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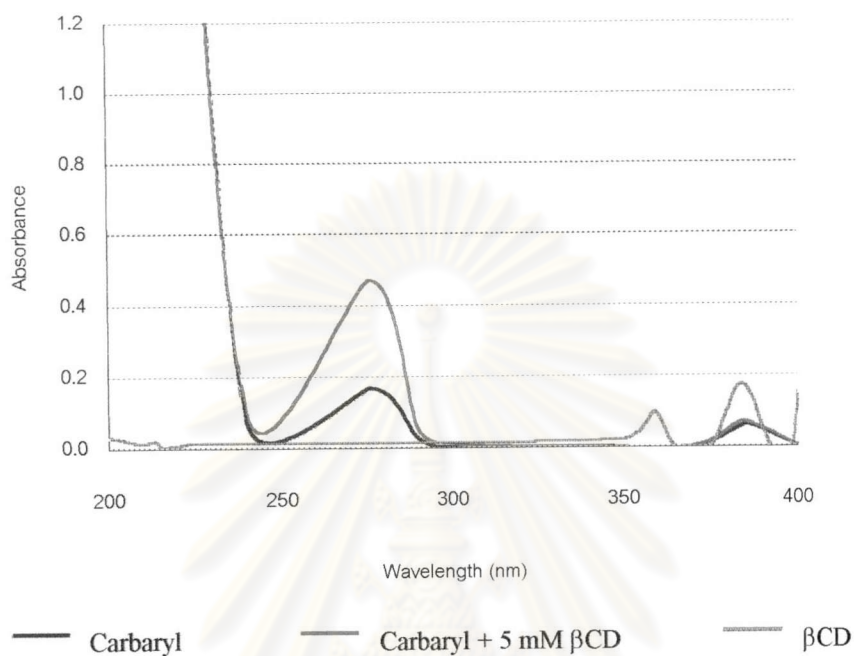
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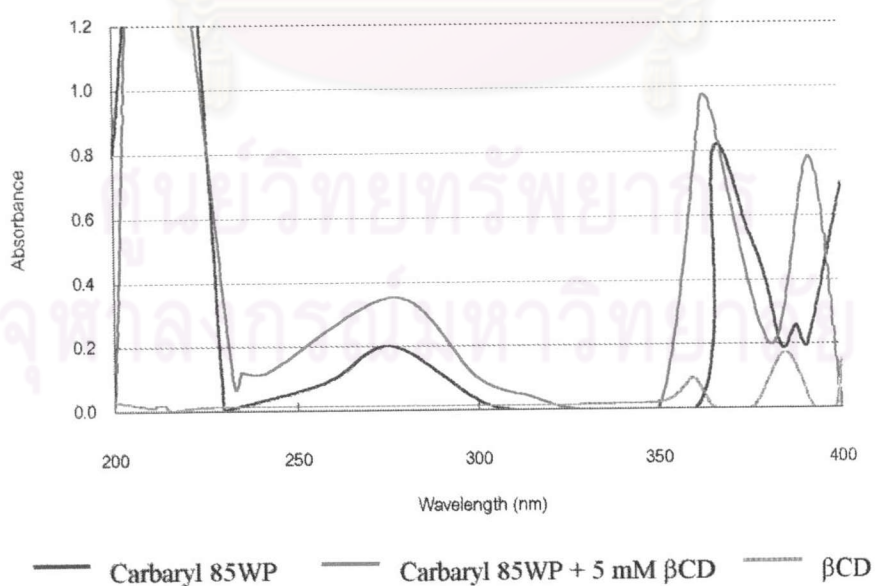
**APPENDICES**

