopn, or chemical adsorption Both organic and inorganic species are involved in these phenomena A large variety of biological factors can also modify the metal bioavailability, which include trophic interactions, biochemical or physiological adaptation, microhabitat utilization, fish size and age, as well as particular species characteristics

The toxicity of weak inorganic acids and bases, such as hydrogen sulfide, cyanide, and ammonia decreases with pH for acids and increase with pH for bases The pH in the gill microenvironment also has a role in determining this relation

Organic toxicants

The fate of organic chemicals in the aquatic environment is generally controlled by the association or interaction of the chemical with the organic matter already in the water Organic chemicals that are freely dissolved in the water, and not bound to or asociated with organic matter, may undergo diffusion-controlled processes such as volatilization, diffusion between sediment and water, and bioconcentration in living organisms On an other hand, those which are associated with organic matter in the water are subject to other processes such as settling, resuspension, deposition, filter feeding, and dietary accumulation

MECHANISMS OF CONTAMINATION

Microbial Uptake by Fish
(from Shuval & al, 1994)

Pathogens are likely to penetrate the fish tissues despite the fish defense system The penetration occurs through the skin, mouth, gills, and thin epithelium (Sniezko, 1974) With exposure to high pathogen concentrations, penetration through the gall bladder, the stomach cavity, and even the eyes is possible (Munro, 1982)

The penetration of the different microorganisms in the fish organs largely depends on the concentration of these microorganisms in the water where the fish is grown When the concentration of these microorganisms reaches beyond certain threshold, they might even reach the muscle When this happens, the fish particularly shows signs of high stress (Buras & al, 1987)

Penetration through the skin

The fish outer layer of skin is covered by a thick layer of mucus which prevents the adsorption and growth of microorganisms within the skin However, skin wounds allow for penetration of bacteria, viruses, and fungi

Penetration through the mouth and the digestive tract

Water and particles reach the stomach through the mouth Bacteria, viruses, and protozoa enter the fish in the same manner Some enter through the intestine and then pass from the digestive tract (DT) to the inner organs such as the spleen, the liver, and the kidneys (Sniezko, 1970, Buras & al, 1985, Fattal & al, 1992) In the DT, there is a high concentration of phagocytes which present the first barrier to foreign bodies that invade the fish After entry in the esophagus, the microorganisms can reach the blood stream or the lymphatic system, and from there they enter the various lymphatic organs In the lymphatic organs, further phagocytic activity is taking place Then, those who are

no yet phagocytized and are still in the blood stream can reach other tissues (Digirolamo & al, 1975)

Canzonier (1971) assumes that the penetration is based on two different but related processes First, it may be from contaminated food into the DT In this case, the bacteria or viruses reach the DT, pass through it, and are excreted in the feces Second, it may be by a long term exposure to small numbers of microorganisms In this case, the microorganisms remain in the DT and in the lymphatic organs They will not be released quickly and, therefore, will stay in the DT for long periods of time

Pain (1986) suggests the existence of cleanliness cells called haemocytes which capture microorganisms from the DT and transfer them to other tissues The physiological system which is responsible for this phenomenon may be different in different species of fish and this has important public health implications

Penetration through the gills

Studies have shown that 2 h after exposure of carp to viruses, they could be detected in the gills and , 5 d later, could be found in the blood system (Ahne, 1975) It appears that the type of cells which existed in the gills serve as proper pathways to the blood system (Chilmoczyk, 1980)

Defense mechanism of the fish

It can be divided into the physiological system and the immune system (Ellis & al, 1976)

The physiological system is composed of the skin and of a thick layer of mucous which prevents the adsorption and growth of microorganisms on the skin This layer protects the skin cells from mechanical, biological, and chemical wounds and provides a selective transmission of metabolites and pathogens between the fish and the environment (Krovacek, 1987)

The immune system includes lymphocytes, granulocytes, and phagocytes all of which are found in the skin, gills, DT, and the lymphatic organs These cells attack the microbiological invaders The lymphatic organs (e g , spleen and kidneys) destroy the different antigens in an efficient manner (Lamers, 1985) In fact, the lymphatic organs that are most active in distancing foreign microorganisms are the spleen and kidneys These organs have the highest concentrations of macrophages and lymphocytes (Buras & al, 1980) The phagocytic cells in the lymphatic organs represent the fish potential initial defense mechanism (Buras & al, 1987)

Chemical Uptake by Fish

Three mechanisms have been proposed to explain how a chemical passes across a cell membrane (a) passive diffusion through the membrane, (b) filtration through membranous pores, (c) specialized transport systems that carry water-soluble and large molecules across the membrane by means of a carrier

Cell membranes are essentially lipoproteins with aqueous pores through which water-soluble molecules can pass Most membranes have an electrical potential that may effectively preclude the ready penetration of charged chemical species Thus, it appears that the absorption of a chemical depends on its physico-chemical properties, molecular size, shape, degree of ionization, and lipid solubility

Passive diffusion is considered to be the principal mechanism by which chemicals can cross cell membranes The rate of passive diffusion of a molecule is proportional to the concentration gradient across the membrane, the membrane thickness, the area available for diffusion, and the diffusion constant (La Du & al, 1972) The rate of passage is related directly to the lipid solubility (Brodie, 1964) However, since absorption requires passage through an aqueous as well as a lipo-phase, the absorption of a chemical with an extremely low solubility in water may be impeded in spite of a high lipid-to-water partition coefficient The passive diffusion also depends on the extent of ionization and the lipid solubility of the ionized and nonionized species (Brodie, 1964)

Filtration is a process by which a chemical passes through the aqueous pores in the membrane , and is governed by the size and the shape of the molecule The bulk flow of water across the membrane produced by an osmotic gradient or hydrostatic pressure can act as a carrier for chemicals

Specialized transport processes are needed to explain the transport and kinetic behavior of large, lipid insoluble molecules and ions Two types of carrier-mediated transport systems have been recognized active transport and facilitated diffusion The carrier in both systems is some component of the membrane that combines with the chemical and assists its pass age across the membrane It has a limited capacity and when it is saturated , the rate of transfer is no longer dependent on the concentration of the chemical and assumes zero order kinetics Structure, conformation, size, and charge are important in determining the affinity of a molecule for a carrier site, and competition for carrier site will occur

Active transport is a carrier-mediated transport system which moves a molecule across a membrane against a concentration gradient or, if the molecule is an ion, against an electrochemical gradient It requires the expenditure of metabolic energy and can be inhibited by poisons that interfere with cell metabolism

Facilitated diffusion is a carrier transport mechanism by which a water soluble molecule is transported through a membrane down a concentration gradient No apparent energy is required and metabolic poisons will not inhibit this process

Uptake and Elimination of Xenobiotics** (from Heath, 1995)

(a) - The route by which a foreign chemical is taken into a terrestrial animal for the most part do not apply to fish This is because several constraints are imposed on the animals by the aquatic environment where they live in One of the most critical is the matter of obtaining sufficient O_2 A fish must breathe roughly 20 times more of its respiratory medium (i e water) than a terrestrial animal (using air) to obtain the

equivalent amount of O_2 Therefore, the gill tissue (which is the main point of entry for dissolved substances) will be exposed to a far greater amount of selected pollutants than a terrestrial animal breathing air Furthermore, the gills have a countercurrent blood/water flow system, very thin epithelial membranes, and large surface areas, these features all facilitate the uptake of materials from the water and their transfer to the blood

There are four possible routes for a substance to enter a fish absorption onto the gills, ingestion with food or with drinking water, and adsorption to the body at the skin Gills and food vary greatly as routes of exposure, depending mostly on the availability of the considered substance (i e , whether it is concentrated in the food or dissolved in the water) The importance of skin varies with the size of the fish and toxicant, and accounts more for small fish because of the large surface to volume ratio

Then, the distribution into various organs is influenced by the route of uptake Xenobiotics that enter the circulation via the gills can be rapidly distributed throughout the body to all organs In fact, once it is absorbed via the gills or gut, a chemical is usually bound to a protein and then transported by the blood to either a storage point (such as fat) or to the liver for transformation and/or storage If transformed by the liver, it may be stored there, excreted in the bile, or returned to the blood for possible excretion by the kidneys or gills, or stored in extrahepatic tissues such as fat On the other hand, xenobiotics that are absorbed via the intestine will pass first to the liver via the hepatic portal system, where some metabolism or sequestering may occur

Thus, the concentration found in different organs after environmental exposure for a particular time depends on several simultaneous dynamic processes The total body burden of a foreign chemical will be a weighted average of all the tissues, which may differ from each other in concentration of this chemical by orders of magnitude

(b) - Depuration of foreign chemical is usually measured by allowing the contaminated fish to reside in non-contaminated water for some specified period of time and then residue analysis is performed on the whole body or specific tissues The latter kind of analysis are far more informative since the rate of depuration varies greatly between different tissues and this is related to their relative abilities for metabolic transformation of the chemical

It is difficult, if not impossible, to draw many generalizations regarding the excretion of foreign chemicals in fish because these compounds are so diverse and the fish is endowed with several excretory modes (gills, skin, mucus, bile, feces, urine) However, it does seem safe to state that the liposoluble xenobiotics are usually metabolized into hydrosoluble form before excretion, and the products of such metabolism are largely excreted via the bile and/or urine The gills can be an important mode of exit for small compounds that require little if any biotransformation before excretion, this is presumably a passive diffusion process

MATHEMATICAL MODELS

Krauss & Hill have presented the possible models for all the terms forming the overall bioaccumulation of a contaminant in fish As summarized from the literature, these different terms are

- uptake via gills
- uptake by adsorption to the skin
- uptake by ingestion (sometimes called "food-chain accumulation")
- elimination from the gills
- elimination by defecation
- loss from growth dilution
- loss from biotransformation
- reproductive loss

The approach for modeling each of these terms is not always consistent, and some models have not included every one

To focus on the elimination process, we shall consider the simplest model, which is a one-compartment open model

where k_a and k_e denote respectively the rate constants for absorption and elimination

In using this model, it is assumed that the chemical equilibrates with all tissues to which it is distributed sufficiently rapidly to preclude kinetic differentiation by the techniques being used to characterize its movement in the organism

Being principally interested in the depuration process, we shall also assume that the rate of elimination of a given contaminant is proportional to its concentration in the considered organ And we shall adopt the most commonly used mathematical model, with zero order intake and first order elimination Thus, the concentration in this organ will be described by apparent first-order kinetics

$$dC(t) / dt = -k_e C(t)$$

where C(t) is the concentration at time t and k_e is the rate constant for elimination

The solution of this differential equation gives, when C(t) is the concentration of the chemical in the organism at time t of the process and C(0) is the initial concentration at the beginning (time t = 0)

$$C(t) = C(0) \exp(-k_e t)$$

DECONTAMINATION / DEPURATION

Summary of the Experiments

Pathogen depuration

Zuaretz and Nasser (1993) were performing a study on Tilapia hybrids (*Sarotherdon aureus* X *S niloticus*) The main purpose of their research was to evaluate the depuration rate of fish organs and to learn the effect of active depuration on these organs Water samples and fish tissues (skin, muscle, liver, spleen, and digestive tract DT) were assayed for three different microorganisms E coli, MS2 bacteriophage, and Poliovirus 1

The study included 3 major controlled laboratory experiments (1) Contamination kinetics (2) Fish purification (3) Fish depuration, by continuous water change

- Experiment (1)
  This experiment was performed in two parts

  The first part lasted 24 h, during which the three types of microorganisms were inoculated once in the fish water tank It was intended to determine the concentration of the various microorganisms in different fish tissues after one inoculation

  The second part lasted 15 d, and consisted of 11 inoculations of these three different microorganisms in the fish water tank It was conducted to determine the accumulation and persistence pattern of the test microorganisms in different fish tissues during the 14 d period when the microorganisms were seeded to the water every 24 h

- Experiment (2)
  This experiment was performed to evaluate the self purification rate of the different fish organs It lasted 21 d during which the three microorganisms were each inoculated once into the medium

- Experiment (3)
  This experiment was conducted to evaluate the effect of continuous water change on the persistence of the tested microorganisms in fish organs It lasted 5 d during which the fish were fed daily (1% of body weight) Two tanks were used Tank A containing clean and continuously aerated water , and Tank B in which the microorganisms were inoculated once and which was containing the fish stock for the experiment itself Every 3 h, 100 l of clean water from tank A passed through the tank B, and this was considered as one water change

Chemical decontamination

(a) - Pesticides residue

Shereif *and al* (1994) attempted to evaluate, under laboratory conditions, the process of uptake bioaccumulation, and elimination of fenitrothion by the fish Tilapia

(*Oreochromis niloticus*) In addition, the relationship between these processes and fish body size was assessed

After determination of the median lethal concentration (LC50) of fenitrothion with a static test (USEPA, 1982), the uptake and elimination experiments were carried out according to the renewal exposure system Test solutions and control water were renewed periodically, usually with 24 h intervals The fish were raised for 15 d in glass aquaria containing the aqueous solution of fenitrothion at a concentration equivalent to the LC50, and then transferred to clean water for another 15 d Commercial dry feed was given once a day, at 4% of fish body weight, throughout the experiment

At each sampling time, two fish of different size were washed with running tap water, weighed and analyzed for chemical residue At the same time, water samples were taken and subjected to residue analysis

(b) - Metals and PAHs

In an other study (Dr El-Samra & al, Task 3 of the Seafood Safety and Fish Decontamination Project), the rates of uptake and elimination of metal contaminants were assessed by using cadmium This metal was selected because of its ubiquity, high toxicity, and ability to accumulate in aquatic organisms

Live fish of *Tilapia sp* were caught and acclimated to laboratory conditions for one week at the beginning of the experiments Groups of active and healthy fish of 7 different sizes were placed for 30 d in glass tanks containing 1 ppm of cadmium Initial concentrations of cadmium were measured in the entire body for small fish and in different organs in the larger fish The mean value of concentrations were calculated For the experiences, fish were removed from the containers at different time periods to measure the Cd concentrations in the muscle, liver, gills, and whole body Using the static-renewal procedure, actual concentrations of Cd in water samples from the medium were determined at the beginning and after 24 h of exposure before water removal

The decontamination experiment started after the 30 d of accumulation, when the alive and active fish were transferred to tanks containing clan seawater The same procedures as those implemented in the uptake phase were followed

Experimental Results and Modeling

Pathogen depuration

- Experiment (1) Contamination Kinetics

We shall not consider herein the results of these experiments, since they are attempting to present the fish contamination kinetics which do not constitute our purpose (as it is stated in the introduction section)

- Experiment (2) Fish Purification

In each case, we shall get an approximate value of k_e which corresponds to the model for C(t) by considering the concentration of the considered microorganism in the DT, where also it has each time a significant value

TABLE 1 - Numeration of E Coli in different organs of fin fish during Experiment #2

