Scheduled Waste in Malaysia

by

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Content:

1. Background

The Malaysian manufacturing industry consists of more than 20,000 registered companies. The majority are small and medium-sized industries (SMI's) with less than 75 employees. Many SMI's serve as sub-supplier to large, often multinational, companies, who have established themselves in the Industrial Parks.

The large industries are well organised with local branches covering the entire country. The small enterprises are registered but usually organised locally, if at all. The business climate for SMI's is highly competitive. They normally do not have the necessary skills or resources for introducing cleaner technologies or proper management of hazardous waste.

The Malaysian Government is well aware of the environmental threats emanating from uncontrolled and scattered generation and disposal of hazardous industrial waste (Scheduled Wastes). In 1989, Environmental Quality Regulations were introduced for the management of Scheduled Wastes (Published as P.U. (A)'s no 139, 140, and 141/1989).

The Regulations were prepared with technical assistance from the Danish Environmental Protection Agency, and bear great resemblance to the corresponding Danish regulations regarding the waste generator's obligation to notify the Department of Environment (DOE) about waste generation and the requirements for storage, collection, packaging, labelling, processing, and disposal of any scheduled waste.

Through its state branch offices, the DOE is responsible for enforcing the regulations on scheduled waste.

2. Malaysian Strategy

The Government of Malaysia has set out its position clearly in respect of a strategy for sustainable development in the Sixth Malaysian Plan 1991-1995.

The Sixth Malaysian Plan contains a clear policy statement regarding the need to fully integrate environmental concerns into the overall planning and development process.

The attention is drawn to the need to maintain a balance between the competing demands of growth and sustainable development. The need for more effective environmental management, especially in urban areas is stressed, as is the need for careful management of natural resources as a further basis for sustainable development.

It is the further aim of the Government to render all scheduled waste harmless at the site of origin or at specially designed treatment plants. Disposal of non-degradable waste and treatment residues must take place in properly constructed, secure landfills.

3. Kualiti Alam Sdn. Bhd. (KA)

The Malaysian Government has decided to privatise the management of scheduled waste, based on "polluters pay" principle. For a period of 15 years from the start of operation of the Waste Management System (1.8.98), the company Kualiti Alam Sdn. Bhd. has been granted the exclusive rights to collect and treat all scheduled waste generated in the Peninsula of Malaysia which can not be treated within the premises of the waste generators (on-site treatment). The treatment facility and the secure landfill is located at Bukit Nanas in Negeri Sembilan.

The service fee for scheduled waste collection and treatment will be monitored by the government, but shall allow for a reasonable return on the investment for Kualiti Alam.

4. KA's Concept

The Integrated Waste Management System consist of:

- one centralised integrated Waste Management Centre (WMC), to treat and dispose of the different types of scheduled waste.

- proposed three regional transfer stations, one each for the Northern, Eastern and Southern Region of which only the Northern may be established in the initial phase. These stations act as focal points for the collection of wastes for industries in the region and transfer these wastes after sorting to the WMC for treatment. Waste from industries in the Central Region will be transported directly to the WMC. Currently, transportation will be by road.

Kualiti Alam will provide complete waste management for collection of scheduled wastes from the waste generator's premises, transportation, and treatment to final disposal.

5. Kualiti Alam - Waste Management Center (WMC) in Bukit Nanas

The WMC is placed in the middle of a palm oil plantation, and covers an area of 56 ha of which 19 has been cleared.

The plant will consist of several independent smaller plants e.g.:

- physical chemical treatment plant
- solidification plant
- poison plant
- incineration plant
- plant for treatment of special wastes
- landfill

all supported by the necessary infrastructure, facilities for receipt, storage and handling of waste. The location of facilities result in a practical and natural flow of the waste through the treatment processes. Furthermore, the location and spacing of the facilities complies with Malaysian safety and fire code requirements.

The construction of the WMC is divided into 4 phases:

Phase 1:	The establishment of the secure landfill for solid inorganic and asbestos waste which is now in operation.
Phase 2:	The establishment of the solidification plant. Target operation date July 97.
Phase 3:	The establishment of the physical/chemical treatment plant. Target operation date January 1998
Phase 4:	The establishment of the incinerator plant for incineration of organic chemical waste. Target operation July 1998.

Solidification plant stabilises neutral inorganic waste from waste generators and internally produced waste (originating from physical/chemical treatment plant in form of sludge or from the incineration plant in form of fly-ash.

In the physical/chemical plant inorganic wastes will be pH neutralised, cyanides will be destroyed by oxidation and heavy metals will be precipitated as very insoluble products, which then after solidification will be placed on the landfill.

In the incineration plant all organic waste classified to be applicable for thermal destruction will treated.

The incineration process produces slag, fly-ash and flue gas cleaning products. These products will be solidified and deposited on the secured landfill, which is constructed and monitored according to all modern environmental requirements.
Emission of flue gases complies with the requirements of the strict Malaysian legislation, which follows similar legislation around the world e.g. EU-legislation.

It ought to be mentioned that internally produces process water and the leacheate from the landfill is treated in the incineration train so release to the environment is avoided.

6. Conclusion.

Malaysia having their own Waste Treatment Facilities will contribute to the cleaner environment for the future generations.

SIMPLE MODEL FOR ESTIMATING THE DISTRIBUTION OF PHENOXY TOLUENE IN THE ENVIRONMENT

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INTRODUCTION

Physical chemical property is a very important parameter in understanding the chemicals' behavior in the environment. This property may characterize the physical state of chemicals under certain environmental conditions. Physical chemical parameters such as vapour pressure (V_p), adsorption coefficent (K_{oc}), partition coefficient (log P_{ow}) and water solubility (S_w) are used to estimate the distribution of chemicals in the environment. Various mathematical models are used to estimate the distribution of these chemicals. The mathematical models require experimental data obtained from laboratory scale experiments like microcosum and physical chemical properties in order to solve thermodynamic problems.

Mathematical model fundamentally consists of a few models. Compartment model or also known as Mackay's fugacity model is commonly used to estimate the distribution of a chemical in the environment. In our study, we measured a few physical chemical properties of 3- phenoxytoluene such as water solubility, vapour pressure, partition coefficient and adsorption coefficient. These measurements were done following OECD Test Guidelines and the distribution of 3-phenoxytoluene in the environment was estimated by using a simple mathematical model based on Mackay's fugacity model.

Physical chemical property is also used for Quantitative Structure Activity Relationships(QSARs). The partition coefficient (Pow) between n-octanol and water is important and can be related to the aquatic toxicity, biodegradability and bioaccumulation. These studies were reported by Vithe, Schultz, *et al.*(1979). The correlation between Pow and adsorption coefficient(Koc) or water solubility (Cs) is studied and reported by Kenaga, *et al.*(1986). Several mathematical and statistical techniques are used to develop QSARs . Various criteria were used to select chemicals for which QSARs are to be developed, such as similarities in physical chemical properties, structure as well as potential interactions within a biological system.

MATERIALS AND METHODS

The chemical structure of 3- phenoxytoluene (Fluka Chemica,Switzerland) is shown in Figure 1. The chemical has a high purity of 98% assay so further purification is not necessary. The 3- phenoxytoluene ($C_{13}H_{12}O$) has a molecular weight of 184.24, with a density of 1.052 and a refractive index of 1.573.

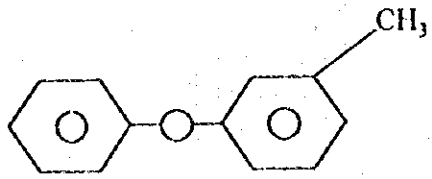

Figure 1. Structure of 3-Phenoxytoluene

Measurement of water solubility

3-phenoxytoluene (0.1g) and deionized water (30ml) were added into a conical flask. The mixture was stirred for 72 h at ambient temperature. The mixture (5 ml) was transferred into a test tube and centrifuged for 10 minutes at 2500rpm. The supernatant was analyzed using high performance liquid chromatography(HPLC). Measurement was done in triplicates.
The HPLC conditions employed are as follows:

HPLC system : LC-10 system (Shimadzu)
Column : L- column (reversed phase) H 150mm, I.D 4.6mm (CITI , Japan)
Mobile phase : 70% (v/v) methanol/water
Flowrate : 1.0 ml/min
Detector : UV detector, wavelength 225 nm

Measurement of partition coefficient

Partition coefficient was measured using HPLC method. It is well established that a good correlationship exists between log Pow and capacity factor, k'. The k' of each reference chemicals were calculated by using the following equation:

$$k' = (t_R - t_0)/t_0 \qquad (1)$$

where t_R is the retention time of the reference chemicals and t_0 is the dead time, i.e the average time a solvent takes to pass through the column or for a chemical to pass through the column without interaction with the packing material. Urasil / 2,4 - Dihydroxypyrimidine, was used to measure the dead time(t_0).
The t_R of seven reference chemicals recommended by the OECD guidelines were measured using the HPLC conditions above..
Reference chemicals of similar group structure, were chosen for calibration purpose.
The t_R of reference chemicals were measured five times and the capacity factor calculated. The regression equation was obtained from the log Pow vs log k'. The correlation between log Pow and log k' is as follows:

$$\log Pow = a \log k' + b \qquad (2)$$

where a and b are constants determined from the calibration graph for reference chemicals recommended by OECD. Then retention time of 3-phenoxytoluene was measured five

times and capacity factor of the 3-phenoxytoluene was calculated. Then the capacity factor of 3- phenoxytoluene was included in the regression equation to obtain the log Pow value.

The reference chemicals and Pow values used in this experiment are as follows:
Toluene (2.7), Ethylbenzoate (3.2), Biphenyl (4.0) , Phenanthrene (4.5), N-Buthylbenzene (4.6), Floranthane (4.7) and Triphenylamin (5.7).
( ) shows log Pow measured using the shake flask method.

Measurement of vapour pressure

Vapour pressure of 3- phenoxytoluene was measured using a gas saturation method. Nitrogen gas was passed through a saturated column for a fixed time. The column was packed with control pour glass (CPG) and 3-phenoxytoluene. The gas saturated with 3-phenoxytoluene was passed through a solvent trap containing methanol. Concentration of 3-phenoxytoluene trapped in the methanol solution was analyzed using HPLC. The amount of 3- phenoxytoluene was calculated using the following equation;

$$Ps = \frac{m\,R\,T}{MV} \qquad (3)$$

where,
Ps : saturated vapour pressure
m : amount of phenoxytoluene traped in the solvent
R : gas constant (8.205 x 10Exp3 m^3Pa/T)
T : absolute temperature (K)
M: molecular weight of phenoxytoluene
V :Volume of gas passed through the column (m^3)

The vapour pressure was determined at 25, 35 and 46°C. Triplicate measurements were done for each temperature.

Measurement of adsorption coefficient

Adsorption coefficient was measured using local soil collected from five different places in Klang Valley. Mixture of about soil (1g) and 3-phenoxytoluene (20ml) in four different concentrations of 0.1, 0.25, 0.5 and 1.0 µg/ml(C_o) were transferred into a stoppered glass tube and agitated for 12 hours. Mixtures were centrifuged at 3000rpm and the supernatant was collected. Concentration of 3-phenoxytoluene (Cw) in the aqueous phase was analyzed by HPLC. Measurement was done in duplicates.
The concentration of 3- phenoxytoluene adsorped on to the soil sample (Cs) was calculated using the following equation:

$$Cs(\mu g/g) = \frac{(Co - Cw) \times \text{volume of aqueous solution (20ml)}}{\text{amount of soil}} \qquad (4)$$

From the Freundlich isotherm, the correlation between log Cs and log Cw as shown in equation 5.

$$\log Cs = (1/n) \log Cw + \log Ko \qquad (5)$$

where ,
Ko : adsorption coefficient
n : constant
Log Cs was plotted against log Cw, and log Ko was obtained from the intercept.

