

Safety Assessment and Monitoring Techniques of Environmental Chemical Substances

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1. Endocrine Disrupting Chemicals

Endocrine disrupting chemicals (EDCs) consist of synthetic and naturally occurring chemicals that affect the balance of normal hormonal functions in animals. Depending on their activity they may be characterized as estrogen modulators or androgen modulators. They may mimic the sex hormones estrogen or androgen (thereby producing similar responses to them) or they may block the activities of estrogen or androgen. (i.e., be antiestrogens or antiandrogens).

2. Environmental Endocrine disruptors

What are the environmental endocrine disruptors (EEDs)? "Our Stolen Future" by Colborn, Dumanoski and Myers lists about 50 suspect EEDs. Another expanded list of about 70 suspect EEDs is published on the Internet by the World Wildlife Fund Canada. In addition, a list of about 50 suspect EEDs from the Center for Disease Control & Prevention (CDC) in Atlanta and another list of about 50 suspect EEDs from the U.S. Environmental Protection Agency were obtained by private communication with scientists there. Many of the same chemicals are on all of these lists but there are also significant differences in additions and omissions of some of these chemicals. This highlights the fact that we really do not have good enough information on this group of chemicals to even agree on the basis question of their definition. The 67 chemicals were selected on the basis of their inclusion of a reasonable amount of information on their chemical, physical and/or toxicological properties (Table). Given that there are about 600 registered pesticides and 80,000 industrial chemicals in commercial use today it is likely that the lists of suspect EEDs will continue to grow and vary over the next several years as the results of current research begin to throw more light on this subject.

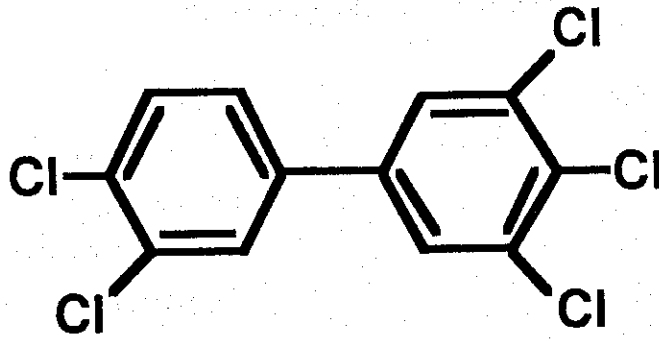
"Environmental Endocrine Disruptors", Lawrence H. Keith, John Willey & Sons, Inc. 1997

3. Dioxins

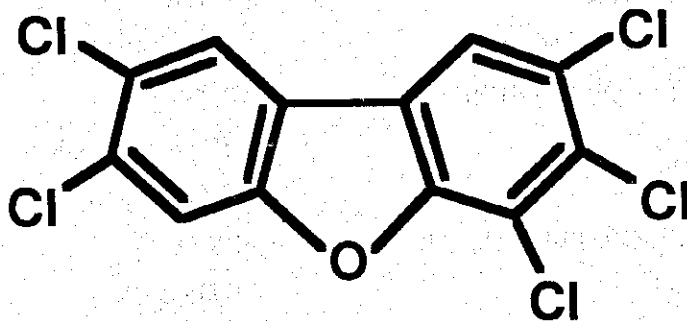
Taking an example of Dioxins, the toxicity (LD50, ADI), modes of toxic ation, sources of production, residue levels in the atmospheres, soil, water, food, and human samples were discussed.

Table. The List of Environmental Endocrine Disruptors

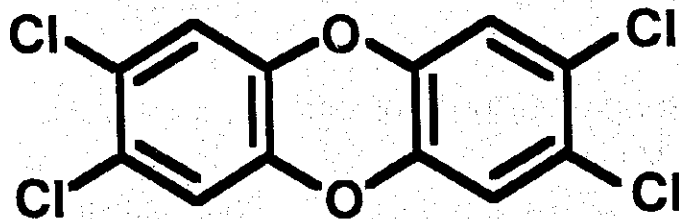
ACENAPHTHENE	ENDRIN
ALACHLOR	HEPTACHLOR
ALDICARB	HEPTACHLOR EPOXIDE
ALDRIN	HEXACHLOROBENZENE
ALLETHRIN	INDENO[1,2,3-C, D]PYRENE
AMITROLE	LEAD
ANTHRACENE	LINDANE
ARSENIC	MALATHION
ATRAZINE	MANCOZEB
BENOMYL	MANEB
BENZA(A)ANTHRACENE	MERCURY
BENZO(A)PYRENE	METHOMYL
BENZO(B)FLUORANTHENE	METHOXYCHLOR
BENZO(K)FLUORANTHENE	METIRAM
BETA-BHC	METOLACHLOR
BISPHENOLA	METRIBUZIN
BUTYL BENZYL PHTHALATE	MIREX
CADMIUM	MITROFEN
CARBARYL	PARATHION
CHLORDANE	PCBs
CHLORPYRIFOS	PENTACHLOROPHENOL
CHRYSENE	PENTACHLORONITROBENZENE
CYPERMETHRIN	PHENANTHARENE
2,4-D	PYRENE
DDD	SIMAZINE
DDE	STYRENE
DDT	2, 4, 5-T
1, 2-DIBROMO-3-CHLOROPROPANE	2, 3, 7, 8-TCDD
2, 4-DICHLOROPHENOL	TOXAPHENE
DICOFOL	TRIFLURALIN
DIELDRIN	VINCLOZOLIN
DI(2-ETHYLHEXYL)PHTHALATE	ZINEB
DI-N-BUTYL PHTHALATE	ZIRAM
ENDOSULFAN	



3,4,5,3',5'-PCB



2,3,4,7,8-Dibenzofuran



2,3,7,8-TCDD

LD50 TCDD Homologus

Homologue	LD50($\mu\text{g}/\text{kg}$ body weight)	
	guinea pig	mouse
2, 8-	300,000	-
2, 3, 7-	30,000	3,000
2, 3, 7, 8-	2	300
1, 2, 3, 7, 8-	3	350
1, 2, 4, 7, 8	1,000	5,000
1, 2, 3, 4, 7, 8-	70	800
1, 2, 3, 6, 7, 8-	70~100	1,250
1, 2, 3, 7, 8, 9-	60~100	1,440
1, 2, 3, 4, 6, 7, 8-	600	-