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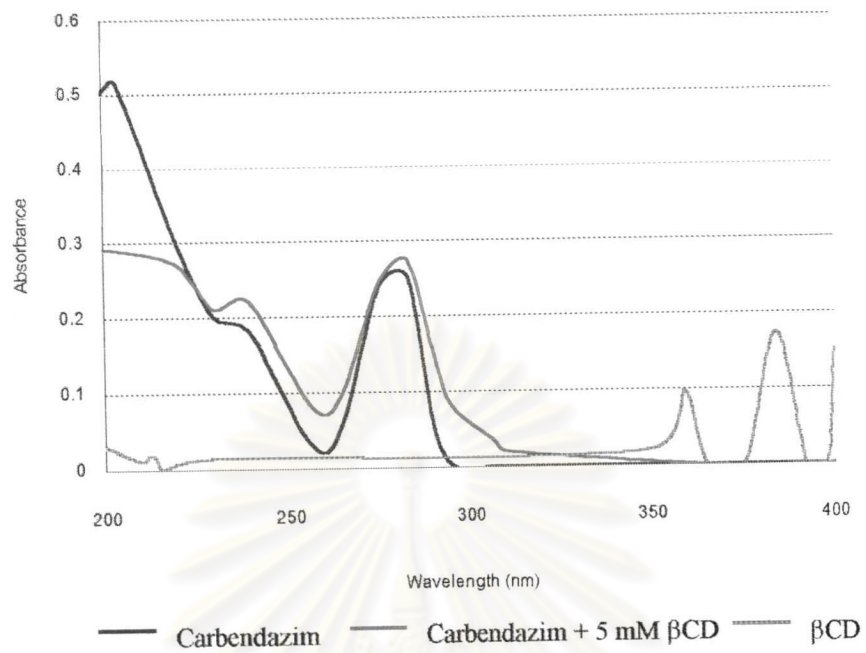
**APPENDIX 1: The UV spectrum of pesticides and pesticide- $\beta$ CD complexes**



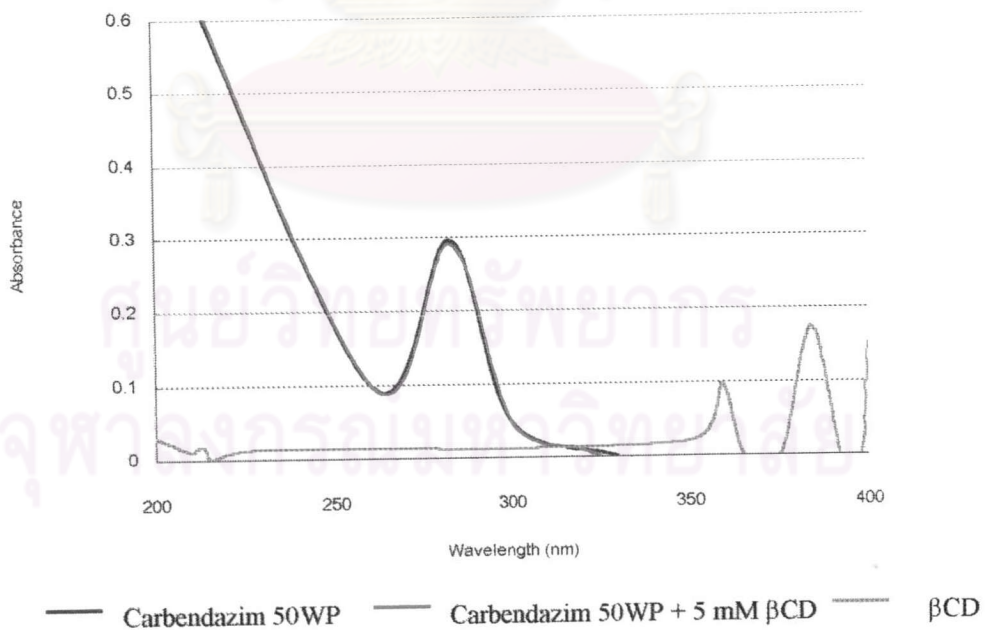
**Figure 25. UV spectra of Carbaryl and Carbaryl in 5 mM  $\beta$ CD solution**



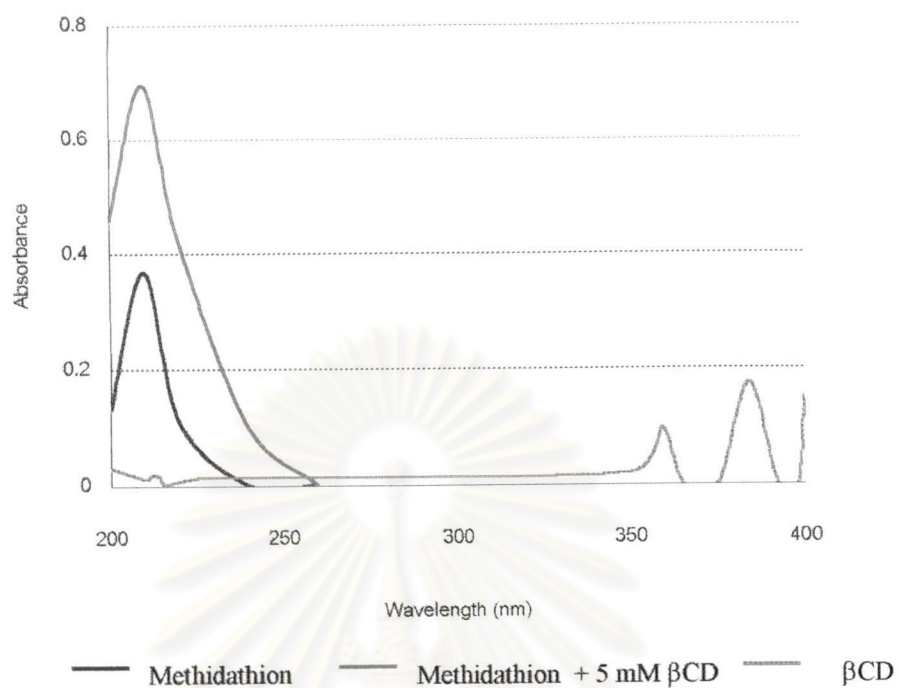
**Figure 26. UV spectra of Carbaryl 85WP and Carbaryl 85WP in 5 mM  $\beta$ CD solution**