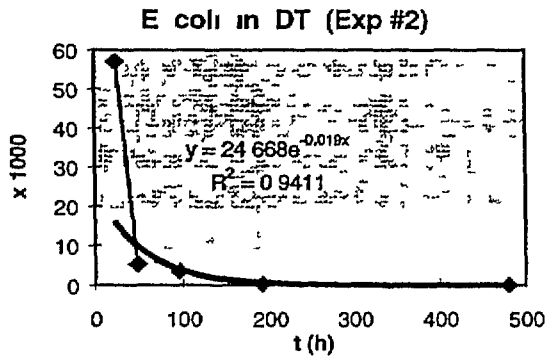
Escherichia coli

t (h)	in water	DT	Spleen	Liver	Muscle	Skin
0*	0	0	0	0	0	0
24	710	57	3 1	0 19	0	0 31
48	160	5 3	0 56	0 042	0	0 25
96	3 4	3 5	0		0	0 034
120	0 19					
144	0 014					
192	0 004	0 25	0	0 004	0	0
216	0 002					
480	0	0 004	0	0	0	0

NOTE all the reported numbers are to be multiplied by 10^3

(*) indicate the inoculation of microorganisms performed during the experiments

FIGURE 1 - Depuration of the DT from E Coli during Experiment #2



The model for E coli in this experiment gives an approximate value $\mathbf{k_e = 0\ 02}$ with a very marked determination coefficient (R^2 = 0 94)

TABLE 2 - Numeration of MS2 Phage in different organs of fin fish during Experiment #2

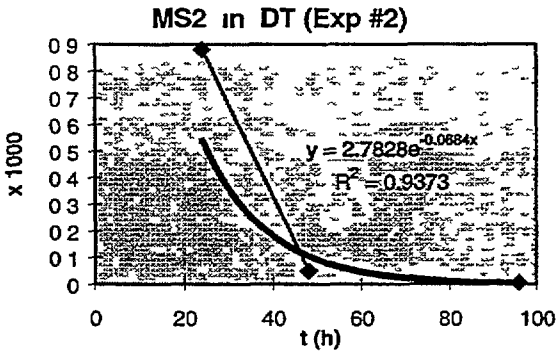
MS2 Phage

t (h)	in water	DT	Spleen	Liver	Muscle	Skin
0*	0	0	0	0	0	0
24	6 1	0 88	0	0 002	0	0 13
48	0 028	0 05	0	0 008	0	0 008
96	0 024	0 005	19	0 51	0	0 11
120	0					
144	0					
192	0	0	0	0	0	0
216	0					
480	0	0	0	0	0	0

NOTE all the reported numbers are to be multiplied by 10^3

(*) indicate the inoculation of microorganisms performed during the experiment

FIGURE 2 - Depuration of the DT from MS2 Phage during Experiment #2



The mathematical model for MS2 phage in this experiment yields a relatively good approximate value (with $R^2 = 0\ 937$) of $\mathbf{k_e = 0\ 07}$

TABLE 3 - Numeration of Poliovirus 1 in different organs of fin fish during Experiment #2

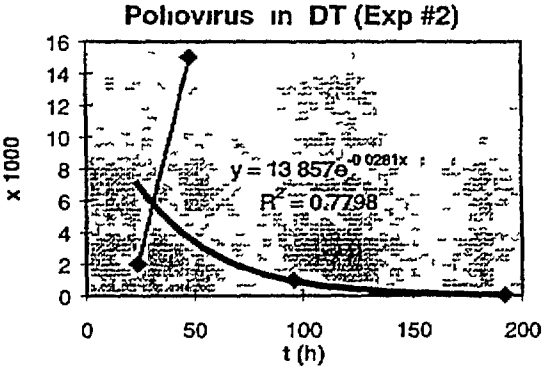
Poliovirus 1

t (h)	in water	DT	Spleen	Liver	Muscle	Skin
0*	0	0	0	0	0	0
24	3 6	2	0	0 54	0	0 15
48	2 8	15	6 5	0 14	0	0 077
96	0 11	1	0	0 27	0	0
120	0 045					
144	0					
192	0 005	0 05	0	0	0	0
216	0					
480	0	0	0	0	0	0

NOTE all the reported numbers are to be multiplied by 10^3

(*) indicate the inoculation of microorganisms performed during the experiment

FIGURE 3 Depuration of the DT from Poliovirus 1 during Experiment #2



In this experiment, according to the model, Poliovirus 1 is given an elimination rate of approximately $k_e = 0\ 03$

However, this approximation is less adequate than for the other microorganisms in the same type of experience (with $R^2 = 0\ 78$)

- Experiment (3) Fish depuration

TABLE 4 - Numeration of E Coli in different organs of fin fish during Experiment #3

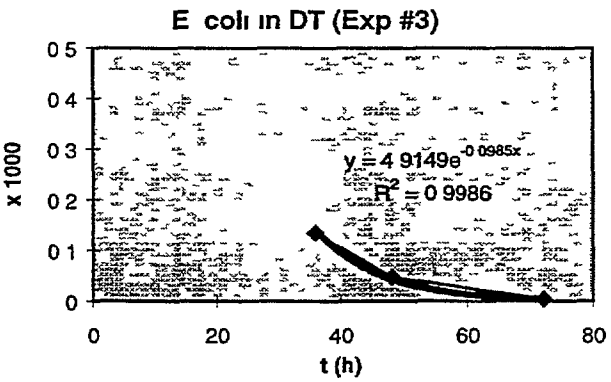
Escherichia coli

# change	t (h)	in water	DT	Spleen	Liver	Muscle	Skin
0	0			0	0	0	0
0*	1	70					
0	12	0 105	0 488	0	0 114	0	0 007
0	24	0 07		0	0	0	0
3	36	0 002	0 135	0	0 001	0	0
8	48	0	0 047	0	0	0	0
16	72	0	0 004	0	0	0	0
24	96	0	0	0	0	0	0

NOTE all the reported numbers are to be multiplied by 10^3

(*) indicate the inoculation of microorganisms performed during the experiment

FIGURE 4 - Depuration of the DT from E Coli during Experiment #3



The model seems to be truncated and does not consider the part fitting before the first water change In fact, abnormally, the obtained values indicate that no microorganism was present anymore in the fish before the first water change and , then, they got contaminated again

However, we can always observe that in this experiment, the approximate value of the depuration rate is $\mathbf{k_e = 0\ 10}$ with a coefficient $R^2 = 0\ 998$

TABLE 5 - Numeration of MS2 Phage in different organs of fin fish during Experiment #3

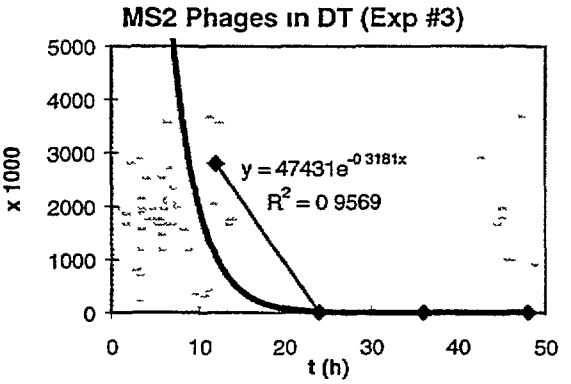
MS2 Phage

# change	t (h)	in water	DT	Spleen	Liver	Muscle	Skin
0	0*			0	0	0	0
0	1	14000					
0	12	140	2800	13000	38	0	490
0	24	5 6	6 6	1600	250	0	0 18
3	36	2 6	0 315	0	0 143	0	0 008
8	48	0	0 023	0	0	0	0 017
16	72	0	0	0	0	0	0
24	96	0	0	0	0	0	0

NOTE all the reported numbers are to be multiplied by 10^3

(*) indicate the inoculation of microorganisms performed during the experiment

FIGURE 5 - Depuration of the DT from MS2 Phage during Experiment #3



The model for MS2 phages in this experiment gives approximately $\mathbf{k_e = 0\ 32}$ (with $R^2 = 0\ 96$)

TABLE 6 - Numeration of Poliovirus 1 in different organs of fin fish during Experiment #3

Poliovirus 1

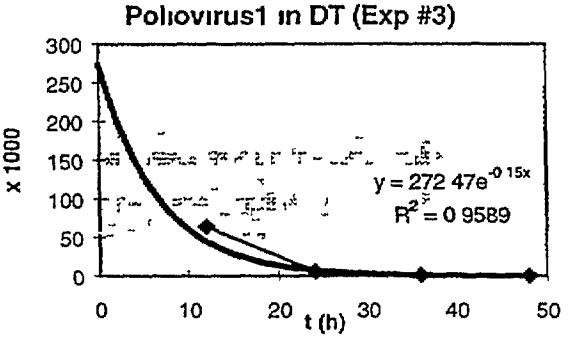
# change	t (h)	in water	DT	Spleen	Liver	Muscle	Skin
0	0*			0	0	0	0
0	1	7 67					
0	12	1	64	0	3	0	0 31
0	24	3 33	5 8	0	2 6	0	0 16
3	36	0 087	0 71	0	0	0	0
8	48	0 006	0 32	0	0	0	0
16	72	0	0	0	0	0	0
24	96	0	0	0	0	0	0

NOTE all the reported numbers are to be multiplied by 10^3

(*) indicate the inoculation of microorganisms performed during the experiment

According to the model deduced from the experiment (cf *FIGURE 6* below), we would have for Poliovirus 1 in this experiment a depuration rate of $k_e = 0\ 15$ In this case, the precision would be relatively good (with $R^2 = 0\ 96$)

FIGURE 6 Depuration of the DT from Poliovirus 1 during Experiment #3



Chemical decontamination

(a) - Pesticides residue

TABLE 7 - Uptake of Fenitrothion

t (d)	1	3	7	10	15
Size 1	0 75	1 02	1 9	2 4	2 41
Size 2	1 05	1 8	2 45	3 04	3 05

The organic insecticide (fenitrothion) concentrations in fish increased gradually until day 10 and nearly reached equilibrium at day 15 of exposure, during the uptake phase Fish tissue showed residues of 2 41 mg/kg in small fish and 3 05 mg/kg in larger fish Thus, accumulation (ratio of the concentration in the fish tissue over that in the water) was 60 for the small fish and 76 for the larger ones

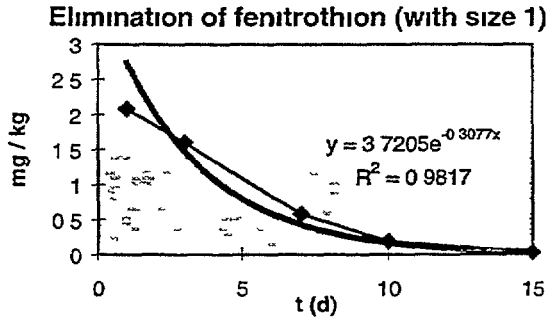
Again, we shall not attempt to model the uptake phase

TABLE 8 - Elimination of Fenitrothion

t (d)	1	3	7	10	15
Size 1	2 08	1 6	0 58	0 19	0 03
Size 2	2 42	1 75	0 85	0 28	0 04

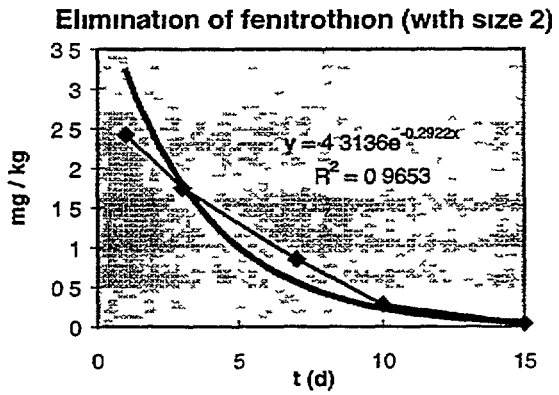
When the fish were transferred to a clean water environment, fenitrothion concentration in the fish decreased gradually At the end of the 15 d period, the chemical residues in both size did not show a significant difference

FIGURE 7 - Decontamination from Fenitrothion of smaller fish



In this experiment, the model for the fish of smaller size gives to the depuration rate a value of approximately $\mathbf{k_e = 0\ 31}$ with a relatively good adequacy ($R^2 = 0\ 98$)

FIGURE 8 - Decontamination from Fenitrothion of larger fish



The model for the same experiment, but done this time with the fish of bigger size, gives to the depuration rate the value of $\mathbf{k'_e = 0\ 29}$, with the same range of adequacy ($R^2 = 0\ 965$)

(b) - Metals and PAHs

The experiences showed that Cd accumulated most rapidly in the gills Accumulation during the first 48 h showed a 12 fold increase over the initial concentration, although the other organs (muscle and liver) showed no response to Cd uptake during the same period After a 30 d period, Cd levels in the gills had increased 65 fold over the initial concentration, while that in the liver had increased to levels as high as 8400 fold No saturation was recorded in the gills or liver tissues after 30 d

No response was recorded in the muscles during the first 10 d of the experiment After this period, a rapid increase (55 fold) was observed up to day 20 The uptake rate slightly decreased to 21 fold over the initial concentration after 30 d It appears that these results were due to detoxification processes

In the whole fish, Cd increased 37 fold over the initial concentration during the first 24 h However, the accumulation reached a plateau between day 1 and day 5 and was then followed by a rapid rise until day 30

After this rapid uptake in the whole fish, the elimination from the tissue was slow

CONCLUSION

The experiences which have been conducted, along with the mathematical models we have derived, allow us to observe a trend concerning the reactions of the fish relative to the chosen microorganisms (E coli, MS2 bacteriophages, and Poliovirus 1) In fact, in the two series, the bacteriophages always have the highest elimination rate after they get in the DT, followed by the Poliovirus 1 *Escherichia coli* has the lowest elimination rate, traducing their tendency to remain longer in the DT once they are ingested by the fish

Furthermore, the experiences done with fenitrothion show that the elimination rate of a contaminant is not significantly influenced by the fish size Such result seems to not confirm the role played by the "surface to volume ratio" during an exchange with the external environment

However, such results obtained from laboratory studies do not allow us to draw a firm conclusion regarding the effectiveness of the studied methods if they were brought on field These experiments might be refined and would benefit in accuracy from other field investigation

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PROCEDURES FOR ASSESSING HEALTH RISKS FROM CONTAMINATED FISH AND SHELLFISH

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INTRODUCTION

Assessing the human health risk associated with consuming fish and shellfish harvested from chemically contaminated waters is of great importance for the protection of human health For this reason, the establishment of public health guidelines and advisories for seafood consumption in Egypt and Isreal, is one of the main objectives of the Seafood Safety Study Based on derived risk assessments health advisories can be issued to the public regarding consumption of fish known to be contaminated with toxic substances Unfortunately, in many cases advisory decisions are currently made on very little testing data for a particular body of water Considerable uncertainty results as to the public health implications from exposure to chemical levels identified in seafood

Adequately assessing risk through toxicological analysis and the application of appropriate models is the critical first step upon which public health protection measures, such as regulations and advisories pertaining to fish harvesting and consumption practices, can be based In every case it is essential that advisory decisions be made on the basis of protecting the public and yet be compatable with the environmental health and socioeconomic factors pertinent to the region

In general, for risk assessment to be meaningful for a particular region, several characteristics of that region must be explored First, it is necessary to identify the types of fish in the fish harvesting areas relevant to the regions, with special consideration to the types of seafood most highly consumed by the population Second, it is critical to analyze the toxicity and pollution levels of the water in which fish harvesting takes place Finally, it is important to acknowledge that each region has cultural and economic differences in the amount of seafood consumed and their methods of preparation and handling Each of these characteristics, along with the average size (body weight in Kg) of the population or populations targeted for health guidelines, is necessary for an accurate risk assessment