Result

The average value of 3-phenoxytoluene water solubility of is **18.28± 1.86 µg/ml** at 25°C± 1°C.

Partition Coefficient of 3- phenoxytoluene obtained from the regression equation of reference chemicals was calculated as to be 5.56 (k'= 14.8186). The capacity factor of 3-phenoxytoluene was calculated and the average value was included in equation 5.

$$\text{Log Pow} = 2.831 \log K' + 2.253 \ (R^2 = 0.967, n = 7) \qquad (6)$$

Vapour Pressure Measurement results were obtained from the gas saturation method at three different temperatures. From the ln P versus 1/T plot in accordance with Clusius-Clapeyron equation, the following equation was derived.

$$\ln P = -(dH \text{ vap})/ R \times (1/T) + \text{const} \qquad (7)$$

where,
R: gas constant
dH : heat of vaporization

A good correlation was obtained between ln P and 1/T. The regression equation is shown in equation 8.

$$\ln P = -17633 \ (1/T) + 62.713 \ (R^2 = 0.970, n = 9) \qquad (8)$$

From equation 8 we can calculate the dH for 3- phenoxytoluene can be calculated.

$$\ln Ps = -17633 \ (1/T) + 62.919$$

$$dH \text{ vap} = (17633 \text{ K}) (8.314 \text{ J/ Kmol})/ (1000 \text{J / kJ})$$
$$= 146.85 \text{ kJ/ mol}$$

The adsorption coefficient (Koc) was obtained from the Freudlich isotherm equation. Adsorption coefficient (Ko) was obtained from the intercept of the plot Cs vs Cw. The correlation between log Cw (concentration in the aquaous phase) and log Cs (concentration in soil phase) is given in equation 5.

$$\text{Log Cs} = 0.902 \log Cw + 1.610 \qquad (R2 = 0.967 , n = 18)$$

The adsorption coefficient of 3-phenoxytoluene was found to be **40.7.**

Physical Chemical Properties obtained from this study are listed in the Table 1.

Table 1. Water Solubility (Cs), Partition Coefficient (log Pow), Vapour Pressure (Ps) and Adsorption Coefficient (Ko) of 3-phenoxytoluene.

	Measured value	Temperature ^0C
water solubility(C)	18.3+/- 1.86(ug/ml)	25 +/- 1
partition coefficient, Pow	5.5 (log Pow)	25 +/- 1
vapour pressure, Ps	49.1	25 +/- 1
	217.1	35 +/- 1
	2404.6	46 +/- 1
adsorption coefficient,Ko	40.74	25 +/- 1

Application Of Physical Chemical Property

Physical chemical properties obtained from the above measurements are frequently employed to estimate the distribution of a chemical in a evaluative environment.This can be done by using mathematical models like the compartment model.The evaluative environment consists of six compartments such as air, water, aquatic organisms, suspended solid, sediment and soil. However, this model does give information on small changes or transformation within the compartment. Some drawbacks in this model are:

1. There is no inflow or outflow of a chemical except the exposed amount.
2. 3-Phenoxytoluene can move into each compartment instantaneously without resistance of transfer and each compartment can reach equilibrium thermodynamically.
3.There is no decomposition like biodegradation, hydrolysis and photolysis within each compartments.

Fugacity (f) is defined as the degree of chemical transfer to other compartments .

$f = f_{air} = f_{water} = f_{sediment}$ and $s.s = f_{soil} = f_{organisms}$

Fugacity is proportional to the concentration of a chemical(Ci); Equation 9 below shows this relationship.

$$C_i = Z_i f \qquad (9)$$

where, Z_i : fugacity capacity (mol/m^3Pa)
i : components of compartments

Population (P_i) of a chemical in each compartment is represented as a function of fugacity capacity and volume.

$$Pi = \frac{Zi \times Vi}{\Sigma (Zi \times Vi)} \quad (10)$$

If the total amount of chemical exposed in the environment is ΣM_i, fugacity is represented by the following equation.

$$f = \frac{\Sigma M_i}{\Sigma (Zi \times Vi)} \quad (11)$$

If fugacity capacity and volume of each component are estimated and calculated using physical chemical properties and the total amount of chemical exposed into the evaluative environment defined, fugacity can be calculated using equation 11.

After calculation of f, chemical concentrations of each compartment is calculated using equation 9 and the amount of a chemical distributed into each compartment is calculated by using equation 12.

$$Mi = Ci \times V i \quad (12)$$

Fugacity capacity (Zi) of each compartment is defined as follows;

Z(air)	= 1/RT	mol/m^3 Pa
Z(water)	= 1/H = Cs/ Pa	mol/m^3 Pa
Z(suspended solid)	= Koc dss /H	mol/m^3 Pa
Z(organisms)	= Pow do /H	mol/m^3 Pa
Z(soil)	= Z(sediment)r	mol/m^3 Pa
Z(sediment)	= Koc ds' /H	mol/m^3 Pa

where H : Henry constant
Cs : Water Solubility
Ps : Saturation Vapour Pressure
Koc : Adsorption Coefficient
R : gas constant
Pow : partition coefficient
r : ratio of the organic content in soil (%) and in sediment(%)

A simple model is employed to estimate the distribution of 3- phenoxytoluene in the environment. Chemical physical properties measured in our study and other properties needed in the mathematical model are listed in Table 2.

Table 2. Physical Chemical Parameters to calculate the distribution of 3-phenoxytoluene in the environment

Parameter	Values	Unit
reference		
molecular weight: MW	184.24	
gas constant: R	8.314	$m^3Pa/kmol$
Measured and Calculated values		
water solubility: Cs	0.0992	mol/m3
partition coefficient : Pow	363078.0	
saturation vapour pressure: Ps	49.1	Pa
adsorption coefficient :Koc	40.7	
Henry constant : H= Ps/ Cs	495.0	m^3Pa/mol
estimated values:		
density of soil :ds	1.5	g/cm^3
density of suspended solid : dss	1.5	g/cm^3
density of organisms in water:do	1.0	g/cm^3
density of sediment: ds'	1.0	g/cm^3

Table 3. Distribution of 3-phenoxytoluene in the environment at 298 K

compartment (phase)	volume of compartment Vi (m3)	fugacity capacity Zi	concentration in compartment Ci (mol/m3)	Present amount in compartments Mi (mol)
air	10000000000	0.00039568	5.42275E-09	54.22747
soil	9000	0.02717046	3.72372E-07	0.00335
water	7000000	0.00088359	1.21097E-08	0.00848
organism*	3.5	313.510313	0.004296669	0.01504
suspended solid	35	0.05434091	7.44744E-07	0.00003
sediment	21000	0.05434091	7.44744E-07	0.01564

For example,
Assume that the amount of 3-phenoxytoluene exposed to the evaluative environment is 55 mol. Fugacity was calculated as 1.371×10^{-5} Pa.

Fugacity Capacities and concentration of 3- phenoxytoluene distributed into each compartment are listed in Table 3. The result shows that about 99% of 3-phenoxytoluene is estimated to be present in air and 0.3 % in water.

Discussion

Various mathematical models have been employed to estimate the distribution of chemicals, however it does not give a complete estimation under the real environmental conditions. Since local environments differ from one region to another, it is difficult to use one model to explain the different local environments. Therefore, physical chemical properties of the local environment should be measured using local soil or local temperature. It is suggested that local model be derived to reflect local states for aquatic effects and exposure assessment.

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Bioaccumulation of Diphenyl Derivatives on Carp and Tilapia

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INTRODUCTION

Tremendous numbers of chemical substances are continuously produced and discharged into the environment. One example is organic mercury which has resulted in minamata disease which occurred in 1953 in Japan. However its after effect is still persisting in the country until now. The disease was officially announced to be caused by contamination of an organic mercury effluent in Minamata Bay only in 1957. This is a typical environmental disruption and also a well-known crucial artificial incident that resulted from industrial waste.

Another similar case happened in 1968 also in Japan which was caused by polychlorinated biphenyls specifically of polychlorinated benzene being taken up into human body from food oil. PCB which was banned in 1972, had been used widely in electrical industries. Despite its ban, the world's environment has long been contaminated with PCB which is detectable in living organisms such fish, birds and some mammals. PCB was accumulated in these species by way of food chain.

Therefore an assessment of the chronic effects caused by the presence of hazardous chemicals is of necessity. The data on chemicals accumulation and depuration by aquatic organisms are useful for the evaluation on their safety to human and their contamination for example of fish in lake, river and sea.

In order to prevent such problems, some significant inspection and testing to chemicals are crucial. Bioaccumulation test is one of the keys in managing chemicals hazardous to health. In OECD countries, bioaccumulation test had been firmly established to evaluate new and existing chemicals to prevent the occurrence of incidents described above. Chemical evaluation is required under the law concerning examination and regulation of manufacture of chemical substance which was enacted in 1973 by two systems, a pre-marketing and a post marketing control system. Malaysia as a growing industrial country should also take a similar step to monitor the safety of its aqua biotic organisms which may have been exposed to hazardous chemicals.

Bioaccumulation test is performed at the SIRIM Environmental Technology Center under a JICA project. The initial aim is to conduct bioaccumulation test and develop the expertise. OECD countries can perform this test independently to determine the degree of chemical accumulation in fish. Each country uses its local fish, for example Japan uses carp. Therefore, our main goal here is to compare BCF (bioconcentration factor) values between carp and a local Malaysian fish, tilapia, to see if there is any difference in the result. In the event that there is no obvious difference, tilapia can be used as the test fish following the same OECD Guidelines practiced in Japan. On the other hand if some differences existed, bioaccumulation test is necessary in order to confirm the chemical safety in Malaysian environment using local fish. This is essential since bioaccumulative information performed in other countries may not be similar in Malaysia.

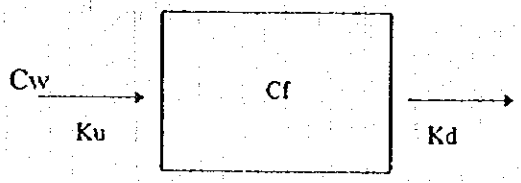
The fundamental principle of bioaccumulation test is to investigate the degree of hazardous chemical accumulation in the fish body. Later in the stage the assessment on the chemical's potential effects can be related or linked to its potential hazards on human and environment. The bioaccumulation test has been conducted according to OECD Guidelines 305C (MITI recommended method) and 305E (EPA recommended method) which are common and relatively practicable.

PRINCIPLE OF BIOACCUMULATION TEST

Bioaccumulative mechanism can be expressed by using a simple compartment model. Uptake of a chemical from water is considered only by input which is assumed to be directly proportional to the exposure concentration of the chemical (Cw). The rate constant of depuration is assumed to be first order which is directly proportional to the concentration in the organism, for example fish (Cf). In this case the concentration in fish is given by:

$$dCf / dt = \text{uptake} - \text{depuration} = Ku\ Cw - Kd\ Cf$$

where Ku is the uptake rate constant and Kd is the first order depuration rate constant.



To integrate the above equation, it is necessary to assume that the rate constant as well as the concentration in water remain constant. The integrated form is :

$$Cf = Ku / Kd \times Cw\ [1 - \exp(-Kd \times t)]$$

This equation describes an increasing Cf at the early stage of uptake, which approach a plateau with time. Steady state occurs when the uptake equals the depuration rate. Therefore after a sufficient duration of exposure, for example if t becomes infinity:

$$Cf = Ku / Kd \times Cw$$

Then the steady state bioconcentration factor (BCF) can be defined as follow :

$$BCF = Cf / Cw = Ku / Kd$$

a) MITI Test Method

MITI method involves a long exposure period of test fish to the test chemical. The test is undertaken until a plateau or steady state is reached. This occurs when the curve in the plot of test substance in fish (Cf) against time becomes parallel to the time axis. Continuous

monitoring by measuring the chemical concentration in fish sample is conducted twice per week.

