## TABLE OF CONTENTS

	Page
ACKNOWLEDGEMENT	iii
ABSTRACT (English)	iv
ABSTRACT (Thai)	vii
TABLE OF CONTENTS	X
LIST OF TABLES	xiii
LIST OF FIGURES	xiv
ABBREVIATIONS	xvi
CHAPTER 1 INTRODUCTION	1
1.1 Statement of problem	1
1.1.1 Dichlorodiphenyl trichloroethane (DDT)	1
1.1.2 Metabolism and tissue distribution of DDT	3
1.1.3 The standard technique for detection DDT and	5
Its derivatives 1.1.4 Immunoassay	6
	6
1.1.4.1 Monoclonal antibody	
1.1.4.2 Antibody Structure	SITV
1.2 Literature review of antibody to DDT and its derivatives	10
production US ReSeRV	
1.3 DDT and its derivatives of the present study	14

	Page
1.4 Hypothesis of the present study	16
1.5 Purposes of the study	16
1.6 Benefits of the study outcome	18
1.7 Key words and definition	18
CHAPTER 2 MATERIALS AND METHODS	21
2.1 Materials	21
2.1.1 Chemicals and reagents	21
2.1.2 Equipments	21
2.2 Methods	21
2.2.1 Preparation of haptens and immunogens	21
2.2.1.2 Preparation of immunogens	23
2.2.1.3 Bradford protein assay	23
2.2.1.4 Hapten density assay	25
2.2.2 Production of antibody	27
2.2.2.1 Immunization	28
2.2.2.2 Detection of antibody by non-competitive	
indirect ELISA	28
2.2.2.3 Production of monoclonal antibody by cell	
fusion method	29 V/ (A)
CHAPTER 3 RESULTS	35
3.1. Haptens and immunogens	35
3.1.1 Haptens	35

Page

3.1.2 Immunogens	36
3.2 Hapten density assay.	36
3.3 Protein concentration of immunogens	38
3.4 Determination of antibody titer in mouse sera	39
3.5 Antibody screening of hybridoma	40
3.6 The inhibition by DDT and its derivatives	44
3.7 Cloning of antibody-secreting hybridoma cells by limiting dilution	46
3.8 Determination of the isotype of monoclonal antibodies	47
3.9 Preparation of antibody for application	47
3.9.1 Purification of Antibody	47
3.9.2 Sensitivity of antibody	48
3.9.3 Specificity and cross-reactivity of antibody	49
3.10 Recovery determination of DCBH in human serum samples	50
CHAPTER 4 DISCUSSION AND CONCLUTIONS	53
4.1 Hapten synthesis and immunogen preparation	53
4.2 Production of antibody	55
4.3 Conclusion and future study	60
CONCHAPTER 5 SUMMARY Chiang Mai Univers	61
A REFERENCES & h t s r e s e r v e	62
APPENDIX A	71
APPENDIX B	78
CURRICULUM VITAE	84

### LIST OF TABLES

Table	Page
1.1 Properties of polyclonal and monoclonal antibody	9
1.2 Common name, chemical name, and structure of DDT and its derivatives	15
3.1 Absorbance of carrier proteins and hapten-protein conjugate at 215 nm and	39
hapten density of immunogen	39
3.2 Protein concentration of immunogens determined by BioRad dye assay.	40
3.3 Relatives cross-reactivity of monoclonal antibody	49
3.4 % Recovery of DCBH added to human serum compared with a standard	51
curve	31
3.5 The recoveries of DCBH $\pm$ SD, Min, and Max and mean recovery $\pm$ SD	52
4.1 Comparison of IC <sub>50</sub> of inhibitors in supernatants	58
5.1 IC50 and %Cross-reactivity of produced mAb	61