A data bank on fish contamination and the resultant public health impacts is in the process for being established for Egypt and Isreal As it is not yet completed, for the pupose of this paper where we could, we extracted relevant data from existing literature to serve as examples for the risk anaylses procedures At other times, national data from the United States is presented It is important to note that these data serve only as examples, as Egypt and Isreal will want to establish guidelines specific to their regions and their people Sometimes it is beneficial to examine policy already in place to serve as models For this reason, when applicable, examples have been provided from the Department of Public Health Fish Consumption Advisories for the State of Michigan

The following is a summary of procedures used in assessing the health risks posed by consumption of contaminated fish These procedures are based on those established by the United States Environmental Protection Agency (U S EPA), set forth in their guidance manual *Assessing Human Health Risks from Chemically Contaminated Fish and Shellfish* [1] Additional information, particularly concerning the application of risk assessment to policy development, comes from *Sport Caught Fish Consumption Advisories Philosophy, Procedures, and Process* issued by the Michigan Deptment of Public Health, Center for Environmental Health Sciences However the procedures and information have been adapted to be applicable to the specific circumstances and needs of Egypt and Israel

Quantitative risk assessment is an important but complicated science This report was put together in order to create an awareness of the complexities of risk management and risk anaylsis It is the intention that this report should be used as an educational tool and will hopefully provide interest for the development of health and national guidelines for safe seafood consumption

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[1] U S Environmental Protection Agency Assessing Human Health Risks from Chemically Contaminated Fish and Shellfish EPA-503/8-89-002 Washington, DC September, 1989

Overview of Risk Assessment and Risk Management

The objective of a risk assessment is to estimate the probability that exposure to a given toxic substance will produce adverse health effects Because direct measurement of human health risks is not usually feasible risk assessment is an invaluable tool that utilizes information on exposure and toxicological data combined with uncertainty analyses, to arrive at an estimate of risk The potential health hazard from consumption of contaminated fish will most likely not be human toxicois from large doses of toxicants over a short period of time Rather it is quite likely that the consumer would be exposed to low levels of indeterminable number of hazardous substances over an extended period of time with no observed effects for years Risk assessment then is particularly useful for identifying these long-term human health risks (e g diseases such as cancer with long latency periods) posed by relatively low concentrations of toxic substances in the environment or food supply A risk assessment is comprised of the following steps

- **Hazard Identification** Qualitative evaluation of the potential for a given substance or substances to cause health effects in humans or animals

- **Dose-response Assessment** Quantitative estimate of the relationship between the dose received and the probability of an adverse health effect

- **Exposure Assessment** Characterization of the populations exposed to the substance of concern the environmental transport and fate pathways of the toxic agent, and the frequency duration and magnitude of the exposure dose

- **Risk Characterization** An estimate of risk based on the quantitative and qualitative results of the first three steps of the risk assessment

Figure 1 depicts the conceptual structure of a quantitative risk assessment

FIGURE 1

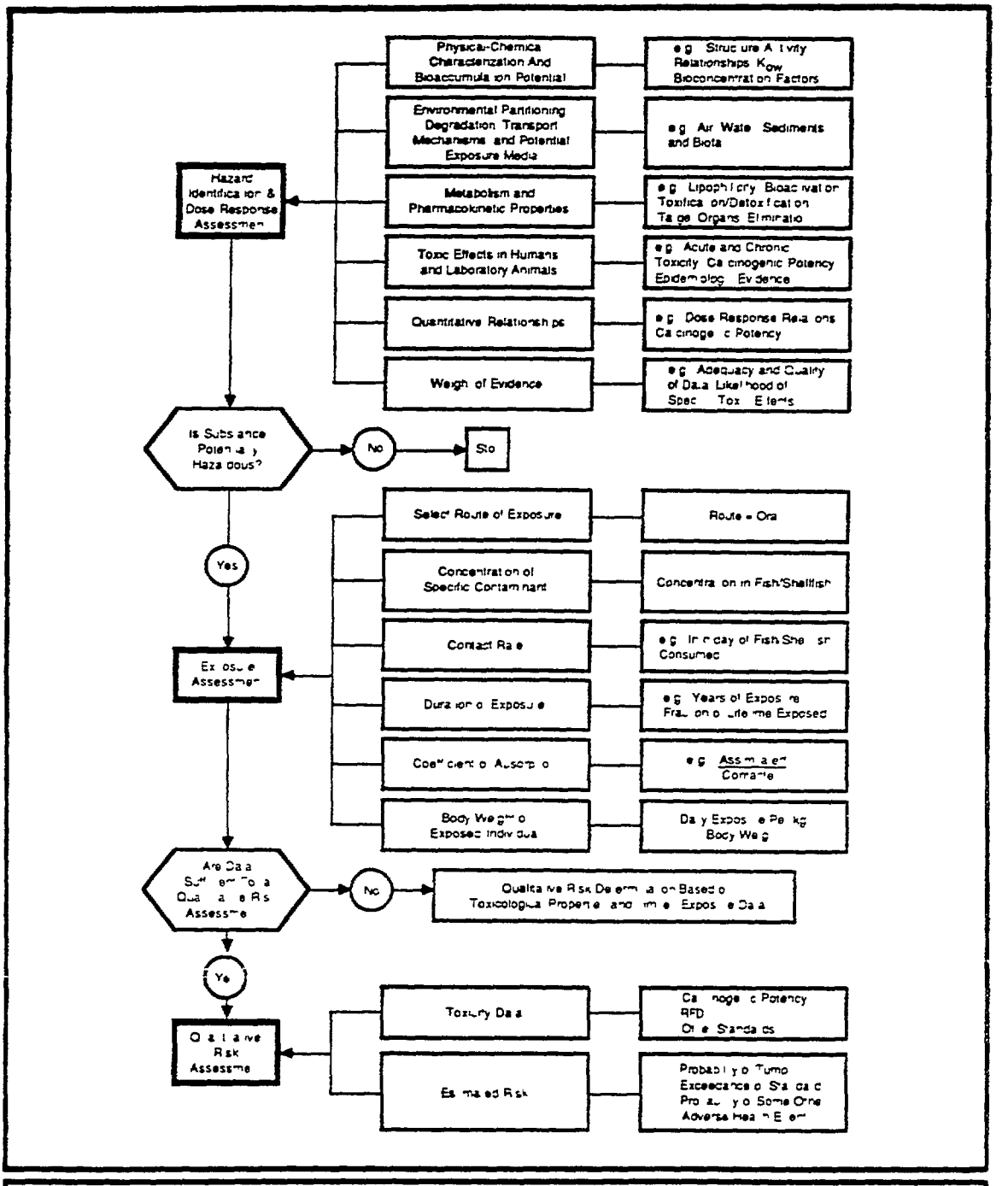


Figure Conceptual structure of quantitative health risk assessment model

Source EPA Assessing Human Health Risks from Chemically Contaminated Fish and Shellfish A Guidance Manual 1989

Applications of Risk Assessment

The objective of risk assessment is to estimate the probability of adverse health effects from exposure to a toxic agent It is particularly helpful for estimating potential health risks from long-term exposure to relatively low levels of contamination The information from risk assessment provides the scientific basis for public policy and regulatory decision-making Estimates of human health risks obtained through risk assessments often provide the rationale for government regulations guidelines or environmental criteria Risk assessments can be used to identify and prioritize toxic contamination problems that pose unacceptable risks to public health Risk assessment can also form the basis for public information and advisories designed to minimize public health impacts by influencing human behavior, thereby modifying exposure or consumption patterns Risk management, on the other hand, is the process of defining what constitutes an acceptable risk and establishing appropriate control measures Risk management decisions are based on information obtained in the risk assessment as well as on other economic social political and technical considerations That is, risk management decisions must be made by examing both the risks and benefits (eg positive health effects, increased economy etc) associated with fish consumption Together risk assessment and risk mangement comprise risk analysis

For example, the Michigan Deptment of Public Health exercises risk mangement when issuing a seafood advisory Not only does the Michigan Department of Public Health consider the quantitative risk assessment but also the available control and monitoring technology, cost-benefit anaylsis acceptable risk policy analysis and social and political factors Other concern are how the advisories will be publicized how they will be modified with the introduction of more data and how they can educate consumers to reduce their own exposure to contaminants by utilizing certain preparation and cooking methods Michigan also assumes different health risks for nursing mothers, pregnant women and children Egypt and Isreal may find the Michigan model helpful for developing their own seafood advisories Of course theirs will be dependent

on their own particular criteria systems for contaminants for what constitutes a health risk, and would also include economic social, and cultural factors specific to each country

One of the primary purposes of completing a risk analysis is to prevent exposure to significant quanties of chemical agents potentially harmful to human health Another purpose of risk analysis is to insure the quality and wholesomeness of the seafood entering Egyptian and Isreali markets Wholesomeness refers to food that is fit to eat, that is clean and uncontaminated, and that has been stored in a sanitary environment (Martian, 1988) Guidelines and tolerances for certain residues in fish should be established as they have been for other agricultural food products Contaminants in seafood include not only chemicals and heavy metals, but also pesticides, parasites, microorganisms, viruses, filth, toxins, and other foreign matter

In addition, the results of the risk assessments from this study can be used to establish water quality standards for the Nile Delta area including lakes, Manzaka, Borullus, Edku, Mariut, and other fishery sources such as the Aswan High Dam Reservoir Combining information on the degree of health risk posed by the contaminant with the bioconcentration factor (BCF) of that chemical in the resident species, a water quality criterion can be established The bioconcentration factor is defined as the ratio of the concentration of the chemical in tissues of the organism to the concentration of the substance in the water The criterion should represent the upper limit concentration of contaminant in the water that will prevent an unacceptable level of the chemical from bioaccumulating in the fish

HAZARD IDENTIFICATION

Preparation of a public health advisory of any type requires consideration of a number of factors which can be grouped into several categories exposure/hazard identification, toxicity evaluation, medical evaluation human exposure assessment and public health considerations Each of these will be looked at in turn Information ideally required for development of a public health advisory is summed up in table 1

Table 1 Information Ideally Required for Development of a Public Health Advisory

I Exposure Identification

a) Identification of Contaminants Present
- Matrices in which found (fish/water/sediments/air)
- Location found (area specific/widespread)
- Sources of contaminant(s)

b) Estimate of contaminant concentrations (mean and range)
- Relationship between concentration, age and size (in the case of fish)
- Distribution of contaminant within the matrix
- Trend of concentration (increasing/decreasing)
- Source reduction controls taken

II Toxicity Evaluation

a) Nature of contaminant
- Chemical properties
- Bioaccumulation/bioconcentration characteristics
- Biochemical interactions, metabolism, and degradation
- Persistance and environmental fate
- Bioavailability

b) Toxicological modes of action
- Reported acute/chronic effects in animals and humanss
- Beneficial effects vs adverse effects
- Metabolic breakdown products and their toxicity
- Site and type of toxic effect
- Effects of interactions with other compounds

III Medical Evaluation

a) Reported exposure experiences
b) Medical outcomes - long term or short term
c) Reported and potential health effects

IV Human Exposure Assessment

a) Number of people potentially exposed
b) Location(s)
c) Range of exposures among exposed populations
d) Contaminant concentrations measured in human tissues and fluids
e) Dose measurements

V Public Health Considerations

a) Selection and use of risk prediction models and end points for estimates of disease outcome probabilities
b) Applicability of existing guidelines policies, opinions or acceptable daily intake guides
c) Judgement of health benefits and health risks
d) Preparation of an advisory/risk communication
e) Issuance of advisory through effective channels

Source MDPH Sport Caught Fish Consumption Advisories Philosophy, Procedures, and Process 1986

The first step in the risk assessment process is to identify the contaminants of concern and their potential adverse health impacts Nearly one thousand chemical compounds have been identified in the Great Lakes aquatic environment Although we do not yet have as much information on the Nile Delta area, we know that many of these chemical compounds, such as lead cadmium, mercury, PCB's, pesticides and petrolium degradation products, have been identified in the Nile Delta area and other major Egyptian fish harvesting areas Specifically, a current study on the River Nile has identified the toxicity levels of six industrial effluents (magnesium cadium, copper, iron, lead and zinc) polluting the river (Abdel-Hamid, 1993) Other chemical contaminants of human health importance are listed in tables 2-3 These tables should just be used as a guide for the types of compounds which should be considered for the purpose of developing fish consumption advisories Although some of these chemicals are naturally occurring at very low concentrations, at high levels of pollution the potential seriousness of chemical contamination of seafood and the magnitude of the public health threat could be substantial

Once identified toxicity profiles are prepared for each contaminant, either based on existing data or by conducting additional toxicological analyses Several criteria exist for the identification of contaminants of concern

- High levels of persistence in the aquatic environment,
- High bioaccumulation potential
- Proven or suspected high toxicity to humans,
- Known sources of contaminant in areas of interest,
- High concentrations in previous samples of fish or shellfish taken from the areas of interest

Information and data on toxicity, bioaccumulation potential and persistence can be obtained from literature and data bases e g IRIS RTECS HSDB CHRIS, OHMTADS, T R I POLTOX

Table 2 A Listing of Chemicals Found in the Great Lakes Which May Impact on Human Health in the Event of High Local Contamination

A Extremely toxic chemicals (LD_{50} <50 mg/kg)

Aldrin
Carbofuran
Dieldrin
2,3,7,8-Tetrachlorodibenzodioxin (2,3,7,8,-TCDD)
Endosulfan
Endrin
Ethion
Methyl mercury (chloride)
Oxychlordane
Toxaphene
1,1,2-Trichloro- 1,2,2-trifluoroethane

B Very toxic chemicals (LD_{50} 50-500 mg oral/kg)

Aniline
Bromochloroethane
Carbon disulphide
Chlordane
2-Chloroaniline
4-Chloroaniline
o-Cresol
DDT
Diazinon
1,2-Dibromoethane
1,2-Dichlorobenzene
2,3-Dichlorobutadiene
2,4-Dichlorophenoxyacetic acid (2,4-D)
1,3-Dichloropropene
2,3-Dichloropropene
Diphenylamine
N-Ethylaniline
Furfural
α-Hexachlorocyclohexane
γ-Hexachlorocyclocyclohexane (Lindane)
Hexachlorobutadiene
Mirex
Pentachlorophenol
Phenol
Photomirex
Tetrachloroethane**
1,1,2,3-Tetrachloropropene
2,4,5-Trichlorophenoxyacetic acid (2,4,5-T)
Vinyl Bromide
Vinyl Chloride

C Elements which form toxic compounds (LD_{50} <500 mg oral/kg)

Arsenic	(trioxide^{3+})
Cadmium	(chloride)
Cobalt	(cobaltous^{2+})
Lead	(alkyl^{4+})
Mercury	(elemental^{0})
Nickel	(acetate^{2+})
Silver	(nitrate^{3+})
Vanadium	(trioxide^{3+})

Source MDPH, Sport Caught Fish Consumption Advisories Philosophy, Procedures, and Progress 1986

Table 3 Chemicals Found in the Great Lakes with Known Effects in Mammals

CHEMICAL NAME	CAS NO.
PESTICIDES	
Aldrin	309-00-2
Chlordane (total isomers)	57-74-9
2,4-Dichlorophenoxyacetic acid(2,4-D)	94-75-7
DDT (DDE, 72-55-9 and DDD, 75-54-8)	50-29-3
Diazinon	333-41-5
Dieldrin	60-57-1
Endrin	72-20-8
Heptachlor	76-44-8
Heptachlor epoxide	1024-57-3
Lindane (γ-hexachlorocyclohexane)	58-89-9
Methoxychlor	72-43-5
Mirex (and photomirex, 39801-14-4)	2385-85-5
Silvex	93-72-1
Toxaphene	8001-35-2
HALOGENATED HYDROCARBONS	
Polychlorinated biphenyls	1336-36-3
Trihalomethane (chloroform)	67-66-03
(bromoform)	75-25-2
(bromodichloromethane)	75-27-4
(chlorodibromomethane)	124-48-1
(iododichloromethane)	594-04-7
ELEMENTS	
Arsenic	7440-38-2
Cadium	7440-43-9
Chromium	7440-47-3
Lead	7439-92-1
Mercury (methyl)	115-09-3
Selenium	7782-49-2
Silver	7440-22-4
ETHERS	
2,3,7,8-Tetrachlorodibenzodioxin	1746-01-6

* Monitoring based on concern for human health.