Bioconcentration factor at steady state is then calculated by the ratio of chemical concentration in fish (Cf) and chemical concentration in water (Cw).

b) EPA Test Method

EPA method consists of two phases: the exposure (uptake) and post-exposure (depuration) phases. During the uptake phase, separate groups of fish of one species are exposed to two different concentrations of a chemical such as the diphenyl derivative. Normally measurements are continued during the uptake phase until a plateau or at least 80% of estimated steady state is achieved. The depuration period is then begun by transferring the fish to the same medium but without the test substance in another clean test tank. This phase is necessary unless uptake of the test substance during the exposure period has been insignificant. In addition to the two test concentrations, a control group of fish is held under identical conditions except for the absence of the test substance.

BCF calculation for this method is as a kinetic bioconcentration factor, which is the ratio of rate constants of uptake (Ku) and depuration (Kd) assuming first order kinetics.

EXPERIMENT

In order to compare the difference in bioaccumulation between carp and tilapia, the chemical chosen should be accumulative at least a certain degree. In our study diphenyl ether, 4-phenoxyphenol and 3-phenoxy toluene are chosen for comparing BCF values. Diphenyl ether is considered medium accumulative. 4-phenoxy phenol and 3-phenoxy toluene are considered low and high accumulative, respectively.

In our study, biaccumulation test is conducted by using both MITI recommended method and EPA recommended method. Figure 1 shows the test apparatus which is called the continuous flow-through system. This apparatus is used for both type of test methods. Test conditions of each chemical are listed in Table 1.

Table 1 Test conditions of each chemical

Chemical	test fish	test concentration (ug/ml) high level/low level	flow rate (ml/min stock dilution solution	solution	dispersant*
Diphenyl ether	carp	0.05 / 0.005	2.0	400	HCO40 (x20)
	tilapia	0.05 / 0.005	2.0	400	HCO40 (x20)
4-phenoxyphenol	carp	0.10 / 0.01	4.0	800	HCO40 (x10)
	tilapia	0.10 / 0.01	4.0	800	HCO40 (x10)
3-phenoxytoluene	carp	0.025 / 0.0025	4.0	800	HCO60 (x5)
	tilapia	0.025 / 0.0025	4.0	800	HCO60 (x5)

*HCO40 and HCO60 were used as emulsifier because the chemicals have poor solubility in water

Test chemicals were purchased from Fluka. These chemicals were more than 97% pure and were used without further purification.

DYNAMIC FISH TEST
[FLOW-THROUGH SYSTEM : MITI METHOD]

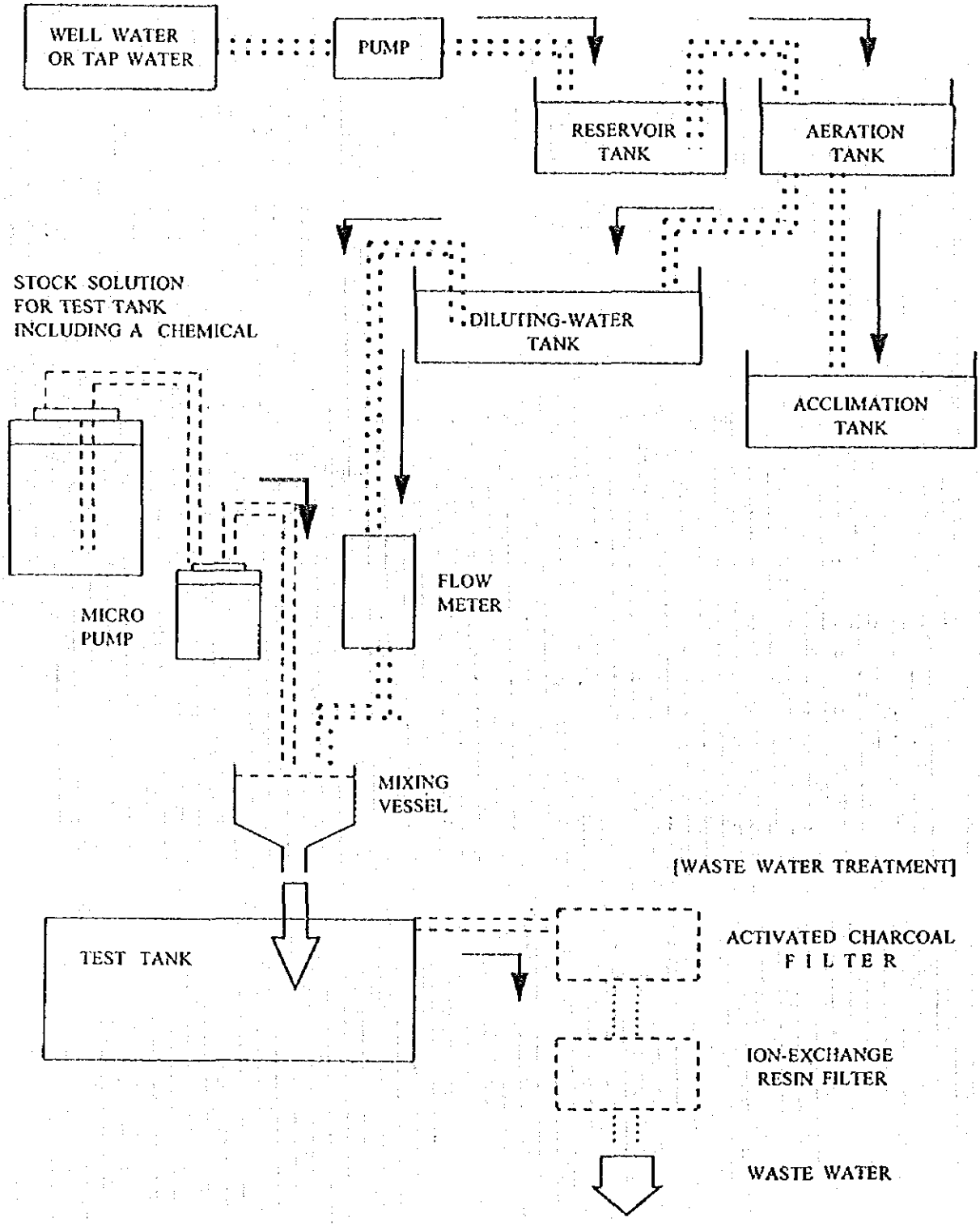


Figure 1: Outline of Apparatus of Accumulation Test

Test fish carp and tilapia are reared in the Center itself. The ideal body length and weight of the test fish were relatively 7-11 cm and 9-15 g, respectively.

The test tank's dimensions are 50 cm width, 30 cm height and 76 cm length, and contained about 30 - 40 test fish. During the test, the flow rate of stock solution and dilution water and temperature of each test tank were maintained every day at 400 or 800 ml/min and 25 (+/-) 2°C, respectively. The supplied dissolved oxygen (DO) was checked twice per week to maintain an acceptable range of 6 - 8 mg/l.

For accumulation experiments, three stock solutions containing a test chemical and dispersant were each diluted continuously 200 times with dechlorinated city water or underground water and supplied to each of the tanks: high level concentration, low level concentration and control tank (contained dispersant only). Measurement of the test water concentration in each tank was analyzed two times in a week. The test water concentration was maintained at least 60% of the calculated concentration throughout the test period. Two fish for analysis were removed from high and low level test tanks each week for MITI method and 3- 4 times per week for EPA method. The recovery rates for both water and fish analysis were more than 60% which were determined before the actual test.

Under the above test conditions, none of the fish showed signs of tiredness and stress during the accumulation and depuration experiments.

Quantitative Analysis of Test Chemicals

(1) Analytical condition and method

High pressure liquid chromatography (HPLC) is used to analyze the chemicals. The analytical conditions are as follow :

HPLC	:	LC-10 (Shimadzu Co. Ltd.)
HPLC column	:	ODS Inertsil C 18, L 150mm x 4.6 mm I.D
Mobile phase	:	9/1 or 8/2 (v/v) acetonitrile/water
		Flow rate 0.8 - 1 ml/min
Detector	:	UV (wavelength 220 - 228 nm)
		sensitivity 0.04 AUFS, responsibility 3
Integrator	:	SPD-10, attenuation 2

(2) General experimental procedure

The experimental procedure is as shown in Figure 2: Flow scheme of bioaccumulation test.

Test Procedure

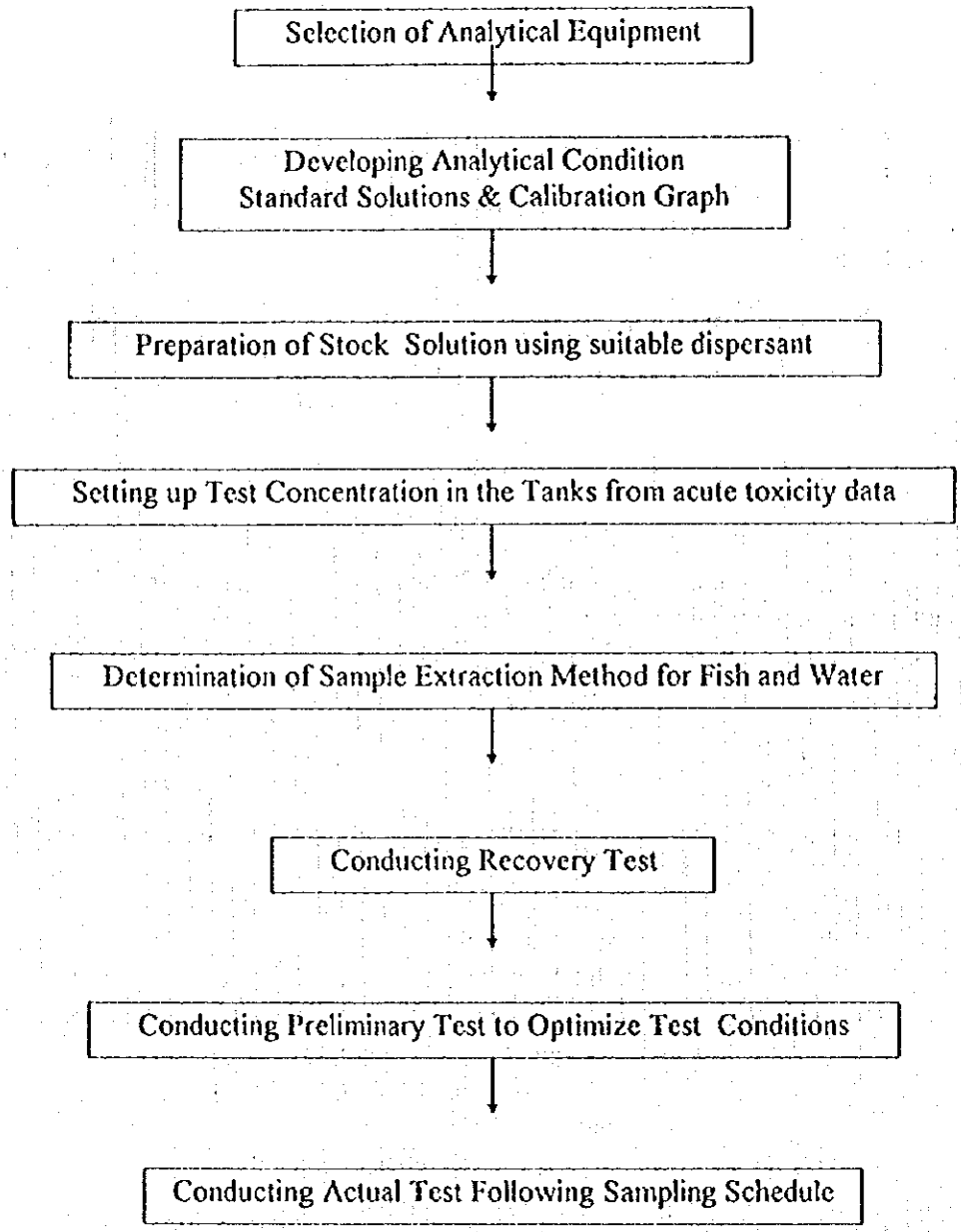


Figure 2 : Flow Scheme of Bioaccumulation Test

RESULTS

BCF values of each chemical are listed on Table-2. BCF values are calculated and averaged at plateau level on MITI test method which were obtained two or three weeks later after chemical exposure.