LD50 of 2, 3, 7, 8-TCDD

animal	LD50	
	$\mu\text{g}/\text{kg}$ body weight	
guinea pig	0.6~	0.2
rat	20 ~	60
hen	25 ~	50
monkey	70	
dog	100 ~	200
rabbit	100 ~	300
mouse	100 ~	600
hamster	1,000 ~	5,000

ADI of PCB and Pesticides

chemical	ADI (mg/kg body weight/day)
PCB	0.005
γ -BHC	0.008
heptachlore epoxide	0.0005
malathion	0.02
fenitrothion	0.005
diazinon	0.002

ADI of 2, 3, 7, 8-TCDD

country	ADI (pg/kg body weight/day)
Canada	10
America	0.01
Holland	1
Sweden	5
Denmark	5
Switzerland	5
U.K.	10
Japan	10

TCDD and Dibenzofurans in the Atmosphere

country	2, 3, 7, 8-TCDD equivalents (pg/m ³)
USA	0.02 ~0.8
Canada	0.03 ~0.9
Holland	0.1 ~0.6
Germany	0.02 ~0.4
Austria	1.2 ~2.3
Japan	0.001~2.9

TCDD in Soils

classification	2, 3, 7, 8–TCDD equivalents (pg/g or ppt) TCDD & Dibenzofurans		
	Europe	Canada USA	Japan
industrial city	120	41	64
city	—	11	17
farm village			
mountain area	2.4	0.4	48

TCDD and Dibenzofurans in Water

water	country	2, 3, 7, 8–TCDD equivalents (pg/L)
river water	Sweden	0.06~0.12
water resources	USA	7.3
tap water	USA	0.13

TCDD and Dibenzofurans of Animals in Baltic Sea and in G. of Bothnia

animal	2, 3, 7, 8–TCDD equivalents (pg/g or ppt)
Fish	
pike	40~ 120
herring	80
salmon	70
Bird	
egg of sea eagle	950~3,800 in industrial area
egg of sea crow	1,300 (pollution area)
egg of osprey	550
sea eagle	190~ 230
osprey	640
Mammal	
seal	14~ 300

TCDD and Dibenzofurans of Food in Canada and Japan

food	2, 3, 7, 8-TCDD equivalents (pg/g or ppt)	
	Canada	Japan
fishes & shellfishes	8.5	1.2
meats & eggs	0.19~0.84	0.14
diary products	0.07	0.16
oils & fats	—	0.18
green & yellow vegetables	—	0.17
other vegetables, seaweeds	0.05	0.01
bean products	—	0.02
cereals	0.05	0.001
rice	—	0.002
fruit	0.07	0.004

TCDD in human body

country	2, 3, 7, 8-TCDD equivalents (pg/g or ppt)
Japan	30~40
USA	30~40
Canada	35~40
Sweden	30~35
China	13
North Vietnam	12
South Vietnam	65

TCDD and Dibenzofurans in mother's milk

country	2, 3, 7, 8-TCDD equivalents (fat body : pg/g or ppt)	
Holland	37~40	
Belgium	34~40	Industrial Europe
U.K.	29~37	
Germany	28~37	
Japan	20~28	
Sweden	20~23	North Europe
Finland	16~18	
Norway	15~19	
Canada	16~23	North USA
USA	17	
Hungary	9~11	East Europe
Yugoslavia	12	
Thailand	5	
India	6	Developing or Agricultural countries
New Zealand	6	

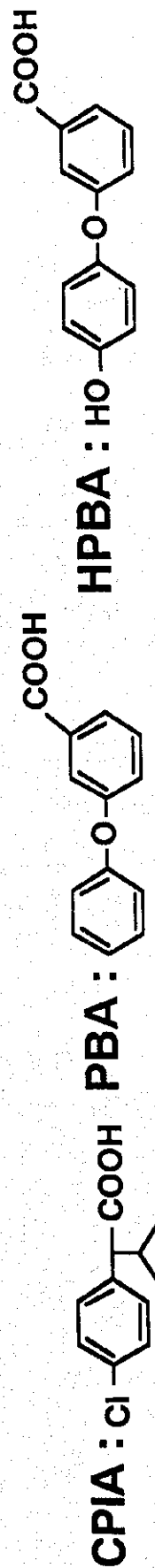
**Recoveries, MDA and MDC of Degradation Products of
Fenvalerate in Human Urine**