Source MDPH, Sport Caught Fish Consumption Advisories Philosophy, Procedures, and Progress 1986

Toxicity profiles summarize the following information for the chemicals of concern

- Physical-chemical properties (e g , vapor pressure, octanol-water partition coefficients),
- Metabolic and pharmacokinetic properties (e g , metabolic degradation products, depuration kinetics),
- Toxicological effects (e g , target organs, cytotoxicity, carcinogenicity, mutagenicity) according to specific uptake route of concern (i e , ingestion)

DOSE-RESPONSE ASSESSMENT

After the potential hazards of each contaminant of concern have been identified, the relationship between the dose of a substance and its biological effect must be determined Dose-response investigations are used to identify the toxicological potency of a substance, which is a quantitative measure of its potential to cause a specified health effect

Dose and Exposure

Exposure is defined as the "contact by an organism with a chemical or physical agent " In the case of human exposure to contaminants in fish or shellfish, the exposure is via the oral route through ingestion The dose is defined as "the amount of chemical uptake by an organism over a specified time as a consequence of exposure " A distinction must be made between *ingested dose* which is the amount of the contaminant taken in through ingestion, and *absorbed dose* which is the amount of the chemical that is assimilated by the organism through absorption across the lining of the gastrointestinal tract Exposure level or exposure concentration is the concentration of the chemical in the edible tissue of the fish or shellfish, usually expressed in mg/kg wet weight Calculations for estimating the absorbed dose are given in the section on exposure assessment

The Dose-Response Relationship

The dose-response relationship for carcinogens is thought to be fundamentally different than that of non-carcinogens, the difference being that carcinogens do not have a demonstrated threshold level This implies that even at extremely low levels of exposure there is a risk of cancer Non-carcinogens, on the other hand, generally exhibit a threshold level below which no adverse biological effects are observed This threshold level is called the No-Observed-Adverse-Effect-Level (NOAEL) A nonzero mean response value can represent a NOAEL if that value is not statistically different from a zero (no response) value The Lowest-Observed-Adverse-Effect-Level (LOAEL) is defined as the lowest dose that results in a statistically significant health outcome

Toxicological potency indices for carcinogens and non-carcinogens are defined as follows

- The **Carcinogenic Potency Factor**, also referred to as a slope factor, is a measure of the carcinogenic potential of a given chemical, estimated as the upper 95 percent confidence limit of the slope of a straight line calculated by the linearized multistage procedure Or more simply put is the excess tumor incidences/mg chem /kg body wt /day
- The **Reference Dose (RfD)** is an estimate of the daily exposure to the human population including sensitive groups, that is unlikely to cause an appreciable risk of adverse health effects over a lifetime RfDs are conceptually similar to Acceptable Daily Intakes

Epidemiological or clinical data are the preferred sources of information on toxicity, but because that type of information is not available for most substances, data from animal studies are widely used In the absence of human data, animal data from the most sensitive species or from the species most similar to humans should be used

Carcinogenic Potency Factors

The linearized multistage procedure, given below can be used to derive the carcinogenic potency factor

$$R(d) = 1 - \exp[-(q_1 d + q_2 d^2 + \quad + q_k d^k)] \qquad (1)$$

where

R(d)	=	Excess lifetime risk of cancer (over background at dose d)
q_i values	=	Coefficients (kg day/mg (i e the inverse of dose units)]
d	=	Dose (mg kg^{-1} day^{-1})
k	=	Degree of the polynomial used in the multistage model

This model assumes that carcinogenesis results from a series of interactions between the carcinogenic chemical and DNA, with the rate of interactions linearly related to dose Other models include the logit probit single-hit and Weibull models (Food Safety Council 1980, 1982, Hogan and Hoel 1982 Cothern et al 1986) For high doses the models produce very similar results, but at low concentrations the linearized multistage procedure and the single-hit model produce higher estimates of risk

To implement the linearized multistage model, it must first be fitted to the data on tumor incidence vs dose The maximum linear term consistent with the data is calculated, which defines the linear portion of the dose-response curve at low doses The coefficient of the maximum linear term (q_1*) is set equal to the slope of the dose-response function at low doses q_1* is usually calculated as the upper 95% confidence limit of the estimate of q_1 in Equation 1 The resulting q_1* serves as the upper-bound estimate of the Carcinogenic Potency Factor, or slope factor

To estimate the plausible-upper-limit risk for low level exposure over a lifetime, the following equation is used

$$R^*(d) = q_1^* d \qquad (2)$$

where

$R^*(d)$ = Upper-bound estimate of excess lifetime risk of cancer (ie excess tumor incidences)

q_1^* = Upper-bound estimate of carcinogenic potency (kg day mg^{-1})

d = Dose (mg kg^{-1} day^{-1})

The value of q_1^* should not be used as a upper-bound potency estimate at high levels of exposure because the slope of the function at high doses could differ significantly from that which was calculated for the low dose range

With the above equation one can easily estimate the quantitative risk of excess cancers in a population for various exposures to a specific carcinogen For example, PCBs have a q1, cancer potency factor of 4 34 (EPA, 1989) When the dose, ie the exposure, to PCBs over a lifetime is 6 5 x 10-7 mg PCB/kg/day, then the upper bound estimate for excess lifetime cancer is 2 8 x 10-6 ((6 5 x 10-7) X (4 34)) That figures represents 2 8 excess cases of cancer in a population of 1 000 000 exposed to 6 5 x 10-7 mg PCB/kg/day over a lifetime of seventy years If that exposure was to increase to 9 8 x 10-6, then the quantitative risk of excess cancers would increase to 42 5 ((9 8 x 10-6) X (4 34)) excess cases per 1 000,000 exposed

When basing the carcinogenic potency factor on animal data, extrapolations must be made either based on body weight or surface area differences between the experimental animals and humans The U S EPA prefers the use of the surface area extrapolations

Reference Doses

While the carcinogenic potency factor (i e , slope factor) is used to estimate the health risk from carcinogens, the reference dose (RfD) is used to predict effects caused by exposure to non-carcinogens The RfD is usually based on the threshold or no-effect exposure level demonstrated through animal bioassays This threshold level corresponds to the NOAEL (or the LOAEL if the NOAEL is indeterminate) The RfD is calculated by applying an uncertainty factor

which generally ranges from 1 to 1000 The purpose of the uncertainty factor is to take into account interspecies variation differing levels of sensitivity within the human population, and variations between different bioassay procedures For example an uncertainty factor of 1000 is based on combining a factor of 10 to account for animal-to-human extrapolation, a factor of 10 to protect sensitive individuals and a factor of 10 to account for use of a LOAEL in place of a NOAEL

More information on established RfDs and CPFs can be obtained from various databases, such as IRIS and HSBD (Hazardous Substances Databank)

EXPOSURE ASSESSMENT

The exposure assessment is the process of determining how much of the contaminant(s) of concern the human population is exposed to It entails determining the fate and transport patterns of the chemical in the environment characterizing the human populations that are exposed to the chemical and calculating the frequency, magnitude and duration of the exposure Exposure assessment pertaining to consumption of contaminated fish and shellfish involves consideration of the factors listed below The information is useful in constructing an exposure profile typical of the exposed population and for setting the course for subsequent stages of the analysis

- Concentrations of the contaminant in the aquatic species of concern,
- Potential transfer of contaminant from source to the human population via fish and shellfish
- Harvesting and consumption patterns dietary factors and other characteristics of the exposed population
- Numerical values to be used in model applications, such as absorption rate and rate of consumption
- Purpose of conducting the assessment e g development of regulations or consumption advisories assessment of health impacts of local environmental contamination etc

Measurement of Contaminant Concentrations in Fish and Shellfish Tissues

General guidelines and issues involved in the measurement of contaminant levels in fish and shellfish will be discussed in this section including sampling procedures and analytical protocols It should be recognized however that the design of any given study will be largely dependent upon the type of assessment (for example, is the focus on sports or commercial fisheries or on fish purchased in the marketplace), the objectives of the study (e g , regulatory purposes for use in sports fishery advisories public health impact assessment, etc) and the means and resources available for use in the study

Sampling Design

Sampling of fish and shellfish can take place either at the source or in the marketplace Samples taken from the source can either be obtained from commercial fishermen or directly from the aquatic environment by study personnel using vessels other than commercial fishing boats Techniques for marketplace sampling are beyond the scope of this discussion In any case proper handling, storage and documentation procedures should be used If samples are obtained from commercial outfits an observer or technician should be on board to ensure proper handling and documentation Sample documentation should include sampling location, species, size weight sex, reproductive status, time and date, and preservation technique

The concentration of a contaminant in tissue samples is referred to as the bioaccumulation survey Bioaccumulation is the "uptake and retention of a contaminant (e g a potentially toxic substance) by an organism ' In cases where the tissue concentration is greater than the concentration levels found in the surrounding aquatic environment the bioaccumulation is called bioconcentration

Developing an analytic study design requires defining the following parameters

- Objectives,
- Target species and size or age class
- Sampling station locations

- Sampling times,
- Type of sample (e g grab vs composite cooked vs raw, fillet vs whole organism)
- Sample replication strategy,
- Analytical protocols and detection limits, and
- Methods for the statistical analysis of data

Other considerations that should be part of the study design development include the spatial and temporal characteristics of harvesting (e g , seasonality, species composition, types and size range and catch rates) and harvesting and food preparation methods that could effect exposure levels An appropriate statistical model should be selected, and stratified random sampling should be used when possible with equal numbers of randomly assigned samples for each stratum-treatment combination The study should include sampling and analysis of species from control or reference areas and a comprehensive quality assurance/quality control program should be implemented

Detailed information regarding sampling procedures can be found in Phillips (1980), Green (1979), Tetra Tech (1985b c 1986b) Phillips and Segar (1986), and Gilbert (1987)

Environmental and Exposed Populations Factors

A complex web of interactions between an array of environmental factors and the characteristics of the exposed population greatly influences the degree of exposure that occurs Environmental factors include conventional water quality (e g salinity pH, temperature, suspended solids) habitat location (e g proximity to contaminant source, depth of habitat, etc), contaminant concentrations in water and sediments, species available for harvest (dependent upon migratory patterns fisheries management practices, etc), organism activity and feeding patterns seasonal biological cycles (e g reproductive cycle) in relation to seasonality in contaminant inputs, organism size age weight and the lipid concentration in the tissues

Characteristics of the exposed population which impact on exposure level and interact with environmental factors include harvesting practices (e g location, timing, methods), methods of preparing fish for market and consumption, types of species and size consumed, and what parts of the organism are consumed

Target Species

The selection of target species depends on the objectives of the study For example, target species selection would differ for a study that aimed to perform a comprehensive analysis of all harvested species vs one that was focused on determining the typical exposure case represented by the dominant harvested species Similarly, characterizing the worst-case exposure scenario from a specified location would require a different species selection rationale than would a study seeking to determine the spatial distribution of a contaminant using an indicator species The advantages and disadvantages for each one of these study approaches -- comprehensive species analysis typical exposure case worst-case species, and spatial pattern indicator species -- should be taken into consideration during the study design process

Considerations for selecting appropriate indicator species include

- Target species should be harvested by the exposed population, or should be representative (in terms of contaminant concentration) of the primary harvested species,
- Species snould be representative oi a specific study area Ideally, they would be relatively non-mobile by nature, or physically restricted by a dam, waterfall, etc ,
- Species should be relatively abundant and easy to obtain and large enough to provide sufficient tissue amounts for analysis,
- Concentrations of contaminants in the water should be reflected in the concentrations found in the target species
- Species should have a weak or non-existent ability to metabolize the contaminant of concern,
- Contaminant interactions should not significantly reduce the usefulness of the species as site-specific indicators even when the contaminant composition is expected to vary from site to site

- Target species should be useful in assessing the effects of contaminant uptake over time,
- The species selected should have a high bioaccumulation potential factor, especially for worst-case analyses

Sampling Locations

Field samples can be obtained either from commercial fisheries or the investigators can conduct independent harvesting missions to obtain samples One advantage of obtaining fish from commercial outfits is that the samples are directly representative of the population of interest i e , they are representative of the fish and shellfish that are consumed by the public However the potential for contamination or insufficient documentation is far greater than if the sampling is conducted by the study team Independent sampling also allows for the collection of sufficient quantities of each particular species size, age group, etc required for variable-specific stratified analysis When conducting independent sampling, every effort should be made to simulate commercial harvesting procedures in collecting the samples In addition, samples should be taken from a closely matched aquatic habitat in a reference (non-contaminated) area for comparison purposes

Sampling stations can be arranged in a variety of probability sampling designs as depicted in Figure 2 Simple random sampling means that each organism in a sample area has an equal chance of being picked and the selection of an organism does not influence the selection of any other organism This is appropriate where there is no observable pattern of contamination in the area under study Stratified random sampling involves random sampling within non overlapping strata, and is useful when distinct geographic areas within the study area differ according to contaminant level or composition Two-stage sampling involves random or systematic subsampling within primary units that have been randomly selected For example, fish could be selected randomly from the aquatic environment, and then subsamples of the tissues obtained could be randomly sampled for analysis Cluster sampling refers to the collection of groups of organisms rather than individuals and analyzing the contaminant

concentration in all members of the group It is similar to random sampling, but is based on clusters of organisms rather than individuals Systematic sampling is sampling done according to a specified pattern, such as the grid pattern depicted in the illustration Grid sampling is particularly useful for identifying environmental contamination from point sources, and has been successfully achieved by using caged mussels as the indicator species Finally, random-sampling-within-blocks is a combination of random and systematic sampling

The U S EPA recommends the use of a combination two-stage and stratified-random (or stratified-grid) sampling strategy for use in exposure assessment studies of fisheries contamination The two stages refer to individual organisms and edible tissue samples Stratification can be according to harvest area, species, size, and other variables as appropriate