Table 2 BCF values of diphenylether, 4-phenoxyphenol and 3-phenoxytoluene at the plateau state during exposure phase

test fish	carp		tilapia	
exposure level	high	low	high	low
diphenyl ether	259 (+/- 47)	282 (+/-178)	268 (+/- 47)	275 (+/- 153)
4-phenoxy phenol	57 (+/- 14.2)	28.5 (+/- 8)	12 (+/- 4.5)	n.d
3-phenoxy toluene	6667 (+/- 1165)	5739 (+/-1527)	on-going test	

[Values in bracket show the standard deviation]

Depuration rate constant and half life of diphenyl derivatives in carp and tilapia using EPA test method are listed in Table-2 below. Table-2 also contain uptake rate constant (Ku) calculated by using the derivatives concentration in fish at the mid-point (tm), and the tangent (dCf/dt), some change of concentration in fish at certain test period, dCf/dt is obtained from a hypothetical uptake curve. BCFs values calculated from estimated and measured uptake rate constant (Ku) and depuration rate constant (Kd) are listed in Table 2a to Table 2c, respectively.

Table 2a Depuration and uptake rate constant, half-time of depuration and theoretical BCFs

diphenyl ether

test fish	test level	depuration rate conts. Kd(day^{-1})	half-time (t1/2)	uptake rate constant Ku (day^{-1})		BCF Ku / Kd	
				mid-point	hypoth.	mid-point	hypoth.
carp	high	0.433	1.6	188.9	200.8	436	464
	low	0.302	2.3	370.8	423.7	1228	1403
tilapia	high	0.376	1.8	269.0	178.9	715	476
	low	0.290	2.4	266.3	276.5	918	953

Table 2b Depuration and uptake rate constant, half-time of depuration and theoretical BCFs.

4-phenoxyphenol

test fish	test level	depuration rate conts. Kd(day^{-1})	half-time (t1/2)	uptake rate constant Ku (day^{-1})		BCF Ku / Kd	
				mid-point	hypoth.	mid-point	hypoth.
carp	high	0.663*1	1.0*1	42.8	40.2	65	61
	low	0.553*1	1.3*1	57.3	27.6	108	52
tilapia	high	0.354*1	2.4*1	4.7	5.5	13	15
	low	no data because of low accumulation property					

*1 Depuration rate constants are calculated from equation Kd=0.693 / t (50%); mid-point.

Table 2c Depuration and uptake rate constant, half-time of depuration and theoretical BCFs.
3-phenoxytoluene

test fish	test level	depuration rate conts. Kd(day^{-1})	half-time (t1/2)	uptake rate constant Ku (day^{-1})		BCF Ku / Kd	
				mid-point	**hypoth.**	**mid-point**	**hypoth.**
carp	high	0.199	3.5	1173	1491	5893	7491
	low	0.234	3.0	1127	1401	4816	5987
tilapia	high	on-going test					
	low	on-going test					

The correlation between the BCF values obtained from MITI method at steady state and EPA method are evaluated by means of regression analysis. Two different Log BCF (EPA)s were calculated from the values at mid-point and the graphically estimated Kd. Following the regression equations obtained from the plot of log BCF (MITI) against log BCF (EPA) in Figure 3, we have:

$$\log BCF\ (estimated) = 0.968 \log BCF\ (MITI) + 0.355 \qquad (R^2 = 0.924)$$

$$\log BCF\ (mid\text{-}point) = 0.892 \log BCF\ (MITI) + 0.523 \qquad (R^2 = 0.916)$$

There is a good correlation between the BCF values obtained from both method. However there is a difference between the BCF values of diphenylether within the 95 percent confidence level.

More detail comparison of different type of test fish is done by using the BCF values obtained from MITI test method of diphenyl ether and 4-phenoxyphenol. Analysis of variance is done to see whether there is any significant difference between the BCF values obtained from the bioaccumulation test using tilapia and carp.

The ratio of variance (F) of sets of data of diphenylether is smaller than 5 per cent probability level. Hence there is no significant difference between the variances within the level of significance, at 5 per cent level. There is a difference, however between the variance of sets of data of 4-phenoxyphenol at 5 per cent possibility level.

Estimation of bioaccumulative properties

There is good correlation between the log BCF and Log Pow which is the partition coefficient between n-octanol and water. The reason is that the mechanism by which a chemical can pass through the membrane such as gel is quite similar to the partition between both solutions. Log BCF value is plotted with log Pow, the following regression equation is obtained:

$$\log BCF(carp) = 0.756 \log Pow - 0.484 \qquad (r^2 = 0.939)$$

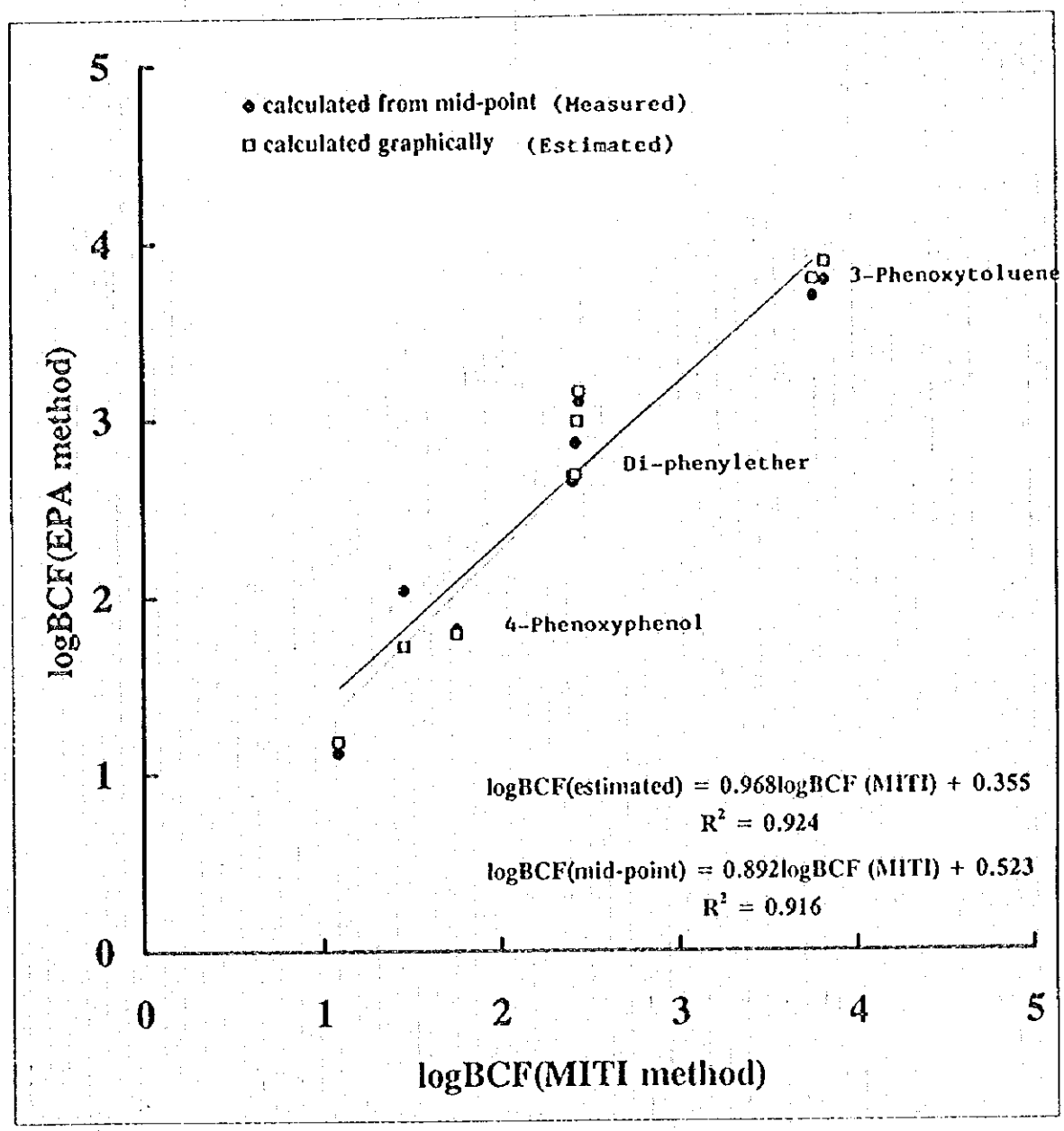


Figure-3 Comparison between logBCFs obtained from MITI method and EPA method.

DISCUSSION

There is an obvious difference between the degree of bioaccumulation of 4-phenoxyphenol using carp and tilapia obtained from our study.

The comparison is based on only three chemicals and further bioaccumulation test is quite complicated. Hence it was difficult to retain good repeatability of data especially in the presence of experimental difficulties such as keeping test water concentration constant.

However BCF values obtained from MITI method and EPA method in our study do not differ significantly except for diphenylether. On kinetic values, such as rate constant and half-time obtained from EPA method a slight difference is observed.

To some degree the data collected for each chemical using either carp or tilapia do differ. However, we still can observe a similar pattern or trend accomplished for a given chemical in either carp or tilapia.

Further experiments should be performed to ensure the reliability and reproducibility of the data obtained.

LITERATURE

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ESTABLISHING THE BIODEGRADATION TEST FOR CHEMICALS, DETERGENTS AND WASTEWATER USING LOCAL STANDARD SLUDGE

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1.0 INTRODUCTION

Environmental fate assessment for test substances such as chemical and detergent play an important role in the development of acceptable manufacturing, use and disposal practices.. To proceed an assessment, a preliminary screening of the test substance is required with the inclusion of biodegradation test.

Biodegradation of test substance by microorganisms is a common breakdown process for organic chemicals deposited in the environment. It is used to predict the aerobic decomposition of chemicals in the natural environment. Many biodegradation test have been introduced internationally, however it is still new to Malaysian researchers.

301C-MITI(I), one of the six screening test methods compulsory for readily biodegradation which is closely regulated by the Chemical Substances Control Law of Japan under the jurisdiction of Ministry of International Trade and Industry of Japan (MITI). It lies in the flow scheme of hazard assessment of new chemicals. It is currently made available to the SIRIM Environmental Technology Laboratory. A chemical is defined as readily biodegradable when the BOD is more than 60 percent when tested by 301C-MITI(I). Such chemicals may not have adverse effects to human health and the environment.

2.0 PRINCIPLE OF THE STUDY

301C-MITI(I) was developed for use in the temperate countries. The inoculum or the standard sludge has been identified as the most critical parameter in 301C-MITI(I), because it is the main element in measuring biodegradability performance. Since the inoculum is made up of microorganisms from different sources and localities in Malaysia, it is believed that the temperature changes may influence the degree of biodegradability.

A series of tests were conducted to establish the inoculum as a reference local standard sludge representative of the malaysian environment The activities involved were:

i. filtration and temperature study on the local standard sludge.
ii. collective study of the fresh sludge from each station.
iii. performance test of the local standard sludge.

