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THESIS

ADSORPTION KINETICS OF CARBAMATE PESTICIDE IN RICE FIELD SOIL

SOONTREE KHUNTONG

A Thesis Submitted in partial fulfillment of
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Two extractive methods: ultrasonic and Soxhlet extraction with petroleum ether:acetone (1:1, v/v) were compared for extraction efficiencies of carbofuran in rice field soil. In this study, Soxhlet provided slightly higher extraction efficiency (83.13%) than ultrasonic extraction (75.55%). The amount of cabofuran was determined by ultrasonic extraction followed by reverse phase HPLC: Intersil ODS as analytical column and 50% acetonitrile-water with flow rate of 1.2 ml/min as mobile phase and detector at 210 nm. The relative error of the method was 0.47% with percentage of recoveries varied from 84 to 77% in the concentration ranges of 10 – 40 mg l^{-1} of spiked soil samples. High amount of residues found in the plots that contained high organic contents. . K_{oc} were 1.91 x 10^{-3} and 7.46 x 10^{-3} mg l^{-1} calculated from K_d and half-life of adsorbed carbofuran and GUS indexes (6.37 and 5.82) calculated from K_{oc} presented an high lixiviation potential. The adsorption of carbofuran in soil reached equilibrium within 23 h. The percentage of adsorption varied from almost 30% to 80% depending on concentrations of carbofuran. The Freundlich isotherms; $q = KC_f^{1/n}$; for the two lines provided the correlation coefficients of 0.9281 and 0.9097, respectively. The distribution coefficients, K were 7.07 x 10^{-5} and 2.79 x 10^{-5}. The Freundlich adsorption exponent (1/n) values which were greater than unity (2.5092 and 2.1248) in the two adsorption time. The positive ΔG indicated nonspontaneous reaction. The adsorption kinetics corresponded to the first order reaction with the half-life of 8.9 days and 0.0799 mg d^{-1} of adsorption rate. The desorption rate was 0.0288 mg kg^{-1}, soil d^{-1}. The percentages of desorption was approximately 55% from the beginning to 21 h.

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Student' signature Thesis Advisor's signature

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Soontree Khuntong

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LIST OF ABBREVIATIONS

atm	=	atmosphere
ADP	=	adenosine diphosphate
AMP	=	adenosine metaphosphate
ATP	=	adenosine triphosphate
BOD	=	biochemical oxygen demand
C	=	Celsius
COD	=	chemical oxygen demand
cm	=	centimeter (s)
DMSO	=	dimethyl sulfoxide
EPA	=	Environment Protection Agency
EU	=	European Union
FAO	=	Food and Agriculture Organization
g	=	gram (s)
h	=	hour (s)
Hg	=	mercury
I.D.	=	internal diameter
in	=	inch (s)
K_d,	=	distribution coefficient
K_F	=	Freundlich adsorption constant
kg	=	kilogram (s)
K	=	reaction rate constant
K_L,	=	adsorption constant
K_P	=	partition coefficient
l	=	liter (s)
lb	=	pound (s)
LC-MS	=	liquid Chromatography-Mass Spectrograph
LD	=	Lethal dose
m	=	meter (s)
mg	=	milligram (s)

LIST OF ABBREVIATIONS (Continued)

min	=	minute (s)
mm	=	millimeter (s)
mM	=	millimolar (s)
ng	=	nanogram (s)
pH	=	power of hydrogen ion
PMT	=	photo-multiplier tube
ppm	=	part (s) per million
psi	=	pound (s) per square inch
PTFE	=	polytetrafluoroethylene
RSD	=	relative standard deviation
TOC	=	total organic carbon
UN	=	United Nations
US	=	United States
UV	=	ultraviolet
V	=	volt (s)
ΔG	=	Gibb's free energy
ΔH	=	enthalpy
ΔS	=	entropy
μg	=	microgram (s)

ADSORPTION KINETICS OF CARBAMATE PESTICIDES IN RICE FIELD SOIL

INTRODUCTION

Soil is the geochemical and biochemical complex material located at the interface between the atmosphere and the earth's crust (Harrison, 1997). Soil is covered most of terrestrial earth's surface and highly heterogeneous in both composition and spatial distribution. Beside the beneficial activities of soil in growing and horticultural crops, spill of petroleum products and chemicals, discharge of air contaminants that fall to the ground and subsistence forming that drains the soil of nutrients cause the deposit of waste to the soil (Lehr *et al*., 2001). The physical and chemical properties of soil such as adsorption and desorption, soil pH, cation and anion exchange capacities affect the behavior of pollutants in soil (Harrison, 1997). According to Food and Agricultural Organization, Global Resources Assessment 2000, agricultural lands contains 50% of the global land use and the application of pesticides are increased by 85.4% from 1961 to 1999 (FAO, 2001). Increasing of pesticides causes their residues in soil which effect the conveying of pesticides and biologically active degradation products to crops grown in later season, biological effects in terrestrial and aquatic ecosystems, including bioaccumulation and transfer to food chain, groundwater contamination and soil fertility. Insecticides and herbicides are taken part of modern agriculture (Manahan, 2004). But unfortunately, only 500 species of the world totally five million species of insects feed on human crops but they cause potentially enormous damage (Spiro and Stigliani, 2003). The residues of pesticides were reported in the Unseen Poison in Asia, the residues of DDT, HCH, organochlorine and endosulfan are persisted in agriculture area in Thailand (Allsopp *et al*., 1998). The soil contaminated with pesticides is one of the most important problems in the environment.

Carbofuran, 2,3-dihydro-2,2-dimethylbenzofuranyl-methylcarbonate was marketed since 1965 under the trade name of Furadan. It is an odorless, white

crystalline solid and available as granules, water emulsifiable liquid (Pinakini and Kumar, 2006). It would be one of the most widely used insecticides, nematicides and acaracides applied to control insects in rice field, sugarcane, tobacco and so on (Yen,1997). It was known as a more persistent insecticides than other carbamates or organophosphorus insecticides. The residues of carbofuran were found in tissues and eggs of hens (0.33 – 0.15 mg kg^{-1}) as well as in milk (FAO, 1997).

Many metabolic pathways including photolysis could degrade carbofuran which hydroxylation and oxidation at the C-3 position yielding 3-hydroxy carbofuran and 3-ketocarbofuran were the major pathways. Photolysis and hydrolysis were two main degradation processes in soil. The natural attenuation at the site occurred in 4-year interval (Campbell *et al*., 2004). Microbial degradation and moisture content of soil affected the persistence of carbofuran in soil (Katsumata *et al*., 2005). Since carbofuran was mostly applied in rice field, it could be reacted with water generating C-N bond and breaking C-X bond (X stands for –OH) (Seiber *et al*., 1978). Factors enhanced the hydrolysis of carbofuran, including high pH of water, microbial communities and light intensities (Siddaramappa *et al*. 1978). The calculated half-life was 320 days under acid and 150 days under alkaline conditions (Pinakini and Kumar, 2006). Different from FAO, Chaudhry and Ali found that the half-life of carbofuran ranging from 3-10 weeks with degradation rate of 7-10 times faster in alkaline soil (pH 7.9) than in acid to neutral alkaline soils (pH 4.3-6.8) (Chaudhry and Ali, 1988).

The accumulation of carbofuran in soil could transport to surface water and groundwater. The methods such as oxidation with ozone, photodegradation, combined ozone and UV irradiation, Fenton degradation, biological degradation, coagulation and adsorption were available for remediation of carbofuran in soil (Burrow *et al*., 2002, Benitez *et al*., 2002, Wang and Lemley, 2003, Bano and Musarrat, 2004).

Adsorption of cabofuran resulted from the accumulation of carbofuran in soil and the adsorption isotherms are mostly corresponded with Freundlich isotherms (Singh *et al*.,1994, Hsieh and Kao, 1998) and also agreed with monolayer Langmuir equilibrium model (Gupta *et al*., 2006). Adsorption in soil may be enhanced by

increasing soil pH, organic matter and clay contents. For adsorption equilibrium kinetics, Gupta reported that adsorption of carbofuran on carbon slurry at 25, 35 and 45 ^{o}C were fitted with pseudo-second order kinetic model. The equilibrium constant, *K* of carbofuran at 15 and 25 ^{o}C were 7.769 x 10^{-9} and 7.367 x 10^{-8} and the positive sign ΔG^{o} of 40.697 and 44.715 kJ/mol indicated that adsorption of carbofuran in sandy clay loam were nonspontaneous with ΔH^{o} of 160.6 kJ mol^{-1} (endothermic reaction) and ΔS^{o} of 402 kJ mol^{-1} (Mear *et al*., 1996).

Since carbofuran is one of carbamate pesticides that long lasting persistence in environment, it has been studied for residue in soil, adsorption characteristics and kinetics and desorption from soil in the demonstrated field of Bureau of Rice Research and Development, Department of Rice, Ministry of Agriculture and Cooperative. For remediation and fate of any pesticides even natural attenuation, it is important to understand the physical characteristics of carbofuran in any media.

OBJECTIVES

1. To screen the suitable extraction and analyze methods for quantification of carbofuran residues in the rice field soils.

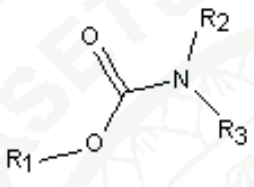
2. To characterize adsorption isotherms as well as kinetic and thermodynamic parameters that control sorption mechanism of carbofuran in rice field soil..

3. To characterize the desorption behavior of carbofuran in rice field soil.

LITERATURE REVIEW

1. Characterization of carbamate pesticides

Carbamate pesticides are groups of synthetic chemicals that have been developed from carbamic acid with the general formula shown in Figure 1.



where R, R_2 and R_3 are alkyl or alryl groups.

Figure 1 General structure of carbamate pesticides.

Source: IPCSINTOX Databank (n.d.)

The modes of action of carbamate pesticides are similar to organophosphate pesticides in the inhibition of cholinesterase enzymes affected nerve impulse transmission. The first carbamate, carbaryl, was initiated in 1956 for lawn and garden because of their relatively low toxicity to mammalian oral and dermal contact.

Carbamate pesticides were classified into three categories (1) the ester derivatives used as insecticides (and nematicides), they were very stable and possessed very low vapor pressure and water solubility, (2) the carbamate herbicides (and sprout inhibitors) had the general structure of $R_1NHCOOR_2$; R_1 and R_2 were aromatic and aliphatic species and (3) carbamate fugicides contained a bezimidazole group.

In spite of low vapor pressure, they might slowly evaporate or sublime at normal temperature leading the volatilization of carbamate from soil or water to air.

The contribution in aqueous was the important route carbamate transportation while distribution via air was the minor route for highly soluble carbamates.

Photodegradation and photodecomposition caused rapid decomposition of carbamate pesticides due to their light absorption characteristics so the long-term contamination might be minimized. Carbamate insecticides were daily applied to the plants and entered the soil while carbamate nematicides and herbicides were directly applied to the soil. Carbamate pesticides could be biodegraded via microorganism activities affected by many factors such as volatility, soil types, soil moisture and adsorption property of soil, soil pH, temperature and photodecomposition (Environmental Health Criteria, EHC 64, 1986).

1.1 Kinetic and metabolism of carbamate pesticides

Fate of carbamate pesticides in plants, insects and mammals were basically the same but the hydrolysis rate via esterase enzymes in mammal was faster than in plants and insects. They were easily adsorbed through skin, mucous membrane, and respiratory and gastrointestinal tracts. The metabolic products were less toxic than the parent materials and rapidly excreted to urine in mammals. Hydrolysis of carbamic acid producing carbon dioxide and amine was the first step of degradation. *N*-methyl and *n*-dimethyl degradation provided different mechanisms. Hydrolysis of *n*-methyl passed through isocyanate intermediate but hydroxyl ion was the additional product from *n*-dimethy- l carbametes yielding alcohol and *n*-dimethyl substituted acids.

Different from hydrolysis, oxidation was another mechanism including hydroxylation, *o*-dealkylation, *n*-methyl hydroxylation, oxidation of aliphatic side chains, and sulfoxidation to the corresponding sulfone. Oxidation involved with mixed function oxidase (MFO) enzymes, the products of *n*-glucolonides, sulfates and mercapturic acid derivatives in mammals and glycosides and phosphates in plants were formed. (EHC, 1986).

1.2 Toxicities of carbamate pesticides

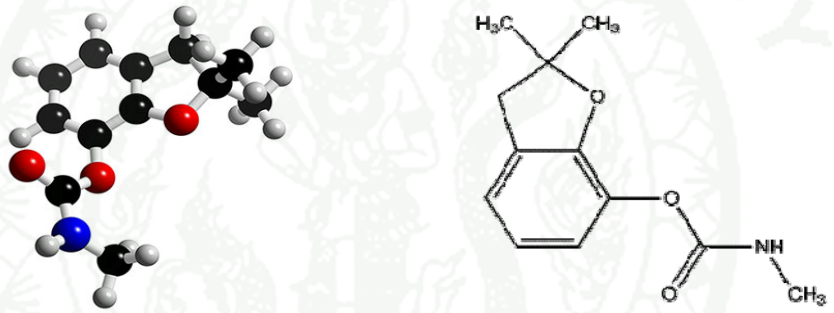
Toxicities of carbamate pesticides depended on their types and physical properties. Normally, they possessed low vapor pressure causing slowly evaporation and sublimation from water and soil. Highly soluble carbamates and light adsorption characteristics rapidly decomposed by photodegradation under aqueous conditions. The others that strongly adsorbed on soil slowly decomposed caused highly toxic to environments (EHC 64, 1986).

1.2.1 Effects on experimental animals. The dose-effect of carbamate poisoning depended on the route and dose of application, the severity of symptoms and the degree of cholinesterase (ChE). The LD_{50} for rat were varied from less than 1 mg kg^{-1} to over 5,000 mg kg^{-1} body weights. Dermal exposure tends to be less toxic route than inhalation or ingestion except aldicarb. It could be concluded that the toxicity of carbamate pesticides including anticholinesterase activity, haemopoietic system, an influence on the functioning of, and, at higher dosages, degeneration of the liver and kidneys, and the degeneration of the tested animals.

Carbofuran is an *N*-methyl carbamate insecticide nematicide and acaracide used to control pest in soil and leaves (Environmental Protection Agency,EPA, 2008 and FAO, 1997). The white crystalline solid was formulated for agricultural applications under the trade name of Furadan in granule by FMC Corporation and Curater since 1967. The liquid form was marketed by various pesticide companies under the trade name of Furadan, Carbodan, Furacarb, Rampart, Yaltex, Brifur, Crisfuran, Kenafuran and Pillarfuron. It was widely used to control soil from dwelling and leaf feeding insects including corn root worms, wireworms, white grubs, alfafa weevils, stem borers, aphids, mosquitoes and several other insects in variety of field crops such as potatoes, corn, soybean, rice, sugarcane and vegetables (Yen *et al*.; 1997, Kale, 2001; López-Blanco *et al*., 2002; Bano and Musarrat, 2004; Katsumata *et al*., 2005; De Vries and Evans, 2008; EPA, 2008).

Cabofuran could be applied to soil with slow-release formulations. The pesticide was entrapped in alginate beads and slowly released active ingredient in the soil over several weeks. From laboratory experiment, persistent of carbofuran was initially slow and completely disappeared after 80 years. The persistence was slightly longer in granular formulation (Pussemier *et al*., 1996).

The chemical formula of carbofuran was $C_{12}H_{15}NO_3$ with IUPAC name of 2,3-dihydro-2,2-dimethylbenzofuran-7-yl methylcarbamate. The CAS name was 2,3-dihydro-2,2-dimethyl-7-benzofuranyl methylcarbamate with CAS number of 1563-66-2. The chemical structure of carbofuran was given in Figure 2.



(a) (b)

Figure 2 (a) Three dimensional structure and (b) chemical structure of carbofuran.

Source: Poisoning Wildlife (2008).
toxipedia (n.d.)

Carbofuran is noncombustible but liquid formulation containing organic solvents might be flammable and generating toxic fume on fire. This compound also had a risk on fire and explosion due to the content of organic solvent in its formulations. Chemical properties of carbofuran were presented in Table 1.

It is highly toxic through inhalation, it causes sweating, papillary constriction, muscle cramp, excessive salivation, dizziness, vomiting, labored breathing and unconsciousness. It irritates skin through dermal absorption. It causes

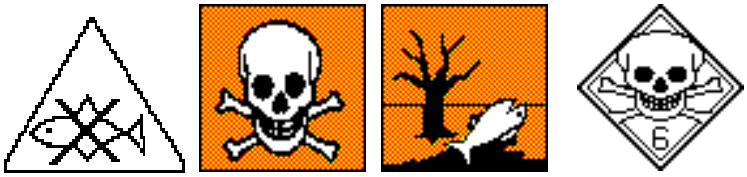
seriously toxic through ingestion, the symptoms of acute toxic exposures are abdominal cramps, diarrhea, headache, nausea, vomiting and weakness. It's half-life in the body varies from 6 to 12 h.

Table 1 Chemical properties of carbofuran.

Chemical properties	Values
Melting point	153 – 154 ^{o}C
Boiling point	200 ^{o}C
Solubility in water	320 mg l^{-1} at 25 ^{o}C
Solubility in acetone	15%
Solubility in benzene	4%
Vapor pressure	$2E^{-05}$ mm Hg at 33 ^{o}C
Molecular weight	221026
Octanol-water coefficient, K_{ow}	log K_{ow} = 2.32
Henry's Law constant	1.02 E^{-10} atm- m^3 mol^{-1}
Soil sorption coefficient	Mean K_{oc} of 29.4
Partition coefficient	1.2304 – 1.4150

Source: DeVries and Evans (2008).

Besides the International Chemical Safety cards, it must be labeled to indicate the type of toxicities including do not transport to food stuffs, marine pollutants, T+ symbol, N symbol, R: 26/28 – 50/53, S: ½ - 36/37 – 45-60-61, UN hazard Class: 6.1 and UN Packing Group 1. The labels were given in Figure 3 and the detail for International Chemical safety Cards were provided in Appendix Table D1. (International Safety Cards, 2004).



(a) (b) (c) (d)

Figure 3 The labels indicate the toxicity of carbofuran (a) Marine pollutant (b) T+ symbol: highly toxic (c) N symbol: dangerous for environment.

Source: NEC-EHWM, n.d.

1.2.2 Animal metabolism of pesticides. Ingestion of the pesticides caused the most lethal and acute toxicity that was directly dependent on the dose to any organisms received. By spraying to the fields, it became directly contact to the target animals with high dose; the actions were very quickly within 20 min. The granules were applied to decrease nematode populations but unfortunately they caused bid kill to birds. The mechanisms were initiated by treating the tested animals (rats, houseflies, laying hens and lactating goats) with radiolabeled carbofuran. The main pathways were the oxidation at C-3 position and hydrolysis of carbofuran ester. The orally treated the single dose of carbonyl- or phenyl labeled ^{14}C-carbofuran, the results indicated 3-hydroxycarbofuran (14%), 3-ketocarbofuran (48%), 7-phenol (20%) and 3-hydroxy-7-phenol (1.4%). Carbofuran (12% internal), 3-hydroxycarbofuran (6%) and conjugated 3-hydroxycarbofuran (11%) were found from the metabolic processes of tropical houseflies treated with radiolabeled carbofuran.

Phenyl-labeled ^{14}C-carbofuran was applied of to hens 3 mg for 7 consecutive day, about 2 mg kg^{-1} body weight/day (approximately 25 mg kg^{-1} in the feed) and then collected eggs and tissues followed by extractions and hydrolysis. The radiolabeled carbofuran were found in residues, liver and eggs ranging from 0.03 to 0.15 mg kg^{-1}. The product of 3-hydroxy-7-phenol (39% of the TRR) was the major

metabolic pathway. Carbofuran was also found in liver and kidneys about 5 % TRR which could be identified as the protease or strong acid treatments.

For the mammalian animals as goats, the ^{14}C-carbofuran at phenyl ring was orally administered for 7 consecutive days at the equivalent rate of 25 mg kg^{-1} of carbofuran in the diet. Milk and excreta were collected daily and tissues were sampled within 24 h of the final dose. The constant residues about 0.10 mg kg^{-1} were found in milk while the residues in fat and tissues were very low (<0.01 mg kg^{-1}). The metabolic products of 3-hydroxy carbofuran (10% of TRR), 7-phenol (15% of TRR) and 3-keto-7-phenol (32% of TRR) were found in milk. Besides animal metabolic wastes of animal, 3-hydroxycarbofuran (11% of the TRR) and 3-hydroxy-7-phenol were found in urine.

1.2.3 Plant metabolism. The selected plants included potatoes, soya beans and maize. It was found that the major metabolisms in potatoes tubers were 7-phenol (45% of TRR) and 3-hydroxy-7-phenol (13%). For immature foliage, 3-hydroxy carbofuran (23% of the TRR) was found together with 5-hydroxy carbofuran (34%) which was the unique metabolic product to potato. The identification of carbofuran (11% of TRR) and 3-hydroxycarbofuran (28%) were found as the major metabolite in soya bean forage (45-day PHI). The residues (40% of the TRR) were remained till a longer pre-harvested interval (139 days) releasing by enzymes as well as acid and basic hydrolysis. Two main metabolites in bean were identified as 3-ketocarbofuran (5% of TRR) and 3-keto-7-phenol (9%). The major compounds in maize forage were carbofuran and 3-hydroxycarbofuran (14% and 13% of TRR, respectively).

Teerakun and Reungsang (2005) found that carbofuran might potentially accumulate in selected plants including (1) grass crops, cat tail and bulrush, (2) upland crops; soybean, ground nut, mung bean, sunflower and corn and (3) vegetable crops; water convolvulus, tomato, aubergin, eggplant, Chinese-kale, chili and cabbage. It was found mostly stems and leaves unless carbofuran residues in soil was lower than the method detectable limit (<0.01 mg l^{-1}).

It can be summarized that the metabolites in plant through the hydroxylation at C-3 position and hydrolysis of the carbamate as provided in animals (FAO, 1997).

Oxidative metabolism of carbofuran is summarized in Figure 4.

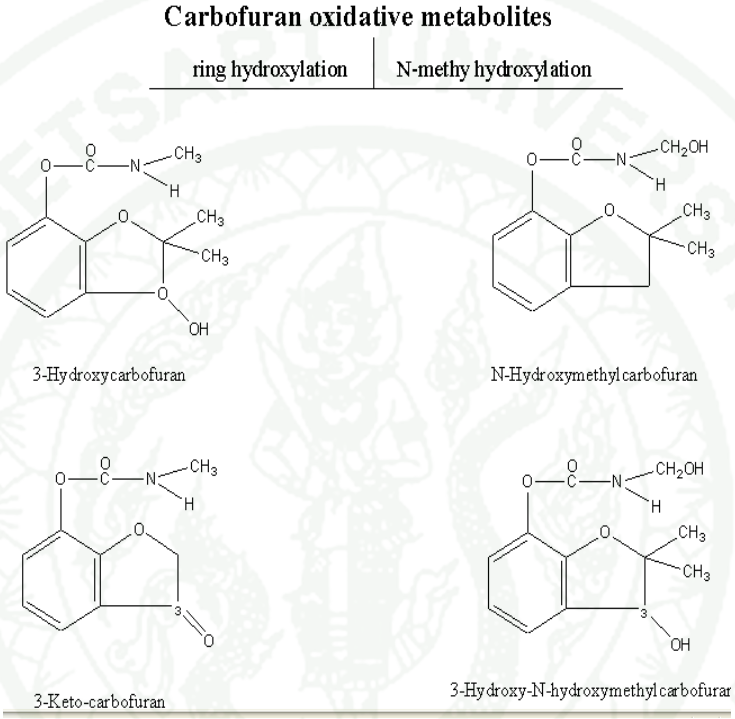


Figure 4 Oxidative metabolites of carbofuran.

Source: DeVries and Evans (2008).