**Figure 27. UV spectra of Carbendazim and Carbendazim in 5 mM  $\beta$ CD solution**

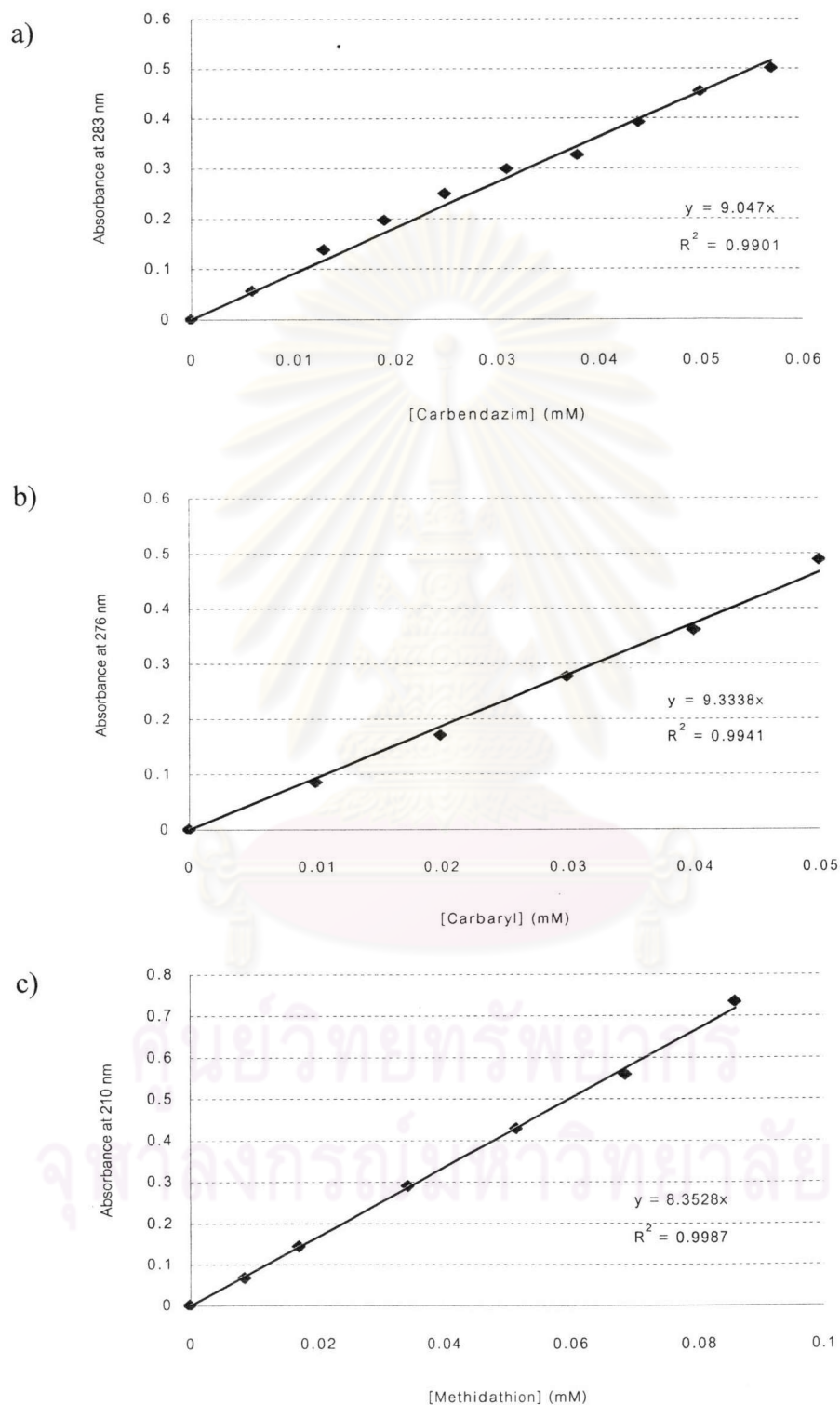


**Figure 28. UV spectra of Carbendazim 50WP and Carbendazim 50WP in 5 mM  $\beta$ CD solution**



**Figure 29. UV spectra of Methidathion and Methidathion in 5 mM  $\beta$ CD solution**

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**APPENDIX 2 : Calibration curve of pesticides****Figure 30. Standard curve: a) Carbendazim, b) Carbaryl, c) Methidathion**

### ***APPENDIX 3 : Carbaryl information profiles (Exttoxnet, 1996)***

**Trade and Other Names** : Product names include Adios, Bugmaster, Carbamec, Carbamine, Crunch, Denapon, Dicarbam, Hexavin, Karbaspray, Nac, Rayvon, Septene, Servin, Tercyl, Tornadao, Thinsec, Tricarnam and Union Carbide 7744.

**Regulatory Status** : Carbaryl is a General Use Pesticide (GUP). However, various formulations vary widely in toxicity. For example, it is categorized as toxicity class I-highly toxic for Tercyl; toxicity class II-moderately toxic for Servin 803; and toxicity class III-slightly toxic for some other products. Products containing carbaryl may bear the Signal Word DANGER-POISON, Warning, or CAUTION depending on the product formulation.

**Chemical Class** : Carbamate

**Chemical Use** : Insecticide

**Formulation** : It is available as bait, dusts, wettable powders, granules, dispersions and suspensions.

**Acute oral LD50** : 255 mg/kg in rat.

**Physical Properties** : (Kidd and James, 1991)

- **Appearance** : Carbaryl is a solid that varies from colorless to white or grey, depending on the purity of the compound. The crystals are odorless. Carbaryl is stable to heat, light and acids. It is not stable under alkaline conditions. It is noncorrosive to metals, packaging materials and application equipment.
- **Chemical name** : 1-naphthyl methyl carbamate
- **CAS Number** : 63-25-2
- **Molecular weight** : 201.23
- **Melting point** : 142°C

- **Water solubility** : 40 mg/L at 30°C