3.0 METHODS AND PROCEDURES

3.1 Biodegradation Test using 301C-MITI(I)

3.1.1 Principle of the test

The test substance in a mineral medium, is inoculated with specially grown microorganisms and incubated for 28 days in a dark enclosed respirometer at 25 ± 1 °C. The respirometer will automatically measured the oxygen uptake produced. Evolved carbon dioxide is absorbed by soda lime. Biodegradation is expressed as the percentage oxygen uptake (corrected for blank uptake) of the theoretical uptake (ThOD) . The percentage of primary biodegradation is calculated from the supplemental specific for each chemical analysis made at the beginning and end of incubation.

3.1.2 Apparatus

a. Automatic electrolytic BOD meter or respirometer normally equipped with 6 bottles, (300 m) and cups to contain CO_2 absorbent.
b. Constant temperature room and/or water-bath at 25 ± 1°C
c. Carbon analyser

3.1.3 Stock Solution of test substances

The test substance can be a chemical, detergent or wastewater. For chemicals, deionised water was added to make up known concentrations of a stock solution.

3.1.4 Preparation of Inoculum

The inoculum is collected fresh from no fewer than ten sites, mainly in areas where a variety of chemicals are used and discharged like sewage treatment plants, industrial wastewater treatment plant, rivers, lakes and seas. The cultured microorganism known as the standard sludge are grown under controlled condition at 25 ± 1°C. It was monitored daily as required by aerobic monitoring practices.

For chemicals and detergents, standard sludge was used as the inoculum. However for wastewater, the inoculum used can also be the sludge which will be used in the treatment.

3.1.4 Sample Preparation

Six bottles containing the following were prepared:

Bottle 1	-	test substance in water at 100mg/L:
Bottle 2, 3 & 4	-	test substance in mineral medium at 100mg/L
Bottle 5	-	reference compound (e.g aniline) in mineral medium at 100mg/L
Bottle 6	-	mineral medium only

Oxygen uptakes for the six bottles were directly recorded using by an appropriate method which produces a BOD curve. At the end of the incubation, normally 28 days, pH of the bottle contents were measured and the concentration of the residual test substance determined. Concentration of known substance were determined by various analysis. For example: chemical may be determined by Gas Chromatography (GC) or High Performance Liquid Chromatography (HPLC) and detergent may be determined by the methylene blue method (ASTM D 2667-89)

3.1.5 Data and Reporting

$$BOD = \frac{mg\ O_2\ uptake\ by\ test\ substance - mg\ O_2\ uptake\ by\ blank}{mg\ test\ substance\ in\ vessel}$$

$$= mg\ O_2 / mg\ test\ substance$$

The percentage biodegradation is obtained as follows:

$$\%\ biodegradation = \%\ ThOD = \frac{BOD(mg\ O_2/mg\ test\ substance)}{ThOD(mg\ O_2/mg\ substance)} X\ 100$$

ThOD is a calculated value which is based on the empirical formula of the test substance. For mixtures, ThOD is calculated from the elemental analysis as a single compound.

3.2 Local Standard Sludge Preparation

Inoculum is the main element in predicting biodegradability of any test substance. Different inoculums may produce different abilities to biodegrade any test substance. In the 301C-MITI(I) test, the inoculum is known as the standard sludge due to it's unique collection, culturing and monitoring programmes. The uniqueness of the inoculum, necessitates of local standard sludge development, to enable the use of the 301C-MITI(I) in Malaysia.

Initially, four sites within the Klang Valley chosen for the collection of fresh sludge. The selected stations were Shah Alam Lake, Sg. Buloh, Pantai Sewage Treatment Plant and Taman Tun Sewage Treatment Plant. The sites were selected after taking into account the sample characterisation, colony count and other various inputs. Finally, ten sites throughout Malaysia were chosen for further test stipulated in 301C-MITI(I).

The sludge was mixed thoroughly and left to settle. The supernatant was collected and aerated at 25°C. For the local standard sludge development study, the supernatant was divided into two portions. Each portion was filtered separately using 1 µm filter and 250 µm sieve. The 1 µm filter was used to remove the protozoa present in the sludge and the sieve was employed to remove floating matter. Two litres of each filtrate supernatant was then aerated in the culturing vessel. The sludge was used for the filtration study and marked as follows:

Tank	Filter Size	Temperature
23	1 µm	25°C
24	250 µm	25°C

After about 23.5 hours, the aeration was stopped for 30 minutes. One third of supernatant was removed and replaced with an equal volume of dechlorinated water. Nutrient (0.1%) containing glucose, peptone and orthophosphate of 1ml/litre cultured sludge was then fed into the tank. Feeding was gradually increased after every 3 days until it reached a maximum of 6.8 ml/litre cultured sludge. The aerobic activities were repeated and monitored daily. The sludge was used as inoculum for the biodegradation test only after a month of acclimatisation.

To maintain the same activities, newly collected fresh sludge were mixed with equal volumes of the old sludge after every three months. The sludge was tested against a reference substance at least every 3 month.

3.3 Biodegradation Test on Detergent

Detergent samples taken from the factory was initially inoculated for acclimatisation in the local standard sludge at 30°C. After 6 days acclimatisation, the inoculated was transferred into a respirometer and incubated for 8 days. The biodegradation was determined by the ASTM D 2667-89 method and the results were compared using HPLC.

3.4 Filtration Study on Local Standard Sludge

3.4.1 Study on Monitoring Parameters

SV30, pH and MLSS of tank 23 and 24 were monitored and compared.

3.4.2 Performance Test on Local Standard Sludge

In this study, 301C-MITI(I) test was used to compare the performance of local standard sludge between the filtered (tank 23) and sieved (tank 24) samples. It was conducted only after a one month period of acclimatisation. The performance was measured by the careful monitoring of biodegradability of reference chemicals as recommended in OECD Guideline.

3.5 Temperature study

3.5.1 Temperature of Water and Ambient Air

The temperature of water and ambient air were recorded during the collection of fresh sludge at collection stations. Besides, the temperature of the river water are also being monitored.

3.5.2 Biodegradation Performance Test

After a series of monitoring on water temperature at the sludge collection stations, the author recommended the inoculum to be cultivated at 30°C. Since 301C-MITI(I) test determined the culturing temperature to be at 25°C, therefore a comparison study was carried out between the two conditions with referred to the biodegradability of OECD reference chemicals.

4.0 RESULTS AND DISCUSSION

4.1 Biodegradation Test on Benzyl Alcohol (Case Study - 1)

Benzyl alcohol was chosen as a sample for the biodegradation test using 301C-MITI(I). It was incubated for 14 days with the local standard sludge at 25°C. Table 1 and Figure 4 shows the biodegradability of benzyl alcohol. The graph shows maximum degradation once a plateau is reached.

Table 2 shows the degree of biodegradability of benzyl alcohol, measured by Total Organic Carbon Analyser (TOC) and HPLC. Biodegradability of benzyl alcohol was found to be between 97-100% and 100% using TOC and HPLC respectively. Benzyl alcohol were sound to be readily biodegradable.

Table 1. Biodegradation Test Result of Benzyl Alcohol

Test chemical:		Benzyl alcohol			
Vessel	Code	7th day		14th day	
		BOD(mg O)	deg(%)	BOD(mg O)	deg(%)
1	control	1.1	-	2.9	-
2	sample 1	61.7	80	71.6	91
3	sample 2	61.9	80	72.5	92
4	sample 3	64.3	84	72.4	92
5	reference	64.4	70	72.1	77
6	sample in water	0.3	-	0.7	-

Table 2 Percentage of Biodegradation of Benzyl Alcohol by TOC and HPLC

Test chemical:	Benzyl alcohol	
Sample	TOC	HPLC
sample 1	97	100
sample 2	99	100
sample 3	100	100

4.2 Biodegradation Test on the Detergent (Case Study - 2)

Table 3 shows the biodegradability of detergent as determined by the Methylene Blue Active Substance (MBAS) Method and HPLC. The degradation was more than 90% and therefore considered biodegradable as stipulated in ASTM D2667-89

Table 3 . Biodegradability of Detergent

Test sample :	Detergent powder	
Sample	**MBAS**	**HPLC**
Sample 1	99	99
Sample 2	99	100

4.3 Filtration Study On Local Standard Sludge

4.3.1 Sludge Volume (SV30)

The different changes of sludge volume (SV30) between tanks 23 and 24 were recorded. Figure 1, shows that tank 23 produced a higher value of SV30 in comparison to tank 24. The unsettle sludge in tank 23 occupied about 80-90% of the total volume of the tank. Hence some sludge would be carried over during the supernatant removal. The study shows that filtered samples contributed to higher growth of sludge.

The filtered supernatant developed a cotton-like sludge which produces large flocs. To confirm this phenomenon, further tests carried out on each station yielded the same results. This maybe due to the absence of protozoa in tank 23 which causes filamentous organisms to dominate the system. One of the most important factor in relation to the settling properties of activated sludge is the relative number of filamentous and flocculent microorganisms. The excessive number of filamentous organisms produce low settling velocities and results higher SV30 values.

To maintain SV30 to less than 50%, the use of a 250μm sieve during the filtration activity is recommended, to allow protozoa to subsist and to minimise the number of filamentous organisms in the local standard sludge.

4.3.2 pH

Figure 2 shows that, all tanks have a pH of between 7.5 to 8.5 throughout the culturing period. The pH range measured is the optimum condition for growth of microorganisms. Filtration activities did not affect the pH of the local standard sludge.

4.3.3 Mixed Liquor Suspended Solid (MLSS)

Figure 3 shows the distribution of MLSS between the two tanks. Tank 24 produced a higher MLSS of 5000 to 6000 mg/L in comparison to tank 23 which

produced MLSS in the range of 3000 - 4500 mg/L. Study on the sludge volume (SV30), the protozoa shows to be present in tank 24 and contributed to the higher MLSS value which is suitable condition for the aerobic activities.

4.3.4 Performance Test on Local Standard Sludge

Table 4 shows the biodegradability of five different chemicals inoculated with sludge from tank 23 (filter) and tank 24 (sieve). The local standard sludge from both tanks were able to biodegrade aminoethanol and aniline to about 50 - 70% and whereas pentaerythritol, polyethylene glycol and trichlorophenol were not degradate. This study shows that filtered or sieved sludge did not affect the biodegradability of test substances since the results were the same.

Table 4 . Biodegradability of OECD Reference Chemicals using local standard sludge

Chemicals	Biodegradability (%)	
	Tank 23 (Filtered)	Tank 24 (Sieved)
Aminoethanol	48	52
Aniline	73	66
Pentaerythritol	0	0
Polyethylene glycol	1	0
Trichlorophenol	0	0

4.4 Temperature Study

4.4.1 Temperature of Collection Sludge

The temperature of water and ambient air was found to be between 29.3 - 30.8°C and 29.0 - 30.3°C respectively. As the temperature of the surrounding water was 30°C, it was most preferable to maintain the conditions of the cultured sludge at this temperature.

4.4.2 Biodegradation Performance Test

Some of the reference chemicals in Table 5 are able to degrade under both incubation conditions, however biodegradation of pentaerythritol and polyethylene glycol is higher at 30°C and trichlorophenol is slightly degradable at 25°C

Table 5. Biodegradability of OECD Reference Chemicals at 25°C and 30°C

Temperature	25°C					30°C				
No. of test	1	2	3	4	5	1	2	3	4	5
Aminoethanol	44	46	46	44	48	40	41	49	54	-
Aniline	68	73	-	71	73	62	71	-	79	-
Hexamethylene glycol	88	89	86	-	-	73	82	112	-	-
Pentaerythritol	0	12	0	0	0	66	51	0	0	-
Polyethylene glycol	-	-	0	43	1	-	-	52	88	-
Trichlorophenol	35	27	0	0	0	0	0	0	0	-

5.0 CONCLUSION

The inoculum or local standard sludge is suitably cultured at 30°C. The temperature study shows that the rate of biodegradation of reference chemical is faster when inoculated with standard sludge at 30°C.