Beside, the environmental fate of carbofuran, hydrolysis is the major metabolic degradation pathway under alkaline condition. Basic catalysis products included 3-hydroxy-7-phenol carbofuran, carbofuran phenol and *N*-methylcarbamic acid. Hydrolysis rate in alkaline solution (pH 10) is 700 times faster than neutral condition (pH 7). Difference of chemical degradation, microbial degradation is an important route in neutral and acidic conditions. In general, photolysis is not considered to be a significant degradable pathway in both soil and water. Photodegradation products comprise 2,3-dihydro-2,2-dimethylbenzofuran-4,7-diol

and 2,3-dihydro-3-keto-2,2-dimethylbenzofuran-7-yl-carbamate (Figure 5). Oxidation and volatilization are normally considered to be a minor degradation pathway (Evert, n.d.).

Maximum Residue Limits (MRL) which is the maximum limit of pesticides residues in food in the unit of milligram of residues per kilogram of food products can be defined for carbofuran. MRLs are available for commercial limits of residues but actually lower than safety levels of residues for the whole life (Acceptable Daily Intake, ADI). MRL is defined as safety level but it is commercial level based on scientific data evaluation. MRL values are different in each country considering from Codex MRL, EU MRL, Asian MRL and experimental data of each individual country.

Codex is the corporation project of Food and Agriculture Organization (FAO) and World Health Organization (WHO) for international standards of food. The standards are based on risk assessment and maximum residues in agricultural products. Codex can be divided into two categories: (1) vertical standard for community standard such as food can, (2) horizontal standard for general subject such as pollutant residues in food. Codex MRLs are the international standards based on The Codex Alimentarius Commission of United Nations. The values are fixed from experimental data, effect of pollutants to human body in chronic and acute exposures (Bayer Crop Science, n.d.).

In Thailand, carbofuran residues in agricultural products are fixed by Thai Agricultural Community and Food Standard, TACFS 9002 – 2006 for pesticide residues: maximum residue limits of carbofuran can be concluded in Table 2 (National Bureau of Agricultural Commodity and Food Standards, 2006).

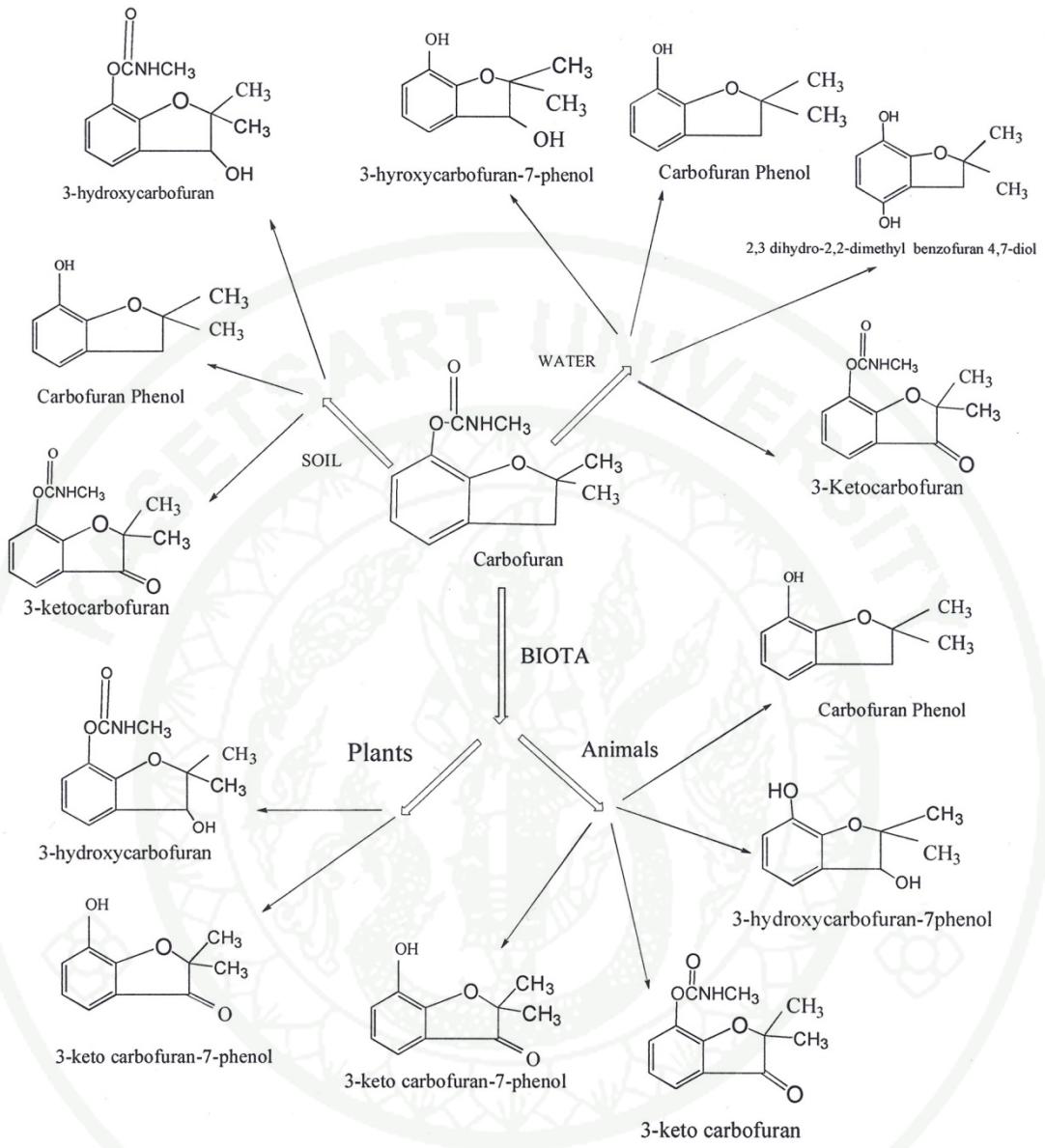


Figure 5 Environmental fate of carbofuran.

Source: Evert, n.d.

Table 2 Thai agricultural commodity and food standard; pesticide residues: Maximum Residue Limits for carbofuran.

Type of residues	Products	Maximum Residue Limit; MRL (mg kg-1)
Carbofuran,	Banana	0.1
3-hydroxycarbofuran,	Corn	0.1
Conjugated-3-hydroxy	Baby corn	0.1
carbofuran	Corn seed	0.05
	Sorghum	0.1
	Rice grain	0.1
	Cucumbers and others (except water melon)	0.3
	Water melon	0.1
	Mung bean	0.2
	Long bean	0.1
	Peanut	0.1
	Soya bean	0.2
	Pigeon pea	0.5
	Pepper	1
	Tomato	0.1
	Cassava	0.2
	Coffee seed	1
	Cotton seed	0.1
	Sugar cane	0.1
	Mammalian meat	0.05
	Mammalian offal	0.05
	Poultry meats	0.08
	Poultry offal	0.08
	Egg	0.1
	Milk	0.05

Source: National Bureau of Agricultural Commodity and Food Standards TACFS 9002-2006

2. Review researches

All review researches can be classified into four categories: (1) toxicity (2) analysis of pesticide residues (3) adsorption, desorption, thermodynamic and kinetics of degradation and (4) treatment and remediation.

2.1 Toxicity of carbofuran

Carbofuran is an antichlorinesterase carbamate and causes consequently adverse health effects and inevitability to humans, animals, wildlife and fish. The overall toxicity include biochemical (cholinergic and noncholinergic), hematological and immunological due to two metabolic pathways of 3-hydroxycarbofuran and 3-ketocarbofuran. Carbofuran and/or its major metabolic products can cross the placental barrier causing very serious effects on the maternal-placental-fetal unit (Gupta, 1994).

The assessment impact of carbofuran on the nontarget cyanobacterium *Anabaena doliolum* in rice field was studied by Hammouda (Hammouda, 1999). The moderate amount of carbamate as well as organochlorine insecticides investigated the influence on cyanobacteria in rice field ecosystem via intermittent flooding and drying cycles corresponding to alternate anaerobic and aerobic conditions. The low levels of pesticide caused the insignificant effects on *A. Doliolum*. The relatively low concentrations at 2 to 10 mg l^{-1} stimulated the growth and increased the level of chlorophyll A due to the utilization ability of carbofuran. Cyanobacteria initially utilized the insecticide whereas the higher concentrations of carbofuran (up to 100 mg l^{-1}) inhibited the growth exceeded more than 50% and initiated the formation of hydrolytic breakdown resulting toxic products. Toxicity of carbofuran was highest at pH 4 – 6 (46 – 59%) and lowest at pH 7 – 10 (12 – 27%) owing to the persistence of the pesticides and the resulting alkaline-catalyzed hydrolysis associated with the water regime of the rice field.

The toxicities of carbofuran had been studied for many kinds of animals such as shrimp, fish, birds, amphipods and mice. In invertebrates, carbofuran inhibited acetylcholinesterase (AChE) on the central nervous system. In insects, AChE inhibition caused hyperactivity, loss of coordination, convulsions, paralysis and death (Kuhr and Dorough, 1976 and US EPA 1988).

Pesticides seemed to be important to ensure good harvests for modern agriculture but overspray and/or runoff of pesticides from agricultural land might contaminate water bodies both surface water and groundwater, resulting critical problems to non-target species including fishes. Toxicity of carbofuran was corresponded to the inhibitory effect on acetylcholinesterase activity at central cholinergic and at neuromuscular junctions (Gupta, 1994). The inhibition effects caused lesser hydrolysis of neurotransmitter acetylcholine (ACh) in synapses. The abnormal amount of acetylcholine leaded the over activation of cholinergic receptors causing toxic effects. Zebra fish (*Danio rerio*) was useful vertebrate as the toxicity modeling because of its suitable characteristics. Besides *in vitro* study, the various amounts of carbofuran in the presence of DMSO provided no significantly different on ATP, ADP and AMP hydrolysis. The effect on ecnonucleotidases was significantly different in millimolar range for ATP hydrolysis in the concentration range from 1 to 5 mM (28 – 59%), ADP hydrolysis 3 and 5 mM (35 and 45%, respectively). There was no significantly different on ADP hydrolysis in all concentration range of carbofuran. For 7 days continuous exposure (50 and 500 $\mu g\ l^{-1}$), hydrolysis of ATP and AMP hydrolysis in brain membranes of carbofuran-treated zebra fish presented no significantly different but ADP hydrolysis was significantly decreased comparing with control groups (Senger *et al*., 2005).

Among avian animals, carbofuran might affected time of flight basically on cholinesterase inhibitors on accidental avian toxicity and mortality which seemed to change the population level in migratory bird species. Pigeons were studied as non-target surrogate species to access the effect of low-dose insecticides. Low levels of carbofuran showed no effect on hematological parameters on pigeons whereas acute high dose carbofuran significantly decreased erythrocytes, leucocytes, hemoglobin

and packed cell counts (Shama and Saxena, 1998). Increasing carbofuran exposure, the time of flight increased. Pigeons gavages with treated water presented the slower flight times from 200 miles by an average of 25 min compared with drinking pigeons. The treated pigeons gavaged at 0.0, 0.25 and 0.50 mg kg^{-1} of carbofuran were circling the sky several times prior to the heading south and the flocks joined together. For 1 mg kg^{-1} dose, the initial acute responses were observed as head bobbing, but on signs of acute over toxicity such as pronounced lethargy or coma. The migratory birds such as pigeons showed the neuronal level causing the adverse effect on altering magnetic field stimuli. Bioenergetic effects paid an important role in carbofuran treated pigeons that mostly returned to the loft until the next day and increased the range and length of flight time as increasing doses. The birds also dropped out of flock and off route at lower non-lethal dose levels (Brasel *et al*., 2007).

Carbofuran could induce alterations in biochemical compositions, lipoperoxidation, Na^+/K^+ ATPase activity and reproductive parameters of *Hyalella pleoacuta* and *Hyalella curvispina*. Na^+/K^+ ATPase were a membrane-bound found in animal cells. It played important role in couple with free energy storing in ATP molecule to the translocation of Na ions. The non-target amphipods were the other species that affected through the physical and energetic processes and they were able to use as sensitive indicators of toxic stress from exposure to pesticides including carbofuran. The organic molecule of DMSO was used to solubilize carbofuran affecting the reproductive parameters, levels of glycogen, total proteins, total lipids, lipoperoxidation and Na^+/K^+ ATPase but the effects were not strong compared with treated animals with carbofuran (5 and 50 μg l^{-1}).The lower levels in glycogen and total lipids were found in carbofuran treated amphipods. The decrease in protein contents was due to formation of lipoperoxidation levels which were independent on species and sex. The treated amphipods presented a lower survival rate comparing to control animals. The concentration of carbofuran exceeded 500 μg g^{-1} caused 100% mortality in 24 h (Dutra, *et al*., 2008).

Toxicities of *N*-methyl carbamate concerned with mammalian animals; mice and rat were the selected animals for examining carbofuran toxicities. Abnormal

ovarian growth in hemicastrated albino mice was investigated for oral administration of carbofuran at doses of 0.4, 0.7, 1 and 1.3 mg (kg day) $^{-1}$ for 15 consecutive days. Treatment with carbofuran significantly decreased the relative ovary mice weight by severe inhibition of ovarian growth in hemicastrated albino mice. It also decreased the number of corpoa lutea and follicles and prolonged diestrus which were due to suppression of secretion/release of gonadotropins or desensitization of the ovary to gonadotropins and releasing of estrogens. The continuous dose of 1.3 mg (kg day) $^{-1}$ for 15 days inhibited the ovarian growth and significantly decreased the number of small, medium and total number of follicles but increased all sizes of atretic follicles. The estrous cycle was significantly decreased whereas duration of diestrus phase increased. It might be hormonal imbalance. The longer treatment with high doses of carbofuran might affect ovarian function and indirectly acted at the level of hypothalamo-hypophysical-ovarian system. The 15-day continuous treatment did not change the body weight in mice. The results were very important to identify the malfunction of reproductive system from nutritional deficiencies or exposure of toxic substances (Baligar and Kaliwal, 2004).

For acute toxic effects, carbofuran induced oxidative stress in slow and fast skeletal muscles were studied by Milatovic, *et al*. (2005) via inhibition of acetylcholinesterase. Single acute injection of carbofuran (1.5 mg kg^{-1}) presented the onset of toxic symptoms including salivation and fine tremors within 5-7 min followed by severe muscle fasciculation, fibrillations and clonic convulsion within 15 – 30 to ~2 h. The increasing of F_2-isoprostances (F_2-Isops) products of lipid peroxidation) occurred within 30 min in soleus and 60 min in extensor digitorum longus (EDL). The levels of F_2-IsoPs in soleus and EDL recovered to control levels in 2 – 3 h and 6 h, respectively. The levels of citrulline as a marker of NO/NOS, in skeletal muscles were slightly higher in control than treated rats. The acute dose of carbofuran increased the levels of the high energy phosphate, ATP and phosphocreatin (PCr) in EDL than in the soleus. Rats pretreated with memantine, MEM (18 mg kg^{-1}) and atropine sulfate, ATS (16 mg kg^{-1}), 60 and 15 min prior carbofuran, respectively presented no signs of toxicity.

Toxicity of carbofuran was studied for time course profiles for brain and red blood cell (RBC) cholinesterase inhibition by Padilla *et al*. (2007). Each animal showed the correlation between red blood cell cholinesterase and brain cholinesterase inhibition. The dose of 0.5 mg kg^{-1} inhibited 55% of cholinesterase activity in red blood cell and 44% of cholinesterase activity in brain by half an hour after dosing. The recovery time required 6 h after dosing for brain but red blood cell could not exceed control level until 24 h.

The subchronic toxicity of carbofuran via drinking water investigated different symptom from gavaged feeding. The rats daily consumed water with total amount of carbofuran of 400 mg l^{-1} for male and 100 and 400 mg l^{-1} for female during 90 days. The erythrocytes and serum ChE significantly decreased in treated animals compared to the controlled one. The dose of carbofuran correlated with alkaline phosphatase, AP activity and related to hystopathological changes liver of tested animals. The activity of serum aspartate aminotransferase, AST and alanine aminotransferase, ALT increased in the 90-day continuously dietary rat with carbofuran. The contribution of carbofuran had significantly effects on hemoglobin concentration and total RBC but significantly decreased white blood cell. The changes in histopathological including liver and kidneys but cell regeneration of liver and kidneys were found in all tested animals (Brkić *et al*., 2008).

2.2 Analysis of pesticide residues

The decomposition of carbosulfan by photolysis, hydrolysis and microbial transformation caused the toxicity of pesticide from the less toxic to the more toxic of carbofuran. Radiolabeled compounds, thin-layer and gas or liquid chromatography with unspecified detectors were the proposed methods with serious weakness of lacking sufficient specificity for identification of the analyte in complex matrices. Soler (2006) investigated LC/MS ion-trap to identify the unknown compounds in the degradation pathways. The fruit samples were blended with anhydrous sodium sulfate for homogenization. The mixtures were extracted with 40 ml of dichloromethane at 100 ^{o}C and 2000 psi, the heating time was 2 min with two cycles and 5 min with static

extraction. The separation achieved in an analytical column Luna C_{18} prior by guard cartridge C_{18}. The methanol/water gradient was selected at the flow rate of 0.8 ml min^{-1}, 20% of methanol was linearly increased to 50% in 20 min and then increased to 90% in 10 min and kept constant for 15 min, and back to the initial conditions in 10 min. The limit of quantification (LOQ) ranged from 0.01 to 0.07 mg kg^{-1} and the recoveries were 55 – 90% with RSD for five replications from 8 to19%.

Flow injection analysis was investigated for determination of carbaryl (1-naphthyl, 1- methylcarbarmate) instead of conventional gas and liquid chromatographic methods. Flow injection-chemiluminescence (FI/CL) methods could enhance sensitivity, increase precision and high sample throughput. Carbaryl was directly measured the chemiluminescence emission generated by oxidation with potassium permanganate in acidic media. The water samples were filtered through 0.45 μm glass fiber filter to removed suspended solids. The carbaryl solution was then injected into the carrier stream of sulfuric acid solution via the sample valve of FI/CL-detection system and it was mixed with CL-reagent stream (potassium permanganate in acidic media). The optimum experimental conditions consisted of 1 mM of permanganate solution, 5 M sulfuric acid solution, flow rate: 1.7 ml min^{-1}, sample volume: 100 μl and PMT voltage: 1400 V. A calibration curve varied at the concentration range from 0.01 – 1 mg l^{-1}. The limit of detection was 14.8 μg l^{-1}. The method provided high precision (RSD. = 2.29%) with standard deviation 0.0023 mg l^{-1} for 10 injections of 0.1 mg l^{-1} (Tsogas *et al*., 2006).

Liquid chromatography – ionspray and thermospray mass spectrometry were investigated to determine carbamate pesticides in sediment samples. Since the carbamate pesticides exhibited the large different in polarity, it could be determined simultaneously in environmental samples. The water samples were filtered through 0.45 μm PTFE glass fiber filters to eliminate particulate matters. The extraction was performed in 500 mg of C_{18} – bonded silica disks of 47 mm diameter and 0.5 mm thickness. The disks were pretreated by washing with methanol, acetonitrile and water and followed by water samples with extraction of 1 h under vacuum. The pesticides were recovered by two times extraction with acetonitrile. After careful evaporation,

each sample was dissolved in 500 μl of acetonitrile prior to LC – MS. The sediment samples were sieved through 125-μm sieves and freeze-dried. To study the recovery, the samples were spiked with standard carbamate solution in methanol, to yield levels of 50 and 500 mg kg^{-1}. The spiked sediment was Soxhlet extracted for 16 h with double – thickness cellulose thimbles and acetone-dichloromethane (1:1). The Soxhlet extracts were concentrated to nearly dryness under vacuum at 35 ^{o}C and achieved under a nitrogen flow to dryness. The Soxhlet extracts were re-dissolved in 500 μl of n-hexane followed by purifying in 15-cm long glass column containing about 2 g of aminopropyl-bonded silica with 20 ml of acetone-dichloromethane (1:3) as an eluant. The eluate was evaporated to dryness and re-dissolved with 1 ml of methanol-water (1:4) prior to LC analysis. Carbofuran was identified of 21 min at a concentration level of 5 μg l^{-1} in water samples from the Ebro delta. Good recoveries of 85-100% were obtained for all carbamate pesticides using LC-TRP-MS (Honing *et al*., 1996).

Vázquez *et al*. (2000) coupled reversed-phase liquid chromatographic switching techniques for determination of *N*-methylcarbamates and their metabolic products in urine. This technique enhanced the sensitivity by the use of injection volume while its technique was selective for carbofuran and its metabolite; 3-hydroxycarbofuran in human urine samples as the biomonitoring of agricultural workers. The dual column contained 30 x 4.6 mm I.D. first separating column (C-1) packed with 5 μm Spherisorb ODS-2 and 100 x 3 mm I.D. second separation column (C-2) packed with Hypersil Shandon Green Env. C_{18} column. An aliquot of 1 ml-urine extract was injected into the sample loop and transferred to the pre-separating column at a flow rate of 1 ml min^{-1}. After 2.9 min, the switching valve was turned to the separating column and then turned back the switching valve to the initial position after duration of 5.7 min. A mixture of acetonitrile-water (5:95) was used as the first mobile phase while the second one consisted of acetonitrile-water gradient elution. Carbofuran and its metabolite were analyzed with UV detector at 210 nm. The limits of detection were between 0.3 and 1 μg l^{-1}. The solid-phase extraction with graphite carbon and a RPLC-LC analysis with UV detection yielded the average recoveries between 84 and 110% (n = 5) with RSD between 4 and 8%.

Continuous ultrasound-assisted extraction coupled to on line filtration-solid-phase extraction-column liquid chromatography-post column derivatization-fluorescence detection was investigated to determine *N*-methylcarbamates in soil and food. The tendency to thermal decomposition of carbamate caused the difficulty for conventional Soxhlet extraction. The extraction efficiencies affected by optimization of the central composite design. The soil or food samples were transferred to the extraction chamber together with glass beads. The mixtures of soil or food were immersed into the water bath at 40 ^{o}C and water as leaching carrier (pH 10) was circulated through solid sample for 2 min under ultrasound radiation (100 W). The selection valve was switched to the filtration-preconcentration manifold to clean up the extracts. The chromatographic separation used a methanol-water gradient at a flow rate of 0.8 ml min^{-1}. The separating column was Ultrabase C_{18} column and the injection volume was 30 μl. The post-column fluorescence derivatization was performed by alkali hydrolysis at 85 ^{o}C and analyzed by fluorescence reagent (OPA). The recoveries of 1 mg kg^{-1} spiked soil were ranged from 77-95%. The detection and quantification limits were12 and 40 μg kg^{-1}, respectively. The relative standard deviations for repeatability and between-laboratory reproducibility were 3.1 and 7.5% respectively (Caballo-López *et al*., 2003).

Microwave assisted micellar extraction could be combined with liquid chromatography for determination of organophosphorous pesticides in soil samples. The techniques were developed for amendments of automation with shorten extraction times and reduction of organic solvent and sample preparation costs. The combination of microwave-assisted micellar extraction (MAME) with nonionic surfactant as micellar media enhanced the fast, low cost, easy handling and non-toxic procedures. Because of difficulty in extraction, the two different surfactants, polyoxyethylene 10 lauryl ester (POLE) and oligoethylene glycol monoalkyl ether (Genapol X-080) were applied on soil contaminated with organophosphorous pesticides. The compounds could be extracted selectively and more quickly with similar or better recoveries. The extracts were analyzed in the conventional UV-LC systems with Nova-Pack C_{18} (150 x 3.9 mm, 4 μm particle diameter) and gradient conditions of methanol-water as mobile phase. The flow rate was fixed at 1 ml min^{-1}.

The two surfactants provided a linear relationship interval 100 – 2,500 μg l^{-1} with the correlation coefficients up to 0.999. The relative standard deviations were equaled and lower than 2.1 and 2.6% for Genapol X-080 and POLE, respectively (Padrón-Sanz *et al*., 2005).