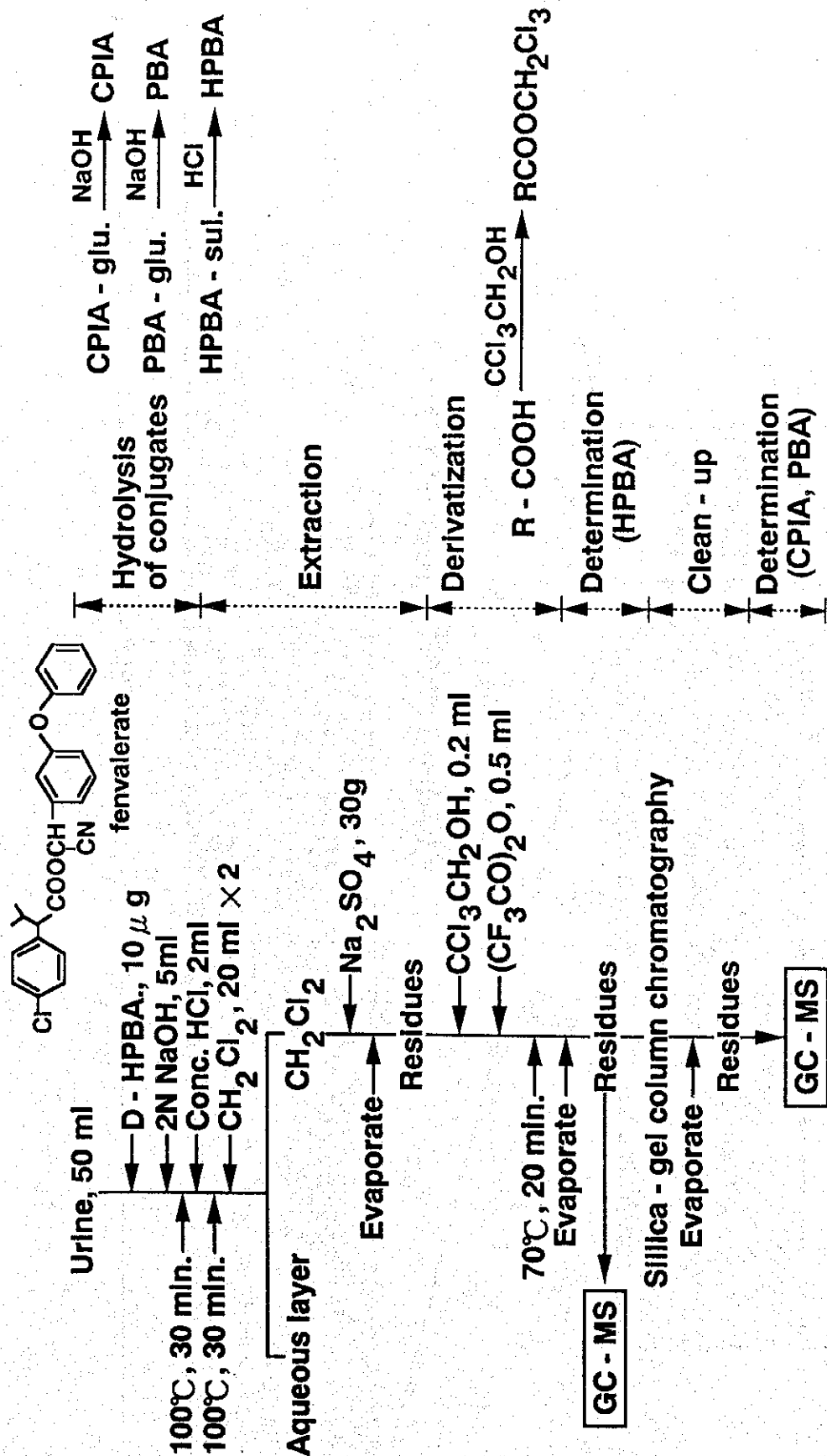
Products	Determination	MDA (ng)	MDC (ppm)	Fortified (ppm)	Recovery (%)
CPIA	GC-MS (MF)	0.2	0.002	0.02	107
				0.2	89
				2	90
PBA	GC-MS (MF)	0.2	0.002	0.02	93
				0.2	88
				2	100
HPBA	GC-MS (MF)	2	0.02	0.2	100
				2	99

MF: mass fragmentography

MDA: minimum detectable amount

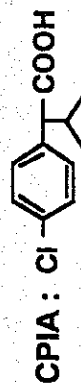
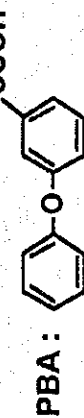
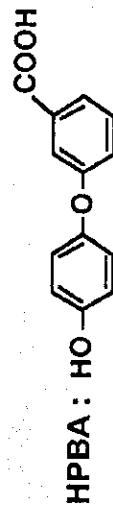
MDC: minimum detectable concentration