Even though the filtration study revealed the same rate of biodegradation for the two filters, it is still necessary to minimise the number of filamentous organisms present in the cultured sludge. The increase in number of filamentous organisms may contribute to a higher value of SV30, low MLSS and the production of large flocs of cotton-like sludge in the culturing vessel. A 250μm sieve is suitable for the collection of sludge.

The purpose of initiating the study for local standard sludge development is to establish a source of reference inoculum for Malaysian representative of the environment and in compliance with the biodegradation test.

Other work that would be carried out in future include:

a. the temperature study
b. investigating the influent of sludge localities to the rate of biodegradation.
c. the changes of bacteria characteristic and strain between the cultivated and the original sludge.

Fig.1 Filtration study on SV30

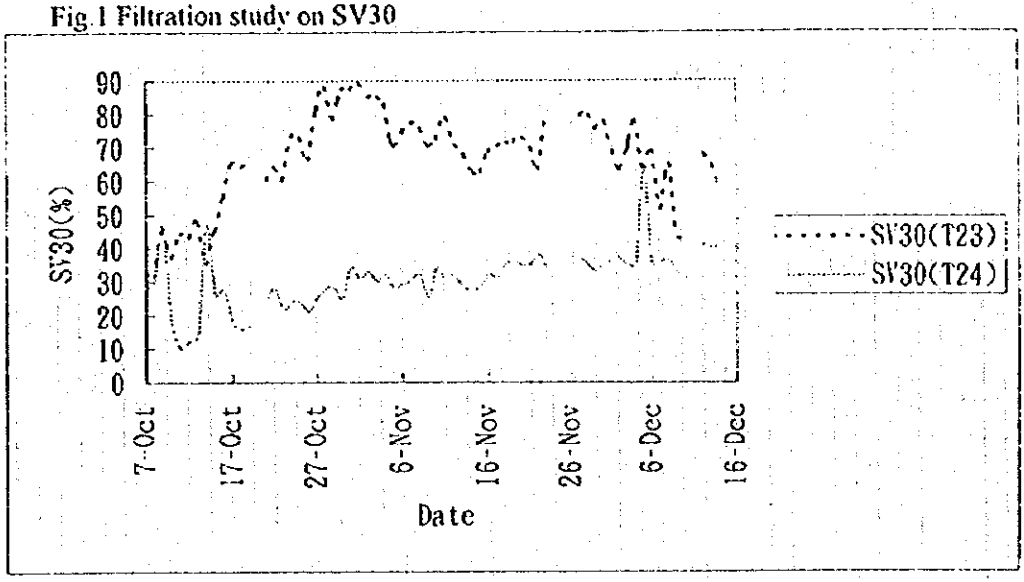


Fig.2 Filtration study on pH

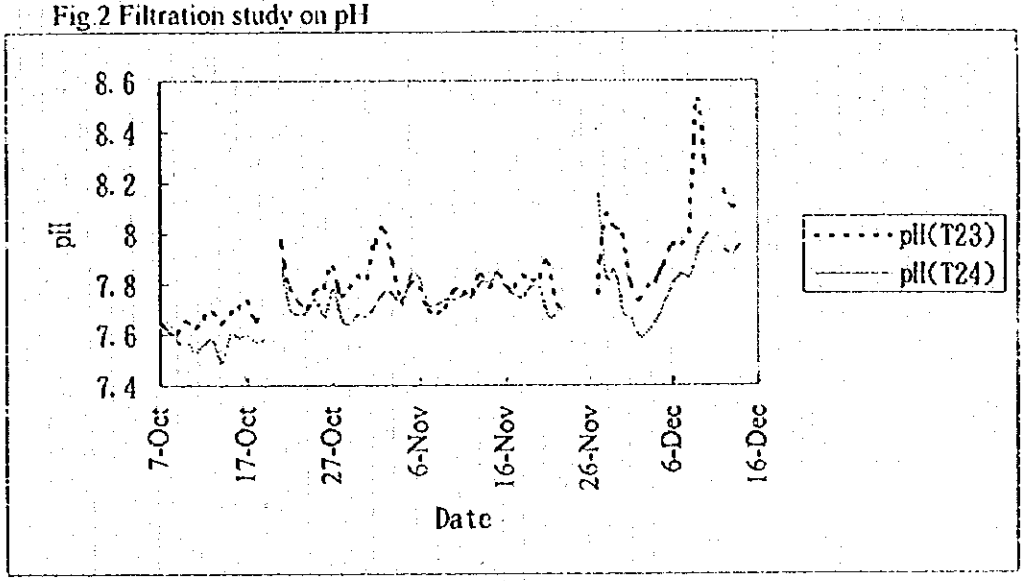


Fig.3 Filtration study on MLSS

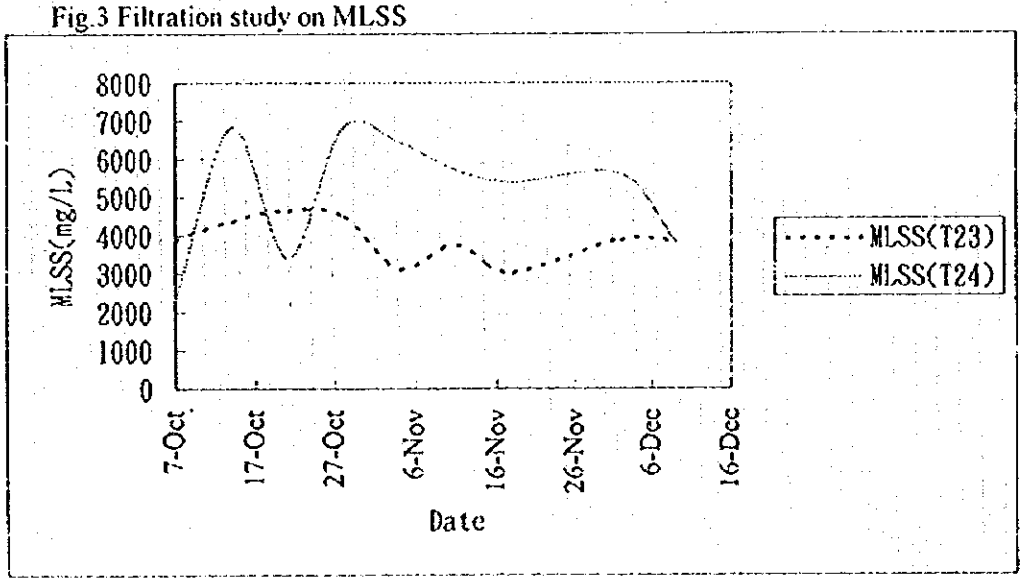
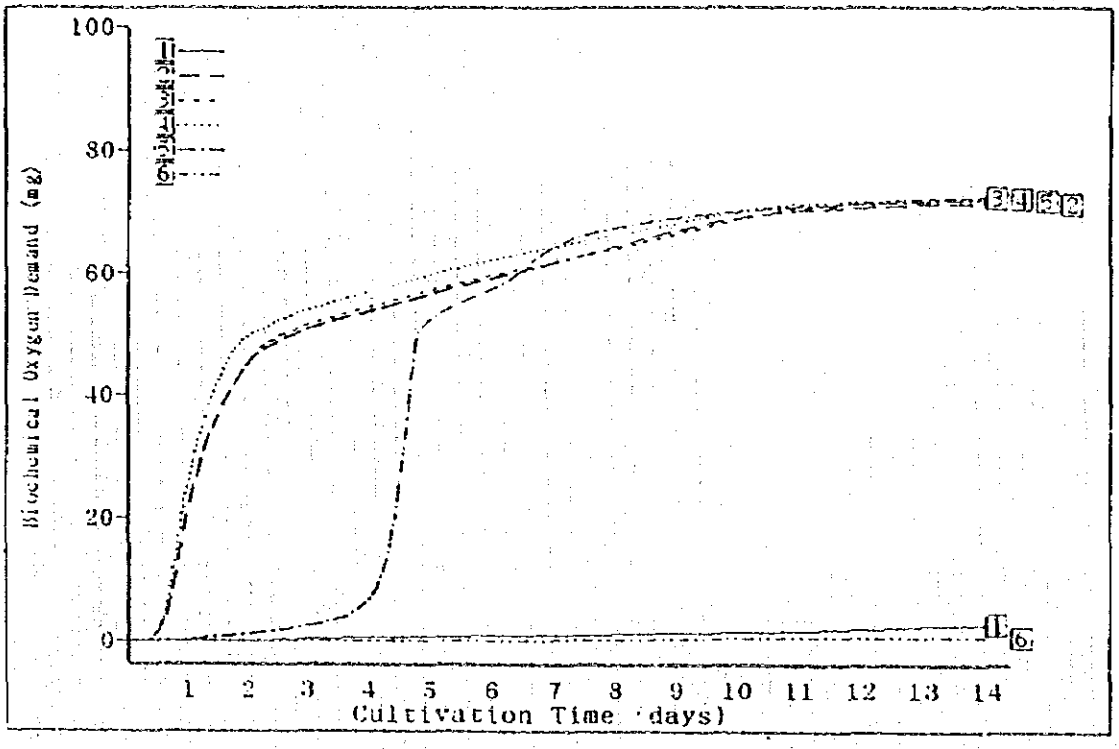


Figure 4 Oxygen uptake versus incubation period



Toxicity Evaluation of Cadmium on the Aquatic Environment Using Local Fish and Crustacean

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1. Introduction

At least three decades ago, the formal conception of ecotoxicology had been a separate science which emphasized on the effects of chemicals and physical agent on fish. Now, the studies have various objectives: the investigation of pollution in rivers, determination of toxic effects in the laboratory especially in the field observations of polluted systems, and more recently screening of a series of chemicals employed in agricultural, industrial or domestic use, which are also used in bioassays with aquatic organisms to monitor the quality of effluents or to give early indication of contamination of water for domestic or industrial supply.

In most cases aquatic organisms, such as fish have been used in ecotoxicological studies. Here, some methodology and some example result are discussed.

2. Ecotoxicological studies using aquatic organisms

2.1 Toxicity determination

One of the most common ecotoxicological studies is to determine the acute lethal toxicity of chemical or product to aquatic organisms under standard conditions. The objective of such a test is to obtain a guidance to indicate the concentration of a substance likely to be hazardous to aquatic organisms in the natural environment. By doing so, a comparison can be made between alternative products for the same function to screen potentially hazardous substances. The tests are most effective when they are performed according to standardized procedures and in well equipped laboratories, so that comparison are free from test to test variability.

2.2 Studies for establishing water quality criteria

Xenobiotic chemicals may enter freshwater as it is widely used in industry, agriculture and the home; some may persist or remain in the aquatic environment for long periods of time. In order to protect freshwater system from such substances, it may be necessary to define the possible risks and then impose water quality standards appropriate to the nature and determine the extent of protection required. Such studies cannot be standardized in detail, because the test program will depend on what is already known about the substance, the receiving environment and its community. Combination of various types of test methods and test organisms is necessary.

3. Acute toxicity test

3.1 Background of the test

Since the 1960's, acute toxicity tests have been used extensively to determine the effects of potentially toxic materials on aquatic organisms during short-term exposure. Fish acute toxicity test is the oldest method which is still used today. Most of the earlier acute aquatic toxicity tests were conducted with freshwater organisms, especially fishes. However the requirement for the assessing the impact of test materials in the estuarine and marine environment has provided the impetus for the development of test methods for saltwater organisms, both invertebrates and fishes.