Tor *et al*., (2006) extracted organochlorine pesticides from soil by ultrasonic solvent extraction. The extraction efficiencies were performed in spiked soil samples by different solvents; n-hexane, ethyl acetate, acetone and a mixture of petroleum ether-acetone (1/1 v/v). The slurry of spiked soil samples in each solvent were placed in an ultrasonic bath operated at frequency of 35 kHz and 320 W. The extracts were filtered and filtrate was reduced under vacuum prior to clean-up activated column (30 x 10 mm). The amounts of extracted pesticides were determined by GC-FID. A mixture of petroleum ether-acetone for 20 min of sonication provided satisfactory extraction efficiency. Recoveries of pesticides from fortified soil samples exceeded 88% in three different levels between 15 and 200 μg kg^{-1}.

2.3 Adsorption, desorption and kinetics.

The adsorption of pesticides described as chemically bonding of pesticides to soil. This phenomenon caused weak biodegradability, weakly mobile and finally less toxicity. The adsorption might be influenced by water infiltration and velocity in soil (Mear *et al*., 1996). Carbofuran was known to be a more persistence than the other carbamates, microbial degradation and soil moisture content affected the persistence of carbofuran in soils. (Rammannas, 1988). The half life of degradation of carbofuran in soils ranged from 3 to 50 weeks and the degradation rate was found to be 7 to 10 times faster in alkaline soils (pH 7.9) than in acidic or neutral soil (pH 4.3 - 6.8) (Chaudhry and Ali, 1988).

Varshney *et al*. (1995) studied the adsorption of carbofuran on the surface of antimony (V) arsenosilicate through a thermodynamic approach. The presence of metal ions played an important role in the nutritional status of soils. Owing to their ion exchange behavior of metal ions, they affected a greater retention of metal ions in

soil thus prevent them from entering into plants and ultimately to food chain. The adsorption isotherms obtained from three different temperatures were closely corresponded to Freundlich equation. The *K* and 1/n values decreased with the increase in temperature. The Gibb's free energy could be calculated from equilibrium constant, K^o and the enthalpy change could be calculated from Van't Hoff equation. A negative value of standard enthalpy changes indicated that the carbofuran-exchange interaction was exothermic. The free energy changes were negative and were accompanied by a positive entropy change; the reactions were spontaneous with a high affinity to carbofuran. Antimony (V) arsenosilicate and tin (IV) arsenosilicate were more selective to carbofuran than antimony (V) silicate.

Adsorption mechanisms of carbofuran on silica were studied for structure, kinetics and solubility influenced by Mear *et al*., (1996). Several factors affected adsorption properties of carbofuran such as nature of soil, presence of organic materials, sizes of particles and solubility of pesticides. Silica was selected to determine the adsorption of carbofuran because it was the basic component of sand and has effective specific surface, be sensitive to pH and not really ionic material. The S-shape of adsorption isotherms with two plateaus indicated that the adsorption phenomena belonged to solute-solvent interaction not from solute-solid interaction. The appearance of two plateaus was generally attributed to a re-orientation of molecules on the surface. The molecule of carbofuran was parallel adsorbed to the silica surface because the interaction between free electrons of oxygen atoms of the surface and antibonding π levels of benzene ring. The ΔG^o was positive because K^o was very weak. The adsorption process was endothermic due to the positive sign of ΔH^o.

Carbofuran insecticide contaminated to groundwater by movement through subtropic soils in different temperatures and moisture contents. The behavior assessment model (BAM) and groundwater pollution-potential model (GWP) were used for assessing the relative fate of carbofuran in soil. Dissipation coefficients in two subtropic soils were determined by degradation and adsorption in the soils. The movement of carbofuran was studied by leaching through the soil column. The

degradation of carbofuran under sterilization was analyzed to obtain the residues in soils. Carbofuran dissipated in Luchu clay and Yuanlin silty clay loam soil during incubation period. The dissipation rate increased with increase soil temperature. The percentage of carbofuran residues significantly decreased in Yuanlin silty clay loam compared with Luchu soil. The residues of carbofuran still retained to both types of soil without autoclave sterilization. Microbial degradation was an important role in degradation of carbofuran in soil; carbofuran was used as carbon and nitrogen sources. The dissipation was seamed to correspond with the first order kinetics. The adsorption characteristic corresponded to Freundlich and linear isotherms. The porosity was an important factor affecting the mobility of chemicals with less adsorption in soils (Yen *et al*., 1997).

The lateritic soil occurring from a violent chemical weathering was rich with ferric and aluminum oxides with a red-brown color. The soil became acidic (pH 4-5) and was lower in cation exchange capacity and organic content. Kaolinite was the main clay mineral contents. The adsorption of carbofuran on the selected lateritic soils was conducted by batch experiments to determine the sorption properties of soils. The effect of pH on adsorption was studied with original and toluene and bromoform treated soil (to remove organic matter). The adsorbed amount of carbofuran in original soil was significantly increased by increase pH. The amount of adsorbed carbofuran on the original soil was slightly higher than treated soil at pH 6-8 with similar patterns. The adsorption of carbofuran increased in alkaline soils. The adsorption isotherms corresponded to Freundlich isotherms with the saturated adsorption coefficient, K_d increased with soil organic content and soil/solution ratio. However, the low values of K_d in lateritic soil indicated the low affinity of carbofuran to lateritic soil (Hsieh and Kao, 1998).

2.4 Treatment and remediation

Carbon-14 carbofuran was the important role to identify the degradation mechanism of carbofuran by measuring the volatile products and $^{14}CO_2$. ^{14}C-labeled carbofuran was used as tracer in the continuous flow system and the mineralization of

carbofuran could be determined by ^{14}C-mass balance. Two experimental procedures consisted of moist and flooded conditions of soils with 60 days for incubation time. The degradation products were extracted and analyzed by silica gel (G) thin layer chromatography. The metabolic products were identified by autoradiographic procedures on X-ray film; the R_f values were compared with the authentic standards. The bound residues were combusted in a biological material oxidizer, $^{14}CO_2$ obtained during combustion was collected in an oxysolve cocktail. The amount of $^{14}CO_2$ was analyzed by liquid scintillation techniques. The results showed more bound residues in moist soil then flooded soil. The mineralization was initially slow on moist soil and reached maximum in the day fifteenth and declined 60 days of incubation. The flooded soil provided slow releasing of $^{14}CO_2$ at the beginning, slightly increased in day-15th and maintained at the same level till the end of incubation. The degradation of carbofuran in flooded soils was slightly higher in flooded than moist soils due to the total amounts of $^{14}CO_2$. Carbon-14 was labeled at C-3 position of carbofuran and $^{14}CO_2$ was found as one of the metabolic products, it could conclude that the biological attack occurred on the furan ring (Kale *et al*., 2001).

The influence of repeated annual field application of carbofuran on carbofuran-degrading microbial communities was studied by Trabue *et al*. in 2001. The repeated field application of carbofuran enhanced degradation on the chemical in the soil by increasing in the number of microorganisms resulting rapid degradation compared to untreated soils. Three groups of microorganisms involved in degradation of carbofuran in soil. Group I and II hydrolyzed carbofuran and utilized methylamine, one of the hydrolysis products as carbon and nitrogen sources for growth. Group III utilized aromatic ring as carbon source and was capable to hydrolyze carbofuran and then utilized the aromatic ring of carbofuran phenol, one of hydrolysis products. The treated field plots were annually treated with carbofuran for four consecutive years. ^{14}C-carbofuran was used to determine community sizes for mineralization of aromatic ring structure or hydrolysis of carbamate linkage. The uniformly ring-labeled (URL) ^{14}C-carbofuran phenol was used to estimate the numbers of microorganisms capable of mineralizing the phenolic structure of carbofuran phenol. The annual applications of carbofuran at 4.5 kg $ha^{-1}y^{-1}$ did not increase the size of the microbial community in

the mineralization of URL ^{14}C-carbofuran (carbofuran ring degraders) in surface soil (0-15 cm depth). The community capable of mineralizing carbonyl-labeled ^{14}C-carbofuran (carbofuran hydrolyzers) in the treated surface soil was significantly increased after the second application. Communities of methylamine degraders in treated and untreated soils were much greater than the communities of carbofuran phenol degraders, but not significantly different in the size of carbofuran hydrolyzers.

The organic amendments could be one possibility to control pesticide leaching through in soils. The strength of sorption of pesticides to any incorporated organic matter was greatly depended on the hydrophobicity of the compounds and types of organic matter. Actually, the sorption was more strongly controlled by the organic matter than the clay content or pH (unless the pesticide was strongly cationic). Carbofuran was investigated to examine the possible mechanisms for leaching through the soils including bypass flow, sorption hysteresis and colloidally enhanced transport. Degradation rates were measured in sterile and non-sterile conditions of each soil layer as well as typical groundwater and irrigation waters. Extraction with methanol followed by reversed-phase HPLC was used to determine the degradation rate of carbofuran. The sorption studied was studied in each of the soil layers spiked with ^{14}C-radiolabeled carbofuran. The sorption of carbofuran was controlled by organic carbon content; the degradation was strongly depended on pH. The degradation was protected by acidic organic layer which sorbed carbofuran in its layer (Worrall *et al*., 2001).

Oxidation with ozone, UV radiation Fenton's reagent and advanced oxidation processes (AOPs) were available for degradation of carbofuran. Advanced oxidation processes constituted by combinations of ozone plus UV radiation, UV radiation plus H_2O_2 and UV radiation plus Fenton's reagent (photo-Fenton system). Ozonation of carbofuran agreed with pseudo-first order kinetics. The degradation could be enhanced by combination of ozone and UV radiation generating hydrogen peroxide as the product. The photolysis of hydrogen peroxide with UV generated hydroxyl radicals and hydrogen peroxide accelerated the deposition of ozone to hydroxyl radicals. Hydroxyl radicals played an important role for photolytic

degradation process and attacked organic molecules. The contribution of the radical pathway in the combination of AOP O_3/UV corresponded with the pseudo-first order kinetics. The concentration of carbofuran decreased by photochemical decomposition as a function of time. The combination of ozone and polychromatic UV radiation, UV radiation and hydrogen peroxide enhanced the degradation rate due to the generation of hydroxyl radicals from photolysis of ozone. Fenton's reagent alone had the lower oxidizing power than single oxidant ozone or UV radiation while hydrogen peroxide and ferrous ions enhanced the degradation rate of carbofuran (Benitez *et al*., 2002).

A new Fenton Technology including Anodic Fenton Treatment (AFT) was investigated for treatment of carbofuran through an ion exchange membrane for salt-bridge to improve AFT efficiency. According to Fenton treatment, hydroxyl radicals were also generated from the reaction between ferrous ions and hydrogen peroxide. AFT comprised with two separately half-cells, ferrous ions was generated from electrolysis of an iron anodic electrode, whereas water was reduced in cathodic half-cell. The advantages of AFT over conventional Fenton and electrochemical Fenton treatments were (1) electrolysis delivery of ferrous ions into the treatment system reduced the difficulty of handling hygroscopic ferrous salts (2) the pH of the treated effluent could be partially neutralized by combining effluents from anodic and cathodic half-cells and (3) the treatment efficiencies were quite high because the Fenton reaction occurred in optimal pH in an anodic half-cell. The higher initially concentration of carbofuran caused the higher degradation efficiencies. The degradation of carbofuran by AFT could be enhanced by increasing temperature. The BOD and COD could dramatically removed by oxidative degradation of carbofuran in wastewater. From GC/MS analysis provided the degradation of carbofuran was initially taken place at the carbamate branch followed by C-3 position of the furan ring (Wang and Lemley, 2003).

Photolysis and hydrolysis are two main degradation pathways of carbofuran in the environment. The degradation of carbofuran under artificial ultraviolet and direct sunlight to determine the persistence of carbouran in distilled deionized water and seawater was studied by Campbell *et al*. (2004). The natural

attenuation of carbofuran occurred during four-year interval with difference half-life. Carbofuran-7-phenol occurred as the major metabolic product in Laysan and Ottawa sands and minor products were identified in the field samples. The dissipation of cabofuran was over a period of 112 days in Laysan and 91 days in Ottawa sand at 30 ^{o}C and agreed with first order kinetics in both Laysan and Ottawa sands. The photodegradation in seawater ($t_{1/2}$ 0.1 h) was 31 times faster than in distilled deionized water ($t_{1/2}$ 3.1 h) under 300 nm light exposure. The direct sunlight photolysis in distilled deionized water and seawater was 10-18 times slower than UV (under 300 nm) radiation.

The excitation of iron (III) aqua-complexes was investigated for removal of carbofuran by photodegradation under UV irradiation. The hydroxyl radicals were photo-generated from photo-Fenton systems and then Fe(III) aqua-complex, $Fe(OH)^{2+}$ absorbed a fraction of solar radiation up to 500 nm. The optimum photo-catalytic degradation of carbofuran was observed at 280 nm, and up to 1.5 m W cm^{-2} of light intensity. The degradation of carbofuran rapidly increased with increasing pH value up to 2.8 and decreased at ph above 2.8 at the practical temperature of 25 ^{o}C. The degradation of carbofuran could be occurred within 60 min in the absence of carbofuran, the degradation rate increased with increasing initial concentration of Fe(III). The photo-catalytic reaction of carbofuran with $Fe(OH)^{2+}$ under these experimental conditions agreed with a pseudo first-order kinetics. TOC was initiated to determine the mineralization of carbofuran. It could be observed that the complete mineralization of carbofuran was not achieved after 60 min although carbofuran was absent in the solution after exposure of light. TOC rapidly decreased with increasing time up to 10 h. The photo-metabolic products included 7-hydroxy-2,2-dimethyl – benzofuran-3-one and 2-2-dimethyl-2,3-dihydro-benzofuran-3,7-diol. The mineralization could be explained that the carbamate group was firstly removed and the hydroxyl radicals were continuously substituted one of H atoms at C-3 position of the furan ring. The oxidation occurred by removal of another H atom at C-3 position forming the carbonyl group. The hydroxyl radicals attacked at C-2 position of furan ring leading ring cleavage of the ring and demethylation. The aromatic intermediate

was presumably further oxidized through ring-rupturing position into aliphatic compounds (Katsumata *et al*., 2005).

The influence of environmental variations such as temperature, moisture and microbial activities could affect the degradation and persistence of carbofuran in sandy loam soils for cotton fields. The leaching of carbofuran caused contamination to groundwater under conventional tillage and high soil permeability. Lysimeters were initiated for representing field conditions and were utilized to investigate the fate and behavior of chemicals in soils. Drainage from the lysimeter was collected from 49, 52, 59, 73, 100, 113 and 119 days against the application of carbofuran on days 37, 63, 82, 108 after sowing of cotton. Carbofuran was detected in drainage with the value of 2.34 $\mu g\ l^{-1}$ for no tillage and 13.4 $mg\ l^{-1}$ for tillage. The kinetics of degradation of carbofuran was performed with non-sterile and sterile soils, the disappearance of carbofuran followed first-order kinetics. It could found that temperature and moisture contents significantly reduced the half-life of degradation of carbofuran in both soils whereas microbial activities were not significantly different due to low level of organic matters in the soils. Carbofuran was persisted in each soil horizon of the soil profile from 0 to 150 cm (Tariq *et al*., 2006).

Semiconductor oxides including titanium dioxide and zinc oxide could be investigated as photocatalytic degradation of carbofuran because of their high photocatalytic activities, resistance to photocorrosion and biologically immunity. The degradation of these compounds occurred via the formation of partially oxidized intermediates that could degrade by fragmentation. The pH of solution ranged from 4 to 7 increased the rate of degradation and decreased above pH 7. The surface of the catalyst was protonated at pH below 7 because pH_{zpc} of TiO_2 was 6.8. The carbonyl oxygen in carbofuran molecules were also protonated at the same pH values. Besides protonation, the electrostatic repulsion between the catalyst and carbofuran caused the reduction of both adsorption and rate of degradation. The degradation rate decreased above pH 7 because the negative charge of carbofuran phenoxide repelled the negative charge the catalytic surface. The degradation rate related to the formation of hydroxyl radicals that were the critical species in the degradation processes. The

optimum concentration of carbofuran should be maintained at 200 mg l^{-1}. Above the optimal concentration, the degradation rate decreased due to the insufficient amounts of hydroxyl radicals. The increasing amounts of catalyst up to 100 mg increased the degradation rate because of increasing surface area. The rate decreased at higher amounts of catalyst due to the decrease in light penetration. The incident light intensity up to 64 W caused the excitation of electrons as well as the recombination increase. The wavelength of light at 356 nm caused less slightly degradation rate than 254 nm. The shorter wavelength at 254 nm promoted electrons to the conduction band with higher kinetic energy; they could reach the solid-liquid interface easily suppressing electron-hole recombination compared with 365 nm. The degradation with zinc oxide showed less efficiency the titanium dioxide. Complete mineralization with titanium dioxide as catalyst was found over 16 h. the rapid decrease within 3 h and slow decrease afterwards indicated the formation of intermediate products that resistant to further degradation. The metabolic products and pathways were similar to any other researches (Mahalakshmi *et al*., 2007).

3. Soil colloids: chemical and physical properties

Colloids were highly reactive material with electrically charged surfaces. Because of their extremely small particle size, the colloid possessed the enormous surface areas. The colloid particle carried both positively and negatively charge ions to attack the electrostatic charge species on its surface. The charge ions or organic matters were more tightly adsorbed and no longer mobile, available for plant uptake, reaction to soil solution or leaching loss to the environment.

3.1 General properties of soil colloids

3.1.1 Size. The size of colloid was less than 1 μm in diameter that had tremendous capacity to adsorb water and other substances. The relative size of soil particles could be classified in Figure 6**.**

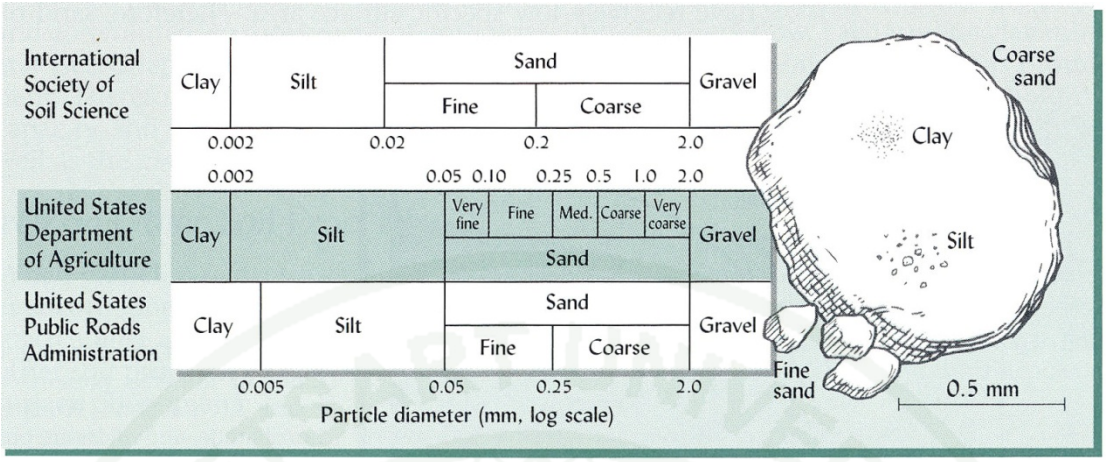


Figure 6 Classification of soil particles according to their sizes.

Source: Brady and Weil (2004)

3.1.2 Classifications of soil colloids. Soils can be classified into four major types. (1) Crystalline silicate clays: -the crystal structures compost with multi-layer like pages of book. Each layer contains two to four sheets of closet packed and tightly bonded between oxygen, silicon, and aluminum atoms. The surface area predominates with negative charges. The kaolinite, fine grained mica and smectite are belonged to this group with intensity of charge, stickiness, plasticity and swelling behavior. (2) Noncrystalline silicate clays; -the tightly bonded silicon, aluminum and oxygen atoms without order of crystalline sheets. The allophone and imogolite are two principal clay types. The properties consist of high amounts of positive and negative charges, high water holding capacity, malleable when wet and low degree of stickiness. (3) Iron and aluminum oxide clays consist mainly of either iron and aluminum atoms coordinated with oxygen atoms that are bonded with hydrogen ions to make hydroxyl groups. Gibbsite (an Al-oxide) and goethite (Fe-oxide) are mostly found in crystalline sheet and oxide minerals are found in noncrystalline structure as amorphous coatings on soil structures. (4) Organic fractions (humus) are mostly in the top soil. It contains convoluted chains and rings of carbon atoms bonded to hydrogen, oxygen and nitrogen atoms. Humus exhibits very high water capacity, no plasticity or

stickiness and low bearing strength because of no cohesive force. It contains high amounts of positive and negative charges with net positive charge.

3.1.3 Fundamental of silicate clay structures. The most important type is silicate layer of phyllosilicates that contain silicon and oxygen in tetrahedral and octahedral sheets.

Tetrahedral sheets consist of two planes of one apical and three basal oxygen atoms with mainly silicon in the space between the oxygen atoms. The basic building block composes of one silicon atom surrounding with four oxygen atoms called tetrahedron. An interlocking array of tetrahedral, each shares its basal oxygens with its neighbor forming tetrahedral sheet (seen in Figure 7).

Octahedral sheets contain the building blocks of the second kind of sheet which construct from six oxygen atoms coordinating with aluminum or magnesium atom at the central of the structure. The shape of an eight-sided geometric solid is called octahedron. The numbers of octahedron units link together horizontally forming octahedral sheets (seen in Figure 7).

The fundamental structural of silicate clay contains tetrahedral and octahedral sheets. Figure 7 showed the stack sandwich-like arrangement of two to four sheets of tetrahedron and octahedron. The nature and combination of sheets in different layers are used to clarify the chemical and physical properties of silicate clays. One unit of silicate clay contains a single eight-sided octahedral unit which aluminum or magnesium is surrounded by six hydroxyl groups or oxygen atoms (variation of each group for the different types of clays).

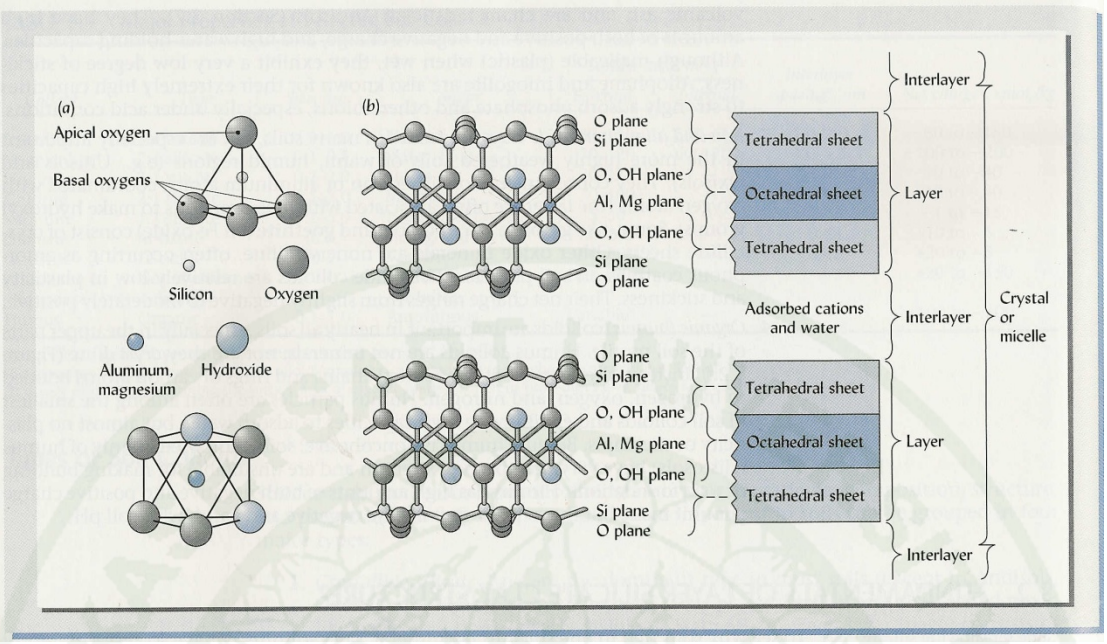


Figure 7 The molecular structure of silicate clays. (a) Single tetrahedral unit of the phyllosilicate minerals contains one silicon atom surrounding by one apical and three basal oxygen atoms. (b) The crystal structure of clay with tetrahedron and octahedron building blocks is connected to silicon and aluminum (or magnesium) ions.