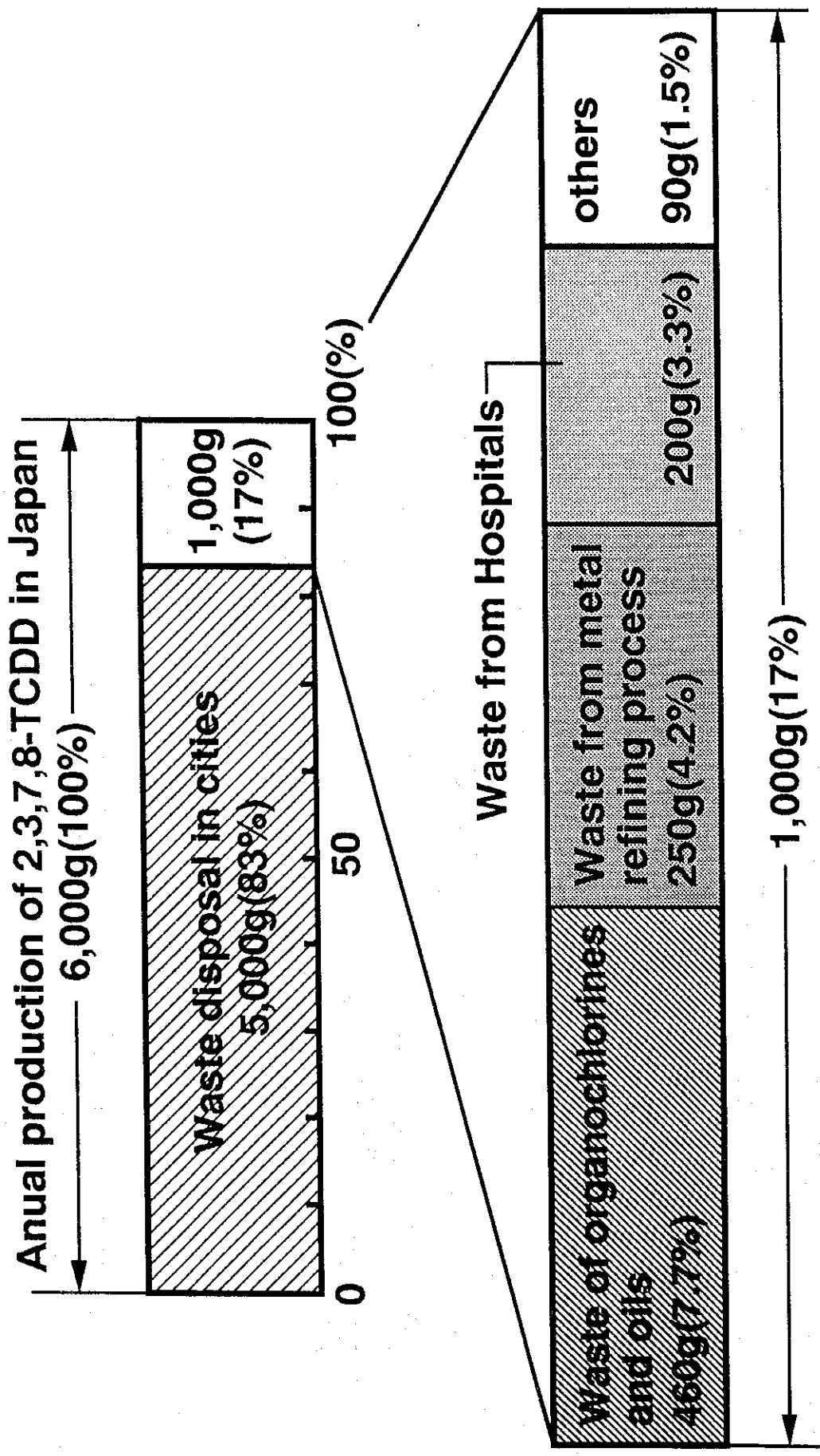


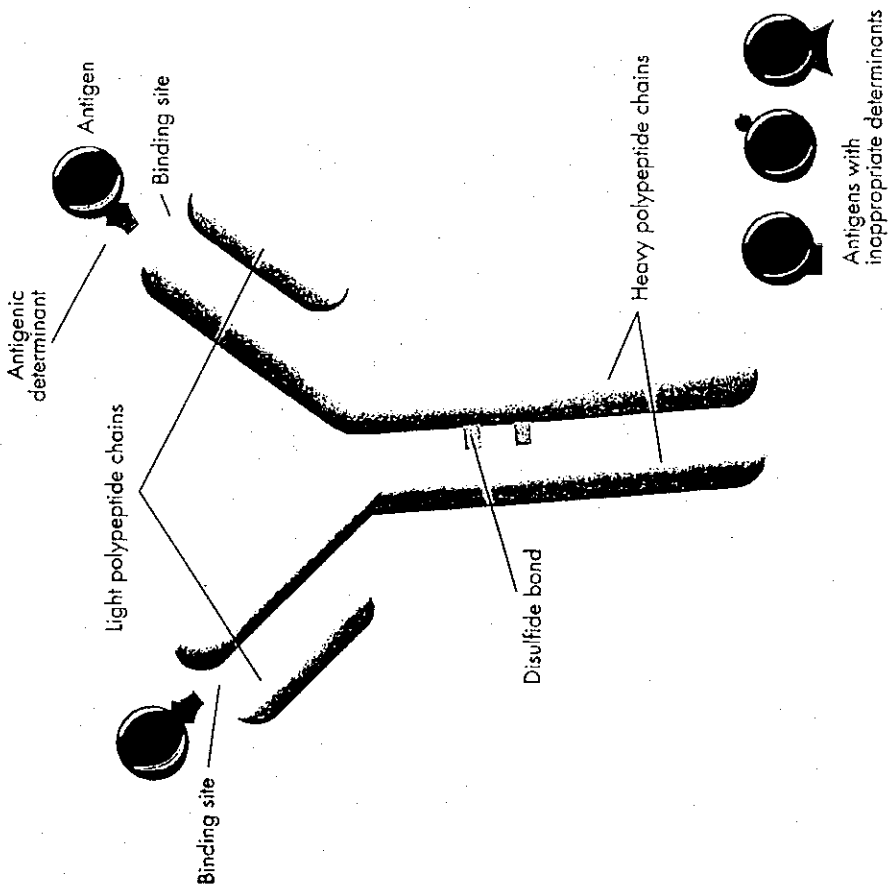


ANALYTICAL METHOD FOR CPIA, PBA, HPBA AND THEIR CONJUGATES IN HUMAN URINE

D - HPBA : Deuterium labeled HPBA (Internal Standard)

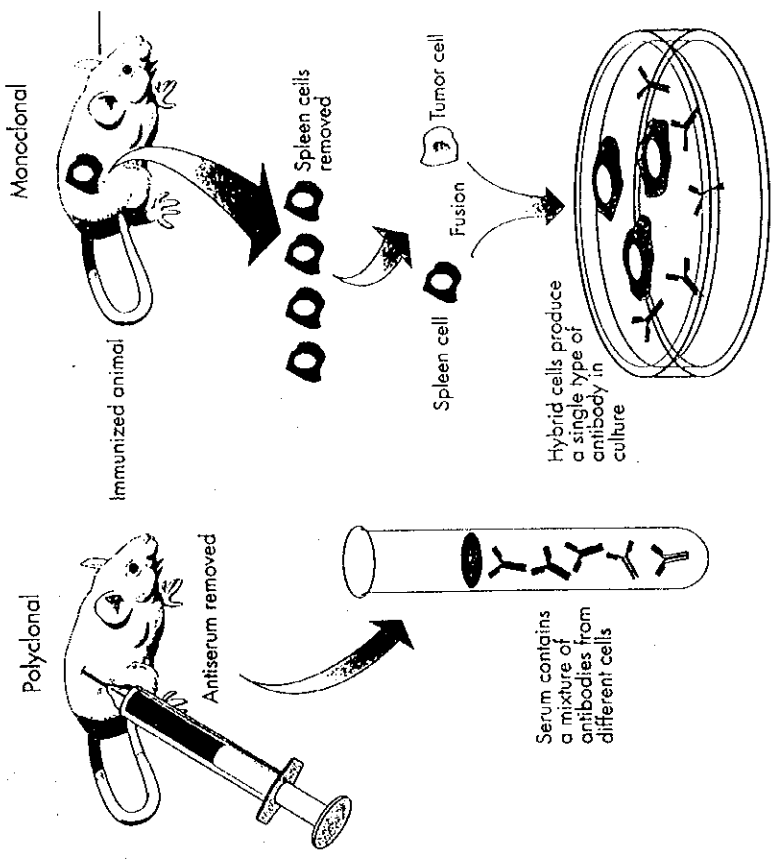




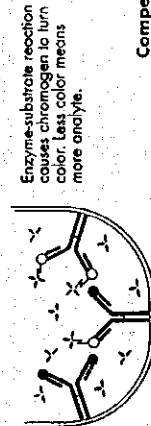
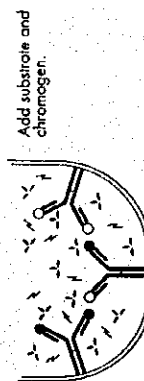
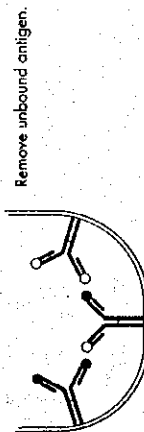
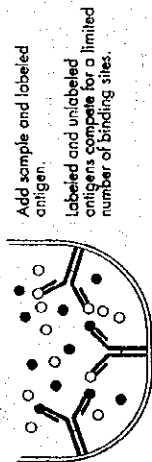
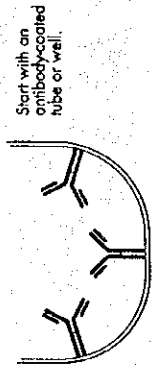


Anatomy of an Antibody

Much like a lock and key, the binding sites on an antibody attach precisely (and non-covalently) to the antigenic determinants of corresponding antigens. This IgG molecule has two binding sites at the top of its "Y" shape.