- **Solubility in Other Solvents**: very soluble in dimethylformaldehyde and dimethyl sulfoxide ; soluble in acetone and cyclohexanone
- **Relative density (water = 1)** : 1.2
- **Vapor Pressure** : <5.3 mPa at 25°C
- **Octanol/water partition coefficient as log Pow** : 2.34
- **Adsorption Coefficient**: 300



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**APPENDIX 4 : Carbendazim information profiles (Extoxnet, 1996)**

**Trade and Other Names** : Commercial names for products containing carbendazim include Agrocit, Benex, Benlate, Benosan, Fundazol, Fungidice 1991, and Tersan 1991. Carbendazim is compatible with many other pesticides.

**Regulatory Status** : Carbendazim is a General Use Pesticide (GUP). The EPA categorizes it as toxicity class IV - practically nontoxic. Carbendazim-containing products carry the Signal Word CAUTION.

**Chemical Class** : benzimidazole

**Chemical Use** : Fungicide

**Formulation** : Formulations include wettable powder, dry flowable powder, and dispersible granules.

**Acute oral LD50** : 10,000 mg/kg in rat.

**Physical Properties** : ([http://www.inchem.org/documents/hsg/hsg/hsg82\\_e.htm](http://www.inchem.org/documents/hsg/hsg/hsg82_e.htm))

- **Appearance** : Colorless crystals or gray to white powder with little or no odor.
- **Chemical name** : Methyl (1H-benzimidazol-2-yl)carbamate
- **CAS Number** : 10605-21-7
- **Molecular weight** : 191.2
- **Melting point** : 250°C
- **Water solubility** : 8.0 mg/L at 25°C
- **Solubility in Other Solvents**: soluble in chloroform, heptane, ethanol and acetone.
- **Relative density (water = 1)** : 0.27
- **Vapor Pressure** :  $<1 \times 10^{-7}$  Pa at 20°C
- **Octanol/water partition coefficient as log Pow** : 1.49

## ***APPENDIX 5 : Methidathion information profiles (Extoxnet, 1996)***

**Trade and Other Names :** Trade names for products containing methidathion include Somonic, Somonil, Supracide, Suprathion and Ultracide. The compound may be found in formulations with many other pesticides.