Source: Brady and Weil (2004)

3.1.4 Types of silicate clays. The different types of silicate clays depend on the arrangement of tetrahedral (Si) and octahedral (Al, Mg and Fe) sheets in the crystal units of the clays. The crystalline clays can be classified into two main groups: 1:1 silicate clays -each unit contain one tetrahedral and one octahedral layer and 2:1 silicate clays -each unit contain one octahedral sandwiched between two tetrahedral layers.

(a) 1:1-Type silicate clays. Kaolinite, halloysite and dickite belong to this group which comprise of one tetrahedral and one aluminum octahedral layer. The apical oxygen atoms are tightly bound with aluminum atom in octahedral sheet. The tetrahedral plane is located in the bottom surface but hydroxyl plane is on the

upper surface. This arrangement causes the positive or negative charges of the soil surface because of the addition or removal of hydrogen ions to the soil and the hydroxyl plane can react with any cations surrounded the surface. The adjacent of oxygens in tetrahedral sheet to hydroxyls of octahedral sheets in alternating layer causes the hydrogen bonding between the two layers. Due to the fixed structure of kaolinite, the clay may not expand when the clay is wetted and cations and water cannot enter the internal layer of the clay.

(b) 2:1-type silicate clays. Four types of this group are divided into smectite and vermiculite in expanding-type and the other two of fine-grain micas (illite) and chlorite in relatively nonexpanding.

Smectite group presents a flake-like crystal with mostly negative charge causing from isomorphous substitution of Al^{3+} from Mg^{2+} in octahedral sheet or some substitution of Al^{3+} for Si^{4+} in tetrahedral sheets. The octahedral layer in the top and bottom planes causes very weak oxygen-to oxygen bonding or cations to oxygen linkages providing the expansion of the internal layers by water. The properties of smectite groups are expanding, high degree of plasticity.

3.1.3 Surface area. Soil colloids possessed a large area per unit mass, more than one thousand times surface area of thee same mass of sand particles. Silicate clays also possessed extensive internal surface area between layers of their plate-like crystal units. The only external surface area of colloids ranged from 10 m^2 g^{-1} to more than 800 m^2 g^{-1} with extensive internal surface area.

3.2 Surface chemistry and adsorption reactions.

3.2.1 Surface chemistry. The most soil colloids were predominated electronegative charges although some mineral colloids provided very acid soils with net electropositive charge. The charge on soil surfaces were basically repulsion or attraction the substances in soil solution. Electrical surface charges on soil were basically from (1) formal charge created from isomorphic substitution or protonation

and deprotonation reactions in organic and organic functional groups; or (2) partial charge expressed through polarity of atoms at the crystal surfaces or in neutral organic functional groups.

Adsorption is a process that occurs when a gas or liquid solute accumulates on the surface of a solid or a liquid (adsorbent), forming a film of molecules or atoms (the adsorbate) (Anonymous, 2009). Adsorption was the most important of physico-chemical processes responsible for retention of inorganic and organic substances in the soil colloids. According the charges on the internal and external surfaces, soil colloids attracted and held a large amount of water molecules into their structures. The polar molecule of waters were attracted the opposite polarity and some water molecules were attracted to the exchangeable cations which were hydrated with layers of water molecules. Adsorption of ions on colloids was presented in Figure 8.

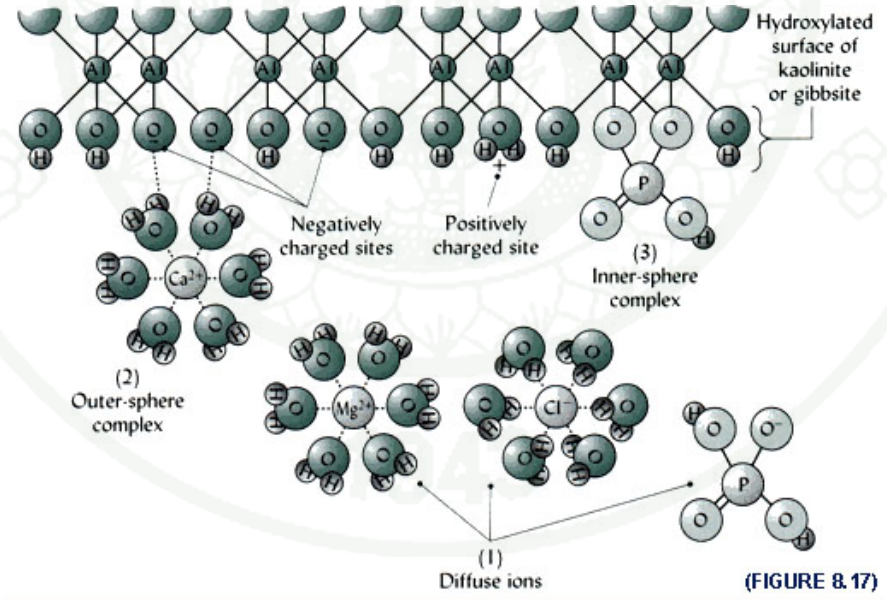


Figure 8 Adsorption of ions on colloid by the formation of outer-sphere and inner-sphere complexes.

Source: Brady and Weil (2004)

Beyond the interlayer surface of colloid, water molecules surrounded diffuse cations and anions (such as Mg^{2+}, Cl^- and $H_2PO_4^-$) in the soil solution. An outer-sphere complex (such as the adsorbed Ca^{2+} ion), water molecules form a bridge between the adsorbed cation and the charged colloid surface. An inner-sphere complex (such as $H_2PO_4^-$ anion) no water molecules intervene and the cation or anion bind directly with the metal atom (aluminum in this case) in the colloid structure.

At equilibrium of adsorption, the amount of substance that had disappeared from the solution phase was assumed to be adsorbed by the solid. The mass distribution of the substances between the solid and solution phases at equilibrium could be characterized by a distribution coefficient:

$$K_d = q/C_{eq} \quad (1)$$

Where q was the equilibrium mass of adsorbed substance per unit mass of adsorbent, C_{eq} was the equilibrium mass of the substance in solution per unit volume of solution and the unit of K_d was volume per mass. The variation in the distribution coefficient with q or C_{eq} was mathematically described the adsorption behavior of a substance in the particular adsorbent (or soil).

3.2.2 The adsorption isotherms. An adsorption isotherm was a graph of equilibrium surface excess or amount of a compound adsorbed (e.g. in unit of mmol kg^{-1} or mg kg^{-1}), designated by q, plotted against the equilibrium concentration of compound in solution (e.g. in units of mmol l^{-1} or mg l^{-1}), designated by C_{eq}, at fixed temperature (termed *isotherm*), pressure, and solution chemistry (pH and ionic strength). There were several different mathematical forms of isotherms.

Langmuir equation defined as the homogeneous of the sorbent surface:

$$q = abC/(1 + bC) \quad (2)$$

where *a*, *b* were constants. The Langmuir equation was developed by the assuming that a fixed number of adsorption site were available and the adsorption was irreversible. The Langmuir equation could be linearized by plotting the inverse values of *q* against the inverse values of *C*.

Freundlich isotherm was an empirical model which could be mathematically expressed:

$$q = KC_f^{1/n} \quad (3)$$

$$q = \text{mass of substances adsorbed (mg)} = \frac{(C_i - C_f) \times V}{\text{mass of adsorbent (mg)}} \quad (4)$$

where V was volume of solution (l), C_i was the initial concentration of adsorbate in solution (mg l^{-1}), C_f was the final concentration of adsorbate in solution (mg l^{-1}) and *K*, *n* were empirical constants. *K* and 1/n could be experimentally determined by logarithmic plot between q (mg g^{-1}) and final concentration of adsorbate in the solution (mg l^{-1}).

3.2.3 Adsorption kinetics. Adsorption kinetics could be explained in two distinct kinetic phases (biphasic kinetics): a rapid and reversible initial stage followed by very slow nonreversible stage. For organic compounds, the rapid phase was characterized by the retention of the compound in a labile form that was easily desorbed. For metals and inorganic ligand, this phase was characterized by easily desorbed, exchangeable forms. For all compounds types, the rapid phase included the retention of the compounds by easily accessible sites on macroparticle and on the edges of layer silicates. The slower reaction phase involved the entrapment of organic compounds in non-labile form that was difficult to desorb. The formation of inner-sphere surface complexes and bond in covalent character occurred during the slow phase. In the slow phase, diffusion of compounds into micropores of the soil organic carbon matrix and inorganic soil components was a rate-determining step.

The entrapment of organic compounds, the diffusion of dissolved ions to adsorption sites within micropores and clay interlayers, and formation of covalent bonds caused the apparent irreversible retention of compounds or ions to the soil. These phenomena leaded to adsorb characteristic of sorption isotherms: desorption hysteresis. Desorption hysteresis could be defined as the apparent increase in the sorption constant (e.g. K_d, K_L, K_F or K_P) in the desorption direction of the equilibrium Brady and Weil, 2004; Essington, 2003 and LaGrega *et al*., 2001).

4. High performance liquid chromatography (HPLC)

High performance liquid chromatography is an analytical technique comprised with two immiscible phases; stationary and mobile. Chromatographic separation was based on specific interactions of the sample molecules with both stationary and mobile phases. The retentive LC achieved through the interaction of the solutes with the support surface or with stationary phase bound to the surface including normal phase, reverse phase and ion chromatography. The non-retentive LC achieved on the basis of solute size through interaction with the pore network of the packing material including size-exclusion chromatography. Affinity chromatography used immobilized biochemical as stationary phase to achieve separation via the “lock” and “key” binding that was prevalent in biological systems.

4.1 Components of a Liquid Chromatograph. The components for HPLC instrumentation included a pump or pumps with pressure gauges and flow meters to deliver the mobile phase through the system; sampling valves and loops to inject the sample into mobile phase; separation column and detector and readout devices.

4.1.1 Mobile-phase delivery systems. HPLC pumps were applied to deliver the mobile phase to the column. The pump was constructed with chemically resistant material to the mobile phase. A degassing unit was necessary to remove the dissolved gases from the solvent. The flow rates of mobile phases ranged from 0.5 to 2.0 ml min^{-1} then the pressure drop up to 6000 lb in^{-2} (414 bar). The reciprocating piston pump was the most popular because of its relatively inexpensive type and

permitting a wide range of flow rates. A flexible diaphragm caused the transmission of hydraulic fluid which minimized the solvent contamination and corrosion problems. The advantages of the pump included (1) variation of flow rate by altering the stroke volume during cycle of the pump or the stroke frequency; (2) continuous solvent delivery; (3) No restriction on the reservoir size or operating time and; accurate gradient elution; (5) available for pulse dampening system and (6) identical for dual-head or triple-head pumps with small pulsation at 180^{o} or 120^{o} angle.

Syringe-Type Pumps characterized as (1) to change the volume by digital stepping water controlled solvent delivery (2) limit solvent chamber capacity (250 to 500 ml) (3) to obtain pulse flow rate (4) high pressure capacity and flexibility for solvent gradients by tandem operation of two or more pumps.

Constant-Pressure pumps drove the mobile phase by pressurize the gas cylinder via a large piston. This type of could be characterized as (1) a low-pressure gas source to generate high liquid pressure; (2) provide pulseless flow; (3) available large flow rates for preparative applications and (4) inconvenient for gradient elution.

Other components of solvent delivery systems included pulse dampers, connecting tubing and sampling valves and loops.

4.1.2 Columns and stationary phases. Separation column was a straight stainless steel in various length (typically from 3 to 25 cm) sometimes coated inside with an inert materials. The internal diameter ranged from 0.5 to 5 mm. Connectors and end fittings must be designed for zero void volume. The guard column acted as a chemical filter to remove strongly retained materials that might destroyed and shorten the life time of analytical column. The mobile phases should be filtered through 0.45 μm micromembrane filter and only HPLC grade-solvents need not be filtered.

The stationary phase was a packing material with uniformly size and mechanically stable. It could be a solid, a liquid or a bonded phase. The bonded-silica

was the phase must be coated on or bonded to particles of a porous solid support. It was prepared from the reaction of alkylchlorosilanes with silica gel in alkali condition. The reactive silanol groups were covalently bound with organic molecules. Bonded stationary phases behaved like liquid so the separation mechanism depended on the partition coefficient instead of adsorption. The polarity of bonded phases could be easily adjusted, constitute the basis of reversed phase partition chromatography. The formation of bonded silica was presented in Figure 9.

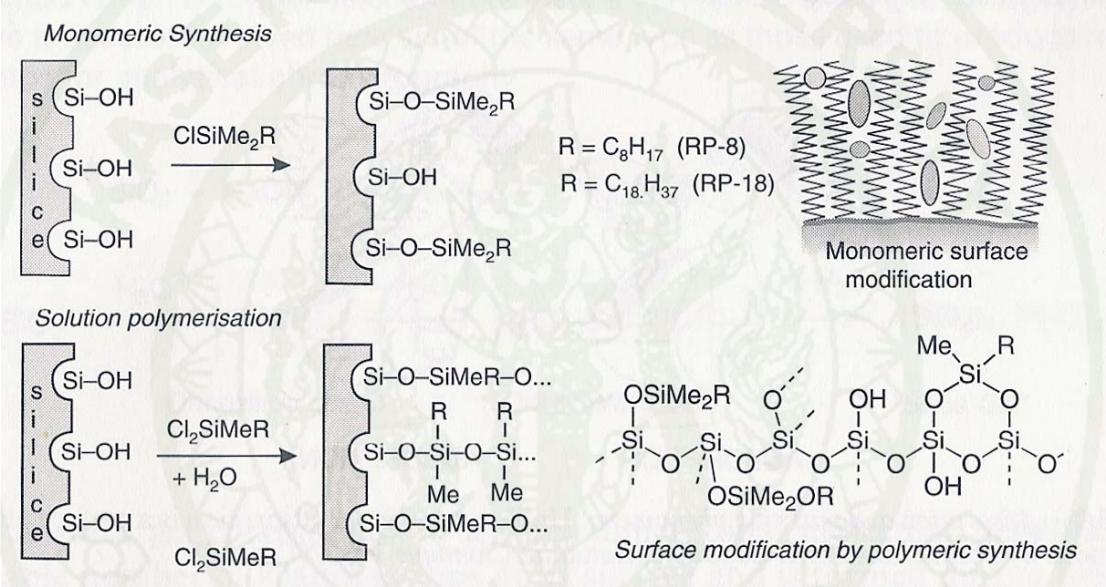


Figure 9 Formation of bonded organosilanes at the interface of silica gel. Representation of organic monomers and polymers at the surface of silica gel. The arrangement Si-O-Si-C was more stable than Si-O-C.

Source: Rouessac and Rouessac (2000)

4.1.3 Ultraviolet-Visible detectors. The principal of optical detectors were basically on ultraviolet absorption in order to detect the compounds that could absorb light in ultraviolet to visible regions. For monochromatic detection, the device consisted with mercury or deuterium light sources, a monochromator used to isolate a narrow band width or spectral line, a flow cell and optical detection usually photomultiplier tube. The intensity of absorption depended on the molar absorption

coefficient of analyte. The chromatogram was plotted between the volume or time (abscissa) and the absorbance (ordinate). The peak areas related to the concentration of analytes in the sample (Christian, 2004; Dean , 1995 and Rouessac and Rouessac (2000).

MATERIALS AND METHODS

Materials

Instruments

1. High Performance Liquid Chromatography (HPLC) consisted of SLC-10A VP system controller, LC-10AT VP solvent delivery modules, SIL-10AD VP autoinjector, CT-10A VP/10AC VP column oven and SPD-10A VP UV-Vis detector. Intersil ODS column packed with 5 μm-packing materials, the dimension was 4.6 x 250 mm equipped with Intersil guard (4.6 mm x 10 mm).

2. Ultrasonic bath, Baudelin Sonorex Super RK 514 BH.

3. Mechanical shaker, Sseriker II Panapolytech.

4. Rotary Evaporator, Buchi Rotavapor R-114.

5. Analytical Balance, sartorious BP 2215 (± 0.0001 g).

6. Centrifuge, Hettich EBA 20 Zentrifugen.

7. Soxhlet extractor (Gerhardt 16).

8. Water purified system, Millipore Simplicity 185.

9. Hot Air Oven, Memmert

10. Sieve mesh (850 nm)

11. Laboratory glassware's such as glass column for sample purification (25 mm x 30 cm), Erlenmeyer flasks, beakers, separatory funnels, glass funnels, centrifuge tubes and any equipment.

Chemicals

1. Carbofuran (99%) was purchased from Dr Ehrenstorfer GmbH (Augsbourg, Germany).

2. All chemicals were high purity reagents including organic solvents: petroleum ether (Lab Scan), hexane (Unilab) and acetonitrile. HPLC grade acetonitrile was purchased from Lab Scan. Water used for HPLC was prepared in house from preparation Millipore Simplicity 185.

3. Alumina (extra pure) purchased from Riedel-de Haën was activated by heating in the hot air oven at 150 ^{o}C for 1 h.

4. Soil samples were collected from the demonstration plot of Bureau of Rice Research and Development, Department of Rice,

Methods

The experimental procedures consisted with soil collection and preparation of soil samples, optimization of soil extraction, analysis of carbofuran residues in soil and adsorption and desorption of carbofuran in soil. The stepwise of experiment was provided in Figure 10.

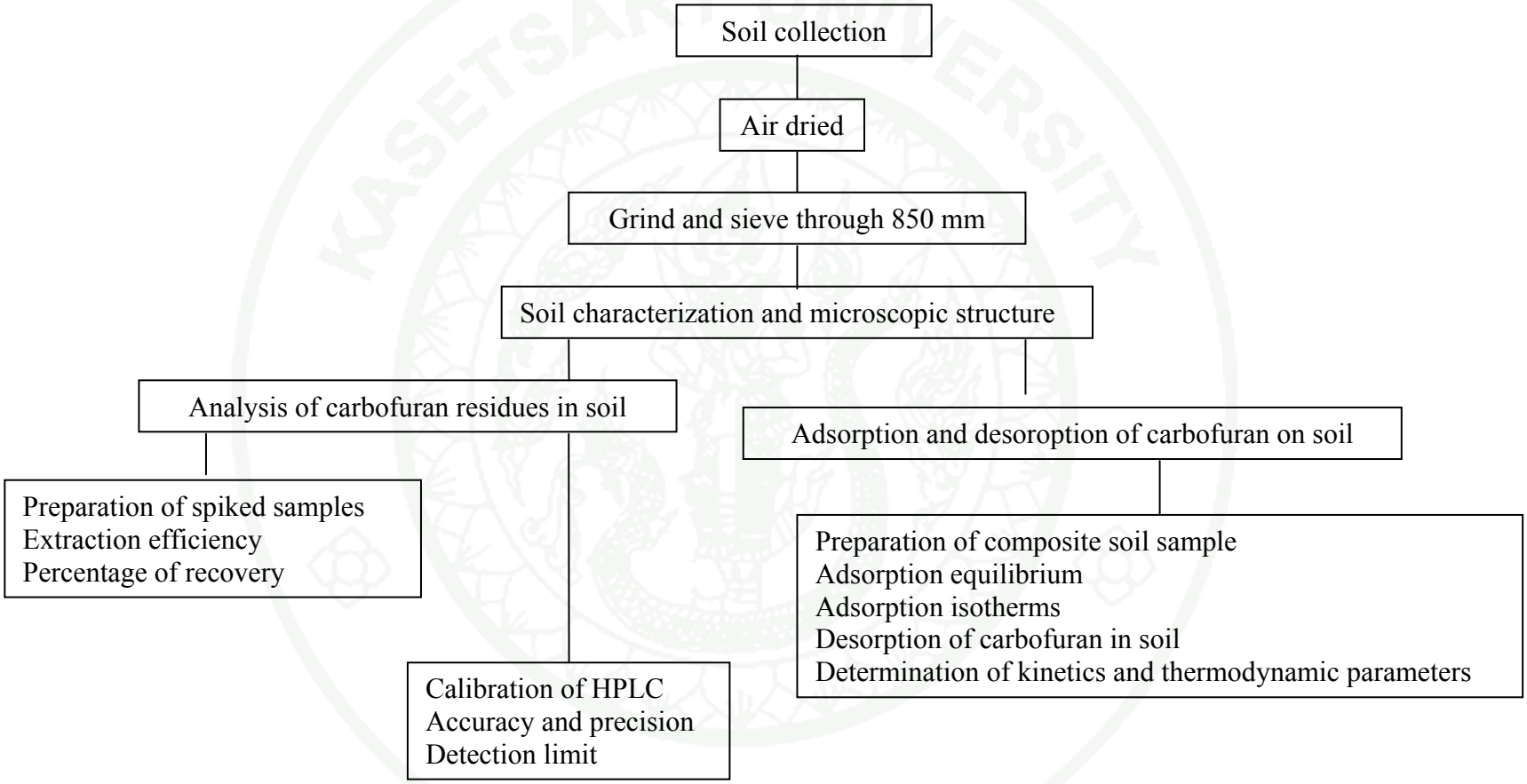


Figure 10 Stepwise of experimental procedure.

1. Site selection for soil sampling and preparation of soil samples

The rice field soils were sampled from the demonstration field of Bureau of Rice Research and Development, Department of Rice, Ministry of Agriculture and Cooperative. The field was located in Kasetsart University, Bangkhen Campus. The field was suitable for studying the physical characteristics of persistence carbofuran since the there was no change in land use of the field itself. All soil was not added or removed except water supply to the field from water channel. Rice was only one crop in this field for more than thirty years and Furadan was repeat applied as nematicde in granule formulation. Each year, the first crop started on May to October and the second crop started from November to April. The soil was considered to be completely homogeneous because of continuously plowing twice a year for more than 30 years. Soil samples were collect at the beginning of the first and second growing season before cropping. Nine sampled-points in the first field and five sampled-points in each second to fifth field (Figure 11) were collected for rice field soil around 0-20 cm depth on October, 2006, May and November, 2007. The first field was used as reference field for determination of adsorption and desorption of carbofuran from soil because it was isolated from any contaminations.

The second field located close to open dumped garbage in the west direction which could generate leached during rain fall. The third, forth and fifth field located close to household wastewater discharge in the east direction. The soils were air-dried and then ground and sieved through 850 nm screen. The ground soil samples were kept dryness until the analysis time.

2. Soil characterization

Some of ground soil samples were homogenized as composite soil samples and test for soil characteristics (percentage of sand, silt and clay, organic matter, Cation Exchange Capacity, CEC) at Department of Soil Sciences, Faculty of Agriculture, Kasetsart University. The physical properties of soil would be seen in Table 3. The microscopic structure of soil colloid was examined by Scanning Electron

Microscope, SEM equipped with Energy Dispersive Spectrometer (Phillips: XL30 & EDAX) at Department of Material Engineering, Faculty of Engineering, Kasetsart University.

Ground soil samples were placed on Pirani 501 device (0.5 x 0.5 cm^2) for SEM and EDS analysis. Electron beams were accelerated at 15 kV and the counting time was 100 seconds. The microscopic structure of soil colloid from Scanning Electron Microscope is provided in Figure 13a. The semi-quantitative analysis of soil compositions are given in Figure 13b.

