# CHAPTER I

# INTRODUCTION

## *1.1 Pesticides*

Pesticides mean any substance, matter or microorganism intended to directly control, destroy, mitigate, attract or repel any organism that is injurious to or noxious or troublesome for humans, animals, vegetation, crops or any other objects (EPA, 2000).

### *Mode of action of pesticides*

Pesticides kill pests in three main ways. They are poisonous when eaten by the pest, these chemicals are called stomach poisons. If killing is by passing through pests' skin or cuticle, they are called contact poisons. If they are breathed in as vapors, these chemicals are called fumigants. (http://www.sarawak.com, 2001)

### *Pesticide terminology*

**Active ingredient** is the actual poison itself – that part of the product which kills the pests. For example: carbaryl 80 is a product containing 80 percents of the active ingredient carbaryl, while the remaining 20 percents is made up of inert materials such as oil, dust, etc.

**Forms of pesticides-** Pesticides are sold in various forms: (1) in dusts or granules (used dry); (2) in wettable powders (W.P.) or dispersable powders (D.P.) or soluble powders (S.P.)-that are mixed with water; and (3) emulsion concentrate (E.C.)-chemicals in liquid form that are mixed with water.

**Formulation** is the way chemical is made up, e.g., as dusts, wettable or dispersable powders or emulsion concentrates.

**Classification** – There are hundreds of different chemicals for killing insects and pests, but only few large groups of toxic chemicals are used for agriculture in Thailand. They can be primary classified on the basis of chemical structure as follows (http://www.sarawak.com) :

1. Botanicals – chemicals obtained from plants, e.g., nicotine, rotenone and pyrethrum.
2. Inorganic compounds- arsenicals, zinc phosphide.
3. Organocompounds
    - organochlorine compounds- DDT, lindane, benzene hexachloride (BHC), cyclodienes, kethane, thiodan, chlodane, toxaphene etc.
    - Organophosphate compounds- malathion, parathion, diazionon, methidathion, dimethoate etc.
4. Carbamates- carbaryl, carbendazim, carbofuran.
5. Fumigants- methyl bromide, ethylene oxide, phosphine, carbon disulphide.
6. Miscellaneous compounds- creosote, metaldehyde, thuricide, warfarin

Morover, they can be classified on the basis of the target used such as insecticides, herbicides, fungicides and other pesticides such as biocides, rotendicides and acaricides etc. Table 1 lists those pesticides classified according to their targets. Our study will focus on the first 3 types since they are reported as residues of pesticide mostly contaminated in soils and water. However, herbicides commonly used such as 2,4-D have been previously studied for their ability to complex with CDs. We are thus interested in insecticides and fungicides. Of all pesticides in the two

groups, carbaryl, carbendazim and methidathion are those commonly used for agricultural purpose in Thailand.

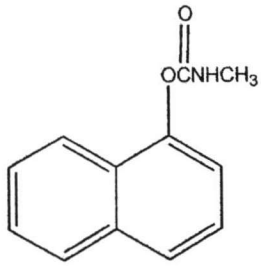
**Table 1. List of pesticides classified according to targets used [PAN Pesticides Database: Chemical classifications (2001), Agricultural Technology Publication Center (1990)]**

| Type of pesticide | Chemical structure | Example |
|---|---|---|
| Insecticide | Organochlorine | aldrin, chlordane, dieldrin, endrin, endosulfan, heptachlor, BHC |
| | Organophosphate | **methidathion,** trichlorfon, dichlorvos, mevinphos, bomyl |
| | Carbamate | **carbaryl**, baygon, zectran, dimetilan, mercaptodimethur |
| | Pyrethroid | pyrethrin |
| | Fumigant | telone, paradow, bromofume, fumazone |
| | Miscellaneous | creosote oil, dowicide G, phthalonitrile, lethane, thanite |
| | Chlorophenoxy and Chlorobenzoic acid | rhothane, methoxychlor, DDT |
| Herbicide | Phenyl urea | 2,4-D, diphenatrile, fluometuron, tebuthiuron, diuron, linuron |
| | Bipyridylium | paraquat |
| | Triazine | canopy, extrazine, sulfentrazone, carfentrazone, atrazine, prometryn, |
| | Amide and thioamide | cyanazine, napropamide, pronamide, propanil, isoxaben |

**Table 1. List of pesticides classified according to targets used (continued)**

| Type of pesticide | Chemical structure | Example |
|---|---|---|
| Fungicide | benzimidazole | **carbendazim**, chlorfenazole, cypendazole, debacarb, fuberidazole |
| | Mercurial compound | chloromethoxypropyl, methyl mercuri guanidine |
| | Inorganic copper | copper, copper carbonate , copper naphthenate, copper potassium sulfide |
| | Organotine | decafentin, fentin, tributyltin oxide |
| | Dithiocarbarmate | diammonium ethyl, metham sodium |
| | Chloronitrobenzene | dyrene, botran, dexon, pentachloronitrobenzene |
| Rotendicide | botanical | scilliroside, strychnine |
| | coumarin | brodifacoum, bromadiolone, coumachlor, coumafuryl |
| | zinc phosphide | zinc phosphide |
| | indandione | chlorophacinone, diphacinone, pindone |
| Acaricide | dinitrophenol | binapacryl, dinex, dinobuton , dinocap |
| | formamidine | amitraz, chlordimeform, chloromebuform, formetanate |

***Carbaryl***




Carbaryl (1-naphthyl methyl carbamate) is one of the most commonly used insecticides in Thailand with an estimated annual use of 150 thousand kilograms (Agricultural Regulatory Division, 2000). It is a broad-spectrum insecticide and is registered for use on more than 100 different crops, animals, ornamental plants, and indoor areas. Many of its carbaryl-containing products are marketed under the brand name Sevin (Cremlyn,1991). The insecticide carbaryl is striking because its use has been associated with such a large number of health problems. From acute toxicity, suppression of immune system functions, and behavioral problems to cancer, genetic damage, and reproductive problems in both males and females, carbaryl's adverse effects span an enormous range. Information on chemical properties is presented in Appendix 4.