**Regulatory Status :** Methidathion is highly toxic compound in EPA toxicity class I. Labels for products containing it must bear the Signal Word DANGER.

**Chemical Class :** Organophosphate

**Formulation :** It is available in emulsifiable concentrate, wettable powder, and ultra-low volume (ULV) liquid formulations.

**Oral LD50 :** between 25 mg/kg and 54 mg/kg in rat.

**Physical Properties :** (Kidd and James, 1991 and Wauchope *et. al.*, 1992)

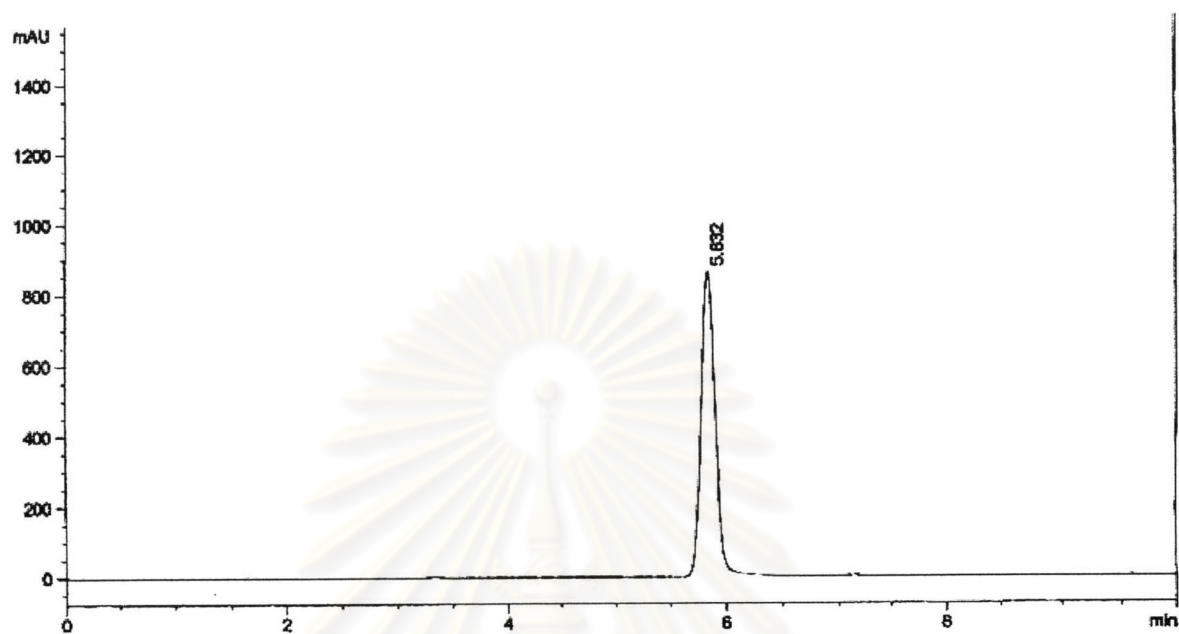
- **Appearance :** Methidathion is a colorless crystalline compound at room temperature.
- **Chemical name :** S-2,3-dihydro-5-methoxy-2-oxo-1,3,4-thiadiazol-3-ylmethyl O,O-dimethylphosphorodithioate
- **Molecular Formula:** C<sub>6</sub>H<sub>11</sub>N<sub>2</sub>O<sub>4</sub>PS<sub>3</sub>
- **CAS Number :** 950-37-8
- **Chemical class:** Organophosphate, thiadiazole
- **Chemical Use:** Insecticide and acaricide
- **Molecular weight :** 302.23
- **Melting point :** 39-40°C
- **Water solubility :** 240 mg/L at 20°C
- **Solubility in Other Solvents :** 53 g/kg (octanol), 260 g/kg (ethanol), 600 g/kg (xylene), 690 g/kg (acetone), 850 g/kg (cyclohexane)

- **Vapor Pressure** : 186 mPa at 20°C
- **Partition Coefficient** : 4.7243
- **Adsorption Coefficient** : 400 (estimated)