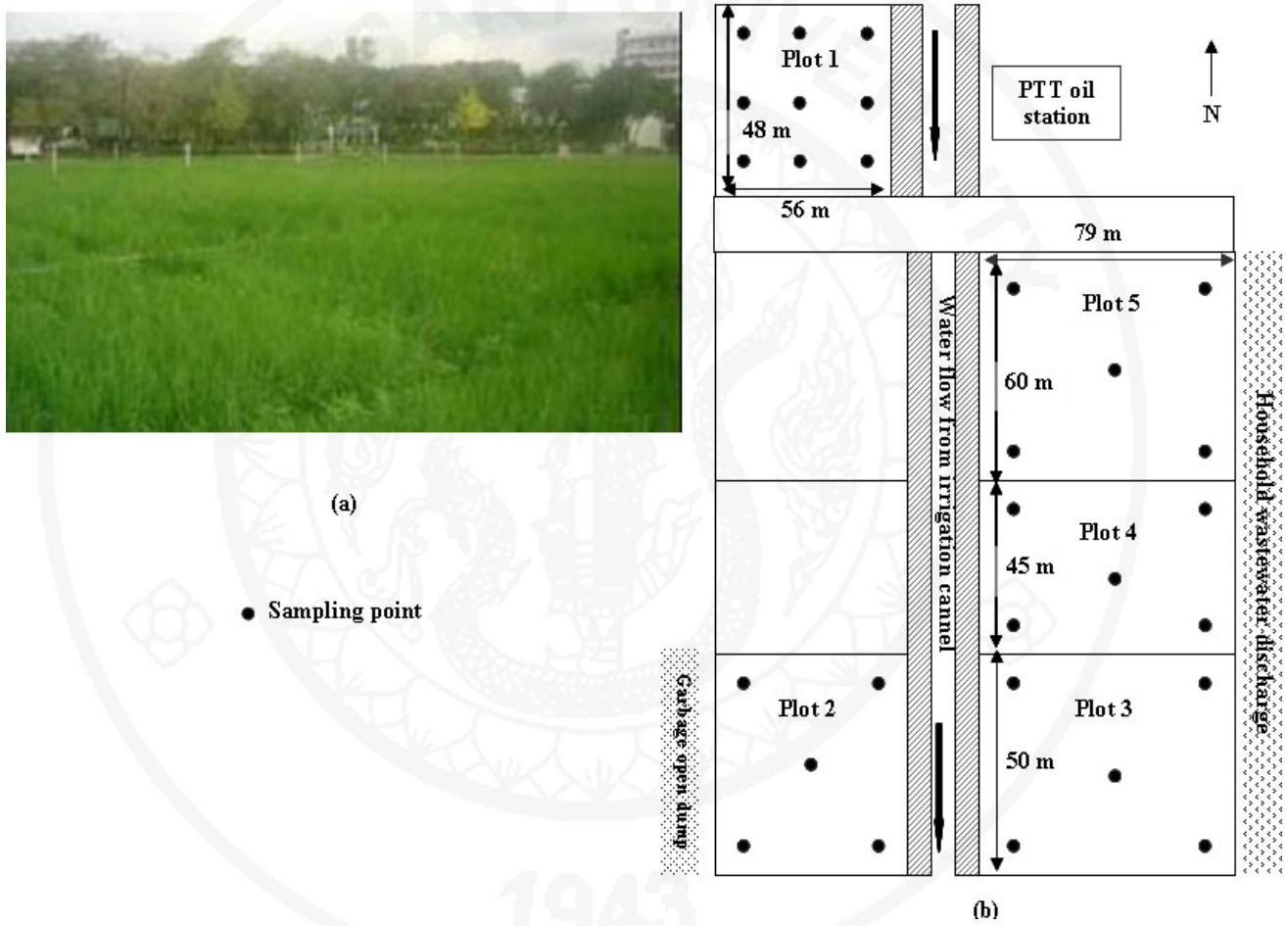


Figure 11 (a) The demonstration rice fields of Bureau of Research and Development Department located in Kasetsart University. (b) The field included five sampling sites for soil collection. The first one was used as a reference site since the location was far away from household wastewater discharge and garbage open dump.

3. Optimization of soil extraction

To determine the amounts of pesticides in soil, two extractive methods; ultrasonic and Soxhlet extraction would be investigated for evaluation of extraction efficiencies as well as time and chemical requirement**.** The selected method was considered from the satisfied efficiency, shorter time requirements and fewer chemicals used. The selected method would be provided for extraction of cabofuran in soil samples. The chromatographic methods for analysis of carbofuran must be examined for good calibration with high precision and accuracy.

3.1 Preparation of spiked samples

The method of soil sample spiking would be modified from Tor *et al*. (2006). Dried soil samples were ground and sieved through 850 micrometer screen and then kept in the desiccators until spiking procedures. Spiked soil samples would be prepared by adding a fixed amount of standard solution of pesticide into 10 grams of dried soil. Ten milliliters of acetone was added to the spiked soil sample, the suspension was mixed for 30 min by a mechanical shaker. After evaporation of solvent at room temperature, the sample was kept in stopper conical flask and stored in darkness at 4 ^{o}C for three days. Spiked soil samples were used to determine the efficiencies and recoveries from two extractive methods.

3.2 Calibration of High Performance Liquid Chromatography

Analytical conditions for carbofuran measurement were modified from Katsumata *et al*. (2005) by using Intersil ODS (4.6 x 250 mm) as analytical column. The eluant was prepared from various ratios of acetonitrile-water (v/v) mixture. The flow rate was varied from 1.2 – 1.5 ml min^{-1}. The signal of carbofuran was detected with UV detector at 210 nm. The chromatogram of carbofuran was presented in Figure 14.

Calibration of High Performance Liquid Chromatography was performed by injection of five concentration ranges of pesticide; 5, 10, 15 20 and 25 mg l^{-1} prepared from dilution of 100 mg l^{-1}-carbofuran solution. The normalization provided from the methods of Skoog *et al*. (1996) and Miller and Miller (1984) in Appendix Table A1. The calibration graph was used to determine detection limit for HPLC. Calibration data were provided in Table 4 and linear regression of the graph between concentrations (mg l^{-1}) of carbofuran (abscissa) against the peak areas (ordinate) was presented in Figure 15.

The precision of measurements was investigated by injecting five replication of standard carbofuran solution at the concentration of 15 mg l^{-1}. The average concentration was calculated from the linear regression line of set of standard solutions, the observed concentrations were compared with the concentrations of standard solutions. The precision was determined from the standard deviation of the observed concentration of five replications.

3.3 Ultrasonic extraction

A mixture n-hexane/acetone or petroleum ether/acetone (1/1 v/v) will be used as extraction solvent. Each ten gram of spiked soil was extracted with 20 milliliter of solvents and sonicated in ultrasonic bath for 20 min. The extracts were filtered through Whatman filter paper and dried under vacuum at 40 ^{o}C to dryness by rotary evaporator. The dried sample was added to exactly 1 ml with acetone prior to clean-up column.

3.4 Soxhlet extraction

Ten grams of spiked samples were introduced into the extraction thimble and extracted with 150 milliliters of petroleum ether/acetone mixture (1/1 v/v) for 4 hr in Soxhlet extractor. The extracted solution was filtered and concentrated to exactly 1 ml prior to clean-up column.

Three replications would be performed for each extraction method.

Before analysis, the extracts had to be cleaned up to eliminate the co-extracted substances which were interfered the analysis and deteriorated the column. The clean-up column was slurry packed with activated alumina in 30-cm long and 10-mm internal diameter column. The 1-ml extract was eluted through alumina column with 100 ml of *n*-hexane:ethyl acetate (7/3 v/v). The eluant was dried under vacuum to dryness and added to exactly 1 milliliter of solvent. The purified samples were analyzed with High Performance Liquid Chromatography.

The extraction efficiencies of ultrasonic were compared with Soxhlet extraction to determine the percentage of recoveries in Table 5. The experimental means from two extractive methods were compared in Appendix Table A2. The selected ultrasonic extraction was performed by spiking of carbofuran in the concentration ranged from 10 – 40 $\mu g\ g^{-1}$ carbofuran soil and the percentages of recoveries were compared in Table 6.

4. Analysis of carbofuran residues in rice field soil

The amounts of carbofuran in contaminated soils were extracted using the selected method and the concentrations were analyzed by the well calibrated HPLC. Carbofuran residues during three consecutive growing seasons were summarized in Table 7. Comparison of carbofuran residues in rice field soils in the three collected period; October 2006, May 2007 and November 2007 were provided in Figure 17.

5. Adsorption and desorption of Carbofuran in soil

Adsorption and desorption of carbofuran in soil were performed by batch methods. The soil sample collected from nine sample points of plot 1 were homogenized as composite sample for adsorption/desorption experiments. Since adsorption was dynamic equilibrium, adsorption isotherms must be examined after adsorption equilibrium. The adsorption data was available to determine kinetic

parameters. Desorption was physical phenomena that reduced the residues from soil sorbent. Experimental variable conditions for determination adsorption and desorption of carbofuran in were concluded in Figure 12.

5.1 Adsorption equilibrium

Composite soil sample was prepared from dried-ground soil collected from nine sample points in plot 1. As explanation in the previous sections, plot 1 is not contaminated by any other pollutants except pesticides and fertilizer applied to the field, the soil is then selected for adsorption and desorption.

The equilibrium time for adsorption equilibrium was performed by adding 50 mg l^{-1} in ten-ml aqueous solution of carbofuran into each one gram of composite soil in each 125-ml Erlenmeyer flask. Each flask with the same initial concentration of carbofuran was mechanically shaken at 1,500 rpm for 2, 4, …, 24 h at room temperature. Each fixed time interval, the suspensions were centrifuged at about 3,000 rpm for 15 min. The supernatants were filtered through 0.22 micron filter and determined the concentration of carbofuran by HPLC. The amounts of adsorbed carbofuran were calculated from the different between the initial concentrations and the concentration in the supernatant liquids. Determination of equilibration time was calculated in Table 8. The equilibrium time for adsorption was determined by the graph between the adsorption time (x) and amounts of adsorbed carbofuran on soil (y) seen in Figure 18. The equilibrium time would be applied for determination of adsorption and desorption of carbofuran on soil.

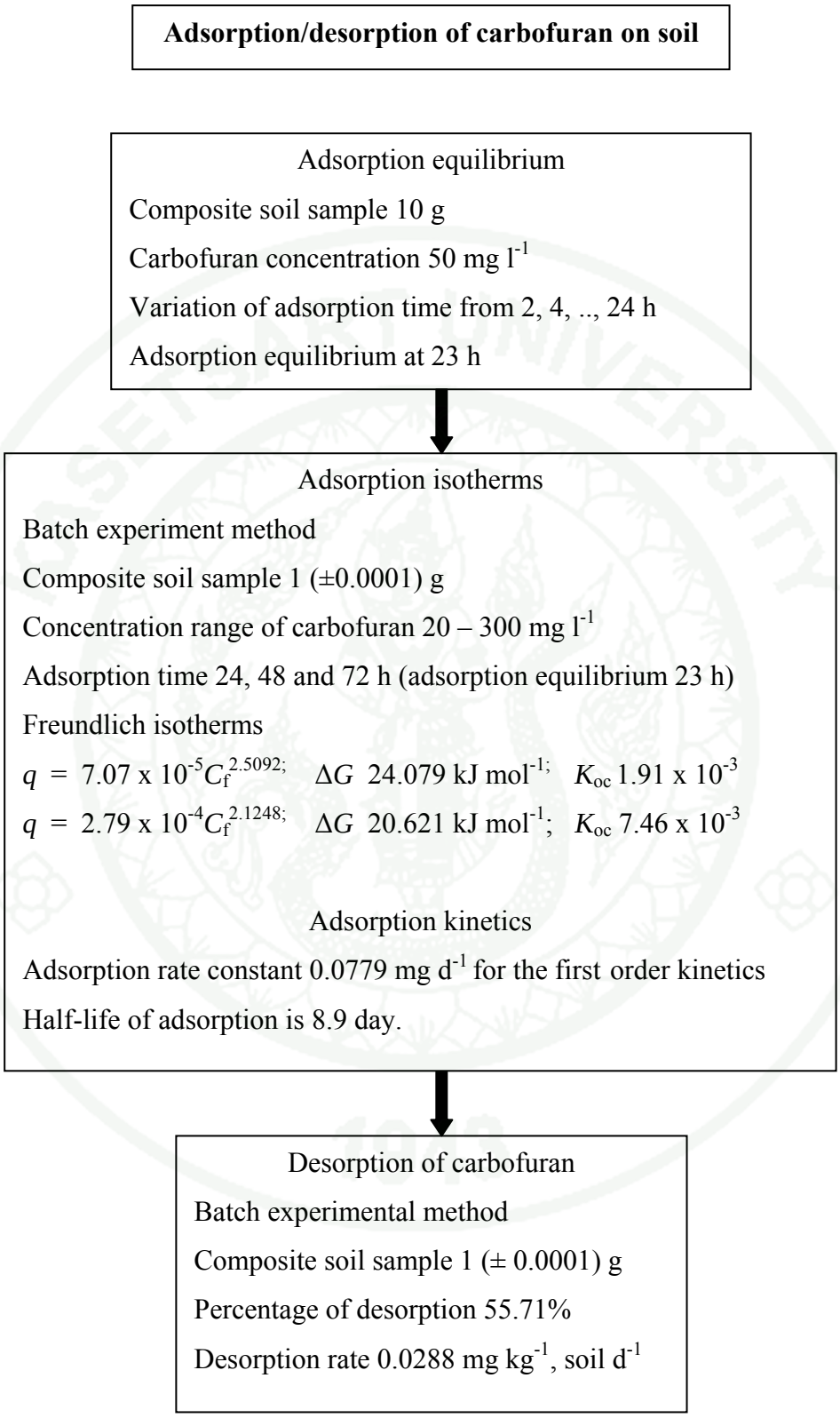


Figure 12 Flow diagram of adsorption/ desorption of carbofuran in soil.

5.2 Adsorption isotherms

Adsorption isotherms were obtained using the batch equilibrium methods. Each one gram of soil sample was weighted into 125-ml polyethylene bottle and then filled with ten-ml aqueous solution of carbofuran at the initial concentrations ranging from 20 to 300 mg l^{-1}. The samples were prepared in five sets of concentration ranges to determine the effect of initial concentrations to the isotherms. The five sets of different concentration ranges were also prepared with the similar method above. The suspensions were mechanically shaken at 1500 rpm for 24, 48 and 72 h at room temperature. After shaking, the suspensions were centrifuged at about 3,000 rpm for 15 min. The supernatants were filtered through 0.22 micron filter and determine the final concentration (C_f) by HPLC. The adsorption data were calculated using logarithmic model from Freundlich isotherm presented in Appendix Table C1 – C3 and Figure 19 - 20. Percentages of adsorption would be shown in Table 9.

The adsorption kinetics of carbofuran would be calculated from the data obtained from different amounts of carbofuran adsorbed in soil in different adsorption times. The adsorption parameters were summarized in Table 10.

5.3 Desorption of carbofuran

The solution of carbofuran with 50 mg l^{-1} was added into each one g of ground soil and mixed well. Each suspension was mechanically shaken at 1,500 rpm for 10 h. After shaking, all of the suspensions were centrifuged at about 3,000 rpm for 15 min. The supernatants were filtered through 0.22 micron filter and determine the concentrations of supernatant liquids by HPLC. The initial concentrations of adsorbed carbofuran on soil were calculated form different between the initial concentration of aqueous carbofuran solution and the concentration of carbofuran in the supernatant liquids. Each soil residue was then filled with ten milliliters of deionized water and continue shaken at 1,500 rpm for 2, 4, 6, …,24 h. Each two h, the suspension was removed and centrifuged at 3000 rpm, the supernatant was determined for amount of carbofuran desorbed from soil. The desorption was characterized by the graph

between the desorption times (x) and amounts of desorbed carbofuran (y) (Figure 21) from data in Table 11. The desorption rate performed by normalization of logarithmic plot of concentrations and times in Figure 22.

RESULTS AND DISCUSSION

1. Physical properties of rice field soil

Physical properties of rice field soil were provided in Table 3. The soil pH was 5.5, 3.74% of organic matters and Cation Exchange capacity, CEC of 23.0 cmol kg^{-1}. The soil texture was 9.6% of sand, 33% of silt and 57.4% of clay; it could be classified as clay by textural triangle (Brady and Weil, 2002). The organic matter content in this field was slightly low at 3.74% because of continuous plowing and growing (twice a year) and no additional of organic materials. Type of soil was classified by soil map and soil classification by Land Development Department as Bang Khen series; Bn. The microscopic structure and composition of soil colloid were presented in Figure 11. The image was similar to flake-like structure. The spot of x-ray spectrum showed the major components of silicate soil contained silicon, oxygen and aluminum. This soil possessed similar properties as smectite groups due to aluminum was dominated in tetrahedral sheet and it seemed to be classified as Vertisols; Swelling Clay Soils (Brady and Weil, 2004). Because of its interlayer expansion, water molecules and ionic species in aqueous phase can be accessible into its internal surface area of dry soil. It possessed relatively negative charge from isomorphous substitutions of Al^{3+} for Si^{4+} in tetrahedral sheet (Brady and Weil, 2004 and Essington, 2003). Adsorption of water to interlayer of soil leaded to high swelling when wet and shrinkage when it dried. Wide crack normally appeared from changing in soil volume.

Table 3 Physical properties of rice field soil.

Properties	Values
pH	5.5
Organic matter (% by weight)	3.74
Sand (% by weight)	9.6
Silt (% by weight)	33
Clay (% by weight)	57.4
Cation Exchange capacity (cmol/kg)	23.0

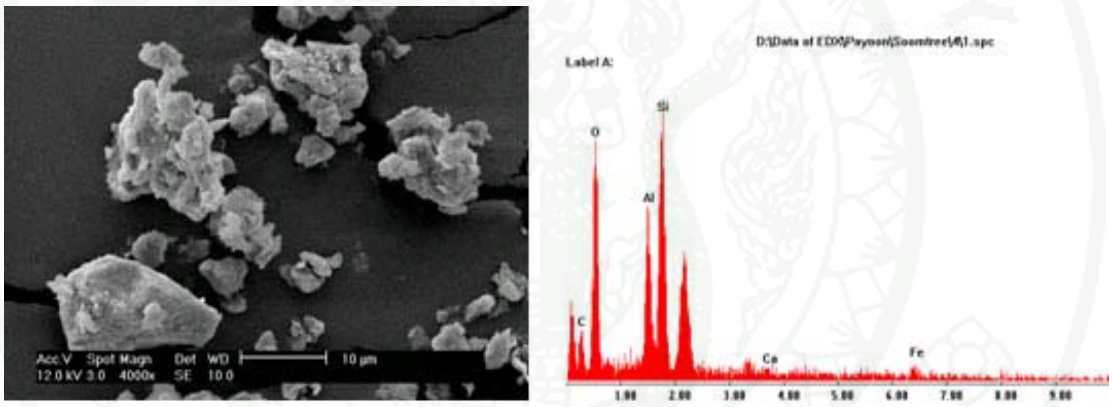


Figure 13 (a) Microscopic image of soil colloid. (b) X-ray spectrum of soil colloid.

2. Calibration of HPLC and efficiencies of extraction

2.1 Determination of retention time from separation with reversed phase HPLC

Besides the variation of compositions and flow rates of mobile phase, the optimum conditions were 50% (v/v) acetonitrile:water with flow rate 1.2 ml min^{-1}. The chromatogram of carbofuran indicated the single peak at 6.4 min (Figure 14). The

linearity of the methods showed the calibration line; y = 820557x + 938462; while y presented for peak area and x represented concentration of carbofuran. Method of least square fit for linear regression line was calculated from the relationship between concentration of carbofuran (x) and peak areas obtained from the chromatograms (y). The calibration data showed in Table 4 and calibration graph was in Figure 15. The high regression coefficient (0.9955) indicated that all coordinate values corresponded to the line. The detection limit of measuring method was 282.83 μg kg^{-1}. Calibration of reversed phase LC was calculated in Appendix Table A1.

The concentrations of five replication measurements were 15.12, 15.23, 14.99, 15.01 and 15.00 mg l^{-1} with the average value was 15.07 ± 0.09 mg l^{-1}. The relative error was calculated from [(15.07 – 15.00)/15.00] x 100 = 0.47%. The calculated relative error lies within ± 5% indicated the high accuracy while the small value of standard deviation indicated the high precision. The measuring conditions were suitable for analysis of carbofuran residues in soil samples. Analysis of blank ultrapure-water and bare soil did not provide any responses to interfere the retention time of carbofuran.

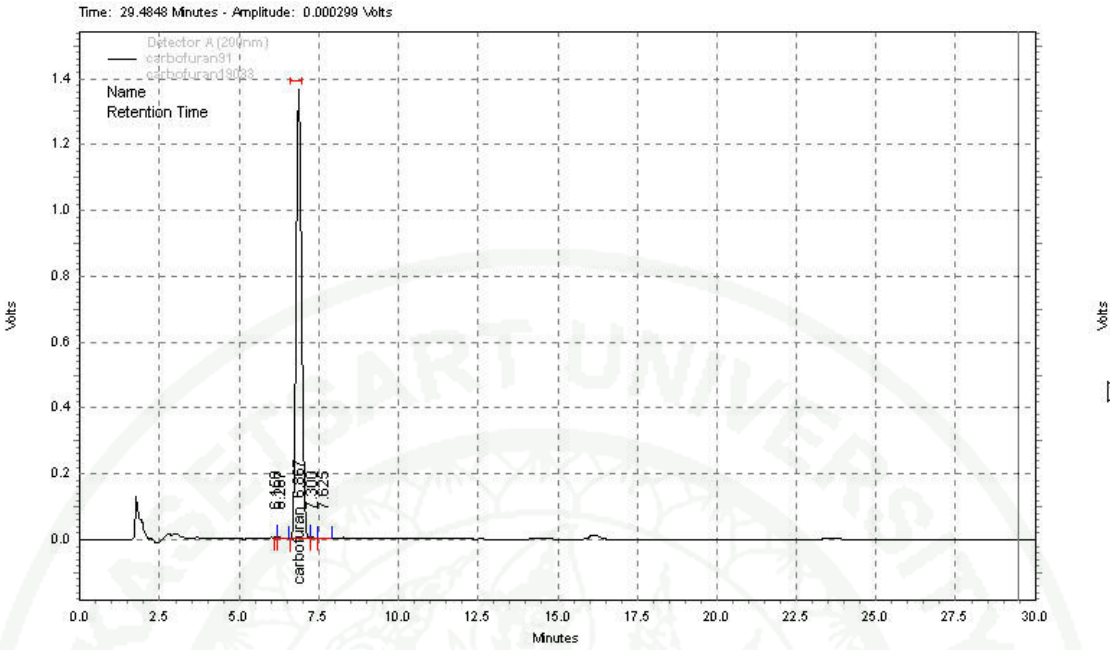


Figure 14 Chromatogram of carbofuran showed the retention time at 6.4 min. The separation was obtained by Intersil ODS column (4.6 x 250 mm) assisted with UV detector at 210 nm, the eluant was 50% acetonitrie:water at the flow rate of 1.2 ml min^{-1}.

Table 4 Calibration data for linear regression line.