Toxic effects may include lethality (mortality), change in growth, reproduction, behavior and so on. The effects may also be expressed by quantifiable criteria such as number of organisms killed, percent egg hatchability, change in length and weight and number of skeletal abnormality. Because mortality is an easily detected deleterious response among them, the most common acute toxicity test is the acute lethality test.

3.2 Principles of acute toxicity test

The objective of an acute toxicity test is to determine the concentration of a test material or the level of agent that produces a deleterious effect on a group of test organisms during short term exposure under controlled conditions. Although toxicity tests with aquatic organisms can be conducted by administering the material directly by injection or incorporating it into food, most tests are conducted by exposing groups of organisms to several treatments in which different concentrations of the material are mixed in water. Different concentrations may result in different strength of deleterious effect. The relationship between concentration and effect may then be established.

Theoretically, a 50% response is the most reproducible measure of the toxicity of a test material, in the relationship. The most frequently used effect parameter is lethality and 96 hours (or less) is the standard exposure time because it usually covers the period of acute lethal action . Therefore, the measure of acute toxicity most frequently used with fish and macroinvertebrates is the 96-h median lethal concentration (96-h LC50).

3.3 Test method

3.3.1 Types of Exposure Systems

In the acute toxicity test there are 3 different systems which can be selected depending on the nature of test substance. The systems are describe are below.

3.3.1.1 Static

Static tests have no solution renewal. The advantages of this test are that it is simple and inexpensive (in terms of facilities, equipment and labour) and requires a minimum of test solution. Some disadvantages are changes in exposure levels (volatility, degradation, adsorption), buildup of metabolic waste products, and maintenance of acceptable dissolved oxygen levels. However the static condition sometimes simulate actual exposure condition.

3.3.1.2 Semi static

A semi static test is similar to a static tests, except for the periodical renewal of test solution. The advantages of this method are similar to the static test. Disadvantages include increased labour costs, increased test solution volume requirements (and corresponding waste volumes), and accumulation of metabolic products. Techniques for performing the solution replacement must not cause stress to the test organisms.

3.3.1.3 Flow-through

Flow-through toxicity tests involves continuous replacement of the test solutions, so that exposure levels and other exposure conditions can be kept at constant level throughout the test. For effluent monitoring , these tests are usually performed on-site because of the large volumes of sample required. In testing pure compounds in the laboratory, concentrated stock solutions can be metered into dilution water to vary concentrations. While flow-through exposures are more representative of true conditions, and reduce problems of dissolved oxygen and metabolic products, they are more costly and more complex to operate and maintain. Since flow rate needs to be high enough to replace the test solution volume several times each day, large amounts of waste material are generated.

3.3.2 Standard documents for fish toxicity test

Because of the importance of fish toxicity testing, test methods for fish toxicity had been developed in an early stage of ecotoxicology. Now in many countries fish toxicity test methods are available and international harmonization in the methodology had been achieved in OECD activities.

3.3.3 Test animals

Test animals should be sensitive, important species indigenous to the area concerned. Fishes, crustaceans like daphnia, shrimp, aquatic insect, alga, etc. are frequently chosen in conducting the test because they are readily available throughout the year, ease of maintenance and conducting the test convenience for testing.

3.3.4 Experimental Design

3.3.4.1 Range finding test

When no information is available about the expected toxicity of a sample, a range finding test is necessary. The range finding test usually uses fewer replicates and minimal test volumes than the definitive test and may not run for the full duration. A series 4 to 5 concentrations is generally prepared, each differing by a factor of 10. A second range finding test working within 1 to 2 orders of magnitude may be required for research-oriented projects where the precise LC50 estimate is required. The purpose of the range finding test is to determine the upper and lower limits of concentrations for the definitive toxicity test.

3.3.4.2 Screening test

Some regulatory agencies may only require that a sample be tested at a single concentration, such as 100% (v/v) for an industrial effluent. If a test substance has very low toxicity, it is enough to show no effect at single high exposure levels such as 1000mg/l and no further testing is required. A definitive test may be required to calculate an EC50 or LC50 value.

3.3.4.3 Definitive test

Definitive tests with aquatic species usually involve testing with a series of concentrations (usually 5-7) of test material in order to determine an EC50 or LC50. The series of dilutions should be logarithmic (e.g., 6.25, 12.5, 25.0, 50.0, 100.0 or 1.00, 1.80, 3.20, 5.60, and 10.0). The ratio between each test concentration must be constant so that the concentrations are evenly distributed when plotting on a logarithmic scale. The EC50 or EC50 value is the concentration of material that elicits a response (lethal or sublethal) in 50% of the test population. To determine the EC50/LC50 value, at least one concentration should demonstrate more than 65% response and at least one other should demonstrate less than 35% response.

The following parameters are necessary; test tank, dilution water and test animal. Usually 10 animals are used for each test concentration. Mortalities are recorded periodically, e.g after 2, 24, 48, 72 and 96 hours.

3.3.4.4 Observation

After exposure for a certain period, the appearance of the test organism in the test vessels change. Under higher concentrations the effects are more serious; death and immobility. On the other hand lower concentrations, are less serious.

3.3.4.5 Calculation

The calculation for the LC50 can be estimated from probit method, graphical method, binomial method, moving average method, Spearman-Karber method, and Trimmed Spearman-Karber method. In this study the probit method to estimate the LC50 was employed

4. The acute estimation of cadmium on local test organism

4.1 Material and method

In this test 5 different test organisms from local species were used. These include common carp (*Cyprinus carpio),* lampam jawa (*Puntius gonionotus*), Tilapia (*Tilapia nilotica*), Suji shrimp (*Palaemon paucidens*) and (*Moina macrocopa*).

These organisms were chosen, because all were local species and lived under tropical condition. They were also easy to obtain, culture and maintain under laboratory conditions. Economically tilapia and carp are one an important source of protein in Malaysia. Carp is distributed almost all over the world, so it is easy to compare the results of local carp with other carp species from other countries. In an ecological point of view suji shrimp and moina are important food for the fish. Moina is used by fish farms as fish food. The data obtained can also be representative of tropical fishes because at present, such data are limited.

Sufficiently aerated dechlorinated tap water was used as dilution water. This water stimulates that of natural river water. All test organism were acclimatized for more than 2 weeks in dilution water. The test procedure used was base on OECD guidelines for testing of chemicals. The guideline were 202 (Daphnia) and 203 (Fish).

The chemical used for the test was cadmium chloride ($CdCl_2$). This chemical was chosen because it is one of the reference test chemical for toxicity testing and is also a common pollutant in our country. For the test cadmium chloride was dissolved in water as stock solution and a desired amount was added to the dilution water to make up the test solution.

4.2 Result and discussion

Table. 1 shows the test results for cumulative mortalities of the test organisms to exposure time and concentration. For the first 2 hours no mortality occurred in all concentrations. At 24 and 48 hours drastic changes in mortalities is apparent. This is a common trend in acute toxicity tests. However after 48 hours it showed only a slightly change in mortalities. The change happened at a concentration of 0.265 mg/l but remained the same in other concentrations.The control showed no mortality after 96 hours, the test fish was in good condition and that the death of the test fish in test solution was caused by test chemical. This point has to be considered because if the mortality of the control organism were higher than 10 %, the results of EC50 or LC50 are invalid.

Table 1. Cumulative Mortality of Lampam Jawa to Cadmium Chloride

Concentration (mg/l)	Cumulative mortalities (%)				
	2 hours	24	48	72	96
1.54	0	100	100	100	100
0.857	0	80	100	100	100
0.476	0	30	60	60	60
0.265	0	0	10	30	30
0.147	0	0	10	10	10
Control	0	0	0	0	0

From the data obtained with test the organisms used, a plot of the mortality against concentration (Fig. 1) was obtained and the slope for all test organism were steep. It shows that the toxicity for each organism is within a narrow range. With that data the LC50 and EC50 are estimated by the probit method (Table 2).

Table 2. LC50 and EC50 of Cadmium Chloride to test organisms

Test organism	96hLC50 (mg/l)
Carp	8.15
Tilapia	16.0
Lampam jawa	0.347
Shrimp	0.015
Moina	0.24*

* The result is in 48h EC50

The result presented in Table 2 shows that each test organism has different sensitivities. Tilapia can tolerate higher concentrations than shrimp.Shrimp is the most sensitive amongst the organisms used. In general crustacean is more sensitive compared to fish amongst fishes, lampam jawa is more sensitive than the other two species. In addition the data presented in Fig. 3 show differences in sensitivity to the various test substances used. It is not always the true that the shrimp is one of highly sensitive organism. From these results we can conclude that one test organism cannot represent all organisms. Fig. 2 indicates that toxicity changes with exposure duration. The changes of LC50 or EC50 were not large for cadmium toxicity.