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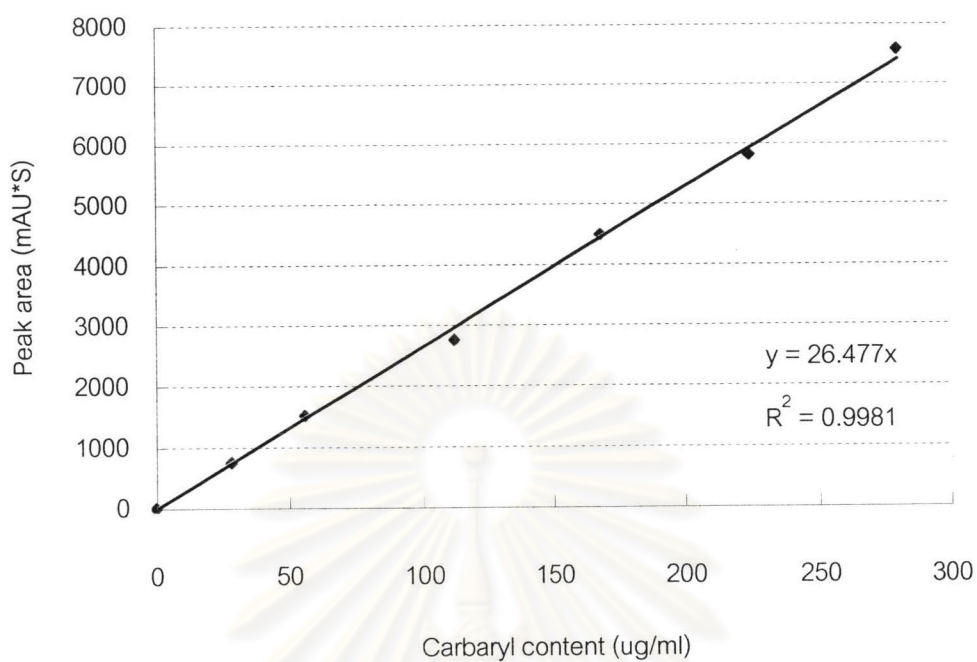
**APPENDIX 6: HPLC chromatogram and standard curve of carbaryl**



**Figure 31. HPLC chromatogram of standard carbaryl at 28 mg/ml**

Wavelength = 278 nm

peak	RT [min]	Area [ mAU*S ]	Height [ mAU ]	Area %
1	5.832	7568.28	866.29	100.00
Total :		7568.28	866.29	



**Figure 32. Standard curve of carbaryl**

Standard carbaryl equation:  $Y = 26.477X$

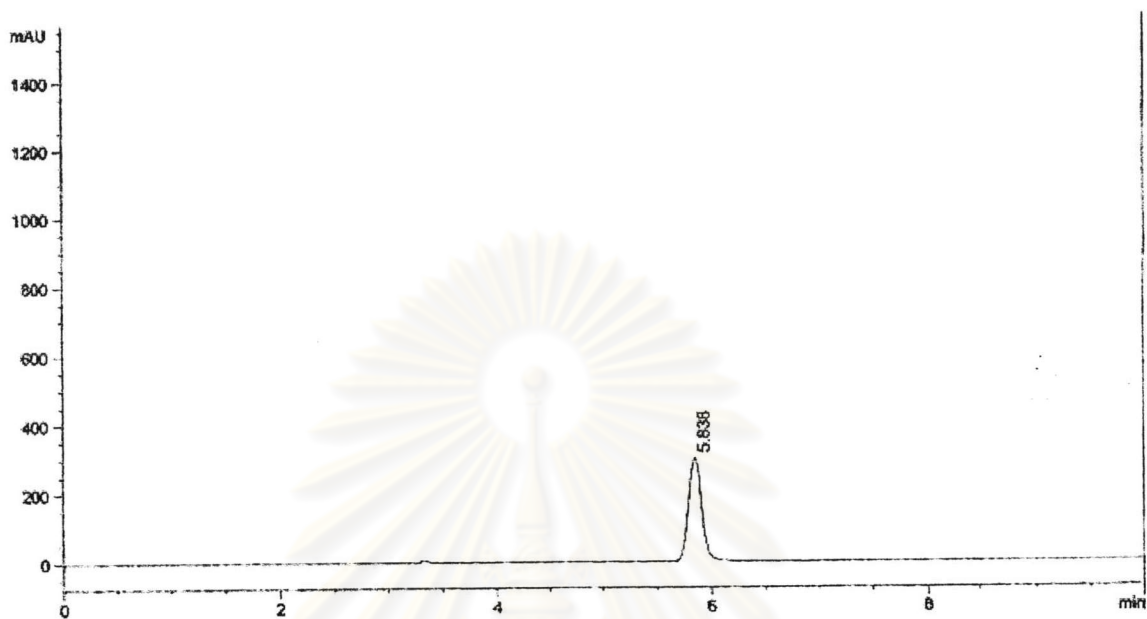
Slope = 26.477

When Y represents peak area (mAU\*S) and

X represents carbaryl concentration ( $\mu\text{g/ml}$ )