Concentration of carbofuran (mg l^{-1})	Peak areas
5	4712473
10	9093867
15	13869279
20	17570318
25	20988184

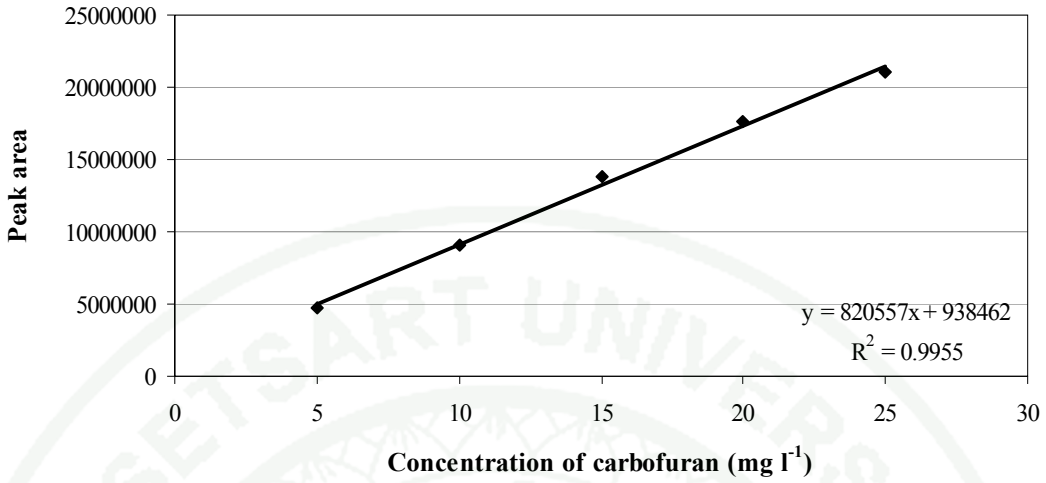


Figure 15 Calibration graph of carbofuran performing from a series of standard solutions of 5, 10, 15, 20 and 25 mg l^{-1} and the peak areas. The linear regression shows the slope of 820557 and the intercept of 938462

2.2 Percentage of recovery of carbofuran in spiked soil samples.

The extraction efficiencies of Soxhlet for five replications were compared with ultrasonic extraction at 20 mg kg^{-1} of carbofuran. The results in Table 5 were compared between ultrasonic and Soxhlet extractions.

Percentage of recovery for Soxhlet extraction was 85.129% (17.0258/20 x 100) while ultrasonic extraction was 74.499% (14.8998/20 x 100). Ultrasonic extraction provided high precision than Soxhlet extraction. The extraction efficiency of Soxhlet extraction was significantly different from ultrasonic extraction; the calculation could be seen in Appendix Table A2. Although the extraction efficiency of Soxhlet was higher than ultrasonic extraction, the ultrasonic extraction with petroleum ether:acetone may be applied for extraction of sample.

Table 5 Amounts of carbofuran between two extraction methods.

Replicate	Amount of carbofuran (mg kg^{-1})	
	Soxhlet extraction	**Ultrasonic extraction**
1	16.784	14.876
2	16.424	14.505
3	17.306	15.171
4	16.932	14.773
5	17.683	15.174
Mean	17.0258 ± 0.4847	14.8998 ± 0.2834

The efficiencies in the different concentrations of carbofuran indicated that the efficiency was slightly higher in lower concentration (10 mg kg^{-1}) and almost similar from 20 to 40 mg kg^{-1}.Ultrasonic extraction was selected for analysis of carbofuran residues in soil because it exceeded the screening criteria: especially satisfied efficiency, less consumption of organic solvent, short time requirement and economics.

Table 6 Percentage recoveries of carbofuran from ultrasonic extraction.

Weight of soil (g)	Measured conc. of carbofuran from HPLC ($mg\ l^{-1}$)	Weight of solution (g)	Amounts of carbofuran (mg)	Observed concentrations of carbofuran ($mg\ l^{-1}$)	Spiked carbofuran ($mg\ l^{-1}$)	% Recovery
9.9934	72.9535	1.1570	84	8.44	10	84
9.9946	132.3947	1.1570	153	15.33	20	77
10.0007	212.2644	1.1076	235	23.51	30	78
10.0003	253.2647	1.2140	307	30.75	40	77

3. Carbofuran residues in rice field soil.

Carbofuran residues in rice field soil were analyzed during three consecutive growing seasons; October, 2006, may, 2007 and November, 2008. The analysis data were given in Table 7. Also carbofuran was prohibited for more than 5 years ago, residue concentrations still remained. Many reasons support this persistence; first, since soil pH was 5.5, carbofuran is stable in acidic condition (Siddaramappa and Seiber, 1979); second, the persistent of carbofuran in rice field soil could be described by the low air-water partition coefficient, K_{aw} of 1.23 x 10^{-7} (de Melo Plese *et al*., 2005) indicated that carbofuran preferred to dissolve in aqueous phase ($K_{aw} \leq 4.0$ x 10^{-6}) and not volatile to gaseous phase under atmospheric temperature (Trapp and Matthies, 1998); third, persistence of carbofuran generated from physical attraction of carbofuran into the interlayer of silicate clay and chemical bonding of carbamate with the ionic sites of silicate clay. Carbofuran possessed its molecular area of 42 $Å^2$ (Figure 16), the molecular structure was almost planar from benzene ring bound with furan ring and the third pseudo ring occurred from H-bonding between hydrogen atom of N-carbamate and oxygen atom of furan ring. The new orientation can be seen in Figure 16. The molecular structure turned to completely planar and it was able to parallel adsorbed to the interlayer of silicate structure. Basal oxygens from octahedral sheet potentially interacted with π electrons in antibonding levels of benzene ring. carbofuran was paralleled to the silicate clay surface because free electrons in oxygen atoms of oxygen-plane in silica layer of silicate clay interacted with antibonding π electrons of benzene ring. The absorbed molecule seemed to loose its third pseudo ring in the surface then hydrogen bound to nitrogen atom forming hydrogen linkage with the surface in different conformation. It could be concluded that one possibility of persistence of carbofuran in silicate clay generated from the bound residues itself with silicate clay (Mear *et al*., 1996); forth, photochemical reactions significantly occur only on soil surface (Scheunert *et al*. 1993); fifth, when the soil is directly exposed to sunlight, temperature exceeds more than 35 ^oC, it may increase the accumulation of the pesticides at the soil surface by convection movement of evaporating water (Bowmer, 1991) and sixth, the lack of oxygen presented in

anaerobic conditions caused the lesser biodegradation by aerobic microorganism (Redondo *et al*., 1994).

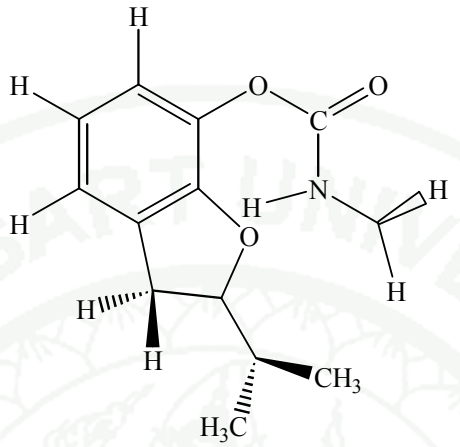


Figure 16 Reorientation of free molecule of carbofuran according to ChemDraw software. The molecular structure was almost completely planar and the molecular area was about 42 $Å^2$ (Mear *et al*., 1986).

However, carbofuran residues were found in rice field soil, it was highly accumulated in stems and leaves of rice (54.4 $\mu g\ kg^{-1}$) dry weight. Accumulation of carbofuran was highest in stems and leaves of the other grass crops such as sunflower, cabbage, cattail and Chinese-kale. The circumference and height of rice (*Oryza sativa* L.) growing in soil contaminated with carbofuran showed similar physical appearance compared with control (rice grew in non-contaminated soil) (Teerakun and Reungsang, 2005).

Table 7 Summarized of carbofuran residues during three consecutive growing seasons; October, 2006, May, 2007 and November, 2008.

Plot no.	Concentration of carbofuran (mg kg^{-1})			
	Oct, 2006	**May, 2007**	**Nov, 2007**	**Mean**
1	0.171	0.162	0.114	
	0.186	0.170	0.086	
	0.214	0.136	0.085	
Mean	**0.190 ± 0.022**	**0.156 ± 0.018**	**0.095 ± 0.017**	**0.147 ± 0.033**
2	0.430	0.376	0.253	
	0.482	0.390	0.328	
	0.470	0.407	0.288	
Mean	**0.461 ± 0.027**	**0.391 ± 0.016**	**0.290 ± 0.037**	**0.380 ± 0.049**
3	1.157	1.189	1.031	
	0.995	1.120	1.134	
	1.164	1.177	1.130	
Mean	**1.105 ± 0.096**	**1.162 ± 0.037**	**1.098 ± 0.058**	**1.122 ± 0.118**
4	2.330	1.852	1.933	
	2.244	2.240	1.814	
	2.171	1.917	2.287	
Mean	**2.249 ± 0.080**	**2.003 ± 0.208**	**2.012 ± 0.246**	**2.088 ± 0.332**
5	4.275	2.993	3.016	
	4.092	3.221	3.344	
	4.547	3.354	3.421	
Mean	**4.305 ± 0.229**	**3.189 ± 0.183**	**3.262 ± 0.213**	**3.585 ± 0.362**

The accumulation of carbofuran was lowest in the first field since it was isolated from any others additional materials except water supply from water channel.

The second field was about two times higher than the first one because the garbage open dump was located at the west site. They would occasionally generate wastewater leachate to the plot. The residues in plot no. three, four and five was greater than the first and second field since this may be affected by organic materials from the household wastewater discharge nearby. The higher clay and organic matter contents may enhance the accumulation of carbofuran in soil also reported by Hsieh and Kao (1998). For twice plowing per year, the soil was directly exposed to sunlight not more than two months since the main degradation pathways are hydrolysis and photolysis (Campbell *et al*., 2004; Soler *et al*., 2006), carbofuran could be broken down with less exposure of sunlight in rice field. From Figure 17, carbofuran residue decreased from the first to the third growing season (October 2006 to November 2007) might be because soil tillage and drainage of water. Soil tillage might increase exposure of soil to sunlight enhancing photodegradation of carbofuran. In this case, carbofuran breakdown could be slow from the lack of direct sunlight exposure in rice field.

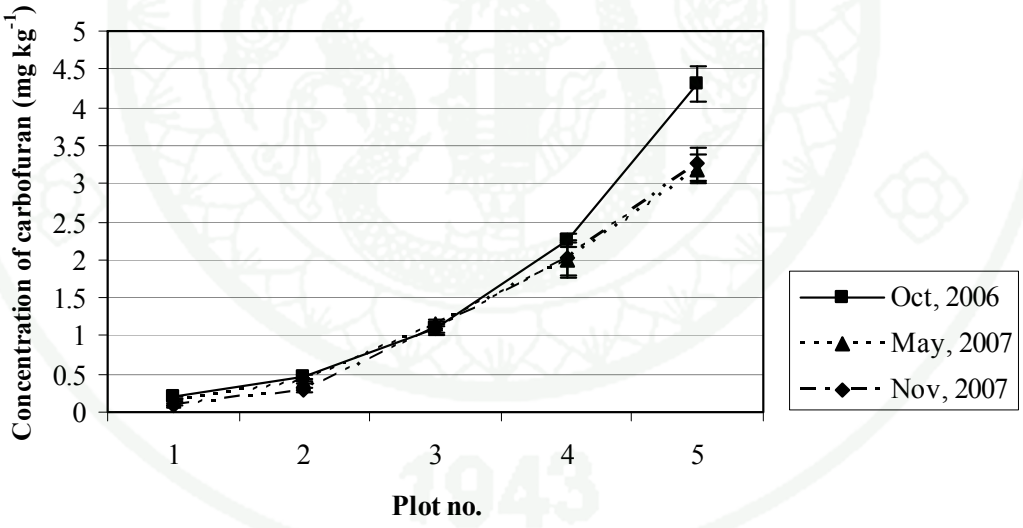


Figure 17 Comparison of carbofuran residues in rice field soils in the three collected period; October 2006, May 2007 and November 2007.

The persistent of carbofuran in rice field soil could be described by the physicochemical properties as followed: the low air-water partition coefficient, K_{aw} of 1.23×10^{-7} (de Melo Plese *et al*., 2005) indicated that carbofuran preferred to dissolve in aqueous phase ($K_{aw} \leq 4.0 \times 10^{-6}$) and not volatile to gaseous phase under

atmospheric temperature (Trapp and Matthies, 1998). The carbon organic partition coefficient, K_{oc} was used to determine the lipophilicity in the assessment of both the uptake and physiological distribution of carbofuran and prediction of their environmental fate (IUPAC Glossary of Terms Used in Toxicology, n.d.). K_{oc} of cabofuran was calculated from Farahani's equation (Farahani *et al*., 2007)

$$K_{oc} = K_d \times 100/OC \quad (5)$$

The calculated values of K_{oc} were 1.91×10^{-3} and 7.46×10^{-3} mg l^{-1}which indicated the low affinity with soil particles and very high mobility to soil solution. The low affinity in soil particle might be due to low content of soil organic matter. Since water supplied to the field was almost standing water; carbofuran may be re-adsorbed to soil colloids (de Melo Plese *et al*., 2005). The Groundwater Ubiquity Score, GUS index, was based on graphical examination of a plot formed by two widely available pesticide properties: half-life in soil ($t_{1/2}$ soil) and K_{oc} (Gustafson, 1989). GUS index could be determined by del Melo Plese *et al*., by:

$$GUS = [4\text{-}\log(K_{oc})] \times \log t_{1/2} \quad (6)$$

In this present work, the half-life of carbofuran adsorbed on soil was 8.9 days (explain later), the calculated GUS index was 6.37 and 5.82 which presented a high lixiviation potential (GUS $\geq$ 2.8). Most of herbicides are relatively persistent and weakly adsorbing, with GUS mostly exceeding 2.8 then the potential to contaminate groundwater can be influenced by other factors, including soil conditions, application methods, and irrigation practices (Gan, 2002). The values were different from del Melo Plese *et al*., (2005) and also agreed with Bosch and Truman (2002) and Cogger (1998) who had detected high amounts of carbofuran residues in soils from Plains, Georgia and Washington, USA, respectively.

4. Equilibrium time for adsorption of carbofuran on soil.

Equilibrium time for adsorption could be expressed as the time that the selected soil adsorbed any species in maximum quantity. Adsorption processes were the dynamic equilibrium of physico-chemical properties. Adsorption equilibrium of carbofuran determined amount of substance that disappeared from the solution phase and assumed to be adsorbed by the soil. The mass distribution of the substances between the solid and solution phases at equilibrium could be characterized by a distribution coefficient. The distribution coefficient, K_d could be considered as equilibrium concentration of carbofuran on soil divided by equilibrium concentration of carbofuran in the solution, K_{eq} might be replaced by K_d in equation 6.

$$K_{eq} = q/C_{eq} \quad (7)$$

Where q was the equilibrium mass of adsorbed substance per unit mass of adsorbent, C_{eq} was the equilibrium mass of the substance in solution per unit volume of solution and the unit of K_{eq} was volume per mass.

The adsorbed amounts of carbofuran were calculated from the difference between initial concentrations of added carbofuran and measured concentrations from HPLC. Equilibrium time might be determined prior adsorption and desorption characteristics. The calculation data for equilibrium were provided in Table 8 and linear plot between time (x) and adsorbed concentration of carbofuran in on soil was given in Figure 18

Table 8 Equilibration time of carbofuran in rice field soil.

	Weight of soil (g)	Concentration of carbofuran (mg l^{-1})	Amounts of carbofuran adsorbed on soil (mg kg^{-1})	Amounts of carbofuran adsorbed on soil (mg kg^{-1}soil)	Average amounts of adsorbed carboforan on soil (mg kg^{-1}soil)
A1/1	1.0010	42.43512445	7.564875554	7.55731824	7.33 ± 0.32
A1/2	1.0015	42.89006696	7.109933038	7.09928411	
A2/1	1.0022	41.29526161	8.70473839	8.68563000	8.62 ± 0.09
A2/2	1.0014	41.43512445	8.564875554	8.55290149	
A3/1	1.0018	39.63068128	10.36931872	10.3506875	10.87 ± 0.73
A3/2	1.0006	38.61263228	11.38736772	11.3805394	
A4/1	1.0024	36.99514573	13.00485427	12.9737174	12.66 ± 0.44
A4/2	1.0012	37.63068128	12.36931872	12.3544933	
A5/1	1.0017	36.89648336	13.10351664	13.0812785	13.04 ± 0.06
A5/2	1.0033	36.96263228	13.03736772	12.9944859	

The rapid adsorption occurred at the beginning since it was only physical affinity between soil and carbofuran and the difference in concentration gradient between bulk solution and surface of soil. Carbofuran was continuously adsorbed to soil and reached equilibrium at nearly 23 h. The adsorption time for longer than 23 h was applied to determine adsorption isotherms and desorption kinetics.

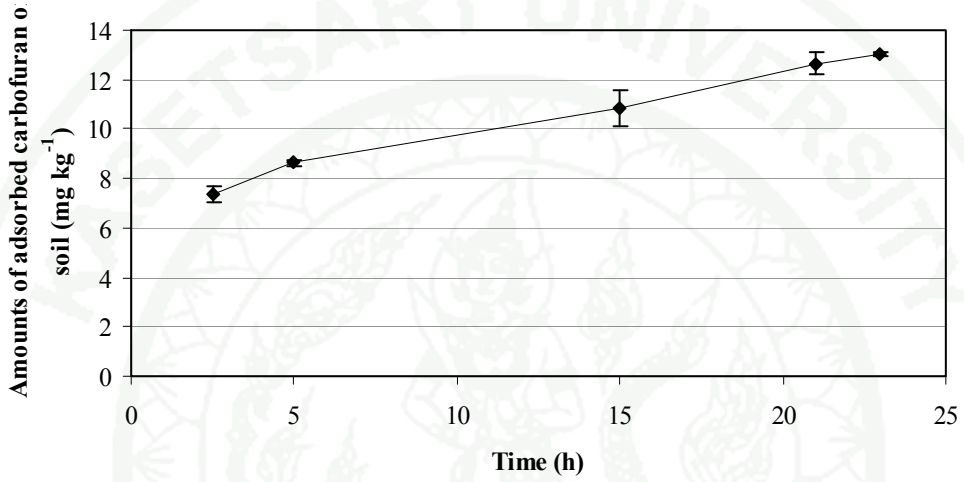


Figure 18 Adsorption equilibrium time of carbofuran of rice field soil. The equilibrium reached maximum within 23 h.

5. Adsorption isotherms of carbofuran on rice field soil.

Adsorption isotherms of carbofuran on rice field soil were performed by variation of concentration ranges from 20 to 300 mg l^{-1} and adsorption times during 1 to 3 days. The isotherms were corresponded to Freundlich isotherm. The adsorption data were calculated in Appendix Table C1 – 3 and the isotherms were provided in Figure 19 – 20.

The amounts of carbofuran adsorbed on silicate clay was given in Table 9, percentage of adsorption depended on concentrations of carbofuran. The concentrations of carbofuran from 20 up to 50 mg l^{-1} corresponded to adsorption of Malaysian clay soil (Farahani *et al*., 2007). From the previous study, high amount of

organic matter paid an important role in high adsorption of carbofuran in clay soil (Heieh and Kao, 1998).

Table 9 Amounts of carbofuran (mg) and percentages of carbofuran adsorbed on soil.

Initial amount (mg)	Adsorbed amount (mg)	Percentage of adsorption
0.2	0.0541 ± 0.045	27.04
0.3	0.0992	33.07
0.4	0.1517 ± 0.037	37.92
0.5	0.2006 ± 0.002	40.11
0.6	0.2826 ± 0.020	47.11
0.8	0.4449 ± 0.008	55.61
0.9	0.5306 ± 0.011	58.95
1	0.6101 ± 0.011	61.01
1.2	0.7788 ± 0.018	64.90
1.5	1.0317 ± 0.029	68.78
1.6	1.1416 ± 0.022	71.35
1.8	1.2948 ± 0.020	71.93
2	1.4939 ± 0.021	74.70
2.4	1.8526 ± 0.026	77.19
2.5	1.9683 ± 0.025	78.73
3	2.4235 ± 0.025	80.78

The Freundlich isotherms obtained from linear regression line between log C_f (mg l^{-1}) and log q (mg kg^{-1}), the equation was presented by $q = KC_f^{1/n}$. The high correlation coefficients of the two lines were 0.9281 and 0.9097, respectively. The Freundlich equations of adsorption were be seen in Equation (8) and (9). The Freundlich adsorption exponent (1/n) obtained from slope of this line and the extent of adsorption K_d from the intercept of the line (Table 10). The 1/n values which were

greater than unity (> 1.0) in the two adsorption time indicated the relatively increasing from the increasing of initial concentrations.

$$q = 7.07 \times 10^{-5} C_f^{2.5092;} \quad (8)$$

$$q = 2.79 \times 10^{-4} C_f^{2.1248;} \quad (9)$$

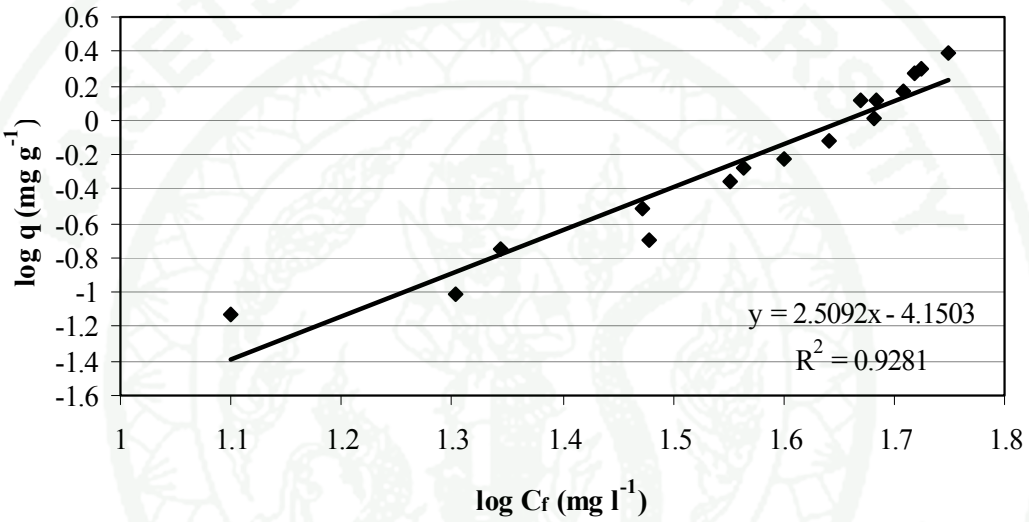


Figure 19 Adsorption isotherm of carbofuran on soil for 24 hrs of adsorption.

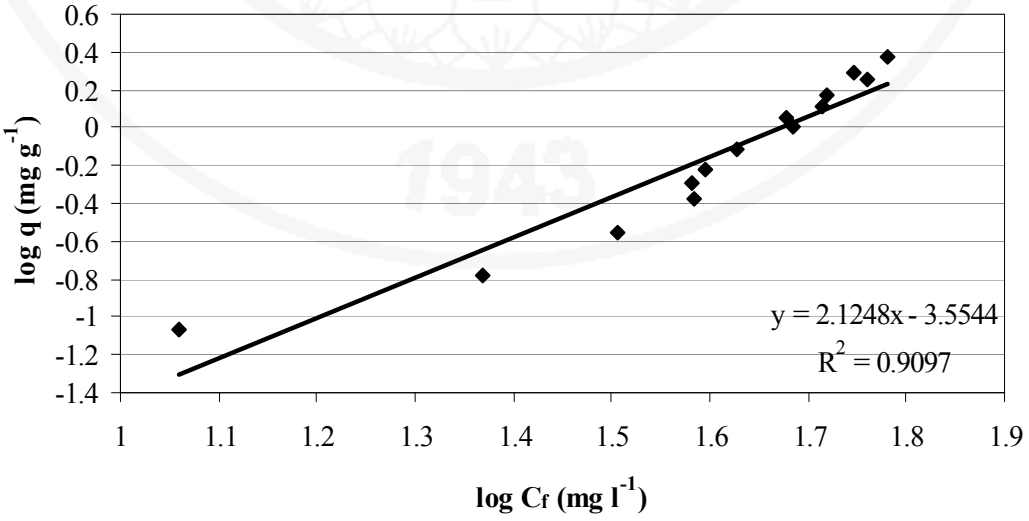


Figure 20 Adsorption isotherm of carbofuran on soil for 72 hrs of adsorption.

Table 10 Correlation coefficients (r^2), adsorption coefficient (K_d), Soil sorption coefficients (K_{oc}) and adsorption exponent (1/n).