Carbaryl is a neurotoxic carbamate insecticide. Like all members of this chemical family, it inhibits the action of an enzyme that is an essential component of insect, fish, bird, and mammal nervous systems. The enzyme, acetyl cholinesterase (AChE), controls the chemical reaction that transforms acetylcholine into choline after acetylcholine has been used to transmit nerve impulses across the junctions between nerves (Morgan,1989). Carbaryl can also affect a number of other enzyme systems in living things. For example, the carboxylesterases (detoxification enzymes) (Gay and Ehrich,1990),lactic dehydrogenase (enzyme that utilizes sugar) (Parafita and

Fernandez-Otero,1984), and serine esterases (enzymes important to the function of certain immune system components) (Murphy,1986) are all inhibited by carbaryl.

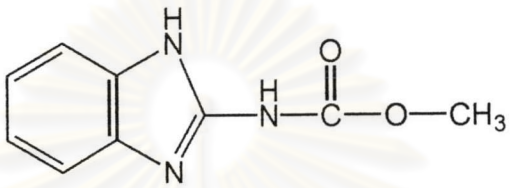
In humans, acute effects of carbaryl exposure include headaches, nausea, incoordination, and difficulty breathing. Carbaryl can cause a variety of behavioral effects, some of which are relatively long-term. It also suppresses several functions of the immune system. Men exposed to carbaryl have more abnormal sperm and lower sperm counts than unexposed men (Smalley, Curtis and Earl,1968). In female laboratory animals, exposure to carbaryl has caused a variety of reproductive problems, including birth defects in beagle dogs and increased rate of miscarriages in monkeys (Cranmer,1986). Exposure to carbaryl has been associated with a higher incidence of the cancer non-Hodgkin's lymphoma in farmers and brain cancer in children. Carbaryl acts synergistically with a number of other insecticides and herbicides. Secret ingredients in carbaryl formulations include petroleum oils and crystalline silica (MSDS reference for crop protection chemicals,1992) associated with the lung disease silicosis and cancer (U.S. Dept. of Health and Human Services ,1991).

Humans are exposed to carbaryl through consuming contaminated food and water, using carbaryl in homes, gardens, and offices, through drift, and through occupational exposure. It has been found in groundwater, surface water, and fog. Carbaryl is well-absorbed by skin, particularly skin of young animals (Street,1981). Protective clothing can be difficult to effectively launder and transmits more carbaryl under hot, sweaty conditions (Shabanov *et al.*,1983).

A wide variety of nontarget animals, plants, and microorganisms are affected by carbaryl exposure. The number of sublethal effects that occur at low exposures is particularly striking. Beneficial arthropods, fish, birds, a variety of crop plants, and

nitrogen-fixing microorganisms are all affected by carbaryl. Ecosystem study indicated that the effects on individual species result in persistent effects on ecosystems (Barrett, 1968).

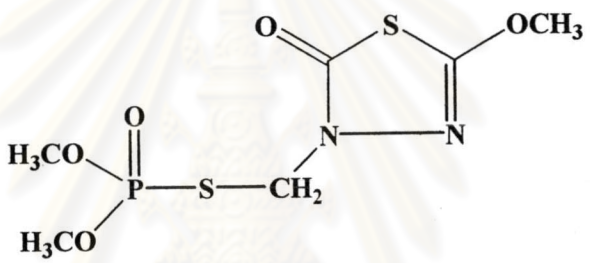
## *Carbendazim*



Carbendazim is a systemic broad-spectrum fungicide controlling a wide range of microorganisms and also invertebrates, especially earthworms. It is used against fungal diseases of field crops, fruits, nuts, ornamentals, mushrooms, and turf. In addition, it can be used as a preservative in textile, paper-making, leathers and painter industry as well as a preservative of fruits and eggs (www.carbendazim technical.htm). The Carbendazim is an internal absorbent germicide used to prevent and control plant diseases caused by various fungi. In 2000, carbendazim was the major imported fungicide to Thailand with an estimated quantity about 537,238 kilograms (Agricultural Regulatory Division, 2000). It is the most widely used member of a family of fungicides known as the benzimidazoles. It is formulated as an aqueous dispersion, aqueous suspension, flowable water dispersible granules, and a wettable powder. Carbendazim is well absorbed after oral, but not dermal, exposure. Absorbed carbendazim is rapidly metabolized and eliminated in the urine and faeces. Because of its stability on plant material, lasting several weeks, carbendazim may become accessible to organisms feeding on leaf litter. However, the strong adsorption of carbendazim on soil and sediment particles reduces the exposure of terrestrial and

aquatic organisms. The toxicity of carbendazim through ingestion is low. Estimated human exposures, based on dietary analysis and crop tolerance values, indicate the expected intake to be below the recommended Acceptable Daily Intake (ADI), based on no-observed-effect levels in animal tests. Given the toxicity levels of carbendazim and the low dietary exposure levels, it is unlikely that it poses a significant health risk for the general population (FAO/WHO,1988b). Information on chemical properties of carbendazim is shown in Appendix 5.

## *Methidathion*




Methidathion is a non-systemic organophosphorous insecticide and acaricide with stomach and contact action (EXTOXNET, 1996). Trade names for products containing methidathion include Somonic, Somonil, Supracide, Suprathion and Ultracide. Information on chemical properties is presented in Appendix 6. The compound may be found in formulations with many other pesticides. It is used to control a variety of insects and mites in many crops such as fruits, vegetables, tobacco, alfalfa and sunflowers. It is especially useful against scale insects. It works by inhibiting certain enzyme actions in the target pests. Methidathion is a highly toxic compound that carries the signal word DANGER on its label. The compound is poisonous to humans, because of its capacity to interfere with enzymes related to breathing and other nervous system activities. Symptoms of acute methidathion poisoning may include nausea, vomiting, cramps, diarrhea, salivation, headache,

dizziness, muscle twitching, difficulty breathing, blurred vision, and tightness in the chest (Integrated Risk Information System, 1993). Acute exposure may cause intense breathing problems including paralysis of the respiratory muscles. The symptoms of acute methidathion poisoning are similar to acute exposure to parathion. The compound is also very highly toxic through exposure on the skin. Moderate to low amounts of methidathion caused a number of adverse reproduction related effects. The EPA has classified the compound as a possible human carcinogen. The committee stated that this one study constitutes only limited evidence of carcinogenicity because it induced common tumors in only one sex of one species and that the mutagenic tests were not supportive of a higher classification (Guidance for the Registration of Pesticide Products containing Methidathion as the Active Ingredient, 1988). Methidathion is rapidly absorbed, broken down and eliminated in animals (Integrated Risk Information System, 1993). The breakdown products of the parent compound are not of toxicological concern. Only very small amounts of various metabolic products of methidathion have been detected in milk from cows (Smith, 1993) and in chicken eggs. The compound is highly acutely toxic to all aquatic organisms (vertebrates and invertebrates) and thus can pose substantial risk to these populations if it gets into surface water through actions like pesticide drift, in surface water run-off or by entry into the sewer system.