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**APPENDIX 7: Calculation of carbaryl content in carbaryl 85WP**



**Figure 33. HPLC chromatogram of carbaryl 85WP at 23 mg/ml**

Wavelength = 276 nm

peak	RT [min]	Area [ mAU*S ]	Height [ mAU ]	Area %
1	5.838	2557.90	300.99	100.00
Total :		28.513	300.99	

From standard carbaryl equation  $Y = 26.477 X$

$$\begin{aligned} \text{Carbaryl concentration } X &= 2557.90/26.477 \\ &= 96.61 \mu\text{g/ml} \end{aligned}$$

Carbaryl 85WP was prepared from carbaryl 85WP 0.0023 g dissolved in 10 ml acetonitrile which was equal to 230  $\mu\text{g/ml}$

Thus carbaryl 85WP 230  $\mu\text{g}$  had carbaryl content = 96.61  $\mu\text{g}$

$$\begin{aligned} \text{Finally, Percent carbaryl content in carbaryl 85WP} &= \frac{96.61 \times 100}{230} \\ &= 42 \% \end{aligned}$$



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***APPENDIX 8: Amount of carbaryl 85WP dissolved from the free or complex form after 15 minutes of dissolution.***

Method of preparation	Amount of carbaryl 85WP dissolved (mg/l)				
	Free carbaryl	Carbaryl 85WP-methyl- $\beta$ CD			(carbaryl 85WP-dextrin)
		1:1	1:2	2:1	
kneaded	66.17	88.3 (7.84)	85.80 (5.27)	94.2 (6.08)	79.59 (11.88)
Freeze-dried, prepared at					
20°C*	66.17	89.96 (5.24)	75.39 (5.68)	92.81 (4.32)	75.22 (4.25)
30°C*	66.17	78.11 (7.56)	67.27 (1.22)	83.82 (2.58)	82.25 (1.71)
40°C*	66.17	134.06 (1.91)	100.70 (5.64)	149.56 (0.73)	95.94 (3.15)
50°C*	66.17	118.28 (1.57)	132.93 (2.49)	140.49 (0.68)	103.60 (2.14)
60°C*	66.17	154.11 (0.49)	130.20 (0.54)	100.10 (0.67)	112.99 (0.62)

\* = Temperature used to prepare complex before freeze-dried process.

( ) = standard deviation, Each figure represents the average value from three determinations at given sampling time.



## APPENDIX 9 : Probit analysis of $LC_{50}$ of free carbaryl 85WP

### DATA Information

4 unweighted cases accepted.

0 cases rejected because of missing data.

1 case is in the control group.

### MODEL Information

ONLY Normal Sigmoid is requested.

Parameter estimates converged after 12 iterations. Optimal solution found.

Parameter Estimates (PROBIT model:  $(PROBIT(p)) = \text{Intercept} + BX$ ):

Regression Coeff. Standard Error Coeff./S.E.

0.36257 0.03737 9.70194

Intercept Standard Error Intercept/S.E.

-1.62612 0.17399 -9.34621

Pearson Goodness-of-Fit Chi Square = .387 DF = 2 P = .824

Since Goodness-of-Fit Chi square is NOT significant, no heterogeneity factor is used in the calculation of confidence limits.

Dose (mg/l)	Total treated	Killed			probit
		observed	expected	residual	
10	62.0	61.0	60.588	.412	.97723
5	60.0	33.0	34.444	-1.444	.57406
1	60.0	7.0	6.192	.808	.10320
0	60.0	3.0	3.118	-.118	.05196