Adsorption time (h)	r^2	K_d	K_{oc}	1/n	ΔG (kJ mol^{-1})
24	0.9281	7.07×10^{-5}	1.91×10^{-3}	2.5092	24.079
72	0.9097	2.79×10^{-4}	7.46×10^{-3}	2.1248	20.621

Adsorption coefficient equaled concentration of solute in stationary phase divided by concentration of solute in mobile phase which was quite similar to equilibrium constant of adsorption. The adsorption coefficients were almost 10^4 times higher than the previous values by Mear, *et al*. (7.367×10^{-8} and 7.769×10^{-9} at 25 and 15 ^oC, respectively) (Mear, *et al*., 1996). The values of *K* could be applied for determination of Gibb's free energy, ΔG by the equation:

$$\Delta G^o = -RT \ln K_{eq} \quad (10)$$

R was universal gas constant (8.314 J mol^{-1} K^{-1}) and temperature of adsorption, T at 303 Kelvin and equilibrium constant, K_{eq}. The positive ΔG was investigated the adsorption of carbofuran in soil was nonspontaneous. It did not agree with Singh (Singh *et al*., 1994). The equilibrium constant, K_{eq} can also be used to determine enthalpy, ΔH and ΔS using two different temperatures by Van Hoff equation:

$$\frac{d \ln K_{eq}}{dt} = \frac{\Delta H}{RT^2} \quad (11)$$

and

$$\Delta S = \frac{-\Delta G + \Delta H}{T} \quad (12)$$

6. Desorption of carbofuran from rice field soil.

The linear plot of desorption from data in Appendix Table C4 was shown in Figure 21. The desorption line presented that carbofuran rapidly desorbed from soil at the beginning because of the much different in concentration gradient between soil and solution. Since the concentration of carbofuran in soil and solution reached equilibrium, carbofuran still continuously desorbed with slow rate than at the initial condition. At the final state, decrease of cabofuran might be due to the degradation from hydrolysis and photolysis pathways.

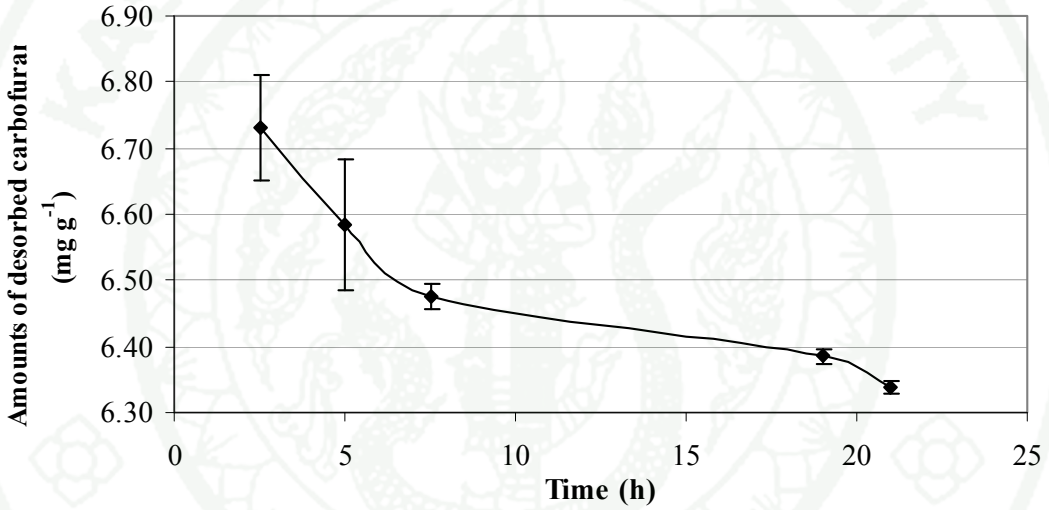


Figure 21 Desorption of carbofuran from rice field soil.

The logarithmic plot of desorbed amounts (mg kg^{-1}) and time (Figure 22) was investigated to determine the desorption rate of carbofuran from soil. The desorption rate obtained from the slope of the regression line. The desorption rate was 0.0288 mg kg^{-1}, soil d^{-1} (0.0012 mg kg^{-1}, soil d^{-1} from slope of the logarithmic plot in Figure 22)

The percentages of carbofuran desorbed from soil were presented in Table 11. The percentages of desorption was approximately 55% from the beginning to 21 h. The high residues of carbofuran in soil may be due to high amount of organic content in silicate clay.

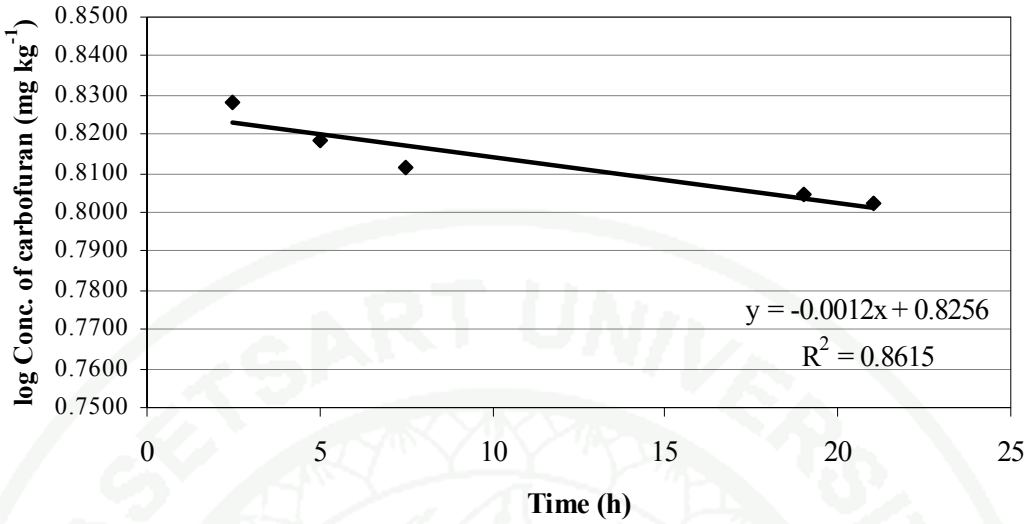


Figure 22 Logarithmic plot between concentrations of carbofuran (mg 1^{-1}) and time (h).

Table 11 Percentages of carbofuran desorption from soil.

Desorbed time (h)	Amount of adsorbed carbofuran (mg l^{-1})	Amount of desorbed carbofuran (mg l^{-1})	Desorption (%)	Amount of carbofuran residues (mg)
2.5	12.42 ± 0.08	6.73 ± 0.08	54.19	5.69 ± 0.11
5	12.14 ± 0.16	6.58 ± 0.01	54.20	4.56 ± 0.16
7.5	10.98 ± 0.59	6.48 ± 0.02	59.02	4.50 ± 0.59
19	11.26 ± 0.33	6.38 ± 0.01	56.66	4.88 ± 0.33
21	11.64 ± 0.33	6.34 ± 0.01	54.64	5.30 ± 0.33

Adsorption kinetics

The adsorption kinetics was expressed from concentration of carbofuran in each concentration range against adsorption time shown in Figure 23. The concentration decreased/ time, dC/dt at average C of each line at initial state was used to construct the graph for determination of adsorption rate constant, *k* seen in Figure

24. The concentration was utilized at the initial state because adsorption was only one mechanism at the initial state indicated by the straight line at the beginning. After initial state, the measured amount of carbofuran may be affected by other mechanisms such as degradation and dissolution.

The reaction rate constant was calculated from the regression of linear line in Figure 23. The line indicated that that the adsorption of carbofuran was the first order kinetics. The adsorption rate constant was 0.0779 mg day^{-1} corresponded to the slope of the regression line.

Following the first order reaction, the half life of adsorption could be determined from:

$$C/C_o = e^{-kt} \quad (13)$$

where C_o and C were the initial concentration and concentration of carbofuran at time t and *k* was the reaction rate constant.

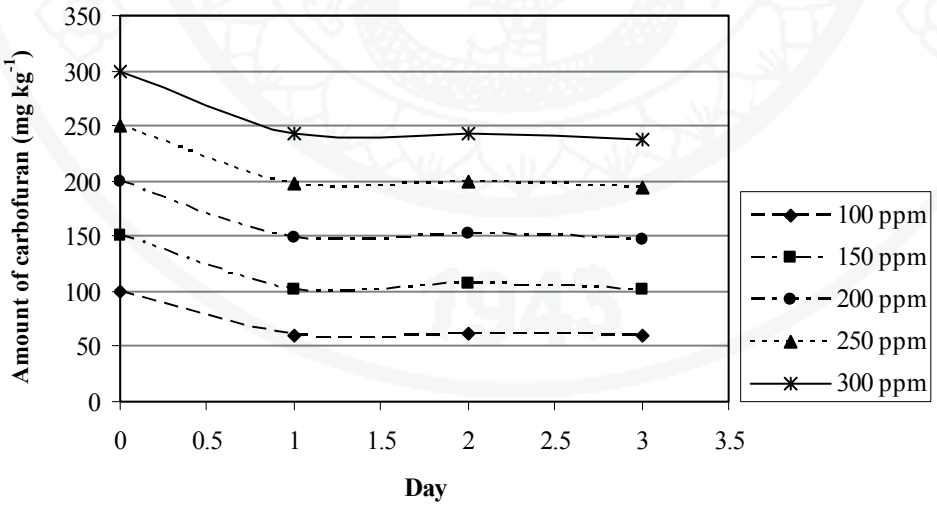


Figure 23 Adsorption kinetics of carbofuran at 30 ^oC, each line corresponded to different concentrations of carbofuran. (a) 100 mg l^{-1}, (b) 150 mg l^{-1}, (c) 200 mg l^{-1}, (d) 250 mg l^{-1} and (e) 300 mg l^{-1}.

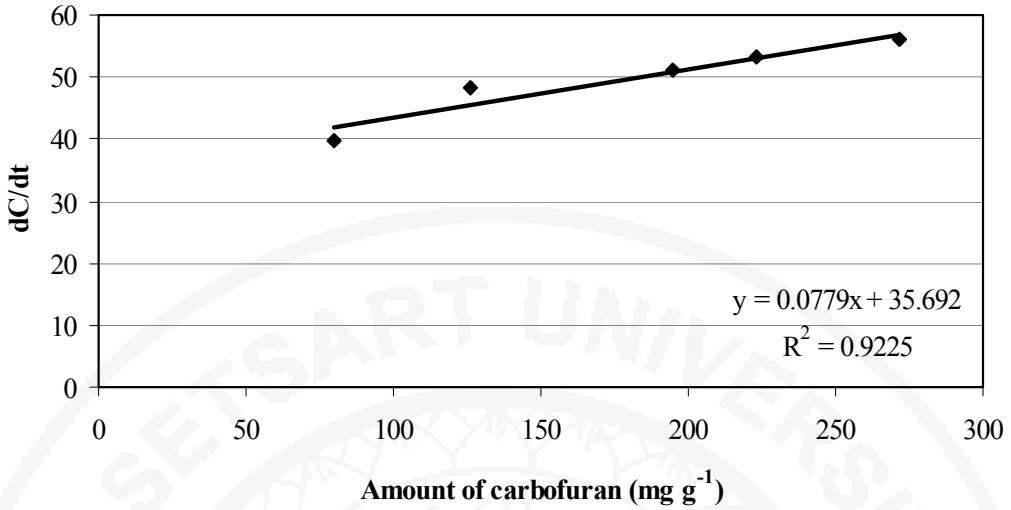


Figure 24 The adsorption rate constant constructed from average concentration of carbofuran and dC/dt at 30 ^{o}C.

The laboratory studied showed the half life of carbofuran adsorbed in rice field soil was 8.9 day which was quite different from Lysan, 112 days and Ottawa sands, 91 days. The half-life which detected in 2 – 20 cm depth of 7 and 14 days was similar with a field study of Johnson and Lavy in 1994 (Johnson and Lavy 1994). Half-life value in rice field also agreed with Mabury *et al*. ranged from 2 to 26 days (Mabury 1996). The first order kinetics agreed with Campbell in both Lysan sand [$X_i = X_o$ exp (-0.0182t + 0.0504) and Ottawa sand [$X_i = X_o$ exp (-0.0151t – 0.0938), where X_i was the concentration at day i, X_o the initial concentration, and t was the time. (Campbell 2004). For very long term and continuous application of carbofuran in this field, the residues were still accumulated and persisted even though it was stopped application for more than five years. Adsorption was one of many mechanisms for persistence of carbofuran it this field. The persistence of pesticides was normally influences by (1) pesticide properties including physical characteristics, sensitivity for chemical biological and microbial degradation, (2) soil properties such as soil pH, moisture content, organic matter and clay content and soil moisture and (3) weathering conditions including temperature, exposure time for sunlight, as well as amount, frequency and intensity of rainfall (Farahani *et al*., 2007).. Carbofuran did not be adsorbed to the soil in large amount but it could be strictly adsorbed on the surface of

clay particles. The natural attenuation of carbofuran was very slow due to residues were still detected. The degradation of carbofuran in this field must be studied.

It could be summarized that the residue of carbofuran still remained in soil unless it possessed high lixiviation potential due to GUS Index. It might be because the standing water in rice field caused re-adsorbed to the soil. The adsorption characteristics were agreed with Freundlidch isotherm. It indicated that the soil presented multilayer properties of the sorbent. In addition with low adsorption coefficients of 7.07×10^{-5} and 2.79×10^{-4} mg g^{-1}, carbofuran could be eliminated from soil by tillage and irrigation but the soil might lost the dissolved nutrients and fertilizers.

CONCLUSION AND RECOMMENDATION

Conclusion

Rice field soil was sampling for three consecutive growing seasons; October 2006, May and November, 2007. HPLC was investigated for analysis of carbofuran residues in soil, the separation conditions comprised Intersil ODS as stationary phase, 50% (v/v) acetonitrile:water as mobile phase, the eluant flow rate was 1.2 ml/min. The concentrations of carbofuran were detected by UV at 210 nm. Ultrasonic extraction with a mixture of petroleum ether and acetone was available for extraction of carbofuran from clay with 75% recovery and the extraction efficiencies were significantly different from Soxhlet extraction. ($p \leq 0.01$). Due to the satisfied recovery, less chemicals and time, ultrasonic method was selected for extraction of carbofuran in rice field soil. Percentages of recovery varied from 77 – 84%.

The three average residual of carbofuran were higher in plot 3 (1.122 ± 0.118 mg kg^{-1}), 4(2.088 ± 0.332 mg kg^{-1}) and 5(3.585 ± 0.362 mg kg^{-1}) than plot #1 (0.147 ± 0.033 mg kg^{-1}) and #2 (0.380 ± 0.049 mg kg^{-1}) due to the high contents of organic matter from wastewater discharge to plot 3 – 5. The residues were slowly decreased year by year.

Adsorption of carbofuran reached equilibrium at 23 hrs. The percentage of adsorption varied from almost 30% to 80% depending on concentrations of carbofuran. The Freundlich isotherms; $q = KC_f^{1/n}$; for the two lines provided the correlation coefficients of 0.9281 and 0.9097, respectively. The distribution coefficients, K_d were 7.07 x 10^{-5} and 2.79 x 10^{-4} mg g^{-1}. The Freundlich adsorption exponent (1/n) values which were greater than unity (2.5092 and 2.1248) in the two adsorption time indicated the relatively increasing from the increasing of initial concentrations. The positive free energy (ΔG) of adsorption (24.078 and 20.621 kJ mol^{-1}) indicated the nonspontaneous in adsorption processes of carbofuran in rice field soil.

Carbofuran rapidly desorbed from soil at the beginning because of the difference in concentration gradient between soil and solution. After equilibrium, carbofuran still continuously desorbed with slow rate than at the initial condition. At the final state, decrease of cabofuran might be due to the degradation from hydrolysis and photolysis pathways. The desorption rate was 0.0228 mg kg^{-1}, soil d^{-1}. The percentages of desorption was approximately 55% from the beginning to 21 h

Kinetic study provided the first order reaction with the reaction rate of 0.0779 mg/d and half-life of 8.9 days.

Recommendation

Analysis of carbofuran were emphasized only the amount of carbofuran residues in rice foil, the other important species were the metabolic products occurred from the metabolic pathways. To determine the intermediate species prior mineralization products (CO_2, H_2O and so on) could meet the knowledge how carbofuran provided very long persistent especially in rice field soil in Thailand. To meet this goal, advance analytical instrument such as HPLC-Mass spectrograph might be used to determine metabolic products occurring during degradation. High magnetic fluxed NMR was important to determine the chemical structure of metabolic products.

For kinetic study, the research could not apply for determination of all kinetic parameters because of the limitation of the instruments such as incubate shaker and high speed centrifuge.

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APPENDICES

Appendix A

Calibration of HPLC and percentage recovery of extraction methods

Appendix Table A1 Calibration data for linear regression line.

	x_i	y_i	$(x_i)^2$	$x_i - \bar{x}$	$(x_i - \bar{x})^2$	$y_i - \bar{y}$	$(y_i - \bar{y})^2$	$(x_i - \bar{x})(y_i - \bar{y})$	$\hat{y}$	$y_i - \hat{y}$	$(y_i - \hat{y})^2$
	5	4712473	25	-10	100	-8534351	7.28E+13	85343510	5041247	328774	108092343076
	10	9093867	100	-5	25	-4152957	1.72471E+13	20764785	9144032	50185	2518534225
	15	13869279	225	0	0	622455	3.8745E+11	0	13246817	622462	387458941444
	20	17570318	400	5	25	4323494	1.86926E+13	21617470	17349602	220716	48715552656
	25	20988184	625	10	100	7741360	5.99287E+13	77413600	21452387	464203	215484425209
Σ	**75**	**66234121**	**1375**	**0**	**250**	**1**	**1.69E+14**	**205139365**			**762269796610**
x	**15**										
y		**13246824.2**									

x_i = concentrations of carbofuran (mg l^{-1})

y_i = peak areas

$\bar{x}$ = (5 + 10 + 15 + 20 + 25)/5 = 15 mg l^{-1}

$\bar{y}$ = (4712473 + 9093867 + 13869279 + 17570318 + 20988184)/5 = 13246824.2

$\hat{y}$ was true y which was calculated from substitution of x_i to linear regression line (820557.46x + 938462).

Correlation coefficient, r $= \sqrt{\frac{\sum(x_i-\bar{x})(y_i-\bar{y})}{\sum(x_i-\bar{x})^2(y_i-\bar{y})^2}}$

$= \sqrt{\frac{205139365}{(250 \times 1.69 \times 10^{14})}}$

$= 0.9980$

Slope, b $= \frac{\sum[(x_i-\bar{x})(y_i-\bar{y})]}{\sum(x_i-\bar{x})^2}$

$= \frac{205139365}{250}$

$= 820557.46$

Intercept, a $= \bar{y} - b\bar{x}$

$= 13246824 - (820557.46 \times 15)$

$= 938462$

Linear Regression line $= 820557.46x + 938462$

$\frac{S_y}{x} = \sqrt{\frac{\sum(y_i-\hat{y})^2}{5}}$

$= \sqrt{\frac{732269796610}{5}}$

$= 390453.58$

$y - y_b = 3S_b = 3S_{y/x}$

$y - 0 = 3 \times 390453.58$

$= 1171360.75$

Detection limit

$1171360.75 = 820557.46x + 938462$

$x = 282.83\ \mu g\ l^{-1}$

Appendix Table A2 Comparison of extraction methods.

Replication	x_s	$x_s - \bar{x}_s$	$(x_s - \bar{x}_s)^2$	X_u	$x_u - \bar{x}_u$	$(x_u - \bar{x}_u)^2$
1	16.784	-0.2418	0.05846724	14.867	-0.0328	0.00107584
2	16.424	-0.6018	0.36216324	14.505	-0.3948	0.15586704
3	17.306	0.2802	0.07851204	15.171	0.2712	0.07354944
4	16.932	-0.0938	0.00879844	14.773	-0.1268	0.01607824
5	17.683	0.6574	0.43191184	15.174	0.2742	0.07518564
Σ			**0.9398528**			**0.3217562**
x_s	**17.0258**					
x_u				**14.8998**		

Comparison between two different experimental means can be calculated from the equations:

$$x_1 - x_2 = \pm ts_{pooled}\sqrt{\frac{N_1+N_2}{N_1N_2}}$$

$$s_{pooled} = \sqrt{\frac{\sum\left[(x_i)_1-\bar{x}_1\right]^2-\sum\left[(x_i)_2-\bar{x}_2\right]^2+\ldots+\sum\left[(x_i)_n-\bar{x}_n\right]^2}{N_1+N_2+\ldots-N_k}}$$

$$= \sqrt{\frac{(0.9398528+0.3217562)}{5+5-2}}$$

$$= 0.397116009498$$

At 99% Confidence Interval, *t* factor for 8 degree of freedoms equals 3.36.

$$\pm ts_{pooled} = 2.31 \times 0.397116009498\sqrt{\frac{5+5}{5x5}}$$

$$= \pm 0.84 \text{ mg g}^{-1}$$

$$\bar{x}_s - \bar{x}_u = 17.0258 - 14.8998$$

$$= 2.126 \text{ mg g}^{-1}$$

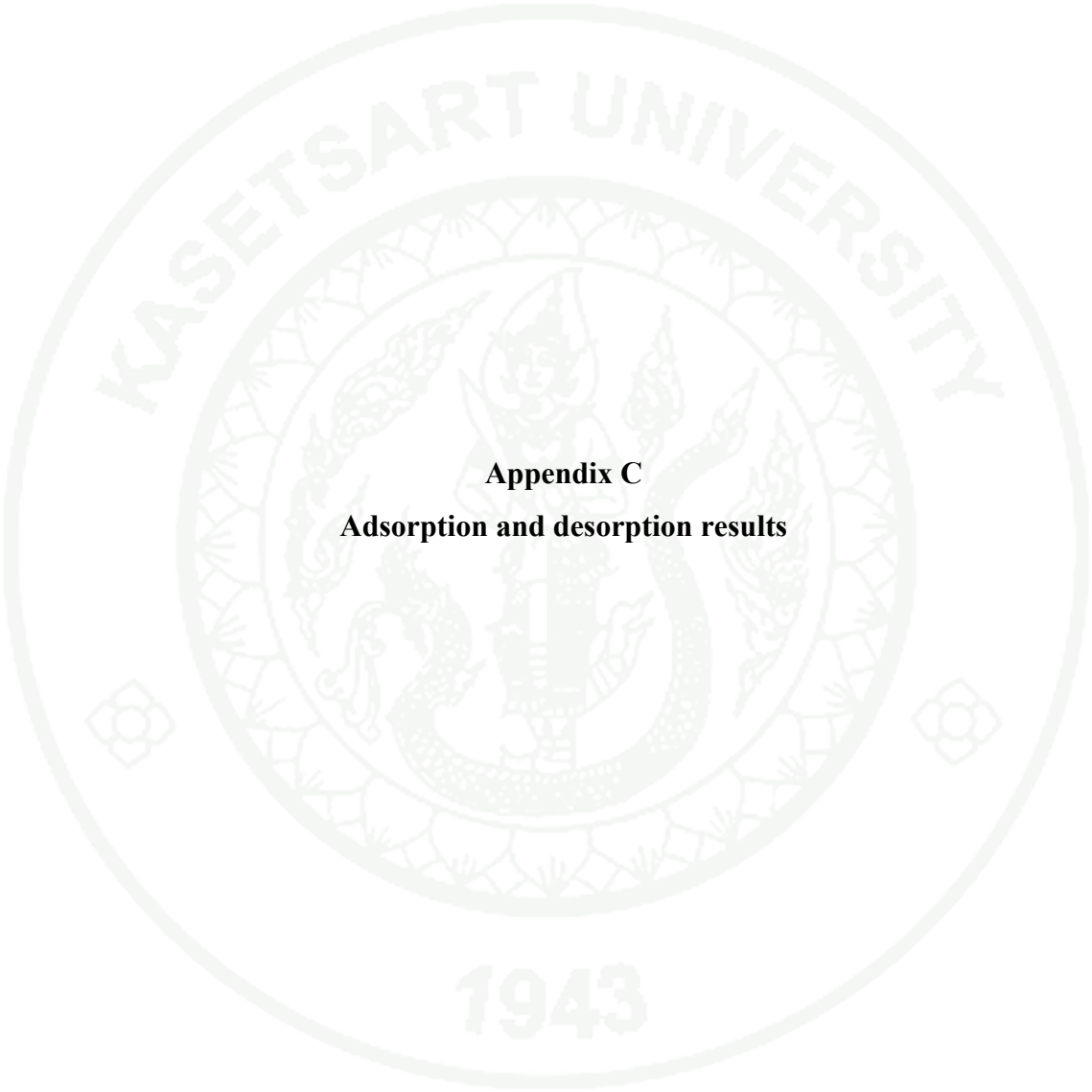
At each1 times in 100, random error will be responsible for a difference as equal as 0.84. The difference in experimental means is 2.126 mg g-1 which is greater than 0.84. At 99% confidence level, it is significantly different between two extraction methods.