## *1.2. Cyclodextrins*

Cyclodextrins, also known as Schardinger dextrins, cycloamyloses, and cycloglucoamyloses, comprise a family of cyclic oligosaccharides obtained from starch by enzymatic degradation. Villers discovered them in 1891, but Schardinger made the first detailed description of the preparation in 1903. In the preparation process, the starch is treated with a group of amylases called cyclodextrin glycosyltransferases (CGTase, E.C.2.4.1.19) the primary product of chain splitting are circular α(1-4)-linked oligosaccharides, called cyclodextrin (Szejtli,1996) see Figure 1.

Cyclodextrins are capable of forming inclusion complexes with various molecules, thus altering their physical/chemical properties. There are three well known naturally produced α-, β-, and γ-cyclodextrin (α-, β-, and γ-CD), consisting of 6, 7, or 8 glucose units, respectively. Cyclodextrins have gained most attention in the areas of pharmaceutics, drug and pesticide delivery by improving solubility and/or stability of various guest molecules. However, the major applications of cyclodextrins include its use in the field of food, textile, paper, separation sciences (*e.g.* chromatographic, electrophoresis, and analytical chemistry), and agricultural industries [Szejtli (1996), Frömming and Szejtli (1994)]. Cyclodextrins containing more than eight glucose units has been reported [Szejtli (1996), Larsen (1998)], but due to low yield and complicated purification, these cyclodextrins are less characterized and have presently no value in commercial applications. Cyclodextrins with less than six glucose units in the ring cannot be formed enzymatically due to steric reasons, but can be obtained by chemical synthesis [Szejtli (1996), Frömming and Szejtli (1994)]. Like the large cyclodextrins, those are also currently of minor commercial interest.

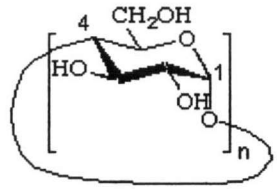



**Figure 1. Cyclodextrin with n glucose units.**

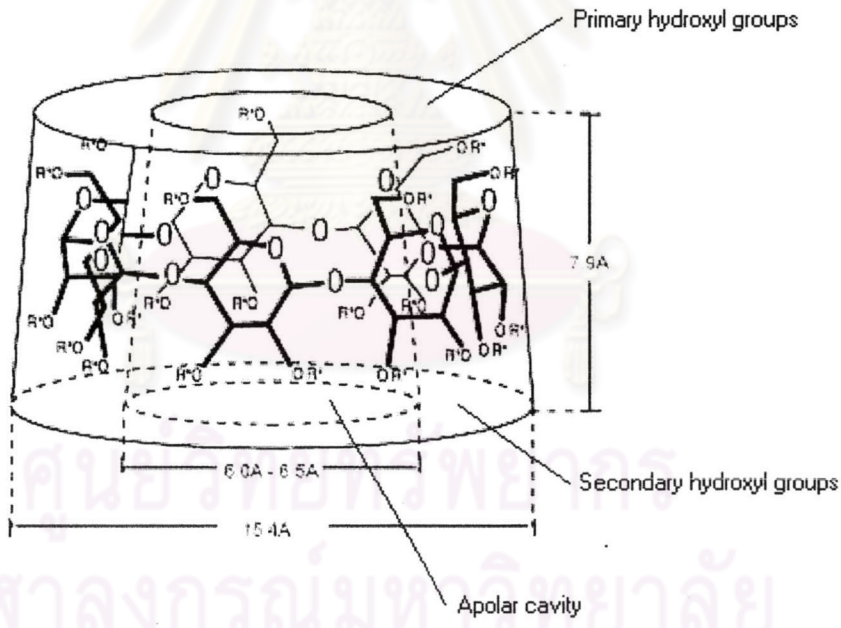



**Figure 2. Toroidal shape of β-CD molecule [http://www.cyclodex.com/indexc.html].**

### *Physicochemical Properties*

Three natural CDs (cyclodextrins), α-CD, β-CD and γ-CD are linked by α-1,4 bonds, as a consequence of the $C_1$-conformation of the α-D-glycopyranosyl residues and the lack of free rotation around glycosidic bonds, causing the formation of torus molecules (cone-shaped). The secondary hydroxyl groups (on the $C_2$ and $C_3$ atoms of the glucose unit) are situated on one edge and all primary hydroxyls on the other, as shown in Figure 2. The cavity of the torus consists only of ring of $C_3$-H group, $C_5$-H group and a ring of glucosidic oxygen. For this reason, the cavity of the torus is non-polar. This makes CDs exterior decidedly hydrophilic where as the interior of the cavity is rather hydrophobic. Free rotation of primary hydroxyls reduces the effective diameter of the cavity on the side they occur, while the secondary hydroxyl groups on the relatively rigid chains cannot rotate (Szejtli,1982). The hydrophilic surface generates relative good water solubility for the cyclodextrins and the hydrophobic cavity provides a favorable environment in which 'to fit' a typically hydrophobic (guest) molecule. This association isolates the guest molecules from the aqueous solvent and may increase the guest's water solubility and/or stability.

### *Cyclodextrin Derivatives*

β-CD is commercially the most interesting of the three natural cyclodextrins due to easy production conditions, cavity diameter, availability, approval status, and price. It is the most widely used and represents at least 95% of all produced and consumed cyclodextrins [Szejtli (1994)]. β-CD possesses a 6-6.8 A° cavity, which is able to accommodate aromatic groups found in many molecules and drugs [Rajewski and Stella (1996)]. Unfortunately, β-CD has the lowest solubility in water compared to α-CD and γ-CD, see Table 2.