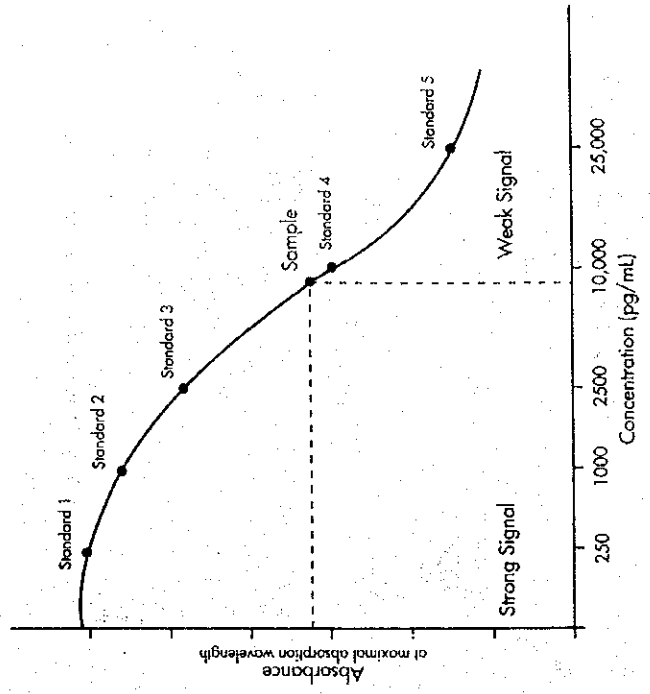
Production of Polyclonal and Monoclonal Antibodies



KEY TO ILLUSTRATION

- Antibody
- Antigen in sample (analyte)
- Labeled antigen
- Substrate
- Chromogen

Competitive-Binding EIA



Example of a Standard Curve for a Competitive-Binding EIA
 The reverse curve illustrates the inverse relationship between analyte concentration and the amount of color.

Environmental Chemicals Currently in Use and The Safety Measures Used Japan

Iori SAKAMOTO

Deputy Director

Environment Department

Hyogo Agricultural Research Institute

I. Environmental Chemicals

1. Chemicals that are released during the production in agriculture and forestry

(1) Pesticides

Pesticides used for the control of diseases, insects pests, weeds, and for the regulation of plant growth contaminate soil, water, and agricultural products.

(2) CO₂ (including CO)

CO₂ and CO are released when fuels are utilized for the operation of agricultural machinery, the heating of green houses and glass houses, as well as the transportation and drying of agricultural products and when these fuels are burned.

2. Chemicals that are released during industrial waste treatments

(1) Dioxins

Dioxin is a general term for polychloride-dibenzo-paradioxin (PCDD) and polychloridedibenzofuran (PCDF). The former has 75 isomers and the latter 135 isomers. These chemicals cannot be artificially formulated.

However, in the heating process of carbon, oxygen, hydrogen, and chlorine, these chemicals are naturally produced. These chemicals are most likely to be released during the incineration of waste materials but they can be also released during a heating process in metal refining.

(2) Polychlorobiphenyls (PCBs)

PCBs are used in transformers for high voltage circuits and condensers.

3. exogenous endocrine disrupters

These chemicals are exogenous substances that are suspected of having an adverse impact on the normal hormone levels and functions when these chemi-

als are ingested and enter the bodies of mammals.

- (1) Chemicals other than pesticides
32 kinds including PCBs
- (2) Chemicals used for pesticides or that were used as pesticides
36 kinds including DDT

4. Chemicals that are released due to daily activities, factory productions, and transportation services

- (1) CO₂, NO_x, SO₂, CO
These chemicals are released due to the use of fuels and electricity (thermal power generatin).
- (2) Fluorocarbon gas
Fluorocarbon gas is used as the cooling catalyst of various cooling machines. Fluorocarbon gas is released into the air when these machines are broken down or during the dissembling of these machines.

II. Impacts of Environmental Chemicals

1. Pesticides

- (1) Residues in crops

Pesticides applied to agricultural crops tend to remain on the surface of crops or to be absorbed and remain inside the crops. Sometimes the residue of pesticides falls out on the soil and is absorbed through the roots of various crops and remains inside the crops.

There are a wide variety of adverse effects on humans through direct oral ingestion of food with pesticide residues as well as the ingestion of milk and meat of cattle that have been fed with pesticide-contaminated feeds. The utmost care is needed for pesticide residues on crops.

- (2) Residues in soil

Not only those pesticides that are directly applied to the soil, but also those sprayed on crops fall out on the soil, remain, and accumulate. These pesticide residues are absorbed by newly cultivated crops and contaminate them. as contamination cases by aldrin and dildrin have been reported, pesticide residues in soil requires the utmost care.

(3) Impact on water qualities

Pesticides are degraded by numerous factors after they are applied, and are gradually displaced. However, regardless of the formulation of pesticides, residues flow into water veins as the result of rainfall and have negative impacts on marine life as well as contaminating ground water. Precautions must be taken to water contaminating pesticides including endosulfan.

(4) Impact on the air.

Highly volatile pesticides, especially fumigant types contaminate ambient air and have adverse impacts on humans, livestock, and agricultural crops in the area in which they settle.

2. CO₂(CO)

CO₂ and CO gases are both the greenhouse gases. The continuous increase in CO₂ and CO gases will raise the atmospheric temperature of the earth and further accelerate global warming. The annual global CO₂ emission for 1994 is said to be 6.4 billion tons (carbon conversion). Along with economic development and the increase in world's population, it has been predicted that the levels of atmospheric CO₂ will increase 3 times over the next 100 years if the current emission rate is maintained.