Timing of Sampling

Design of the sampling procedure should take into consideration timing of the harvest season as well as the biological cycles of the target species Bioaccumulation can be significantly affected by biological cycles such as reproduction and in the case of crustaceans molting

Sample Types

The precision, sensitivity and representativeness of an exposure assessment is greatly influenced by the kind of tissue that is sampled, and whether composite or individual samples are used in the laboratory analyses Composite sampling means combining subsamples of tissues usually from several organisms of the same species which were obtained from the same location, into a composite sample which is subsequently analyzed This is a cost-effective approach particularly when the procedure is expensive and the target organisms are readily obtained It is necessary in cases where the organisms are too small to provide sufficient amounts of tissue for individual analysis The result of composite sampling is an estimate of the average concentration of the contaminant in individual organisms found in that area at that time

FIGURE 2

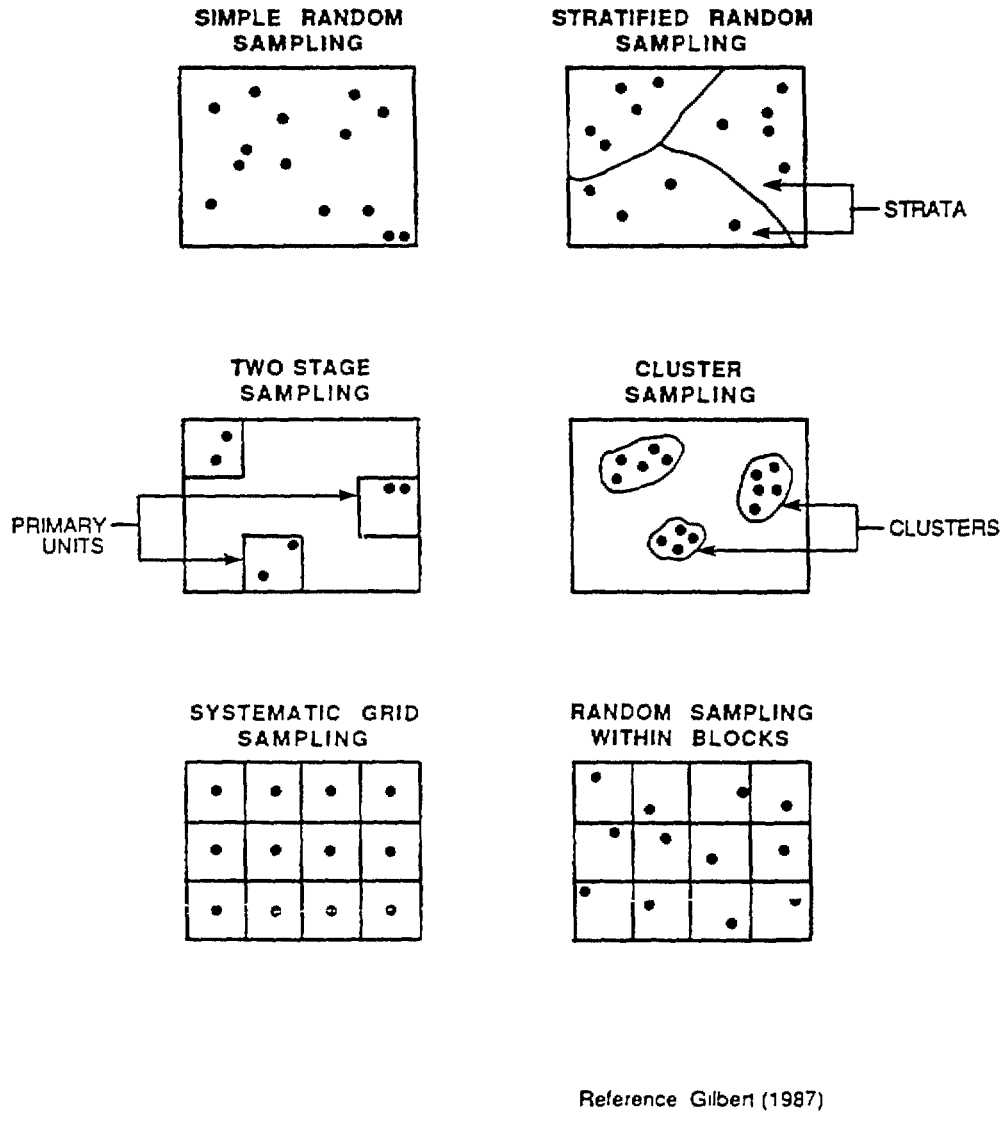


Figure General sampling station layouts for probability sampling in two dimensions

Source EPA Assessing Human Health Risks from Chemically Contaminated Fish and Shellfish A Guidance Manual 1989

The major drawback of composite analysis is that it is not possible to accurately assess the variability and range of concentrations in individual organisms in a study area Lumping together tissues from sample organisms will mask any individual variation which could otherwise serve as a signal that contamination is increasing, or is highly localized

Rohde (1976) and Tetra Tech (1986b) provide a means for estimating the variance among individuals in the population if the variance of the composite samples is known, based on the assumption that replicate observations from individual and composite samples are normally distributed The equation is as follows

$$\text{Var } X = n\,(\text{Var } Z) \qquad (3)$$

where

Var X = variance of the mean of individual samples from all composites

Var Z = variance of the mean of composite samples,

n = number of subsamples constituting each composite sample

It is important that the subsamples are of equal mass (i e , the same amount of tissue is used from each organism in creating the composite), or the variance of the composite samples can increase significantly

Two variations on composite sampling are 'space-bulking" and "time-bulking " Space-bulking means taking tissue subsamples from organisms collected from several locations and combining them for analysis Time-bulking means creating a composite from subsamples of organisms taken from the same location but at different times Because exposure assessments are usually based on averages over time time-bulking may be appropriate in some instances Space-bulking may also be useful in some cases, but the application of either strategy should be carefully evaluated because important information on spatial and temporal variations can be lost

Sample Preparation

Specific protocols for sample preparation should be established and consistently followed The protocol should address

- Type of tissue used, (e g , fillet, whole organism, internal organs),
- Location of tissue in the organism,
- Removal of any or all skin fat, shells or scales,
- Cooked vs raw samples including cooking method if applicable,
- Homogenization method,
- Minimum sample mass required for each analysis

The part or parts of the organism used or whether the whole organism is used, will affect the applicability of the exposure assessment Most humans consume the fillets and not the internal organs of fish Sampling tissues taken from internal organs, which in general exhibit higher contaminant concentrations may lead to a relatively high estimate of exposure Cooking methods too can affect the concentration or composition of the contaminants through decomposition or transformation processes caused by heat

EPA recommendations include conducting a pilot study and consulting guidance manuals to aid in the selection of an appropriate sample preparation procedure, and to assess, in most cases samples of the raw edible portion of the organism

Sample Replication

In most cases, five replicate samples of individual organisms are required to achieve minimal statistical power Replicates greater than ten in number generally are not cost-beneficial Replication is necessary for uncertainty analyses (i e , assessing the precision of the mean concentration estimates) and for conducting various statistical tests

Selection of Detection Limits and Analytical Procedures

Analytical methods may be selected based on the need to achieve a certain level of detection associated with a relevant minimum risk level or as specified by risk managers or government guidelines Financial considerations also play a large part in deterring which analytical procedures will be used The analytic detection limit is selected based on the objectives and needs of the particular study, the financial resources available, and the existing technology for detecting and quantifying the contaminants of concern

Quality Assurance/Quality Control

A comprehensive QA/QC program should be established as part of the study design It should include

- Study objectives,
- Project organization and personnel,
- QA objectives for precision, accuracy and completeness for each type of measurement,
- Summary of sampling procedures (e g , sample containers preparation, preservation),
- Forms for documenting sample custody, station locations, sample characteristics, sample analysis request, and sample tracking during laboratory analysis,
- Detailed description of analytical methods,
- Calibration procedures for chemical measurements,
- Internal QC checks for analytical laboratories,
- Performance and system audits for sampling and analysis operations,
- Preventive maintenance for equipment,
- Procedures for data management, data QA review, and data reporting for each kind of measurement,
- Corrective actions
- Procedures for QA/QC reporting and responsible national and local officers,

Statistical Analyses

Tissue concentration data should include sample size estimates of arithmetic mean concentration range and variance (i e 95% confidence interval or standard error) Geometric means are useful when only estimates of tissue burden or exposure dose are needed, but arithmetic means are necessary to compare exposure estimates with RfDs and to calculate the risk of chronic health effects because long-term consumption calculations involve an averaging process If data is available on the composition of the diet (i e , the proportion of each species consumed), mean tissue concentrations and variances may be calculated for a mixed-species diet

The one-way ANOVA or the multifactor ANOVA analyses are useful for testing for differences in tissue concentrations between species between locations or between sampling times For small data sets for which ANOVA cannot be used, other models such as the Wilcoxon rank sum test for two treatments or the Kruskal-Wallis test for multiple comparisons can be applied and hold the added bonus of being relatively insensitive to a few missing data points Time series analyses can identify trends in long-term data sets (EPA, 1989)

Tissue concentration observations often fall below the detection limits In such instances the means and variances for the concentrations should be calculated both by using the detection limit as the value for undetected observations and again by using zero as the concentration value This method is preferred because it provides a range for the estimated values

Deciding what contaminant concentration value to use in estimating exposure, which is ultimately used in estimating risk is essentially a risk management decision The EPA guidelines discourage use of the upper 90-95% confidence limit values because the resulting estimates of risk may be unreasonable high particularly when the cumulative effect of other conservative estimates throughout the process are considered, such as the use of high-range estimates of fish consumption rates The EPA recommends use of arithmetic mean

concentrations of contaminants in the edible portions of the fish or shellfish tissue for estimating exposure

Sources, Transport, and Fate of Contaminants

Exposure pathways are the routes via which contaminants are transferred from the environment to aquatic species, and subsequently passed on to humans Analyzing the sources, transport mechanisms and fate of contaminants is necessary for identifying these pathways, and ultimately for assessing the risk to human health posed by environmental contamination Because data on the transport and fate of environmental contaminants is often limited or nonexistent mathematical modeling is often used in these analyses Modeling is particularly useful in assessing the movement of contaminants from the environment into the tissues of fish or shellfish Analyzing the fish-to-human transport of contaminants is usually more straightforward because information on fisheries practices and human consumption patterns are more readily available

Modeling is useful in assessing the spatial and temporal distribution of contaminants in fish and shellfish It is also instrumental in identifying sources of the contamination, and in developing appropriate control or remedial measures

When information on tissue concentration is lacking, mathematical models can be used to estimate the contaminant concentrations in aquatic species based on observational data or by modeling the amounts and distribution of the chemicals in the water and sediments Laboratory-derived bioconcentration factors (BCF) are instrumental in estimating tissue concentrations based on ambient concentrations Spatial and temporal distributions are important for identifying hot spots or seasonal fluctuations (EPA 1989)

Analysis of Exposed Populations

A key component of the exposure assessment is identifying and characterizing the exposed population, and arriving at an estimate of average consumption This process involves the following steps

- Identify potentially exposed populations and map the location of fisheries,
- Characterize the potentially exposed populations by dividing them into subpopulations based on age sex and ethnicity Determine the size of the population and each subpopulation,
- Analyze population activities such as harvesting frequency, seasonal and daily patterns of harvest activities, and time per harvest trip Identify the type of activity, e g fishing crabbing, clamming (Note these considerations pertain to recreational fisheries only),
- Analyze the catch/consumption patterns for the total population and for each subpopulation by collecting information on the numbers and weight of species harvested the number of consumers sharing each catch, the time since last meal of locally harvested species the parts of the organism that are consumed, and the methods of food preparation
- Estimate the arithmetic average consumption rate by species and by total catch for the total exposed population and for the subpopulations

Estimates of consumption rates can be based on extensive data gathering on local harvesting and consumption patterns or can be based on national averages or assumed values The former method should be used whenever possible, but time, financial and methodological constraints make using the ideal process sometimes impractical or impossible Consumption patterns are frequently estimated by annual fish production divided by the number of fish consumers in the population The more accurate the data, the better the quality of the risk assessment but very good health advisories can be issued when the limitations of the data are considered Another approach which eliminates the need for exhaustive research on actual consumption patterns is to calculate the exposure and health risks for all possible consumption levels and present the information in a graph This is particularly useful for supplying information to the public, as individual consumers can then identify their estimated risk based on their

personal consumption patterns A third approach would be to identify an "acceptable risk" level and then provide guidance on how much of specific species of fish and shellfish an individual could safely consume without exceeding that level

Catch and Consumption Analyses

Assessing consumption patterns can be accomplished through field survey methods, food diaries or consumer dietary recall, or can be based on existing national or regional dietary information Average consumption rates should be expressed in g/day and meals/year Meals per year in the United States can be calculated from grams per day by assuming an average meal of 150 g (0 33 lbs) It is estimated that in Egypt 8 kg fish/capita/year is consumed This may be translated to 22 g/day or 52 meals per year based on an average meal of approximately 150 g of fish These figures are just estimates and may not reflect recent changes in local seafood consumption Nor should they be used for risk ananlysis without further investigation

Average consumption rate for recreational fisheries can be arrived at as follows

- For each successful harvesting trip, calculate the harvest (weight and number) for each species per household
- Calculate the mean harvest weight consumed per person per time by dividing the total harvest weight for each species by the number of consumers in the household and by the time elapsed (in days) since the last meal of species from the same location was consumed Multiply that value by a factor to account for the difference between harvest weight and prepared (cleaned) weight (Landolt et al 1985 provide factors of 0 5 for squid and crabs 0 3 for fish, and 1 0 for shucked clams Local data is not currently available for these factors and will require further investigation into cultural differences in preparing and eating seafood)
- Calculate mean consumption rate per person by geographic harvest area, by subpopulation and by total exposed population

The model for the mean daily consumption rate (I_{ijk}) for fishery species i, subpopulation j, and area k is

$$I_{ijk} = 1/N_{ijk} \Sigma I_{ijkl} = 1/N_{ijk} \Sigma W_{ijkl} P_i / H_{jkl} T_{jkl} \qquad (4)$$

where

I_{ijkl} = Mean daily consumption rate of species i for subpopulation j, area k, and household l (kg/day)

N_{ijk} = Number of households (successful harvest trips) for species i, subpopulation j, and area k

W_{ijkl} = Weight of species i harvested by household l of subpopulation j in area k (kg)

P_i = Proportion of cleaned edible weight of species i to total harvested weight

H_{jkl} = Number of people in household l of subpopulation j in area k

T_{jkl} = Time elapsed since last meal by household l of subpopulation j in area k (days)

Summary calculations on consumption rates across subpopulations, species or areas can be made but it is important to remember that an overall mean derived from the mean daily consumption rates for individual species is not an accurate representation of diet patterns for populations with mixed-species diets To calculate mean consumption rates for mixed species diets, all I_{ijkl} should be summed across species within households before mean consumption rates are calculated across households

Where detailed information on catch and consumption patterns is not available and the purposes of the study do not warrant extensive investigation mean consumption rates can be based on existing standard values

U S EPA has used a value of 6 5 g/day for national average consumption of fish and shellfish from estuarine and fresh waters and 20 g/day when saltwater species are included Values have been shown to range up to 165g/day in some studies (EPA, 1989)