**Table 2 . Physical properties of the CDs and some derivatives. [Szejtli (1996), Jozwiakowski and Connors (1985)].**

| | α-CD | β-CD | γ-CD | DM-β-CD[1)] | HP-β-CD[2)] |
|---|---|---|---|---|---|
| Number of glucose Residues: | 6 | 7 | 8 | 7 | 7 |
| Cavity dimensions(A°) | | | | | |
| cavity diameter : | 5 | 6 | 8 | 6 | 6 |
| height of torus : | 7.9 | 7.9 | 7.9 | 10 | |
| diameter of periphery : | 14.6 | 15.4 | 17.5 | | |
| Molecular weight | 973 | 1135 | 1297 | 1331 | 1300 |
| Aqueous solubility [3)] | 14.5 | 1.85 | 23.2 | 57 | >50 |
| Melting point (°C) | 275 | 280 | 275 | 295-300 | |
| pKa [4)] | 12.3 | 12.2 | 12.1 | | |
| Half-life of ring Opening[5)] (h) | 6.2 | 5.4 | 3.0 | 8.5 | |
| Enzymatic hydrolysis[6)] | Negligible | slow | rapid | | |

1) heptakis-2,6-di-O-methyl-β-CD
2) 2-Hydroxypropyl-β-CD
3) in grams per 100 ml water at ambient temperature
4) pKa : by potentiometry at 25 °C
5) half-life of ring opening : in 1 N HCl at 60°C
6) by *Aspergillus oryzae* α-amylase

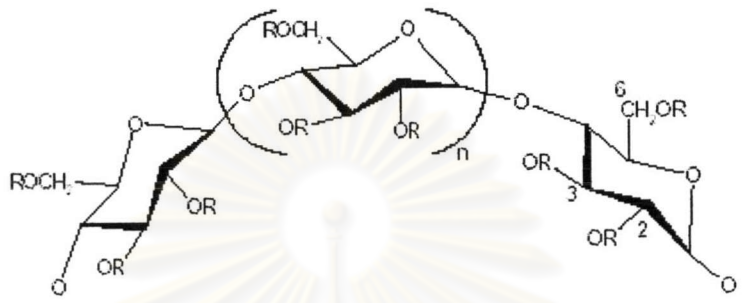
Cyclodextrins are less water-soluble than their comparable linear saccharides in water [Szejtli (1996)]. The lower solubility of cyclodextrins compared to linear dextrins is probably due to a strong crystal lattice energy between each molecule and the inability of some of the hydroxyl moieties to interact with water molecules. For example, the C-2-OH group of one glucose unit can form a strong hydrogen bond with the C-3-OH group of the adjacent glucose unit, which reduces the ability to form hydrogen bonds with the surrounding water molecules [Szejtli (1996), Frömming and Szejtli (1994)].

The secondary hydroxyl groups of the nearby glucose unit in the molecule of β-CD can form seven hydrogen bonds, called the secondary belt, which gave β-CD the most stable but the lowest soluble form. The hydrogen belt is incomplete for the α-CD and thereby only four hydrogen bonds can be fully formed. The hydrogen belt is also incomplete for γ-CD but it possesses a more flexible structure, γ-CD is therefore the most soluble of the three cyclodextrins [Szejtli (1996), Frömming and Szejtli (1994), Szejtli (1998)].

Table 3 shows physical properties of natural and some derivatized CDs. Each CD has a different capability of inclusion complex formation with different sizes and polarity of guest molecules. The natural CDs can be modified for many different purposes, for example to improve the aqueous solubility of β-CD or to decrease the toxicity in parenteral applications. This characteristic has been obtained by alkylation of the hydroxyl groups (methyl-, hydroxypropyl- and also hydroxyethyl-CDs) by substitutions of primary hydroxyl groups with saccharides (glycosyl- and maltosyl-CDs), or by polymerization of cyclodextrins.

Unfortunately, the ideal cyclodextrin derivative does not exist [Szente and Szejtli (1999)]. It means the cyclodextrin derivative that is needed, which after

**Table 3. General structure of cyclodextrin and their major derivatives with pharmaceutical and agricultural interest. The derivatives may have different degree of substitution of the 2nd, 3rd, or 6th position of the glucose unit [Rajewski and Stella (1996), Szejtli (2000)].**




| Cyclodextrin | Abbreviation | R | n |
|---|---|---|---|
| α-Cyclodextrin | α-CD | H | 4 |
| β-Cyclodextrin | β-CD | H | 5 |
| γ-Cyclodextrin | γ-CD | H | 6 |
| 6-O-Glycosyl-β-Cyclodextrin | G-β-CD | Glucosyl Or H | 5 |
| 6-O-Maltosyl-β-Cyclodextrin | $G_2$-β-CD | Maltosyl Or H | 5 |
| Dimethylated-β-Cyclodextrin | DIMEB | $CH_3$ or H | 5 |
| Trimethylated-β-Cyclodextrin | TRIMEB | $CH_3$ or H | 5 |
| Randomly methyl-β-Cyclodextrin | RAMEB | $CH_3$ or H | 5 |
| 2-Hydroxypropyl-β-Cyclodextrin | HP-β-CD | $CH_2CHOHCH_3$ or H | 5 |
| Hydroxybutenyl-β-Cyclodextrin | Hben-β-CD | $CH_2CHOHCHCH_3$ or H | 5 |
| Sulfobutylether-β-Cyclodextrin | SBE-β-CD | $(CH_2)_4SO_3Na$ or H | 5 |

fulfilling its task (*i.e.* solubilizing and carrying a poorly soluble drug to the required site), will be destroyed by ring opening and be eliminated from the body without any accumulating effects (Szejtli, 1996). Even that the ideal cyclodextrin derivative not exist, several cyclodextrin derivatives have still of pharmaceutical and agricultural interest, see Table 3.