3. Dioxins

Dioxin is an organic chlorine compound which causes cancer and deformity, and disturbs reproductive functions. Since dioxin is not broken down easily, minuscule amounts of dioxin are likely to stay in the air, water, and soil.

4. PCBs

PCBs persist in the environment and causes bio-accumulation. Studies have also found that PCBs have chronic toxicities. Fish and birds accumulate PCBs in their bodies, and humans, once poisoned by PCB, develop acne and paralysis as well as pain in their arms and legs.

5. Endocrine disrupters

Scientifically, little has been known about endocrine disrupters. However, several symptoms have been reported including abnormalities occurring in reproductive functions, reproductive behaviors, and the feminization of males as well as the weakening of fertilization among wild animals. These symptoms may indicate a possible linkage between the symptoms and the exposure of organisms

to suspect chemicals that have been present in the habitat of the organisms. In cases of human exposure, certain chemicals are suspected of disrupting thyroid hormone functions.

6. NO_x • SO_2 • Fluorocarbon gas

These chemicals pollute the air and causes photo-chemical smogs as well as destroy an ozone layer.

III. Safety Measures for Environmental Chemicals

1. Pesticides

Registration of pesticides is mandated by law before these pesticides become commercially available. Newly developed pesticides are examined thoroughly in terms of the safety for users, agricultural products, and for environment. Extremely safe pesticides are the only ones that can be registered.

The use of pesticides that tend to reside in crops, soil, and water is limited by government regulations. Especially a governor's approval is required when water contaminating pesticides are to be used.

Every pesticide has its own tolerance level of pesticide residue. If a pesticide is to be used beyond the tolerance level, the registration of the pesticide is suspended. In addition, safety use standards are set for every crop.

2. CO_2 (CO)

Efforts are made at an industrial level as well as a household level to reduce CO_2 and CO emissions by developing energy-saving instruments, designing buildings in a way that CO_2 and CO gases' emissions are reduced, and reducing the use of automobiles as well as properly adjusting the temperature of air-conditioners and heaters.

3. Dioxins

5-year plan for the control of dioxin was set last year, and this plan aims at building up scientific data to clarify pollution sources and pollution levels affecting the environment and human. In order to prevent adverse impacts on human health and ecosystems, possible countermeasures are to be put into practice.

4. PCBs

In order to have the most effective and appropriate treatment systems, social

and practical problems have been isolated and discussed.

5. Endocrine disrupters

This year, "Strategic program on endocrine disrupters '98" have been set. Methods for screening (presence or absence of hormone disrupters) are now being developed and scientific data on suspect chemicals are being collected.

6. NO_x • SO₂ • Fluorocarbon gas

The current air pollution is largely attributed to the excessive use of automobiles. Therefore, the control of gas emission from automobiles is to be reinforced, while the use of less pollution producing cars will be promoted.

◎Specified Pesticides in Japan

1. Traceable Pesticides in Crops

Lead arsenate, Endrin. Both already have been cancellation of a registration.

2. Traceable Pesticides in Soils

Dieldrin, Aldrin. Both already have been cancellation of a registration.

3. Water Contaminating Pesticides

Isobenzan, Endrin, Endosulfan, PCP, Rotenone.

Endosulfan and Rotenone have been registered. The other Chemicals already have been cancellation of a registration.

◎Trace-Standard of Pesticides in Agricultural Products

131 Pesticides have been established about all of agricultural products (as foods).

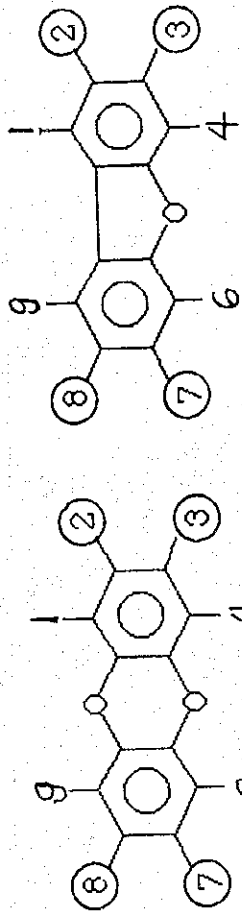
◎Detection-Standard (limited levels) of Pesticides in Water to drink

4 Pesticides have been established.

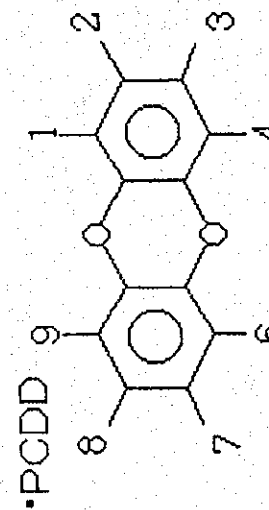
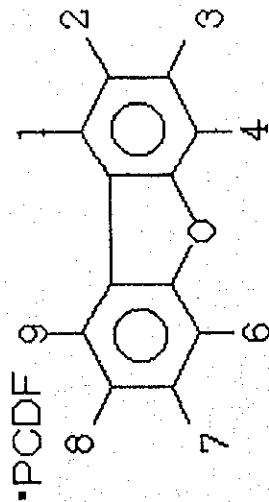
Exogenous Substances that are suspected of having an adverse impact on the normal hormone

Substances	Substances
<p>Dioxin PCB HCB 4-Nitrotoluene n-Butylbenzen Benzophenon Tributyltin Triphenyltin Trifluralin Atrazine Alachlor Simazine(CAT) Parathion Carbaryl Heptachlor Chlordane 1, 2-Dibromo-3-chloropropane(Nemagone) DDT Dicofol(Kerthane) Amitrole</p>	<p>PCP (2, 4, 5-Trichlorophenoxy) acetic Acid (2, 4-Dichlorophenoxy) acetic Acid Endosulfan Malathion Methomyl Methoxychlor Aldrin Endrin Dieldrin Benomyl Mancozeb Maneb Metribuzin Cypermethrin Fenvalerate Permethrin Vinclozolin Zineb Ziram</p>