Exposure Dose Determination

Estimates of chemical intake (dose) are obtained by combining the information on tissue concentration with the average consumption rates Model for single-species and mixed-species diets are given below

Single-species Diets

Calculation of chemical intake from a single-species diet is as follows

$$E_{ijkm} = C_{ikm} I_{ijk} X_m / W \qquad (5)$$

where

E_{ijkm} = Effective ingested dose of chemical m from fishery species i for human subpopulation j in area k (mg/kg day averaged over a 70-year lifetime) Same as dose "d" in Equations 1 and 2

C_{ikm} = Concentration of chemical m in edible portion of species i in area k (mg/kg)

I_{ijk} = Mean daily consumption rate of species i by subpopulation j in area k (kg/day averaged over 70-year lifetime)

X_m = Relative absorption coefficient, or the ratio of human absorption efficiency to test-animal absorption efficiency for chemical m (dimensionless)

W = Average human weight (kg)

Values of subscripted terms may be averages or uncertainty interval values (e g , 95% confidence intervals) depending on the exposure scenario being used such as worst-case or average-case scenarios Absorption coefficients (X_m) are assumed to be 1 0 unless data indicates otherwise The term X_m can also be used to transform the dose from an ingested dose to an absorbed dose, if that coefficient is available and the purposes of the assessment warrant it Average body weight (W) is generally assumed to be 70 kg for the "reference" man used by the U S EPA in its calculations

An example for calculating chemical intake can be found in the section Issuing Health Advisories

Mixed-species Diets

Because of interpersonal variations in the amounts and types of species consumed in any given population the estimation of chemical exposure in a mixed-species diet is complex Nevertheless the following method can be used to arrive at an estimate

$$E_{hjkm} = \Sigma\ C_{jkm}\ I_{hijk}\ X_m\ /\ W \qquad (6)$$

where

E_{hjkm} = Effective exposure dose of chemical m from a mixed-species diet eaten by individual human h in subpopulation j in area k (mg/kg day averaged over a 70-year lifetime)

I_{hijk} = Average consumption rate of species i by individual h in subpopulation j in area k (kg/day averaged over a 70-year lifetime)

Other terms are described as above

The average exposure dose for mixed-species diets is

$$E_{jkm} = \Sigma_h\ E_{hjkm}\ /\ H_{jk} \qquad (7)$$

where

E_{jkm} = Average effective exposure dose of chemical m from mixed-species diet for subpopulation j in area k (mg/kg day)

H_{jk} = Number of persons in subpopulation j in area k

RISK CHARACTERIZATION

During the risk characterization stage of a risk assessment, the information from the hazard identification, dose-response and exposure assessment stages is coalesced into an estimate of the probability and extent of adverse human health effects associated with consumption of contaminated fish and shellfish In general, carcinogenic risk and non-carcinogenic risks are handled separately

The risk estimate is quantitative, but numerical estimates of risk should never be presented without providing information about the assumptions, uncertainties and procedures that were used in generating the estimate In particular, it should be made clear whether the risk estimate is a realistic assessment of risk, or whether it was based on a series of conservative estimates and thus is very unlikely to represent true risk A summary of the methods and assumptions used in the first three stages of the risk assessment should accompany the risk characterization as well as the weight-of-evidence classification and other supporting information

Carcinogenic Risk

Carcinogenic risk is expressed in one of three ways The first method of expression utilizes the Unit Risk which is the excess lifetime risk corresponding to continuous lifetime exposure to a unit carcinogen concentration, such as 1 mg of carcinogen per kilogram of edible fish or shellfish tissue The second way risk can be expressed is as the dose or concentration that corresponds to a given level of risk This presentation of risk is often found in guidelines that establish maximum allowable concentrations for a given medium based on what is considered acceptable risk The third means of expressing carcinogenic risk is based on individual or population-based risk Upper-limit estimates of excess lifetime cancer risk are expressed as probabilities for individuals, and as the number of expected excess cases per population measure (e g per 100,000 people) per generation for populations

The plausible upper-limit to excess lifetime cancer risk at low doses for a single-species diet is estimated as follows

$$R^*_{ijkm} = q1^*_m E_{ijkm} \qquad (8)$$

where

R^*_{ijkm} = Plausible upper-limit risk of cancer associated with chemical m in fishery species i for human population j in area k (dimensionless)

$q1^*_m$ = Carcinogenic Potency Factor for chemical m [(mg kg^{-1} $day^{-1})^{-1}$] estimated as the upper 95% confidence limit of the slope of a linear dose-response curve

E_{ijkm} = Exposure dose of chemical m from species i for subpopulation j in area k (mg kg^{-1} day^{-1})

This equation is only valid for estimated risks below 10^{-2} (one in one hundred) Calculating the upper limit risk for mixed-species diets follows the same general procedure, but the average effective dose (E_{jkm}) obtained from Equation 7 above replaces the species-specific dose (E_{ijkm}) used in Equation 8 All E_{ijkm} are calculated as in the exposure Dose Determination in Exposure Assessment

Noncarcinogenic Risk

The ratio of the estimated chemical intake to the RfD provides an estimate of noncarcinogenic risk

$$H_{ijkm} = E_{ijkm} / RfD_m \qquad (9)$$

where

H_{ijkm} = **Hazard Index** of a health effect from intake of chemical m associated with fishery species i for human subpopulation j in area k (dimensionless)

E_{ijkm} = Exposure dose of chemical m from species i for subpopulation j in area k (mg kg^{-1} day^{-1})

RfD_m = Reference Dose for chemical m (mg kg^{-1} day^{-1})

When exposure from all possible routes is taken into consideration the estimate of total exposure replaces E_{ijkm} in the equation and the resulting hazard index is compared to 1 0 to

evaluate chemical hazard Values of this total exposure hazard index greater than one indicate exposure that is of potential concern and hazard increases as the values increase However the hazard index is not an estimate of risk

Because the hazard index derived from Equation 9 is for chemical intake associated with consumption of only one species fish or shellfish and does not take into consideration consumption of other species or exposure from unrelated sources, values of H_{ijkm} do not indicate overall risk To obtain a hazard index for a mixed-species diet the average effective dose (E_{jkm}) for mixed-species diets can be used To account for exposure in addition to that resulting from fish and shellfish consumption, the sum of the exposures from all sources should be compared to the RfD If this type of information is not available, an alternative method is to assign a relatively small fraction of the RfD to intake from consumption of contaminated fish and shellfish, and use that fraction as the denominator in the hazard index equation (Equation 9) in place of the RfD The resulting index could then be compared to 1 0 to determine the potential for concern More research is needed in evaluating hazard from total exposure

Chemical Mixtures

Assessing risk from chemical mixtures is nearly impossible in most cases due to lack of information on specific effects and concentrations of contaminants and an insufficient understanding of the myriad possible synergistic or antagonistic effects However, the additive-risk or response-additive model has been used to provide a rough estimate of upper-limit risk It entails adding up the upper-limit risks for carcinogens or hazard indices for noncarcinogens Noncarcinogenic risks should only be summed for chemical that affect the same target organ The additive-risk method is primarily useful for comparative uses, e g , comparing fisheries in different locations Because it is not generally feasible to test fish or shellfish tissue samples for all potentially toxic chemicals, the chemical mixture risk estimates should not be interpreted as the total chemical risk associated with ingestion

PRESENTATION AND INTERPRETATION OF RESULTS

In this section formats for the presentation of risk assessment results are provided, as well as guidance in documenting assumptions and uncertainties contained within the estimates Interpretation and application of the results is the purview of risk management and is beyond the scope of this discussion

Presentation Format

Risk assessment results can be presented in tables or graphs All final estimates should be rounded to one significant digit or an order of magnitude if appropriate Supporting information including assumption and uncertainties should appear in text adjacent to the table or graph Individual and population-based estimates of risk should be presented as plausible upper limits based on the stated exposure conditions and assumptions Examples of a summary table and a summary graph are shown in Figures 3 and 4, respectively

Uncertainty analysis such as sensitivity analysis, can be performed in a spreadsheet for presentation in tabular form by calculating exposure estimates for low, mid, and high values of key variables Spreadsheets also allow for presenting exposure and risk information associated with more than one consumption level as shown in the example If a graphic format is used, uncertainty analysis can be achieved by specifying the probability distributions for key variables

Graphic presentations can include plots of estimated risk vs consumption rate, plots of estimated risk vs contaminant concentration in edible tissues, summary maps of risk estimates for different locations or sampling stations and histograms of risk by fish species, human subpopulation or geographic location Uncertainty can be indicated graphically by plotting lines corresponding to maximum and minimum concentrations, as well as the mean values

TABLE 6
Example Tabular Format for Display of Quantitative Risk Assessment for Consumption of Fish and Shellfish

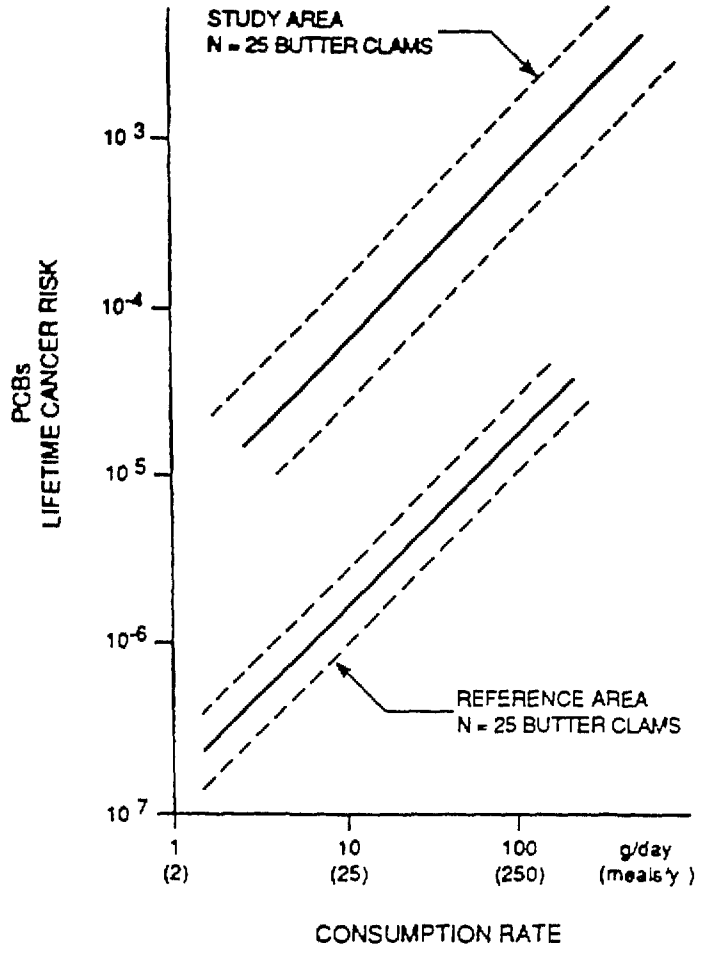
	Exposure Determination							Risk Determination				
								Carcinogens			Noncarcinogens	
Substance	Concentration in Medium	Contact Rate (mg/kg)[a]	Total Daily Contact (g/day)[b]	Exposure Duration (mg/day)	Absorption Coefficient (years)	Body Weight (0 1 0)[c]	Exposure Value (kg)	Potency Factor (mg/kg/d)	Upper Limit U(mg/kg/d)	Weight of Risk	RfD Evidence	Hazard (mg/kg/d)Index
PCBs	0 007	6 5	4 6E -05	70 0	1 0	70	6 5E -07	4 34	3E-06	B2	N/A[d]	N/A
	0 004	6 5	2 6E -05	70 0	1 0	70	3 7E -07	4 34	2E -06	B2	N/A	N/A
	0 010	6 5	6 5E -05	70 0	1 0	70	9 3E -07	4 34	4E -06	B2	N/A	N/A
PCBs	0 007	20 0	1 4E -04	70 0	1 0	70	2 0E -06	4 34	9E -06	B2TN/A	N/A	
	0 004	20 0	8 0E -05	70 0	1 0	70	1 1E -06	4 34	5E -06	B2	N/A	N/A
	0 010	20 0	2 0E -04	70 0	1 0	70	2 9E -06	4 34	1E -05	B2	N/A	N/A
Hg	0 157	6 5	1 0E -03	70 0	1 0	70	1 5E -05	N/A	N/A	[e]	2 9E-04	5E-02
	0 008	6 5	5 2E -05	70 0	1 0	70	7 4E -07	N/A	N/A	[e]	2 9E-04	3E-03
	0 478	6 5	3 1E -03	70 0	1 0	70	4 4E -05	N/A	N/A	[e]	2 9E-04	2E-01
Hg	0 157	20 0	3 1E -03	70 0	1 0	70	4 5E -05	N/A	N/A	[e]	2 9E -04	2E-01
	0 008	20 0	1 6E -04	70 0	1 0	70	2 3E -06	N/A	N/A	[e]	2 9E -04	8E-03
	0 478	20 0	9 6E -03	70 0	1 0	70	1 4E -04	N/A	N/A	[e]	2 9E-04	5E-01

[a] Concentration of contaminant in fisheries species of concern (mg/kg = ppm by mass wet weight)
[b] Amount of fish/shellfish ingested per day prior to accounting for absorption efficiency etc
[c] Ratio of g of contaminant absorbed per g of contaminant ingested or correction factor to account for differential absorption by humans and bioassay animals (see text Exposure A sessment, Exposure Dose Determination)
[d] N/A = not applicable
[e] Carcinogenicity of methyl Hg has not been evaluated by EPA Carcinogen Assessment Group Hg is typically treated as a noncarcinogen in risk assessment

Source EPA, Assessing Human Health Risks from Chemically Contaminated Fish and Shellfish A Guidance Manual 1989

FIGURE 3

FIGURE 4



PCBs Weight of evidence classification
PROBAB E HUMAN CARCINOGEN [B2]

All cancer risks are plausible upper limit estimates of excess risk based on linearized multistage procedure and assump ions summa ized in the text Solid lines are risks associated w th ave age PCB concen ra ions in bu er clams Dashed lines are for uncertainty range (e g 95 percent confidence limits) for average concentrations of PCBs not the total uncertainty Actual risks are likely to be lower than those shown above and may be zero

Figure Example graphic forma' for display of quantitative nsk assessment results for hypothetical study area and reference area

Source EPA Assessing Human Health Risks from Chemically Contaminated Fish and Shellfish A Guidance Manual 1989

Risk Comparison

Interpretation or risk assessment results for fish and shellfish consumption can be facilitated by the use of comparisons either with the same species from a reference (non-contaminated) area or by comparing the risk associated with the fish or shellfish with that of consuming alternative foods This comparative approach is extremely useful for providing the public with a sense of perspective regarding the potential risks Consistent methods, assumptions and uncertainty factors should be used in assessing the risk of both the target species and the reference foods

Summary of Assumptions

The assumptions upon which the risk assessment was based should be presented in a clear, concise manner The risk assessment process can involve assumptions regarding