***Methylated Cyclodextrins***

DIMEB (heptakis(2,6-di-*O*-methyl)-βCD), TRIMEB (heptakis(2,3,6-tri-*O*-methyl)-βCD), and RAMEB (randomly methylated-βCD), see Table 3, are the three most common and studied methylated cyclodextrins (Frömming and Szejtli,1994). Methylated cyclodextrins show increasing water solubility with increasing degree of substitution until 2/3 of the hydroxyls are methylated while a decrease is observed as methylation approaches 21 methoxy substitutions per β-CD molecule (*i.e.* 100%) (Szente and Szejtli, 1999). DIMEB and TRIMEB are up to 30 and 16 times more aqueous soluble than β-CD, respectively.

***Hydroxyalkylated Cyclodextrins***

The hydroxyalkylated cyclodextrins are non-crystalline material with high water solubility. They are prepared by a chemical reaction between reactive alkylene oxide and the free hydroxyls groups (C2, C3, and C6) on each glucose unit. 2-Hydroxypropyl-β-cyclodextrins (HP-β-CD), see Table 3, is produced from β-CD by hydroxypropylation of the hydroxyl groups of the cyclodextrin in the presence of a strong base. The multicomponent character prevents any crystallisation and thus makes it on average 27 times more soluble in water than β-CD. Hydroxybutenyl-β-cyclodextrin (Hben-β-CD) is the newest candidate of the potential guest carriers. With a DS (degree of substitution) of 6.5, it is very water soluble and it was found to

be equal or more effective in solubilising a number of guest molecules than HP-β-CD [Buchanan *et al.* (2000), Szejtli(2000)].

***Branched Cyclodextrins***

Branched cyclodextrins comprise a group of cyclodextrins where the macrocyclic has been derivatised by an α(1→6)-linked with mono-, di- or polysaccharides (Jicsinszky *et al.*,1996). The branched cyclodextrins are often enzymatically prepared and can easily be obtained as homogenous preparations, in contrast to the chemically modified heterogeneous cyclodextrin derivatives, *e.g.* hydroxyalkylated cyclodextrins. They can be categorized into two different groups, homogeneous branched and heterogeneous branched cyclodextrins. Homogeneous branched cyclodextrins have one glucose (G1), or malto-oligosaccharides such as maltose (G2) or maltotriose (G3), etc. attached directly to the cyclodextrin. Heterogeneous branched cyclodextrins have various types of sugar moieties, such as galactose or mannose, in the side chains of the homogenous branched cyclodextrin or directly attached to the parent cyclodextrin [Jicsinszky *et al.*(1996), Abe *et al.*(1986)]. Due to steric hindrance of the attached groups, the branched cyclodextrins are more resistant to enzymatic degradation, *e.g.* by amylases, than their parent cyclodextrin (Yamamoto *et al.*,1989). Two homogeneous branched cyclodextrin derivatives, 6-O-glycosyl-β-cyclodextrin (G-β-CD) and 6-O-maltosyl-β-cyclodextrin (M-β-CD), see Table 3, have been commercially available since mid-1990s. They have significant increased water solubility compared to β-CD.

## *1.3 Cyclodextrin-Guest Complexation*

The various cyclodextrins can be considered as an empty cavity of molecular size. When the cavity is filled with a molecule of another substrate, it is called an "Inclusion Complex". Inclusion complexes are systems that consist of two or more molecules, in which one of the molecules, the host, includes entirely or partially the guest molecule. The molecules in the inclusion complex are only in contact by physical forces and without covalent binding [Szejtli (1996), Duchêne and Wouessidjewe (1996)]. The name "Inclusion Complex" seems to be the most appropriated, since no covalent bond is established between the host and guest. Moreover, the dissociation-association equilibrium is probably the most characteristic feature of the host-guest association (Szejtli, 1996). Almost all applications inside the cyclodextrin technology area involve complexation and therefore this is a very important factor to investigate [Szejtli (1996), Duchêne and Wouessidjewe (1996)].

***Overall Inclusion Complex Stability Constant***

The equilibrium between the guest and cyclodextrin is fundamental in the measurement of the inclusion complex stability constant. The general equation can be expressed as:

$$n[Guest] + m[CD] \Leftrightarrow [Guest_nCD_m] \qquad \text{Eq. 1-1}$$

where $n$ and $m$ are the number of mole for the guest molecule and cyclodextrin, respectively. The equilibrium constant $K$ or inclusion complex stability constant for the process can be defined as:

$$K = \frac{[Guest_nCD_m]}{[Guest]^n[CD]^m} \qquad \text{Eq. 1-2}$$

The simplest formation of a complex between the cyclodextrin and a guest molecule is in stoichiometric proportions of 1:1. Cyclodextrin complexes with two guest molecules per cyclodextrin or vice versa are also quite common (Connors, 1996).

***Mechanism of Inclusion***

The minimum requirement for inclusion complex formation is that the guest molecule must fit, entirely, or at least partially, into CD's cavity. Stable complexes will not be found if guest molecules are too small because they would slip out of the cavity. Complex formation is also possible with bulky molecules if certain groups of side chains of those molecules can penetrate into CD's cavities. (Szejtli, 1982).

The inclusion process is a result of the ability of one compound, owing to its suitable steric properties and partially also polarity, to enclose spatially another compound. The term's host and guest were used to clarify their functions. An important characteristic property of the host is its ability to form a structure with free cavities with dimensions that permit the enclosure of a guest molecule. The formation of inclusion compounds is not dependent on the chemical affinity or the presence of certain groups, but rather on the spatial arrangement and interactions, where primitively van der Waals forces and oriented dipole interactions are important (Smolkava, 1980). Since the cavity of cyclodextrins is hydrophobic, the inclusion of a molecule in the cyclodextrins cavity is basically a substitution of the water inside the cavity with a less polar substance. The substitution of water from the cavity with a more non-polar guest is energetically favorable for both the cyclodextrin and the guest, which is illustrated in Figure 3. The "driving force" in the complexation is not fully understood, but it seems to be a combination of different effects depending on the specific guest and cyclodextrin. These effects can be hydrophobic interaction,