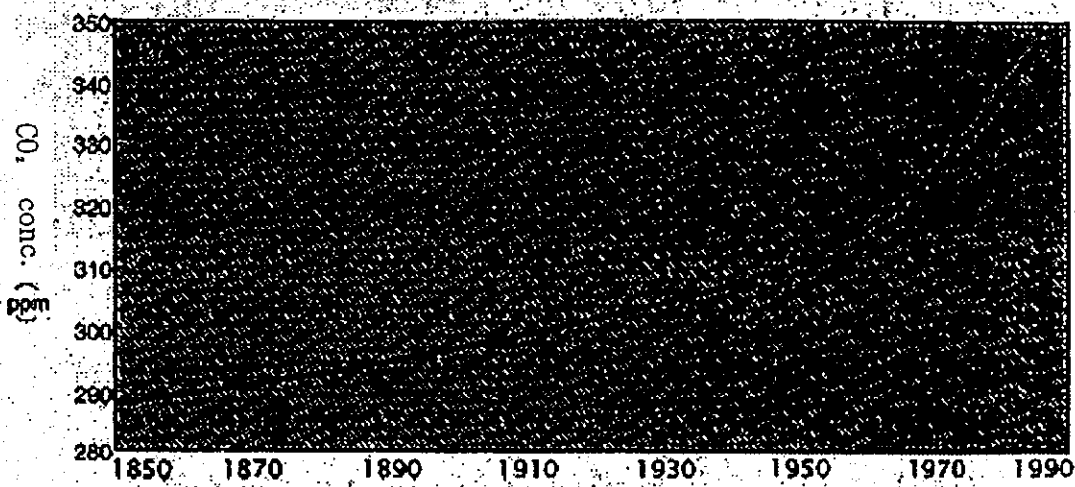
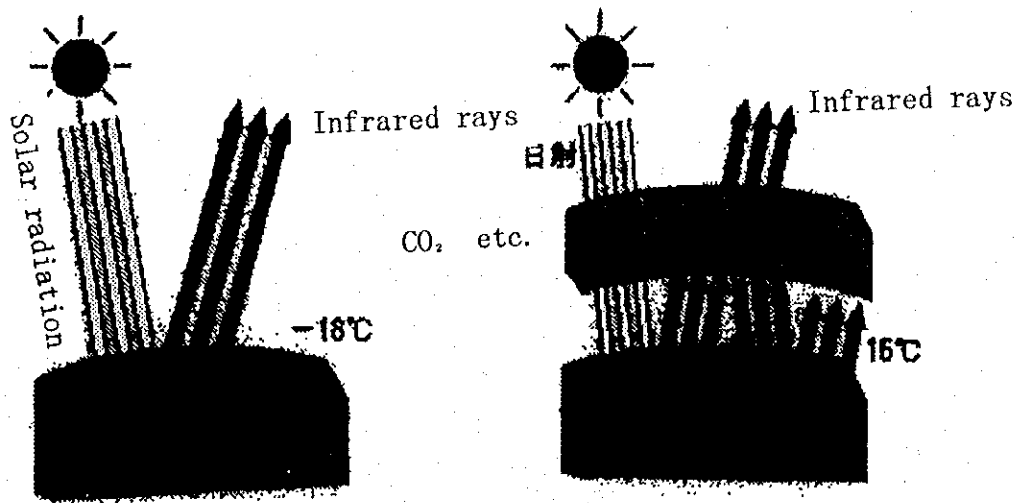
dioxin(2, 3, 7, 8-TCDD)



dibenzofuran(2, 3, 7, 8-TCDF)

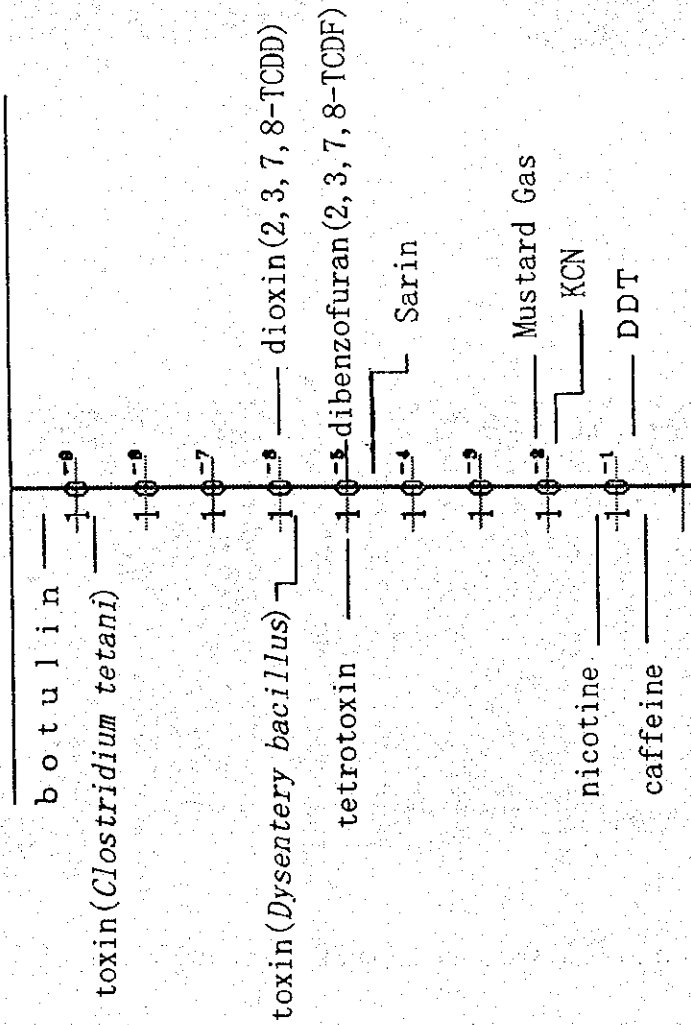


A Structural Formula of Dioxin



(R.A.Houghton and G.M.Woodwell (1989), Scientific American)

LD₅₀
 natural substances (g/k g) artificial substances



Toxicities of Toxic Substances

(Rat are tested)

**The Follow-up Study Team for the Monitoring
of Environmental Contaminants Course
at**

Hyogo International Centre(HIC)

Japan International Cooperation Agency(JICA)

5-10, 4-chome, Ichinotani-cho, Suma-ku, Kobe, 654-0076 Japan

QUESTIONNAIRE

You are kindly required to complete this questionnaire after the meeting for the presentation of the course in Monitoring for Environmental Contaminants.