\*\*\*\*\* PROBIT ANALYSIS \*\*\*\*\*

Confidence Limits for Effective VAR00001

95% Confidence Limits			
Probit	LC value	Lower	Upper
.01	-1.93129	-3.47869	-.85271
.02	-1.17944	-2.55725	-.20646
.03	-.70241	-1.97554	.20648
.04	-.34356	-1.53985	.51903
.05	-.05166	-1.18690	.77471
.06	.19679	-.88766	.99352
.07	.41463	-.62631	1.18639
.08	.60968	-.39321	1.35998
.09	.78707	-.18203	1.51868
.10	.95036	.01161	1.66552
.15	1.62642	.80411	2.28267
.20	2.16373	1.42069	2.78643
.25	2.62469	1.93768	3.23060
.30	3.03866	2.39081	3.64062
.35	3.42225	2.80026	4.03100
.40	3.78625	3.17905	4.41118
.45	4.13842	3.53653	4.78801
<b>.50*</b>	<b>4.48500</b>	<b>3.88009</b>	<b>5.16712</b>
.55	4.83159	4.21610	5.55377
.60	5.18376	4.55066	5.95352
.65	5.54775	4.89012	6.37302
.70	5.93135	5.24199	6.82099
.75	6.34531	5.61610	7.31003
.80	6.80628	6.02715	7.86013
.85	7.34359	6.50049	8.50714
.90	8.01965	7.08935	9.32793
.91	8.18293	7.23069	9.52706
.92	8.36033	7.38390	9.74373
.93	8.55538	7.55199	9.98234
.94	8.77322	7.73930	10.24925
.95	9.02167	7.95245	10.55415
.96	9.31356	8.20227	10.91296
.97	9.67241	8.50861	11.35486
.98	10.14944	8.91469	11.94343
.99	10.90130	9.55256	12.87325

**APPENDIX 10 : Probit analysis of 2:1 molar ratio of carbaryl 85WP-  
methyl- $\beta$ CD complex**

DATA Information

4 unweighted cases accepted.

0 cases rejected because of missing data.

1 case is in the control group.

MODEL Information

ONLY Normal Sigmoid is requested.

Parameter estimates converged after 12 iterations. Optimal solution found.

Parameter Estimates (PROBIT model:  $(\text{PROBIT}(p)) = \text{Intercept} + \text{BX}$ ):

Regression Coeff. Standard Error Coeff./S.E.

0.34908 0.03437 10.15602

Intercept Standard Error Intercept/S.E.

-1.76532 0.18161 -9.72011

Pearson Goodness-of-Fit Chi Square = .157 DF = 2 P = .925

Since Goodness-of-Fit Chi square is NOT significant, no heterogeneity factor is used in the calculation of confidence limits.

Dose (mg/l)	Total treated	Killed			probit
		observed	expected	residual	
10	10.00	63.0	60.340	-.340	.95778
5	57.0	29.0	28.047	.953	.49206
1	64.0	5.0	5.015	-.015	.07835
0	60.0	2.0	2.325	-0.325	.03876

\*\*\*\*\* PROBIT ANALYSIS \*\*\*\*\*

Confidence Limits for Effective VAR00001

95% Confidence Limits			
Probit	LC value	Lower	Upper
.01	-1.60717	-3.15130	-.50863
.02	-.82626	-2.20586	.16816
.03	-.33080	-1.60905	.60060
.04	.04191	-1.16205	.92787
.05	.34509	-.79993	1.19556
.06	.60314	-.49292	1.42461
.07	.82939	-.22475	1.62647
.08	1.03198	.01445	1.80811
.09	1.21623	.23118	1.97413
.10	1.38583	.42992	2.12770
.15	2.08801	1.24375	2.77258
.20	2.64608	1.87770	3.29795
.25	3.12486	2.41018	3.76006
.30	3.55481	2.87789	4.18553
.35	3.95323	3.30154	4.58954
.40	4.33129	3.69446	4.98200
.45	4.69707	4.06617	5.37014
<b>.50 *</b>	<b>5.05705</b>	<b>4.42418</b>	<b>5.75993</b>
.55	5.41702	4.77498	6.15694
.60	5.78280	5.12477	6.56701
.65	6.16086	5.48009	6.99706
.70	6.55928	5.84866	7.45615
.75	6.98924	6.24074	7.95725
.80	7.46801	6.67164	8.52095
.85	8.02608	7.16788	9.18403
.90	8.72827	7.78519	10.02541
.91	8.89786	7.93334	10.22958
.92	9.08211	8.09393	10.45173
.93	9.28470	8.27011	10.69640
.94	9.51096	8.46643	10.97011
.95	9.76901	8.68980	11.28280
.96	10.07218	8.95159	11.65082
.97	10.44490	9.27258	12.10410
.98	10.94035	9.69804	12.70789
.99	11.72126	10.36626	13.66190

## BIOGRAPHY

Miss Ruedeekan Saikosin was born on August 12, 1977, at Bangkok province. In 1998, she graduated with the Bachelor degree of Science (Biotechnology) from King Mongkut's Institute of Technology Ladkrabang with GPAX 2.98. She continued to study the master degree in Biotechnology Program at Chulalongkorn University in 1999.



ศูนย์วิทยทรัพยากร  
จุฬาลงกรณ์มหาวิทยาลัย