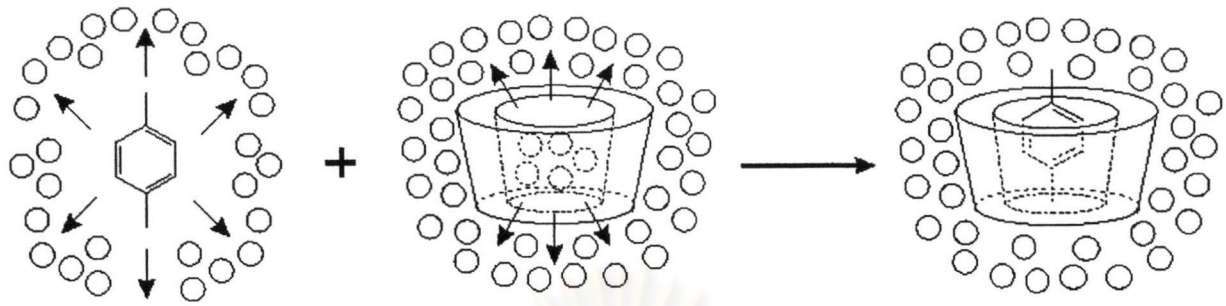


**Figure 3. Schematic illustration of inclusion complexation of *p*-xylene by a cyclodextrin. The small circles represent the water molecules (Szejtli ,1996).**

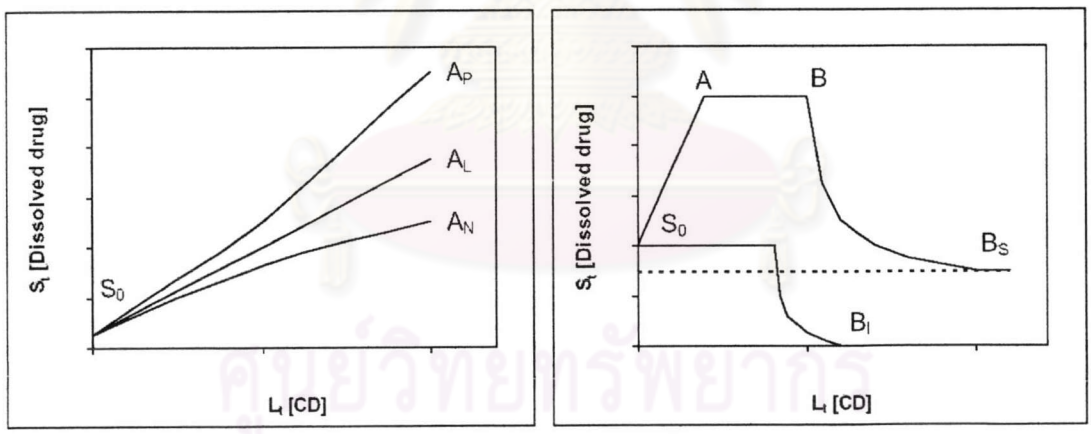



**Figure 4. Types of solubility diagram of CD and guest (Higuchi and Connors, 1965)**

van der Waals interaction, hydrogen bonding, dipole-dipole interaction, and release of "enthalpy-rich" water [Szejtli (1996),Duchêne and Wouessidjewe (1996), Connors (1997)].

***Van der Waals Interaction***

The interaction includes both permanent induced dipole-dipole interactions and London dispersion forces between the hydrophobic moiety of the guest and the cyclodextrin cavity. When two molecules are brought close together, they both attract and repel each other depending on the distances that separate them. The attraction force of the molecules is caused by instantaneous and short-lived imbalance in the electron distribution of an atom that generates a temporary dipole. These short-lived induced dipoles result in an induction electron distribution of the neighboring atom that generates a temporary polarization. This polarization minimizes the electron-electron repulsion between the atoms also known as induced dipole-dipole interaction or London dispersions forces. Other forces involved are dipole-induced dipole and permanent dipole. Common for all these repulsive and attractive forces, known as van der Waals forces, are that they neither are non covalent nor no ionic [Van Holde *et al.* (1998), Martin (1993)]. These force are usually weak for all kind of interactions, but are likely to be numerous in the cyclodextrin cavity and thereby have to be taken into consideration [Szejtli (1996), Connors (1997)].

***Hydrogen Bonding Interaction***

The interaction involves hydrogen bonding between the polar functional group of guest molecules and the hydroxyl groups of cyclodextrin. If hydrogen is close to an atom that is very good at attracting electrons (like N, O, or F) the hydrogen end of the bond becomes very positively charged and the other atom becomes negatively

charged (*i.e.*, polar). Hydrogen is the smallest atom in the periodic table, which makes it possible for hydrogen atom and the other atom to get very close together. The combination of high polarity and close approach result in the interaction being particularly strong due to the force of attraction between two opposite charges, is proportional to the magnitude of their charges divided by the square of the distance between them. In fact, the interaction is so strong that it dwarfs all other dipole-dipole attractions (Chang, 1990). The hydrogen bonding is considered to play an important role in the stability of the cyclodextrin complexes in aqueous solution. It may furthermore contribute to a conformational change either in the cyclodextrin, the guest, or both, which results in a more stable complex (Easton and Lincoln, 1999).

***Release of "Enthalpy-rich" Water***

Release of high energy water molecules in CD's cavity by guest molecules, resulting in a favorable enthalpy change and release of strain energy in the macromolecular ring of CD (changing from the high-energy conformation of the CD-water complex to the lower energy conformation of the CD-guest complex).

When water is substituted from the cavity of the cyclodextrin, a decrease in the energy occurs. This is due to an increase in solvent-solvent interaction, since the surface contact between solvent and cyclodextrin cavity, as well as between solvent and guest molecule, are reduced. Furthermore, water inside the cyclodextrin cavity cannot possess its tetrahedral hydrogen bonding capacity compared to those in the surrounding solvent, and it is therefore often reported as "high energy" water or "enthalpy rich" water. One of the main driving forces for complexation could therefore be the release of this "high energy" water from the cyclodextrin cavity, which allowing them to form their full compliment of hydrogen bonds with the surrounding water [Szejtli (1996), Loftsson and Brewster (1996), Connors (1997)].