Appendix B

Analysis data of carbofuran residues in soil

Appendix Table B1 Analysis data for determination of carbofuran residues in soil.

Sample plot	Weight (g)	Amount of carbofuran		Weight (g)	Amount of carbofuran		Weight (g)	Amount of carbofuran	
		Observed ($mg\ l^{-1}$)	Amount of carbofuran ($mg\ kg^{-1}$, soil)		Observed ($mg\ l^{-1}$)	Amount of carbofuran ($mg\ kg^{-1}$, soil)		Observed ($mg\ l^{-1}$)	Amount of carbofuran ($mg\ kg^{-1}$, soil)
1.1	10.0030	0.171	0.171	10.0023	0.162	0.162	10.0011	0.114	0.114
1.2	10.0000	0.186	0.186	10.0000	0.170	0.170	10.0004	0.857	0.857
1.3	10.0005	0.214	0.214	10.0018	0.136	0.136	10.0006	0.846	0.846
2.1	10.0022	0.482	0.482	10.0018	0.376	0.376	10.0016	0.253	0.253
2.2	10.0013	0.470	0.470	10.0000	0.390	0.390	10.0021	0.328	0.328
2.3	10.0030	0.430	0.430	10.0026	0.407	0.407	10.0019	0.288	0.288
3.1	10.0000	1.157	1.157	10.0010	1.189	1.189	10.0030	1.031	1.031
3.2	10.0003	0.995	0.995	10.0003	0.120	0.120	10.0018	1.134	1.134
3.3	10.0022	0.164	0.164	10.0013	1.178	1.177	10.0000	0.126	0.126
4.1	10.0032	2.331	2.330	10.0017	1.853	1.852	10.0005	1.934	1.934
4.2	10.0001	2.171	2.171	10.0000	2.240	2.240	10.0008	1.815	1.815
4.3	10.0024	2.245	2.244	10.0009	1.917	1.917	10.0017	2.287	2.287
5.1	10.0018	4.276	4.275	10.0000	2.994	2.993	10.0018	3.017	3.016
5.2	10.0011	4.092	4.092	10.0031	3.222	3.221	10.0000	3.344	3.344
5.3	10.0002	4.548	4.548	10.0000	3.354	3.354	10.0003	3.421	3.421



Appendix C

Adsorption and desorption results

Appendix Table C1 The calculation results of Freundlich isotherm for 24 hrs of adsorption.

M, soil (gm)	C_i (mg l^{-1})	C_f (mg l^{-1})	$X = (C_i - C_f)V$	q (mg g^{-1})	log C_f	log q
1.0039	20	12.589	0.07411	0.07382	1.1000	-1.1318
1.0036	30	20.08	0.0992	0.09884	1.3028	-1.005
1.0054	40	22.064	0.17936	0.1784	1.3437	-0.7486
1.0013	50	30.112	0.19888	0.19862	1.4787	-0.702
1.0042	60	29.606	0.30394	0.30267	1.4714	-0.519
1.0015	80	35.574	0.44426	0.44359	1.5511	-0.353
1.0017	90	36.634	0.53366	0.53275	1.5639	-0.2735
1.0041	100	39.849	0.60151	0.59905	1.6004	-0.2225
1.0018	120	43.702	0.76298	0.76161	1.6405	-0.1183
1.0036	150	48.161	1.01839	1.01474	1.6827	0.0064
1.0022	160	46.647	1.13353	1.13104	1.6688	0.1174
1.0039	180	48.265	1.31735	1.31223	1.6836	0.118
1.0021	200	50.976	1.49024	1.48712	1.7074	0.1723
1.0021	240	52.194	1.87806	1.87412	1.7176	0.2728
1.0011	250	53.142	1.96858	1.96642	1.7254	0.2937
1.0011	300	56.141	2.43859	2.43591	1.7493	0.3867

Appendix Table C2 The calculation results of Freundlich isotherm for 48 hrs of adsorption.

M, soil (gm)	C_i (mg l^{-1})	C_f (mg l^{-1})	$X = (C_i - C_f)V$	q (mg g^{-1})	log C_f	log q
1.0015	20	19.715	0.00285	0.00285	1.2948	-2.5458
1.0051	40	29.045	0.10955	0.10899	1.4631	-0.9626
1.0013	50	29.775	0.20225	0.20199	1.4739	-0.6947
1.0051	60	33.505	0.26495	0.26361	1.5251	-0.579
1.0020	80	34.645	0.45355	0.45264	1.5396	-0.3442
1.0032	90	35.998	0.54002	0.5383	1.5563	-0.269
1.0007	100	37.707	0.62293	0.62249	1.5764	-0.2059
1.0039	120	40.233	0.79767	0.79457	1.6046	-0.0999
1.0021	150	43.497	1.06503	1.0628	1.6385	0.0265
1.0044	160	43.313	1.16687	1.16176	1.6366	0.0651
1.0017	180	51.485	1.28515	1.28297	1.7117	0.1082
1.0030	200	48.374	1.51626	1.51172	1.6846	0.1795
1.0001	240	54.566	1.85434	1.85415	1.7369	0.2681
1.0010	250	50.693	1.99307	1.99108	1.7049	0.2991
1.0017	300	56.327	2.43673	2.43259	1.7507	0.3861

Appendix Table C3 The calculation results of Freundlich isotherm for 72 hrs of adsorption.

M, soil (gm)	C_i (mg l^{-1})	C_f (mg l^{-1})	$X = (C_i - C_f)V$	q (mg g^{-1})	loq C_f	log q
1.0071	20	11.471	0.08529	0.08469	1.0596	-1.0722
1.0025	40	23.386	0.16614	0.16573	1.3690	-0.7806
1.0053	60	32.097	0.27903	0.27756	1.5065	-0.5566
1.0054	80	36.310	0.4369	0.43455	1.5832	-0.363
1.0035	90	38.193	0.51807	0.51626	1.5820	-0.2871
1.0017	100	39.408	0.60592	0.60489	1.5956	-0.2183
1.0073	120	42.413	0.77587	0.77025	1.6275	-0.1114
1.0015	150	48.841	1.01159	1.01007	1.6856	0.0044
1.0029	160	47.555	1.12445	1.1212	1.6772	0.0497
1.0011	180	51.804	1.28196	1.28055	1.7144	0.1074
1.0000	200	52.479	1.47521	1.47521	1.7200	0.1689
1.0012	240	57.462	1.82538	1.82319	1.7594	0.2603
1.0025	250	55.673	1.94327	1.93842	1.7456	0.2874
1.0018	300	60.492	2.39508	2.39078	1.7817	0.3785

Appendix Table C4 Desorption of carbofuran from soil.

Replications	Weight of soil (g)	Conc. of carbofuran (mg l^{-1})	Amounts of carbofuran adsorbed on soil (mg kg^{-1})	Amounts of carbofuran adsorbed on soil (mg kg^{-1} soil)	Amounts of desorbed carbofuran from soil (mg kg^{-1})	Average amounts of desorbed carboforan from soil (mg kg^{-1})
D1/1	1.0026	37.64	12.36	12.33	6.68	6.73 ± 0.08
D1/2	1.0011	37.52	12.48	12.47	6.79	
D2/1	1.0013	37.75	12.25	12.24	6.59	6.58 ± 0.1
D2/2	1.0015	37.98	12.02	12.00	6.58	
D3/1	1.002	38.60	11.40	11.37	6.49	6.48 ± 0.02
D3/2	1.0003	39.44	10.56	10.56	6.46	
D4/1	1.0007	38.97	11.03	11.02	6.38	6.38 ± 0.01
D4/2	1.0034	38.51	11.49	11.45	6.39	
D5/1	1.0016	38.59	11.41	11.39	6.35	6.34 ± 0.01
D5/2	1.0012	38.13	11.87	11.86	6.33	
Average amount of carbofuran adsorbed on soil 11.67 ± 0.15 mg kg^{-1}						

Appendix D

International Chemical safety Cards

Appendix Table D1 International Chemical safety Cards.

International Chemical Safety Cards

CARBOFURAN

ICSC: 0122

2,3-Dihydro-2,2-dimethylbenzofuran-7-yl methylcarbamate

2,3-Dihydro-2,2-dimethyl-7-benzofuranyl methylcarbamate

2,2-Dimethyl-2,3-dihydro-7-benzofuranyl-N-methylcarbamate

$C_{12}H_{15}NO_3$

Molecular mass: 221

ICSC # 0122

CAS # 1563-66-2

RTECS # FB9450000

UN # 2757

EC # 006-026-00-9

April 22, 2004 Peer reviewed

TYPES OF HAZARD/ EXPOSURE	ACUTE HAZARDS/ SYMPTOMS	PREVENTION	FIRST AID/ FIRE FIGHTING
FIRE	Not combustible. Liquid formulations containing organic solvents may be flammable. Gives off irritating or toxic fumes (or gases) in a fire.		In case of fire in the surroundings: use appropriate extinguishing media.
EXPLOSION	Risk of fire and explosion if formulations contain		

	flammable/explosive solvents.		
EXPOSURE		PREVENT DISPERSION OF DUST! STRICT HYGIENE! AVOID EXPOSURE OF ADOLESCENTS AND CHILDREN!	IN ALL CASES CONSULT A DOCTOR!
•INHALATION	Sweating. Pupillary constriction, muscle cramp, excessive salivation. Dizziness. Vomiting. Laboured breathing. Unconsciousness.	Ventilation (not if powder), local exhaust, or breathing protection.	Fresh air, rest. Artificial respiration may be needed. Refer for medical attention. See Notes.
•SKIN		Protective gloves.	Remove contaminated clothes. Rinse and then wash skin with water and soap.
•EYES		Safety spectacles, or eye protection in combination with breathing protection.	First rinse with plenty of water for several minutes (remove contact lenses if easily possible), then take to a doctor.
•INGESTION	Abdominal cramps. Diarrhoea. Headache. Nausea. Vomiting. Weakness. (Further see Inhalation).	Do not eat, drink, or smoke during work. Wash hands before eating.	Give a slurry of activated charcoal in water to drink. Refer for medical attention. See Notes.

SPILLAGE DISPOSAL	**STORAGE**	**PACKAGING & LABELLING**
Sweep spilled substance into containers; if appropriate,	Provision to contain effluent from fire extinguishing.	Do not transport with food and feedstuffs.

moisten first to prevent dusting. Carefully collect remainder, then remove to safe place. Do NOT let this chemical enter the environment. Personal protection: self-contained breathing apparatus.	Separated from food and feedstuffs Keep in a well-ventilated room.	Marine pollutant. T+ symbol N symbol R: 26/28-50/53 S: 1/2-36/37-45-60-61 UN Hazard Class: 6.1 UN Packing Group: I

SEE IMPORTANT INFORMATION ON BACK

ICSC: 0122

International Chemical Safety Cards

CARBOFURAN

ICSC: 0122

IMPORTANT

PHYSICAL STATE; APPEARANCE:
COLOURLESS CRYSTALS.

PHYSICAL DANGERS:

CHEMICAL DANGERS:
The substance decomposes on heating producing toxic fumes including nitrogen oxides

OCCUPATIONAL EXPOSURE LIMITS:
TLV: 0.1 mg/m㎥ A4 BEI issued (ACGIH 2004).
MAK not established.
OSHA PEL†: none
NIOSH REL: TWA 0.1 mg/m^3
NIOSH IDLH: N.D. See: IDLH

ROUTES OF EXPOSURE:
The substance can be absorbed into the body by inhalation and by ingestion.

INHALATION RISK:
Evaporation at 20℃ is negligible; a harmful concentration of airborne particles can, however, be reached quickly on spraying or when dispersed, especially if powdered.

EFFECTS OF SHORT-TERM EXPOSURE:
The substance may cause effects on the nervous system , resulting in convulsions and respiratory depression Cholinesterase inhibitor. The effects may be

A T A	INDEX	delayed. Exposure may result in death. Medical observation is indicated. **EFFECTS OF LONG-TERM OR REPEATED EXPOSURE:** Cholinesterase inhibitor; cumulative effect is possible: see acute hazards/symptoms.
PHYSICAL PROPERTIES	Decomposes below boiling point at 150§C Melting point: 153§C Density: 1.2 g/cm3	Solubility in water, g/100 ml at 25§C: 0.07 Vapour pressure, Pa at 33§C: 0.0027 Octanol/water partition coefficient as log Pow: 2.32
ENVIRONMENTAL DATA	 The substance is very toxic to aquatic organisms. This substance may be hazardous to the environment; special attention should be given to soil organisms, honey bees and birds. This substance does enter the environment under normal use. Great care, however, should be given to avoid any additional release, e.g. through inappropriate disposal.	

N O T E S

Specific treatment is necessary in case of poisoning with this substance; the appropriate means with instructions must be available. Carrier solvents used in commercial formulations may change physical and toxicological properties. Do NOT take working clothes home. If the substance is formulated with solvents also consult the ICSCs of these materials.

Transport Emergency Card: TEC (R)-61GT7-I

ADDITIONAL INFORMATION

ICSC: 0122 **CARBOFURAN**

IMPORTANT LEGAL NOTICE:	Neither NIOSH, the CEC or the IPCS nor any person acting on behalf of NIOSH, the CEC or the IPCS is responsible for the use which might be made of this information. This card contains the collective views of the IPCS Peer

	Review Committee and may not reflect in all cases all the detailed requirements included in national legislation on the subject. The user should verify compliance of the cards with the relevant legislation in the country of use. The only modifications made to produce the U.S. version is inclusion of the OSHA PELs, NIOSH RELs and NIOSH IDLH values.

Pesticide Residues in Food - 1996. Report Sponsored Jointly by FAO and WHO. (FAO Plant Production and Protection Paper - 140)

Carbofuran (096) (T)**

** Evaluation in CCPR periodic review programme

TOXICOLOGY

Carbofuran was evaluated for toxicological effects by the Joint Meeting in 1976, 1979, 1980, and 1982. The 1980 Meeting established an ADI of 0-0.01 mg/kg bw, which was confirmed in 1982. The compound was re-evaluated at the present Meeting within the CCPR periodic review programme.

Carbofuran is rapidly absorbed, metabolized, and eliminated, mainly in the urine, after oral administration to mice and rats. After oral administration of *[phenyl-*^{14}C]carbofuran to rats, 92% of the radiolabel was eliminated in the urine and 3% in the faeces. Most of the radiolabel was eliminated within 24 h after treatment. With the [^{14}C]carbonyl-labelled compound, 45% was eliminated as [^{14}C]carbon dioxide. The metabolic pathway involves hydroxylation, hydrolysis, oxidation and conjugation.

Carbofuran is highly toxic after acute oral administration. The oral LD_{50} values in various species ranged from 3 to 19 mg/kg bw. Carbofuran had no sensitizing potential in guinea-pigs, and no local irritation was found in rabbits after repeated dermal applications over 7 or 21 days. WHO has classified carbofuran as 'highly hazardous'.

In a 13-week study in dogs fed diets providing 0, 10,. 70, or 500/250 ppm carbofuran (dose reduced because of marked toxicity), an NOAEL was not identified because inhibition of erythrocyte acetylcholinesterase activity and some clinical signs were observed at the lowest dose. In a subsequent four-week study in dogs, the only dose administered was 5 ppm, equal to 0.22 mg/kg bw per day, which was the NOAEL for clinical signs, mortality, body weight, food consumption, and cholinesterase activity in plasma and erythrocytes. In a one-year study in dogs at dietary concentrations of 0,

10, 20, or 500 ppm, the NOAEL was 10 ppm, equal to 0.3 mg/kg bw per day, on the basis of histopathological testicular changes in a single male at 20 ppm; similar changes were observed in animals at 500 ppm. There was no inhibition of erythrocyte or brain acetylcholinesterase at concentrations of 10 or 20 ppm. The overall NOAEL in these short-term studies in dogs was 5 ppm, equal to 0.22 mg/kg bw per day.

In two-year studies of toxicity and carcinogenicity at dietary concentrations of 0, 20, 125, or 500 ppm in mice and 0, 10, 20, or 100 ppm in rats the NOAELs were 20 ppm, equal to 2.8 mg/kg bw per day, in mice and 20 ppm, equivalent to 1 mg/kg bw per day, in rats, on the basis of inhibition of erythrocyte and brain acetylcholinesterase activity. There was no evidence of tumorigenicity.

In a three-generation study of reproductive toxicity in rats at dietary concentrations of 0, 20, or 100 ppm, the NOAEL was 20 ppm, equal to 1.6 mg/kg bw per day, on the basis of reduced body-weight gain in parental animals and reduced pup growth and pup survival at 100 ppm.

In an early study of developmental toxicity, rats were given carbofuran at doses of 0, 0.1, 0.3, or 1 mg/kg bw per day by gavage. An NOAEL could not be identified in this study. Dose-dependent transient clinical signs (chewing motions) were observed in the dams. In a later study in rats at oral doses of 0, 0.25, 0.5, or 1.2 mg/kg bw per day the NOAEL for maternal and fetal toxicity was 1.2 mg/kg bw per day, the highest dose tested. In a further study of teratogenicity in rats, with dietary administration of 0, 20, 60, or 160 ppm carbofuran, the NOAEL for maternal toxicity was 20 ppm, equal to 1.5 mg/kg bw per day, on the basis of a reduction in body-weight gain at 60 ppm. The NOAEL for pup toxicity, based on reduced pup weight, was 60 ppm, equal to 4.4 mg/kg bw per day. None of the studies showed teratogenic potential.

The results of an early study of developmental toxicity in rabbits at oral doses of 0, 0.2, 0.6, or 2 mg/kg bw per day showed an NOAEL of 0.6 mg/kg bw per day for maternal toxicity on the basis of clinical signs, and an NOAEL of 2 mg/kg bw per day for fetotoxicity and teratogenicity. In a subsequent study in rabbits at doses of 0, 0.12, 0.5, or 2 mg/kg bw per day, the NOAEL was 0.5 mg/kg bw per day on the basis of

slightly reduced body-weight gain in dams and a slightly increased incidence of skeletal variations in pups at 2 mg/kg bw per day. These studies provided no evidence of teratogenicity.

In a 90-day study of neurotoxicity in rats at dietary concentrations of 0, 50, 500, or 1000 ppm, systemic toxicity (reduction in body-weight gain) was observed at all doses. Clinical signs of neurotoxicity were observed at 500 and 1000 ppm. No histopathological lesions in the nervous system were observed.

In a study of developmental neurotoxicity, carbofuran was administered in the diet to provide concentrations of 0, 20, 75, or 300 ppm from gestation day 6 through lactation day 10. Reductions in body-weight gain in dams and pups and in pup survival and some evidence of delayed pup development were found at 75 ppm and higher. The NOAEL was 20 ppm, equal to 1.7 mg/kg bw per day, on the basis of reduced body-weight gain in dams and signs of fetotoxicity at higher doses.

Carbofuran has been tested for genotoxicity in a wide range of tests *in vivo* and *in vitro.* The Meeting concluded that it is not genotoxic.

An ADI of 0-0.002 mg/kg bw was allocated on the basis of the NOAEL for erythrocyte acetylcholinesterase inhibition of 0.22 mg/kg bw per day in a four-week study in the most sensitive species, the dog, using a 100-fold safety factor. The use of a short-term study to determine the ADI was justified because the effect observed was reversible and acute.

A toxicological monograph was prepared, summarizing the data received since the previous evaluation and including summaries from the previous monograph.

TOXICOLOGICAL EVALUATION

Levels that cause no toxic effect

Mouse: 20 ppm, equal to 2.8 mg/kg bw per day (two-year study of toxicity and carcinogenicity)

Rat: 20 ppm, equivalent to 1 mg/kg bw per day (two-year study of toxicity and carcinogenicity)

20 ppm, equal to 1.2 mg/kg bw per day (three-generation study of reproductive toxicity)

1.2 mg/kg bw per day (highest dose tested in a study of developmental toxicity)

20 ppm, equal to 1.5 mg/kg bw per day (study of developmental toxicity)

20 ppm, equal to 1.7 mg/kg bw per day (study of developmental neurotoxicity)

Rabbit: 0.6 mg/kg bw per day (study of developmental toxicity)

Dog: 5 ppm, equal to 0.22 mg/kg bw per day (four-week study of toxicity)

Estimate of acceptable daily intake for humans

0-0.002 mg/kg bw

Studies that would provide information useful for the continued evaluation of the compound

Further observations in humans.

Toxicological criteria for setting guidance values for dietary and non-dietary exposure to carbofuran

EXPOSURE	RELEVANT ROUTE, STUDY TYPE, SPECIES	RESULT, REMARKS
Short-term (1-7 days)	Oral toxicity, rat	LD_{50} =6-14 mg/kg bw
	Dermal toxicity, rat	LD_{50} >500 mg/kg bw
	Inhalation toxicity, rat	LC_{50} = 0.088-0.1 mg/litre
	Dermal irritation, rabbit	Not irritating
	Ocular irritation, rabbit	Not available
	Dermal sensitization, guinea-pig	Not sensitizing
Medium-term (1-26 weeks)	Repeated oral, 4 weeks, toxicity, dog	NOAEL = 0.22 mg/kg bw per day
	Repeated oral, reproductive toxicity, rat	NOAEL = 1.6 mg/kg bw per day, parental and pup toxicity
	Repeated oral (gavage), developmental toxicity, rat	NOAEL = 1.2 mg/kg bw per day (highest dose tested). No evidence of teratogenicity
	Repeated oral (feeding), developmental toxicity, rat	NOAEL = 1.5 mg/kg bw per day, maternal toxicity
	Repeated oral, developmental toxicity, rabbit	NOAEL = 0.6 mg/kg bw per day, maternal toxicity. No evidence of teratogenicity
	Repeated oral, developmental neurotoxicity, rat	NOAEL = 1.7 mg/kg bw per day
Long-term (> one year)	Repeated oral, two years, carcinogenicity, mouse	NOAEL = 2.8 mg/kg bw per day, cholinesterase inhibition. No evidence of carcinogenicity
	Repeated oral, two years, carcinogenicity, rat	NOAEL = 1 mg/kg bw per day, reduced body-weight gain and cholinesterase inhibition. No evidence of carcinogenicity.

CIRRICULUM VITAE

NAME : Ms. Soontree Khuntong

BIRTH DATE : January 7, 1961

BIRTH PLACE : Bangkok, Thailand

EDUCATIION :

YEAR	INSTITUTION	DEGREE
1993	Srinakarinwirot Univ.	B. Sc. (Chemistry)
2001	Kasetsart Univ.	M.S. (Analytical Chemistry)

POSITION/TITLE : Assistant Professor

WORKPLACE : Faculty of Resources and Environment
Kasetsart University, Si Racha Campus

AWARDS : Good Quality Thesis Award in the title of "Determination of Inactive Molybdenum and Molybdenum-99 in Sodium Pertechnetate (Technetium-^{99}m) for Intravenous Injection" Graduate School, Kasetsart University, 1990

Consolation Prize in the Applied Research for the topic "Utilization of Sewage Sludge in Agriculture" Department of Agriculture.