Fig 1. Mortality of test organisms (96 hour) in CdCl2 acute toxicity test

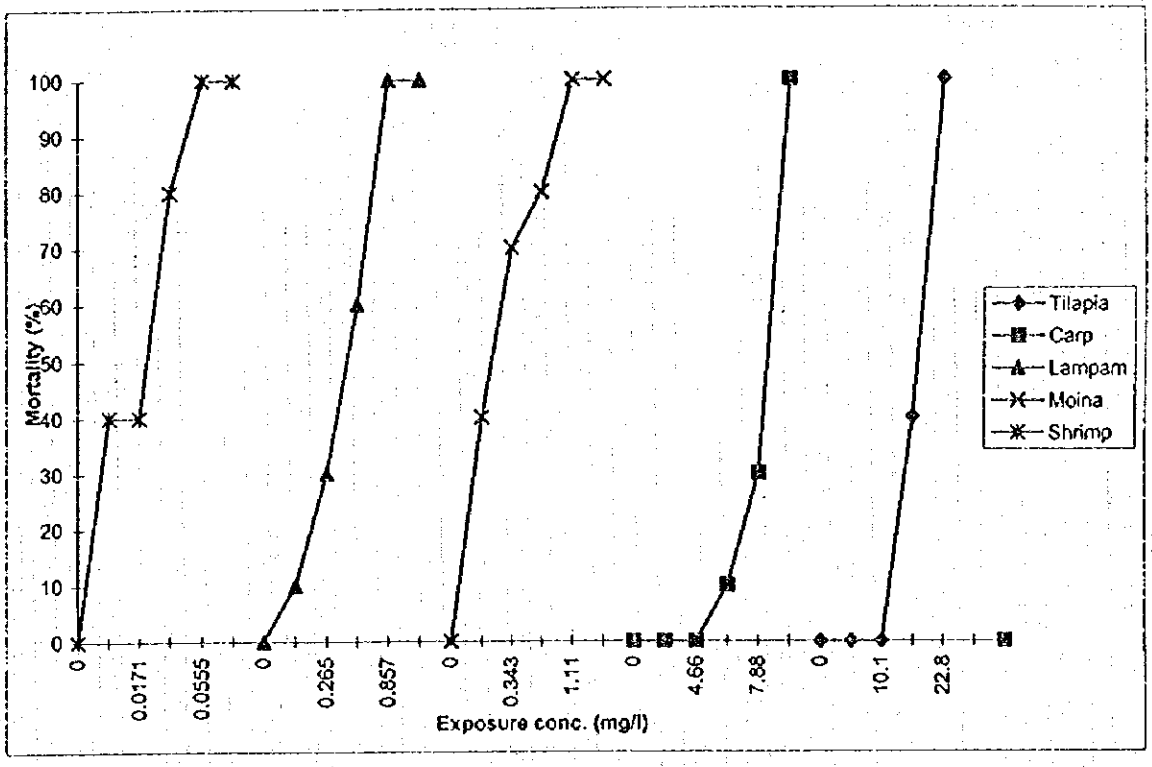


Fig. 2 LC50 or EC50 of CdCl2 for five test organisms

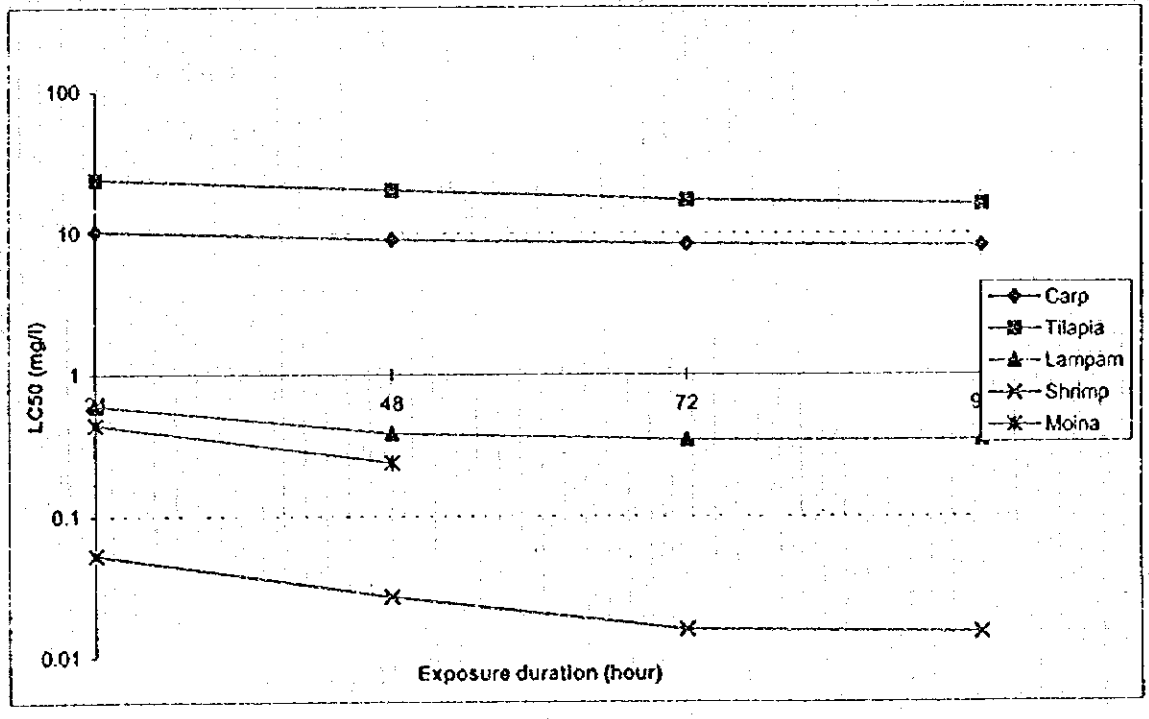
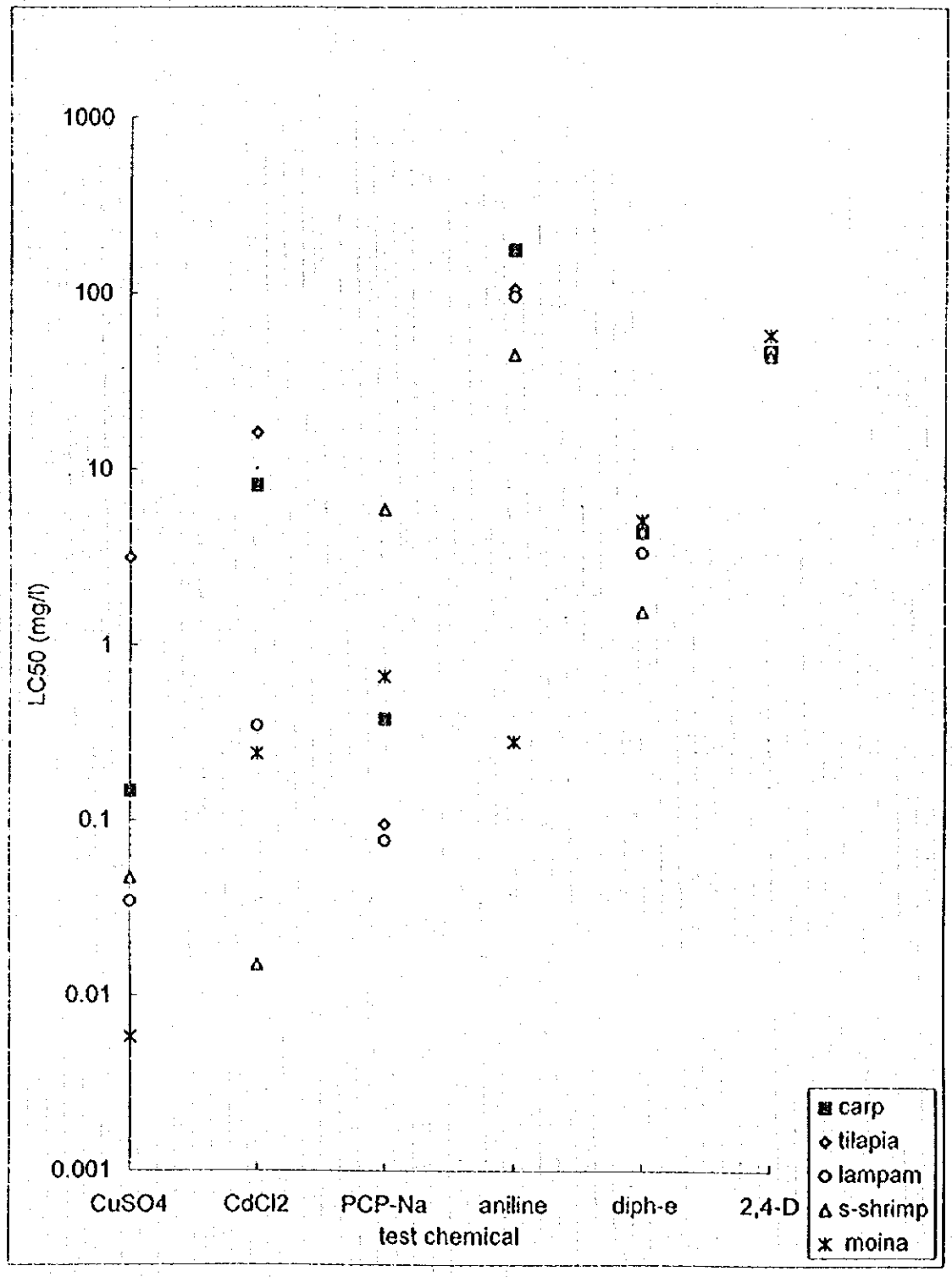


Fig. 3 Susceptivilities of five test organisms to toxicities of six chemicals



4.2 Comparison with another reference

By comparing data from reference (Table 3), the results obtained quite close to each other. For example, the results for carp and tilapia were 8.15 and 16.0 mg/l of cadmium respectively. Converted to cadmium chloride they became 13.3 and 26.1 mg/l. For some crustaceans the results were almost similar, for example the result was 0.24 mg/l cadmium for moina and when converted to cadmium chloride the result was 0.39mg/l. Only for lampam jawa the toxicity was higher (0.57mg/l cadmium chloride) than other fishes tested. This shows that lampam jawa is the most sensitive in this comparison.

Table 3. Toxicity test of Cadmium Chloride from reference books

Organism	Group	Test	mg/l
Daphnia magna	Crustacean	48hEC50	0.1
Charyb	Jellyfish	48hLC50	0.25
G. puls	Crustacean	48hLC50	0.68
Crangon crangon	Crustacean	96hLC50	1.0
Asterias forbesi	Starfish	96hLC50	0.82
Fundulus heteroclitus	Fish	96hLC50	49
Agonus cataphractus	Fish	96hLC50	33
Mytilus	Shell fish	96hLC50	25

5. Criteria of toxicity data

Using the example criteria (Table 4 and 5) we can find out how toxic cadmium is. The toxicity data showed that cadmium can be ranked as medium for fish and highly toxic to crustacean. Consequently, cadmium can be a serious toxicant, especially to crustacean.

Table 4. Criteria for concern used in assessing ecological hazard (ASTM)

	Criteria used for ranking concern		
Hazard properties	High	Medium	Low
Aquatic acute toxicity (ppm)	≤ 1	$> 1 \leq 100$	> 100
Aquatic chronic (ppm)	≤ 0.1	$> 0.1 \leq 10$	> 10
Bioconcentration factor (BCF)	≥ 1000	$\geq 100 < 1000$	< 100

Table 5. Criteria for concern used in registering pesticide under FIFRA

LC50 (EC50) ppm	Category Description
< 0.1	very highly toxic
0.1-1	highly toxic
> 1 ≤ 10	moderately toxic
> 10 ≤ 100	slightly toxic
> 100	practically non-toxic

Theoretically, possibilities of adverse effects of chemical substance can only be calculated in relation to effect concentration. For instance, if a LC50 of a chemical is 1 mg/l and the predicted environmental concentration is 0.001 mg/l, the margin of safety is 1000 for acute toxicity and it can be said that the possibility of acute effect on environmental organisms are very low.

However, experiences have been accumulated and scientists working in the area of ecotoxicity has reached an agreement that although scientists may say that chemicals having LC50 lower than 1 mg/l is toxic or at least not likely non toxic. Therefore, qualitative statement for toxicity strength may be possible using the agreement.

Table 4 and 5 are examples of the classification of toxicity strength. The classification is not valid for legal decision making unless a regulatory agency of the government authorizes it. Therefore, we can say ' generally speaking the test chemical is classified in a very toxic category or toxic and so on."

In some cases acute toxicities to more than one fish species or crustacean are available. Common selection is to choose the lowest toxicity value. If toxicity values of a chemical are largely different from each other, the chemical may have species-specific toxicity and further tests have to be considered.

Another possibilities is to make qualitative evaluation comparison of the toxicity value of a test chemical with that of a known toxicant or a chemical that has been used for the same purpose as the chemical tested. Then we can say that a chemical tested is not as toxic as a known toxicant. For example with dioxin, we can say that the toxicity of a chemical tested is lower than that of the chemical presently used.

6.Conclusion

Acute toxicity tests has been used for a long time and still useful as a tool in ecotoxicology. Usefulness of the test can confirm not only toxic chemicals but can also can be applied for effluent as well. In order to protect our environment it is necessary to conduct ecotoxicity tests, such as the fish acute toxicity test described in this paper.

Thank you Dr.Najimah. Dr. Chong, our colleges,Ladies and Gentlemen,

This seminar is successfully approaching to the conclusion. On behalf of Japan International Cooperation Agency, as a part of the organizer of this seminar, And as a JICA Evaluation Team,I would like to express our appreciation to all the participants for their contribution, especially for the wonderful presentation from our colleagues.

The management of hazardous chemicals and wastes has vital importance not only in Malaysia but in all the country in the world.In modern society, various chemical substances has been introduced and offered many benefits to human kind. But at the same time, it increased a risk to us and an impact to the environment. Japan experienced one of the earliest break-out caused by some of hazardous chemicals and set up legal and social structure to control them after that.

Following to Chapter 19 of Agenda 21 agreed in the UNCED meeting held in Rio de Janeiro in 1992, all the keen countries have reformulated respective national and international efforts to solve chemicals problems and made up respective national agenda. In Malaysia, the 6th and 7th Malaysian plan
include those aspects.

JICA has been conducting the project-type technical cooperation program with SIRIM Berhad on "Hazardous Chemical Substances and Biological Treatment of Hazardous Waste " since September 1993.
The project also has been implemented fruitfully just the same way with this seminar.

As one of the starting member of the Japanese Society of Eco-Toxicology, it is my pleasure to have had an international collaboration in this important field. JICA and myself certainly continue our cooperation and work with all of you to decrease risks caused by hazardous substances but to increase socio-economic sustainability.
I would like to finish my remarks by expressing my appreciation to all of you again. Thank you.