The geometric capability, the polarity or charge of guest molecules, the medium and the temperature are the most important factors for the complex formation. If the guest is too small, it will easily pass in to and out the cavity with little or no bonding at all. Inclusion compound with the guest molecules larger than the cavity may also be possible, but the complex is formed in such the way that only certain groups of side chains penetrate into the cyclodextrin cavity. The extent of the complex formation also depends on the polarity of the guest molecule. Strongly hydrophilic molecules (very soluble in water) strongly hydrated and ionized groups are not, or only very weakly complexable. Only molecules which are less polar than water can be complexed by cyclodextrin. The stability of an inclusion compound is proportional to the hydrophobic character of the guest molecule (Li and Purdy, 1992)

## *1.4 Measurement of Inclusion Complex Stability Constant*

A range of methods has been applied for the determination of inclusion complex formation constants between cyclodextrin and guest molecules. These are *e.g.* solubility isotherms, UV/visible spectroscopy, circular dichroism, fluorescence, nuclear magnetic resonance, potentiometry, calorimetry, refractive index, optical rotary dispersion, kinetics, and chromatography [Connors (1996), Repta (1981)]. In this study, phase solubility technique, DSC and FTIR were chosen as the tool for the determination of the of inclusion complex formation constants between cyclodextrin and guest molecules.

***Detection of inclusion complexation in solution***

Generally, all studies of the interaction between cyclodextrin and guest start with solubility measurements of the guests by a phase solubility experiment. The phase solubility diagram is then constructed by plotting the sparingly soluble substrate, $S_t$, designated as the guest, against the ligand, $L_t$, the cyclodextrin. From this diagram, it is then possible to estimate the inclusion complex stability constant (Connors,1987). Higuchi and Connors (1965) presented a procedure for constructing and analyzing phase solubility diagrams. The phase diagrams or solubility isotherms are classified as either an *A-type* or a *B-type*. The *A-type* diagrams are defined by the absence of any form of precipitation of the inclusion complexes after equilibrium has been attained and by an increase in solubility of the guest as a result of complexation with cyclodextrin. The *B-type* solubility diagrams on the other hand indicate precipitation of a guest-cyclodextrin complex at a certain concentration of the cyclodextrin. As seen in Figure 4, there are three *A-type* diagrams, AL, AP and AN. The AL solubility diagram is the simplest, which depicts a linear relationship between the cyclodextrin concentration and the total concentration of the dissolved guest (SL). This indicates that if all the complexes present are first-order in relation to the concentration of the cyclodextrin, L, then the solubility diagram will be linear. However, the complex may be of higher order (*e.g.* $S_2L$, $S_3L$, *etc.*), but a linear diagram is often taken as evidence for the presence of first order complexes. The non-linear plot of a concave-upward curvature (AP solubility diagram) represents the presence of higher order complexes with regard to cyclodextrin, which means that there are two or more cyclodextrins molecules that are bound to the same guest molecule (*e.g.* $SL_2$, $SL_3$, *etc.*). The non-linear plot with the concave downward curvature (AN solubility diagram) is believed to be either due to the presence of higher

order complexes with respect to the guest or a change in the guest-cyclodextrin solvent interactions as a function of the cyclodextrin concentration or self association of ligand [Connors (1987), Repta (1981), Pedersen (2000)].

There are two *B-type* solubility diagrams, Bi and the Bs diagrams. The Bi solubility diagram is represented initially by a straight horizontal line with increasing cyclodextrin concentration until a certain concentration of the cyclodextrin is attained, followed by a sharp drop in the concentration of the dissolved guest. This indicates that the total concentration of the dissolved guest remains constant despite the formation of any inclusion complex, which leads to the believe that the formation of a guest-cyclodextrin complex under these conditions is insoluble. The end of this plateau indicates the total consumption of the solid guest. At this concentration, the cyclodextrin form complexes with the already sparingly dissolved guest. The guest molecules initially present in the solution therefore leave the solution as a solid inclusion complex and as there are no more solid guest molecules to compensate this reduction, a drop in the concentration of the dissolved guest is observed. The Bs solubility diagram is similar to the Bi diagram with the exception that the initial complex formed is soluble to some extent and therefore an initial increase in the solubility of the guest is seen. However, at a certain cyclodextrin concentration, the solubility of the inclusion complex limits the solubility of the dissolved guest, resulting in the formation of a plateau [Connors (1987), Repta (1981), Pedersen (2000)]. The solubility constant (Kc) of 1:1 can be calculated from the slope and intercept of the initial straight line portion of the diagram from the following equation:

$$Kc = \frac{\text{slope}}{S_0\,(1\text{-slope})}$$

***Detection of inclusion complexation in solid state***

Although there are many techniques to investigate the inclusion complex formation, this section reviews some techniques for identification in this work.

- ***Differential scanning calorimetry (DSC)***

When the guest molecules are included in the cyclodextrin cavity or in the crystal lactice, their melting, boiling and sublimation points are usually shifted to a higher temperature or disappear within the temperature range that the cyclodextrin decomposed.

- ***Fourier Transform Infrared spectrometry (FTIR)***

IR spectroscopy is used to access the interaction between cyclodextrin and guest molecules in solid state. The application of IR spectroscopy is limited to guests having some characteristic bands, such as carbonyl or sulfonyl groups. CD or guest bands often change slightly upon complex formation, and if the formation of guest encapsulated is less than 25%, bands which can be assigned to the included part of the guest are easily masked by bands of CDs (Baker *et al.*,1991)

## *1.5 Cyclodextrin in Agriculture*

Pesticides can be complexed with cyclodextrins just as the guest molecules and the " molecular encapsulation" of pesticides may result in interesting effects. The chief objectives are to develop pesticide formulations that will maintain or increase efficacy on target organisms when applied and that will not adversely impact the environment for groundwater while maintaining effective pest control. Cyclodextrins have found particular application for the formulation of poorly water soluble, volatile or unstable herbicides. Among the advantages of CD complexes of pesticides are enhanced stabilization, reduced volatility, decreased bad odor, enhanced wettability, solubility or bioavaibility and controlled-release properties (Szjetli, 1985).