# ลิขสิทธิ์มหาวิทยาลัยเชียงใหม่ Copyright © by Chiang Mai University All rights reserved

### LIST OF FIGURES

Figure	Page	
1.1 Metabolic pathways of p,p'-DDT in mammals.	4	
1.2 Metabolic pathways of o, p'-DDT in mammals.	5	
1.3 Monoclonal antibody productions (www.biotech.uiuc.edu_fusion)	9	
1.4 Structure of IgG Abs and their fragments	10	
1.5 The structure of hapten by Burgisser et al. in 1990	11	
1.6 The structure of hapten by Abad et al. in 1997	12	
1.7 The structure of hapten by Beasley et al., 1998	13	
1.8 The structure of haptens by Hong et al., 2002	14	
1.9 Conceptual framework of the present study	17	
2.1 Synthesis of hapten. using DCBH as precursor	22	
2. 2 Preparation of immunogen from hapten I and II	24	
2. 3 Diagram of hapten synthesis	26	
2. 4 Diagram of prepared immunogen	24	
2.5 Summary diagram of production and characterization of antibody	34	
3.1 The structure of hapten I resulted from the conjugation reaction of DCBH	ħIJ	
and succinic anhydride linker	35	
3.2 The structure of hapten II resulted from the conjugation reaction of		
DCBH and glutaric anhydride linker.	36	
3.3 Structure of immunogen.	37	
3.4 Wave length scanned for maximum absorbance of DCBH.	38	

Figure	Page
3.5 Antibody responses against 5 immunogens.	41
3.6 Antibody titers in the sera of mice	42
3.7 Supernatant from 4 selected hybridomas were tested for inhibition activity	43
with DDT and its derivatives.	
3.8 Inhibition of antibody by DDT and its derivatives	45
3.9 ELISA inhibition curves for DDT and its derivatives	46
3.10 Isotypes of anti- hapten monoclonal antibody produced by 3B8.10D9 hybridomas.	47
3.11 Purified Ab (10µg/ml) from clone 3B8.10D9 was tested by inhibition	48
ELISA assay against DDT and its derivatives.	48
B.1 Chromatogram of GC/MS used for confirmation molecular weight of haptens I.	78
B.2 Chromatogram of <sup>1</sup> H NMR used for confirmation number of hydrogen in haptens I.	79
B.3 Chromatogram of <sup>13</sup> C NMR used for confirmation number of carbon in haptens I.	80
B.4 Chromatogram of GC/MS used for confirmation molecular weight of haptens II.	81
B.5 Chromatogram of <sup>1</sup> H NMR used for confirmation number of hydrogen in haptens II.	82 W e 0
B.6 Chromatogram of <sup>13</sup> C NMR used for confirmation number of carbon in haptens II.	83

#### **ABBREVIATIONS**

Ab Antibody

Ag Antigen

BSA Bovine serum albumin

CFA Complete Freund's adjuvant

DCBH 4,4-dichlorobenzhydrol

DDA bis (p-chlorophenyl) acetic acid

DDD dichlorodiphenyldichloroethane

DDE dichlorodiphenyldichloroethylene

DDT dichlorodiphenyltrichloroethane

ELISA Enzyme-linked Immunosorbent Assay

FBS Fetal bovine serum

Fc Antibody fragment containing the crystallizable fragment

GC/MS Gas- chromatography/mass- spectrometry

GC-ECD Gas chromatography – electron capture detection

HRP Horse radish peroxidase

IA Immunoassay

IC<sub>50</sub> Concentration of Analyte that Causes 50% Inhibition

IFA Incomplete Freund's adjuvant

Ig Immunoglobulin

IMDM Iscove's modified Dulbecco's medium

KLH Keyhole limpet hemocyanin

mAb Monoclonal antibody

NMR Nuclear magnetic resonance spectroscopy

*o,p*'-DDE 1, 1-dichloro-2(2-chlorophynyl)-2-(4-chlorophynyl)ethane

o,p'-DDT 1, 1,1-trichloro-2(2-chlorophynyl)-2-(4-chlorophenyl) ethane

o,p'-DDT 1,1-trichloro-2(2-chlorophynyl)-2-(4-chlorophenyl) ethane

°C Degree of Celsius

OPD Orthophenylene diamine

OVA Oval albumin

*p,p*'-DDA 2,2-bis(4-chlorophenyl)- acetic acid

p,p'-DDD 1,1-dichloro-2,2-di(4-chlorophenyl)) ethane

p,p'-DDE 1,1-dichloro-2,2-di(4-chlorophenyl) ethylene

*p,p* '-DDT 1,1,1-trichloro-2,2-di(4-chlorophynyl) ethane

pAb polyclonal antibody

PBS Phosphate buffer saline

ppb part per billion (ng/ml level )

rAb recombinant antibody

TLC Thin-layer chromatography

UV-visible spectrophotometer

λmax The wavelength of maximum absorbance