- the average rates of consumption
- the effect or lack of effect resulting from cooking or other food preparation method, upon the concentration of the contaminant,
- the efficiency with which the ingested dose is absorbed by the body
- the average body weight of the exposed population,
- the applicability of the multistage linearized model, which contains the inherent assumption that for risks less than 10^{-2} Risk = Exposure x Potency,
- the appropriateness of extrapolations from animal bioassays,
- the appropriateness of using the upper bound of the 95% confidence interval as an estimate of potency
- the use of extrapolations beyond the range of the dose-response data set to yield plausible upper-bound estimates of risk at low doses
- the lack of a threshold dose for carcinogens
- the existence of a threshold dose for noncarcinogens
- the appropriateness of using the most sensitive animal species to estimate potential effects in humans

- average doses for carcinogens are an appropriate measure of exposure, even when actual exposure varies widely over time,
- risks from multiple exposures are additive
- the absorption efficiency for humans is equal to that of animals for individual chemicals,
- for chemical mixtures the risks for individual chemicals are additive, although the sum of all identified chemicals does not necessarily represent the total risk

Uncertainty Analysis

Uncertainty can enter into an assessment of risk in a variety of ways, including

- uncertainties in estimating the Carcinogenic Potency Factors or RfDs, due to the use of extrapolations from animal bioassays, the low-dose extrapolation model used, and limitations in the quality of study design or implementation,
- variations in consumption rates and contaminant concentrations by site or over time,
- selection of average consumption rates in the absence of site-specific data,
- using the rate of absorption (as a fraction of ingested dose) obtained from animal studies as an estimate of the human absorption rate,
- Variation in individual diets cooking methods, sensitivities etc

Uncertainty in risk estimates can be significant Uncertainty ranges around estimates of mean risk such as 95% confidence intervals can span several orders of magnitude or more The standard EPA approach is to estimate a plausible upper limit to risk, thereby reducing the likelihood that a risk will be underestimated This method also ensures consistency which facilitates comparisons between risks

Analysis of uncertainty in a risk assessment includes both quantitative uncertainty, e g , variations in numerical estimates resulting from changing the inputs in the models and equations and qualitative uncertainty which includes limitations in the study or the weight of the evidence in support of toxicity estimates

Some approaches (in descending order of certainty) to dealing with uncertainty in the variables used in risk analyses include (Morgan, 1984)

- Perform analysis using **single-value-best-estimates** for model variables **without uncertainty analysis**
- Perform **single-value-best-estimate** analysis, **with sensitivity calculations** and discussion of uncertainty,
- Estimate some **measure of uncertainty** such as standard deviation for each model variable and use **error propagation** methods to estimate uncertainty of final exposure or risk value
- Characterize subjectively the **probability distribution** of each model variable and **propagate error** through stochastic simulation,
- Characterize important model variables using a **parametric model** and perform risk analysis using various **plausible values** of each of the variables,
- Determine upper and lower **bounds** on model variables to yield **order-of-magnitude estimates** and range of possible answers

Issuing Health Advisories

Setting Desired Levels for Carcinogens in Fish

When setting safe or advisory levels of exposure for a carcinogen in seafood, the first step is to set an accepted level of risk Risk is expressed as the number of expected excess cases of cancer (tumor incidences) per population measure from exposure to a carcinogen over a lifetime Acceptable risk is usually set to one excess case of cancer per 100,000 (10-5), 1,000 000 (10-6), or 10 000 000 (10-7)

Before continuing with the risk assessment, certain assumptions need to be made regarding the characteristics of the seafood consumers, such as their body weight (in kg) average fish consumption and average life span By convention the United States and the WHO as well as many other countries with set legal limits on contaminants, use 70 kg for the standard body weight and an exposure lifetime of 70 years Fish consumption, however varies

widely among countries and regions within countries The average fish consumption in Egypt for example is 8 kg fish/person/year This figure is probably much greater (2x3 times) in Northern Egypt where seafood is more prominent in the diet Likewise, seafood consumption is expected to be less in the regions of the country where fishing is not as accessible For comparison sample advisories for low consumption (4 kg fish/person/year), average consumption (8 kg fish/person/year) and high consumption (20 kg fish/person/year) will be developed

Summary of assumptions

Risk = 10-6 excess cancers (tumor incidences)
Average consumer body weight = 70 kg
Average consumer life expectancy = 70 years
Average fish consumption low = 4 kg fish/person/year
average = 8 kg fish/person/year
high = 20 kg fish/person/year

One method for setting safe or desirable levels for carcinogens in fish is to calculate the Total Lifetime Dose ingested of a carcinogen and compare it to an established or acceptable risk Total Lifetime Dose includes the total lifetime dose ingested from fish as well as from water air and other foods besides seafood Total dose over a lifetime is computed from the chemical concentration in the substance the amount of substance consumed the duration of the exposure, and the absorption effeciency for that substance

For example,

Fish Lifetime Dose	=	Chemical Concentation (mg/kg fish)	X	Contact Rate (kg fish/day)	X	Exposure Duration (days)	X	Absorption Efficiency (assumed 1)

It has been estimated that at one time fish harvested in the Mediterranean Sea had an average DDT concentration of 4 2 mg DDT/kg fish (Krauss 1986) Using this contaminant concentration lifetime ingested DDT dose from fish may be calculated,

Lifetime DDT Dose for avg fish consumption	Chem Conc	Contact Rate	Exposure	Absorption Rate
	4 2 mg DDT/kg fish	$\frac{(8\text{ kg fish})}{(\text{year})}$ $\frac{(\text{year})}{(365\text{ days})}$	$(70\text{ yr})\frac{(365\text{ days})}{(1\text{ year})}$	1

= (4 2 mg DDT/kg fish) X (022 kg fish/day) X (25550 days) X (1)

= 2361 mg DDT for average consumption over a lifetime

at low consumption

= (4 2 mg DDT/kg fish) X (011 kg fish/day) X (25550 days) X (1)

= 1180 mg DDT

at high consumption

= (4 2 mg DDT/kg fish) X (055 kg fish/day) X (25550 days) X (1)

= 5902 mg DDT

To estimate total lifetime dose of DDT, estimations must also be calculated for total lifetime dose from food, total lifetime dose from air, and total lifetime dose from water These calculations are similiar

Food Lifetime Dose	=	Chemical Concentation (mg/kg food)	X	Contact Rate (kg food/day)	X	Exposure Duration (days)	X	Absorption Efficiency (assumed 1)
Water Lifetime Dose	=	Chemical Concentation (mg/l water)	X	Contact Rate (l water/day)	X	Exposure Duration (days)	X	Absorption Efficiency (varies)
Air Lifetime Dose	=	Chemical Concentation (mg/m3 air)	X	Contact Rate (m3 air/day)	X	Exposure Duration (days)	X	Absorption Efficiency (varies)

Total lifetime dose is the sum of these individual doses,

Total Lifetime Dose	=	Fish Lifetime Dose	+	Water Lifetime Dose	+	Air Lifetime Dose	+	Food Lifetime Dose

Assuming total exposure of DDT from air, water and food = 750 mg DDT, total DDT intake including fish equals

at low consumption 1930 mg DDT
at average consumption 3111 mg DDT
at high consumption 6652 mg DDT

When estimating the risk associated with an intake of a carcinogen, it should be expressed as the amount of chem intake (mg) per kg body weight per day That is,

$$\text{The Risk Associated Intake (RAI)} = \frac{\text{Total Lifetime Dose (mg Chem)}}{\text{Body wt (kg) X Lifetime (days)}}$$

The RAI for DDT from low fish consumption,

$$= \frac{1930 \text{ mg DDT}}{(70 \text{ kg}) \text{ X } (25550 \text{ days})} = 1\ 1 \times 10\text{-}3 \text{ mg DDT/kg/day}$$

RAI for DDT from average fish consumption,

$$= \frac{3111 \text{ mg DDT}}{(70 \text{ kg}) \text{ X } (25550 \text{ days})} = 1\ 7 \times 10\text{-}3 \text{ mg DDT/kg/day}$$

And RAI for DDT at high fish consumption,

$$= \frac{6652 \text{ mg DDT}}{(70 \text{ kg}) \text{ X } (25550 \text{ days})} = 3\ 7 \times 10\text{-}3 \text{ mg DDT/kg/day}$$

DDT has a cancer potency factor (Q) of 8 422 (excess cancers/mg chem/kg body weight/day) therefore the risk associated with low average, and high consumption of fish contaminated with 4 2 mg DDT/kg fish is

Risk = RAI x Q

at low fish consumption

= (1 1 x 10-3 mg DDT/kg body weight/day) X (8 422 excess cancers/mg chem/kg body weight,/day)
= 9 3 x 10 3 excess cancers

at average fish consumption

= (1 7 x 10 3 mg DDT/kg body weight/day) X (8 422 excess cancers/mg chem/kg body weight,/day)
= 1 4 x 10-2 excess cancers

at high fish consumption

= (3 7 x 10-3 mg DDT/kg body weight/day) X (8 422 excess cancers/mg chem/kg body weight,/day)
= 3 1 x 10 2 excess cancers

Setting Advisory Limits on Consumption of Fish

The method for calculating total dose intake can be utilized to set health advisory limits on consumption of fish For example if the fish has a known concentration of carcinogen, then by establishing an acceptable level of risk, advisories may be issued on safe levels of consumption such that the amount of contaminant ingested does not exceed this risk

Summary of assumptions

Risk = 10-6
DDT concentration in fish = 4 6 mg/kg fish
Cancer potency factor (Q) for DDT = 8 45
Average consumer body weight = 70 kg
Average consumer life expectancy = 70 years (25550 days)

Risk = Risk Associated Intake (RAI) x Q

$$RAI = \frac{\text{Total Lifetime Dose (mg chem)}}{\text{Body wt (kg) x Lifetime (days)}}$$

RAI = Risk/Q 10-6/8 45 = 1 18 x 10-7 mg DDT/kg body wt /day

Total Lifetime Dose (mg DDT) = (RAI) X (Body wt (kg)) X (Lifetime (days))

= (1 18 X 10-7mg DDT/kg body wt / day) X (70 kg) X (25550 days)
= 21 mg DDT

From the fish lifetime dose equation,

$$\text{Chemical Conc in fish (mg DDT/kg fish)} = \frac{\text{Fish Lifetime Dose (mg DDT)/}}{\text{(Contact Rate) x (Exposure Duration) x (Absorption Efficiency)}}$$

4 6 mg DDT/ kg fish = 21 mg DDT/ (Contact Rate) X (1 day) X (Aborption Ratio 1)
Contact Rate = 21 mg DDT/4 6 mg DDT/kg fish
= 046 kg fish/day or 32 kg fish/week

Based on the above information an advisory could be made to alert the consumers of seafood that ingesting more than 32 kg of fish per week (ie 16 8 kg fish/year), could result in an increased risk of cancer from exposure to DDT Sometimes it is beneficial to compare safe levels of seafood to number of meals consumed For example, based on an average portion of approximately 16 kg (160 g) fish per meal 32 kg (320 g) fish may be related to the consumer as two fish meals per week

Seafood health advisories may also respond to established guidelines or trigger levels set for specific contaminants Trigger levels represent contaminant concentration levels which, when exceeded are considered to pose greater health risks Trigger levels may be established through regulatory guidelines already in place If no guidelines exist then it is up to the regulatory agencies to establish their own regulatory guidelines based on risk analysis or established legal limits from other countries and/or the World health Organization Legal limits, however may vary widely between countries For example legal limits for lead vary from 5 ppm (5 mg/kg fish) in Canada and Germany, but are as high as 10 ppm (1 mg/kg fish) in the United Kingdom (Krause 1986)

The Michigan Department of Public Health establishes fish consumption advisories based on the detection of contaminants in a particular species of fish and its location Severity of the advisory depends on the percentage of fish sampled that exceed certain trigger values of the known contaminants Trigger levels represent the maximum amount of contamination that may be present in a particular species For example an advisory for reduced consumption to *no more than one meal per week* (based on an 8 oz (227 g) serving) is issued when any of the advisory trigger levels are exceeded by more than 10% but less than 50% of the specimens tested for contamination and the mean concentrations do not exceed the trigger levels for the contaminants found A reduced advisory of *no more than a serving per month* will be issued when any of the advisory trigger levels are exceeded by 50% or more but fewer than 90 of the specimens tested and the mean concentration is no greater than two times the trigger Finally if 90 - 100% of the fish tested exceed the trigger or the mean concentration is equal to or greter than 2 times the trigger then consumers are advised not to eat the fish at all

When providing an estimate of human risk from contaminated seafood, there are a number of uncertainties that are introduced into the estimation of human effects These include, but are not limited to extrapolation from high doses used in the laboratory to lower doses found in the environment extrapolation of adverse effects observed in animal species to humans, extrapolation of results from one exposure pattern to another and estimates of human exposure

or dose levels Nevertheless, fish consumption advisories may be confidently established based on the most currently accepted risk assessment procedures and assumptions Ideally each contaminant needs to be considered individually because of differences in toxicological characteristics and modes of action

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Sample Calculations Of Health Advisories For Chemical Contaminants In Fish In Israel And Egypt

Introduction

Chemically contaminated fish poses a big hazard for populations who heavily rely on fish Fish is a major source of low-fat protein food in developing countries Therefore, many countries have turned to fish farms in order to satisfy the heavy demands of fish in these populations In countries such as Israel and Egypt, the fish farms are being supplemented with wastewater or animal manure These supplements can be a source of microbial and chemical contamination Industry can produce numerous chemicals that may directly or indirectly find their way into wastewater (U S GAO, 1988) It is important to note that water pollution will exhibit a more harmful effect on aquaculture then on natural water systems because the cultured fish has a limited capacity to adapt, avoid or escape pollutants

Exposure to a low level of fish contaminants over a long period of time can result in significant effects in the human population Lifetime risks for diseases such has cancer would be increased Currently, scientists do not know the health risks from exposures to 90 percent or more of all chemicals that get into the environment This is due to the difficulty in establishing direct relationships between levels of many chemical contaminants and human illness

Health advisories are developed in order to protect human health from exposures to chemically contaminated fish For noncarcinogenic chemicals, health advisories are based upon the identification of adverse effects associated with the most sensitive and meaningful noncarcinogenic endpoint of toxicity (NOEL of LOAEL) This is established by relating a particular exposure dose over a specified period of time, most often

determined from the results of an experimental study (Thallium Health Advisory, 1991) In addition, Reference Doses (RfD's) which are similar to Acceptable Daily Intake (ADI's) are used

Health advisories for carcinogenic compounds are determined using a linear dose response model, an accepted level of risk (e g , 10^{6} excess lifetime risk of cancer), exposure information, and carcinogenic potency factors (Q's) Integrated Risk Information Systems (IRIS) will be used as the primary source of information for RfD's, NOEL or LOAEL, and Q's

Methods

Steps for Lifetime Health Advisory Calculations

Non-carcinogenic

1) Find exposure of population

Exposure	=	Chemical Concentration	x	Contact Rate
mg of X		(mg of X/kg of fish)		kg of fish/day

2) Is that exposure acceptable? Find out by comparing to Reference Dose

- Determination of Reference Dose (RfD) or Acceptable Daily Intake (ADI)

$$RfD = \frac{\text{(NOEL or LOAEL)}}{\text{uncertainty Factor}}$$

Maximal Permissible Intake =	(RfD)	x	(Body Weight)
mg of X/day	mg chem/kg/day		kg

3) Calculate Health Advisory

$$\text{Contact Rate (kg of fish/day)} = \frac{\text{Intake (mg of X/day)}}{\text{Fish Consumption (kg of fish/day)}}$$

Carcinogenic

1) Fish Lifetime Dose = RAI x Body Weight x Lifetime
mg of X — mg of X/body weight/day — kg — days

2) Contact Rate = Lifetime Dose / (Chemical Concentration x Exposure Duration)
kg of fish/day — mg of X/kg of fish — day

Basic Assumptions	
Body weight	70 kg
Facts	
Exposure Duration	1 day
Lifetime days	25550 days

Results

Non-carcinogen

RfD	0005 mg of Cadmium/kg body weight/day
Fish Consumption	028 kg/person/day
Chemical Concentration	015 - 027 mg of Cadmium/kg of wet fish

Calculation for Cadmium

1) Exposure = C x Intake

Exposure = 015 - 027 mg of Cd/kg of fish x 028 kg of fish/day

Exposure = $4\ 2 \times 10^{-4}$ - $7\ 56 \times 10^{-4}$ mg of Cd per day

2) Is this exposure acceptable?