Several effects attainable with cyclodextrins can be summarized, as follows

1. The cyclodextrin complexes of poorly wettable, slowly and sparingly soluble substances are fairly wettable, dissolved faster and in higher degree, when compared to uncomplex hydrophobic substances. Consequently the bioavailability is improved, i.e. the same dose is required. This enables a reduction in the cost of expensive substances, and is often important from the environmental protection aspect.

2. Volatile liquids of sublimable crystalline substances can be transformed into stable, solid powders and loss through volatilization can be reduced. There are many highly effective substances with an intolerable odor. When they are complexed, they become rather odorless without altering their effect. A further advantage is that the entrapped substances are released by water e.g. rain, and liberated from their complex just at the due time from the view point of their action.

3. The degradable substances, sensitive to light, heat, oxygen and ions, can be stabilized, and become compatible with other constituents of a formulation.

4. Substances in cyclodextrin complex form are "packed" into a hydrophilic cover, so that their affinity to hydrophilic surfaces such as the intestinal tract, increase; while conversely it is decreased to hydrophobic surfaces. The contact effect of insecticides is therefore reduced, whilst its stomach poisoning effect is enhanced. The poisoning power of insecticides to non-phytophage insect e.g. bees, can be reduced, while increasing it to the herbivorous ones. Since this carbohydrate has a slightly sweet taste the cyclodextrin "packing" reduces the repellent effect of poisons.

5. The biological effectiveness of solid insecticides used in powders or suspensions is generally enhanced by decreasing the particle size. The grinding to fine particles, micronization, is an expensive and energy consuming procedure, and often

appears to be unsuccessful. The micronised particles stick together due to electric charging. Cyclodextrin complexes are formed as microcrystalline powders in aqueous systems. Due to their hydrophilic character they are easily suspended in water, and there is no need to use any organic solvent. (Szejti J., 1982).

Known, published examples of the work on the application of cyclodextrins to pesticide formulations were shown to be useful for improvement of pesticides. Several examples will be mentioned in order to illustrate these applications.

The insecticide DDVP (O-(2,2-dichlorovinyl)-O,O-dimethylphosphate) is unstable in its pure form but can be better used after inclusion within β-cyclodextrin (Teijin *et al.*,1974). Improvement of stability against the light and the atmosphere was reported. The work on pyrethrins was also studied. Pyrethrins are yellowish, light-sensitive oils and their use is therefore limited. Inclusion in β-cyclodextrin gives a powder which is easy to handle, very stable, and toxic for insects long after its application [Mifune *et al.* (1977), Yamamoto *et al.* (1976)]. In 1988 Szejtli reported on the application of βCD in the fungicide formulation (benomyl 50WP). A relatively small CD content in the fungicide formulation resulted in improvement of fungicide activity in biological tests. Dailey *et al.* (1993) studied in details on the preparation and spectroscopic characterization of aldicarb, sulprofos and thiodicarb β-CD complex. They found that β-CD could form inclusion complex with aldicarb and sulprofos but complexing with thiodicarb was unsuccessful. A true inclusion complexes of aldicarb-βCD was comfirmed by spectral study. However, in the case of sulprofos-βCD, spectral difference with its free form was small while the DSC thermogram differed markedly, an external associative complex of sulprofos with β-CD was then suggested. Formation of inclusion complexes of organophosphorothioate

pesticide such as parathion, parathion-methyl and fenitriothion with βCD and methylated βCD helped in the reduction of alkaline hydrolysis of these pesticides. Kamiya and Nakamuru (1997) reported that chemical modifications such as the methylation of the ring hydroxyl groups are effective in altering the stabilizing effect of βCDs upon pesticides. In 1996, Juan *et al.* demonstrated that incubation of the herbicide 2,4-D with βCD resulted in the formation of 1:1 stoichiometry inclusion complex. The spray-drying processing method was found suitable to increase the aqueous solubility of 2,4-D. They later found that methylated-βCD was more effective than βCD (Juan *et al.*, 1999). Szente (1998) reported that molecular encapsulation of volatile organophosphorous pesticides (malathion, DDVP, sumithion, chlorpyriphos and sulprofos) with βCDs resulted in the improvement of the solid state properties such as flowability, wettability and thermal stability. Barboto *et al.* (2000) recently reported that βCD can be successfully used to increase the water solubility of carbaryl, a carbamate insecticide. The phase solubility diagram was of $A_L$ type and X-ray analysis indicating the formation of a 1:1 stoichiometry. The characterization by DSC and FTIR of solid complex suggested that the freeze-dried product had a high degree of amorphization which means an inclusion complex was formed. FTIR showed that in carbaryl-βCD, there was the interaction between the carbonyl group of the carbaryl molecule and the hydroxyl group of βCD.

The application of CDs is thus known for the enhancement properties of the pesticides such as chemical and physical stability, dissolution rate or solubility, wettability and consequently their bioavailability, i.e. the same dose results in higher effect; reduced dose is sufficient to reach the same effect. To be able to develop pesticide formulations that will maintain or increase efficacy on target organisms

when applied and enable reduced expenditure on these substances and for environmental awareness, applicable work on the use of CDs is one possible answer. Owing to a high usage of pesticides for agricultural purpose in Thailand, we would like to apply the use of CDs on the improvement of some properties of certain pesticides. This study was designed to select the pesticide (among a few groups of widely used: carbaryl, carbendazim and methidathion) which best forms the complex with βCD and derivatives (methyl- and maltosyl-) in soluble form. The most appropiate derivative of βCD in complex formation with the selected pesticide will also be investigated from phase solubility study. Inclusion complex in solid form will then be prepared through various methods (co-precipitation, kneading and freeze-drying methods) with the aim of developing a formulation suitable for agricultural use. In addition, evidence confirming the formation of true inclusion complex by DSC and FTIR will be presented. The properties such as dissolution, thermostability, photostability and toxicity of the pesticide-CD product will be assessed in comparison with the original pesticide.

## *1.6 The objectives of this thesis*

Aims of the present study are:

1. To investigate the effects of βCD and their derivatives on soluble complex formation with three groups of widely used pesticides.
2. To determine the suitable method for preparation of inclusion complexes in solid form.
3. To confirm the formation of true inclusion complexes by DSC and FTIR.
4. To determine the dissolubility, thermostability, photostability and toxicity of the complexes.