Maximal Permissible Intake = RfD x Body Weight

= 0005 mg of Cd/kg body weight/day x 70 kg
= 035 mg of Cd/day

The exposure of the population is well under the acceptable exposure

3) Health Advisory

Fish Consumption = Intake/Concentration
= 035 mg of Cd per day/ 015 - 027 mg of Cd per kg of fish
= 2 3 to 1 3 kg of fish per day

Carcinogen

Data for Lindane

Chemical Concentration	$1x10^{-4}$ mg of Lindane/kg of wet fish
Fish Consumption	028 kg of fish/day
RAI	7 54 x 10^{7} mg of Lindane/kg body weight/day
Q	1 326

1) Actual Fish Lifetime Dose = C x contact Rate x Exposure Duration x Absorption
Dose = $1x10^{-4}$ mg/kg of fish x 028 kg of fish/day x 25550 days x 1
Dose = 072 mg of Lindane

2) Recommended Fish Lifetime Dose = RAI x Body weight x Lifetime days
= (7 54 x 10^{7} mg/kg body weight)(70 kg) (25550 days)
= 1 35 mg of Lindane

Conclusion Actual dose is lower then recommended

3) Contact Rate = Fish Lifetime Dose/ Concentration x Exposure Duration x Absorption
= 1 35 mg of Lindane/$1x10^{-4}$ mg of Lindane/kg of fish x 1 day x 1
= 1 35 x 10^{4} kg of fish/day

Discussion

Fish advisory levels are an important tool for controlling health hazards in the population from exposure to chemically contaminated fish The two contaminants in fish that were focused in this study were the heavy metal Cadmium and the organochlorine insecticide Lindane Cadmium is a heavy metal known to be hazardous Cadmium adversely affects several important enzymes In addition, cadmium can cause kidney damage and painful osteomalacia (Manahan, 1995)

Health advisories were based on safe levels of chemical contaminant exposures (RfD and RAI values) However, current exposure to both of the chemical contaminants were found to be "safe" Therefore, the population in Israel is at no risk of hazardous effects from exposure to the heavy metal Cadmium and the organochlorine insecticide Lindane

Seafood health advisories may be established through guidelines or trigger levels set for specific contaminants Trigger levels represent contaminant concentration levels at which, when exceeded pose a health risk If no guideline exist, then it is up to regulatory agencies to establish guidelines based on limits from other countries or WHO

The Michigan Department of Public Health establishes fish consumption advisories based on the detection of contaminants in a particular species of fish and its location Severity of the advisory depends on the percentage of fish sampled that exceed certain trigger values of the known contaminants For example, an advisory for reduced consumption *to no more than one meal per week* (based on an 8 oz serving) is issued

when any of the advisory trigger levels are exceeded by more than 10% but less than 50% of the specimens tested for contamination and the mean concentrations do not exceed the trigger levels for the contaminants found A reduced advisory of *no more than a serving per month* will be issued when any of the advisory trigger levels are exceeded by 50% or more but fewer than 90 of the specimens tested, and the mean concentration is no greater than two times the trigger Finally, if 90-100% of the fish tested exceed the trigger or the mean concentration is equal to or greater than two times the trigger, than consumers are advised *not to eat the fish at all*

When providing health advisories, numerous uncertainties exist These include, but are not limited to extrapolation from high doses used in the laboratory to lower doses found in the environment, extrapolation of adverse effects observed in animal species to humans, extrapolation of results from one exposure pattern to another, and estimates of human exposure or dose levels Nevertheless, fish consumption advisories may be confidently based on the most currently accepted risk assessment procedures and assumptions

SEAFOOD SAFETY CONCERNS AND REGULATORY APPROACHES

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Fresh Fish

- Organoleptic Tests (Subjective Tests)
- Chemical Tests
- Microbiological Tests
- Safety Assurances of Fresh Seafood

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Introduction

The estimated 1995 fish production in Egypt was 407,141 metric tons (mt), of which 70 6% (287,456 mt) from freshwater (the Aswan High Dam, the Nile, and irrigation and drainage canals), and 15 2% (61,815 mt) from fish farming (1, 2, 3) The total fish supply in 1995 was 547,952 mt (of which 26% was imported) This amounts to an average consumption of 9 kg/cap/yr The Government of Egypt (GOE) aims to increase consumption to 10 kg/cap/yr by the year 2000

Fish harvested from the northern coastal areas and the delta lakes exhibits high levels of contaminants, in comparison to fish caught in the Aswan High Dam, the Nile river, and irrigation canals The main reason is water pollution with sewage effluents, agricultural drainage and industrial waste Some of the hot spots are the coastal areas off Alexandria and Lake Manzala

Aquacultural development has a high priority in the GOE plans, which includes the increase of fish farming in various governorates However, the GOE policy prohibits the use of fresh water from the Nile and from irrigation canals for fish farming In addition, the use of groundwater is permitted mainly for fish hatcheries Water use for fish farming is currently restricted to irrigation drainage water (which contains variable levels of contaminants, e g pesticides, fertilizers, and domestic and industrial waste) and coastal and delta lakes

We have provided the relevant authorities in Egypt with research results indicating that the reuse of treated domestic wastewater is highly preferred over the reuse of agricultural drainage water in fish farming These research findings were based on a full scale demonstration of the public health and economic advantages of using treated sewage effluents for aquaculture-agriculture applications The treatment of domestic wastewater in Egypt should include the option of reusing these treated effluents in fish farming applications The effluents from the fish farms can be further used in irrigation applications

Fish farming practices in Egypt are largely unregulated In 1995, 17% of the fish farms were run by the government, and only 20% of the remaining private farms were licensed Sustainable development of fish farming in Egypt will largely depend on the close coordination of the policies of the Ministry of Water Resources & Public Works and the Ministry of Agriculture Fresh water resources allocations should be based on integrated aqualculture-agriculture systems, and cost-benefit analysis This should include considerations for water quality management and pollution prevention

Fresh Fish

Until today, there are no reliable and standardized specifications to ensure degree of freshness in fish In general, the following methods of quality assurance are adopted

Organoleptic Tests (Subjective Tests)

Ensuring the freshness of fish depends primarily on the consumer's judgment or any trained person through subjective inspection of the fish product

Organoleptic inspections include

1-General appearance of fish

2-Brightness of eyes

3-Structure
4-Color of gills
5-Smell
6-Adherence of scales to skin
7-Condition of blood
8-Color of flesh meat on the backbone
9-Floating tests of fish in water

Chemical Tests

This method depends on the estimation of some components in the fish such as T M A (Trimethylamine), U B (volatile organics), T U (Tyrosine number), T U N (volatile Nitrogen), PH U (acidity number), U F A (volatile organic acids), and V R S

Microbiological Tests

This test relies on the estimation of microbial load per gram of fish size or per 1 cm^2 of fish surface A relation exists between the number of fish and the degree of spoilage, i e , the less the number of fish the longer shelf-life of the fish

It is important to recognize generally the following information regarding the freshness of fish

1 Flat bodied fish such as sole remain fresh for longer periods than others
2 White fish meat remain fresh for longer periods than red fish meat
3 Benthic fish can often be stored for longer periods than surface fish
4 Fish that have their intestines and gills removed directly after fishing can be stored for longer periods than others
5 Fish with higher fat content spoils more quickly than those with lower fat content
6 Some species of fish remain naturally alive for 12 - 24 hours after being caught Such fish remain fresh longer than those that d e directly after being caught

Safety Assurance of Fresh Seafood

Fresh seafood destined for commercial sales should have no unpleasant smell The structure of fish should be firm and lean, not soft and flaky, and the scales should cling tightly to the skin The eyes of fresh whole fish should be bright and clear, not cloudy or sunken Gills should have a bright red color and be clean with an acceptable smell

A more general approach to differentiate between fresh and spoiled fish is to immerse the fish in water-filled bucket Fresh fish will eventually sink to the bottom, whereas, spoiled fish will float to the surface

Some fishermen and retailers resort to fraud practices to conceal spoiled fish Some common practices include painting gills with red coloring, removal of fish

eyes, and mixing of fish with large amounts of ice to conceal the smell or mixing fresh fish with stale ones

The main problems encountered with fish manufacturing is assurance of quality and safety to consumers with regard to the rapid spoilage of fish In Egypt, the problems are more prominent due to lack of advanced storage systems in fishing vessels and boats, length of fishing shores, and unavailability of highway transport

It has been proven that the gills area of fish is the first site for microbial invasion The next areas of bacterial attack, in order of progression are kidneys, peritoneal lining, outer surface of skin, flesh surrounding the skeletal bones

It is well known that not all fresh fish are suitable for consumption Certain kinds of fish are poisonous and can harbor or accumulate natural toxins in specific areas of the fish such as eggs, stomach, liver, skin or even sometimes in fish meat Such toxins are heat resistant and remain after cooking Poisonous fish are spread across coral reef areas, and in some tropical areas such as the West Indian Ocean and equatorial regions of the Pacific Ocean and the Red Sea The severity of poison in such fish can vary according to area, seasonal variation, and specific locations

Eliminating poisonous fish from entering commercial markets depends greatly on the expertise of fishermen who can recognize them and remove them upon sorting

Processed Fish

Fish refrigeration, fish freezing, salting, and canning are various processing techniques in use today for fish preservation

1 Fish Refrigeration The refrigeration temperature ranges between 0°C and 7°C The temperature should be adjusted as to not allow glazing to occur in fish cells To retard spoilage, the preferred temperature range is -3°C to -2°C
2 Fish Freezing The temperature ranges between -10°C and -40°C Rapid freezing is more advantageous than slow freezing as it does not allow for spoilage and the product is of a better quality Frozen fish can be stored for 1 - 2 months at 14°C, for 2 - 4 months at -20°C, and for 6 - 8 months at -23°C
3 Salting It is one of the oldest and cheapest methods for fish preservation Salt can be applied as soft or dry salt
4 Canning It is the best way for preservation as it retains the fish's natural flavor, provides ease of transportation of fish, and cans can be kept at room temperature without spoiling Salmon, tuna, mackerel, sardine, and cod are some of the important types of seafood being processed by the canning industry

Seafood Safety Regulations in Egypt

The responsibility of seafood safety regulations in Egypt is shared by the following governmental agencies

1 Ministry of Health
Responsible for food safety

Sampling and analysis of marketed food

2 Ministry of Agriculture
Responsible for fish production and fish diseases

3 Ministry of Supply and Foreign Trade
Quality control of exports and imports of fishes and fish products

4 Ministry of Tourism
Quality control of tourist facilities, e g hotels, restaurants, etc

5 Ministry of Industry
Responsible for setting standards

Currently in Egypt, there are no comprehensive, specific legislation regarding the safety of fresh fish, but rather a very general, crude legislation applicable to all foods destined for human consumption (1) Established laws for foods in general are quite insufficient for the protection of fish consumers, and enforcing the law is limited, unreliable, and sometimes unfeasible The following food laws have been established Law No 48 of 1941, established for the prevention of cheating and fraud, and Law No 10 of 1977, established for the oversight of foods and its distribution Both laws have been amended with Law No 106 of 1980 Penalties for violation of these laws are lax and insufficient

Currently, there are no standardized specifications for fresh fish, in contrast to processed fish

The General Egyptian Organization for the Unification of Standards and Quality Assurance (GEOUS&QA) has issued a number of standardized specifications codes for fish and seafood that include canned salmon, canned anchovy, salted fish, canned sardines, frozen fish, canned tuna, canned shrimps and crab meat, frozen shrimps, dried shrimps canned mackerel, frozen squid, and smoked fish The GEOUS has issued a quality of assurance mark ES which stands for Egyptian Standards This mark on commercial products designates that the product complies with Egyptian standardized specifications

A Canned Salmon Code No 1472 – 1980
B Canned Anchovies Code No 808 – 1988
C Salted Fish Code No 1725 – 1989
D Canned Sardines Code No 287 – 1990
E Frozen Fish Code No 889 – 1991
F Canned Tuna and Bonito Code No 804 – 1990
G Canned Shrimps, Prawns, and Crab code No 414 – 1993
H Frozen Shrimps Code No 516 – 1993
I Dried Shrimps Code No 546 – 1993
J Canned Mackerel Code No 1521 – 1995
K Frozen Squid Code No 2800 – 1995
L Smoked Fish Code No 288 – 1991

Recommendations

An effective seafood safety program must include both the public and private sectors, i e government, industry and consumers It has been repeatedly proven that government controls alone do not work The new trends in environmental health and safety regulations emphasize prevention of food contamination over the inspection and end product testing

It is proposed to adopt HACCP-based seafood safety programs (2) HACCP is a "Hazard Analysis Critical Control Point" nongovernmental management system It is a system of preventive controls for food safety that industry operates to keep health hazards from occurring or keep the likelihood of their occurrence to a minimum It is not a Government operated system The Government, through its inspections, verifies that industry systems are adequate and are working, and obtains remedial actions when they are not

HACCP is the mirror image of end-product testing as a means of ensuring safety End-product testing attempt to catch problems after they have occurred It has a hit-or-miss quality that can require the sampling of large amounts of food to overcome In contrast, HACCP can provide a food processor with immediate and relatively inexpensive feed-back about whether hazards are being controlled It is science-based because it relies on a knowledge of what the reasonably likely food safety hazards are for a given product and process and what the controls for those hazards are

It is important to note that HACCP does not place new responsibility on to the industry to produce safe seafood That continues to be the responsibility of the processor What is new is that the processors will be responsible to demonstrate that they have correctly identified the hazards and are controlling them as a matter of design The burden of identifying food safety problems will shift from government inspectors to the processors The government shall continue to verify compliance

HACCP offers the opportunity for government-industry cooperation to achieve efficient safety systems

Finally, there exists a pressing need for public education programs to advise consumers on how to minimize the health risks from eating contaminated seafood This includes (a)advisories on eating salted raw fish or improperly prepared fish, (b)guidelines for purchasing, storing and cooking and (c)information on the nutritional values of seafood, economic fraud and other matters related to the industry

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