I. Personal Data :

1. Name in full : _____ Age _____

(Please underline family name)

2. Name of institution where currently employed :

Address : _____

II. Employment/Work Experience

Please describe briefly your current position and responsibility

III. Evaluation of the JICA training program :

1. When did you participate to the course in Pesticide
Utilization and Safety?

Fiscal Year _____

2. To what extent can you apply and make use of the knowledge acquired during the training in your present job?

Please check by ✓

Completely Highly Somewhat

Hardly Not at all

Please explain your answer briefly :

3. Did you achieve some improvements in your work after you attended the training at JICA?

Please check by ✓

No improvements Yes, there is/are improvements

If yes, what improvements did you achieve?

Please check by ✓

Professional recognition Wider international contacts

Greater responsibility Better Prospects of the future

Obtaining another(better) job Salary increase

Others

Please explain your answer briefly :

IV. Expectation to the new course (Monitoring for Environmental Contaminants)

The course in Pesticide Utilization and Safety was abolished in fiscal year '98.

In stead of the course our centre will implement the course in Monitoring for Environmental Contaminants for acquiring indepth knowledge of the technology for evaluating safety of environmental contaminants to human beings and environment through understanding of principles and technology for monitoring environmental contaminants, instrumental analysis, and

practice of immuno—chemical measurement, thus contributing to the improvement of crop and environmental monitoring technology.

The course is for Intermediate Level Engineers.

The training period is 7 months from the beginning of February in 1999.

Please give us your impression which you heard the outline of the course at the meeting.

Describe it briefly :

V. Any other comments.

Please describe it briefly :

Thank you in advance for your kind attention and cooperation to this matter.

一般特設「環境負荷物質分析技術」コースの新設について

1. 研修コース名

環境負荷物質分析技術 (Monitoring for Environmental Contaminants)

2. 背 景

残留農薬など環境負荷化学物質による土壌、水質、農産物の汚染は先進国と開発途上国における共通の問題であり、環境や農産物の安全性を確保するために環境負荷物質の安全性評価及びモニタリング技術を整備することは緊急性を要している。

3. 目 的

開発途上国の当該中堅技術者が環境負荷物質の安全性評価及びモニタリング技術について知識ならびに技術を習得する。

4. 到達目標

- (1) 環境負荷物質の環境及び人に対する安全性評価技術について理解する。
- (2) 環境負荷物質の環境試料及び食料におけるモニタリングの原理及び技術について理解する。
- (3) 環境負荷物質の機器分析、免疫化学測定及び生物検定の技術を習得する。

5. 協力年限

平成10年度から平成14年度まで (計5回実施)

6. 受入期間

7ヶ月間 (平成10年度：平成11年2月8日～平成11年9月5日)

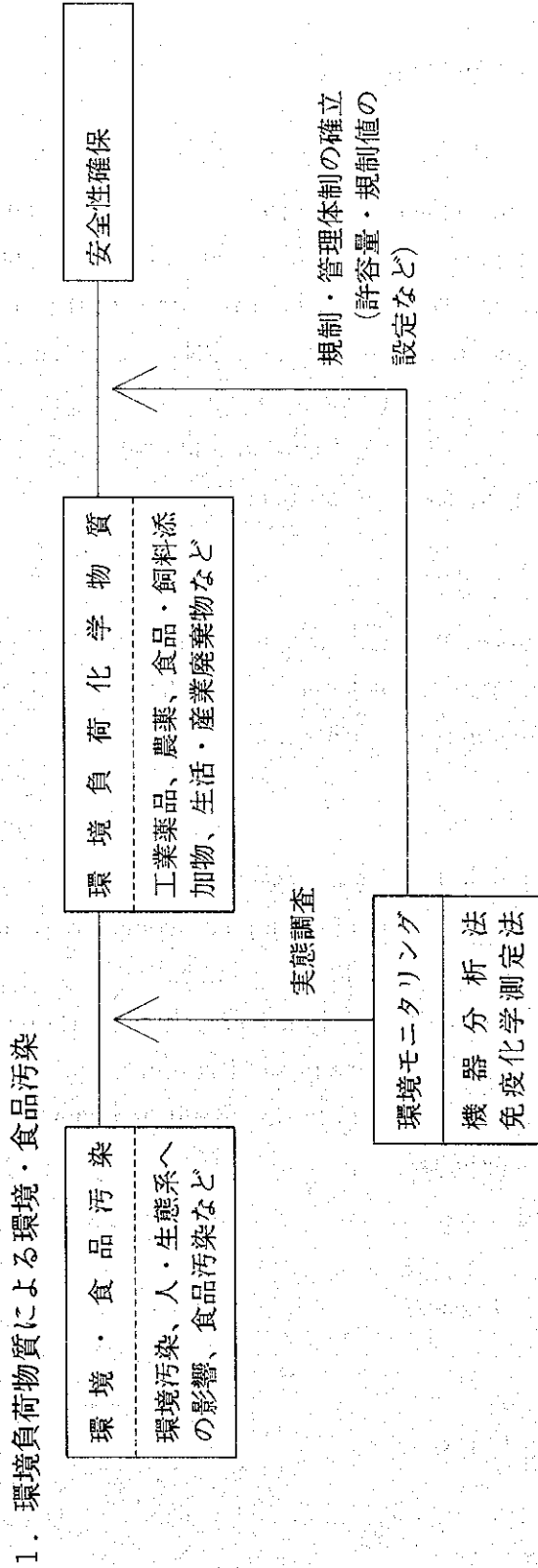
7. 定 員

8名

8. 研修実施機関

神戸大学農学部
兵庫県立中央農業技術センター
兵庫県立公害研究所
厚生省神戸検疫所
(株)住化分析センター
日製産業(株)

環境負荷物質分析技術コース



2. コースの研修概要ならびに到達目標

研修概要	講義	実習	見学	到達目標
環境負荷物質の人および生態系に対する安全性	○		○	安全性に関する評価技術についての理解
環境負荷物質のモニタリングの原理と技術	○		○	モニタリングの原理と技術についての理解
農産物・環境試料における環境負荷物質の分析		○		機器分析法および免疫化学測定法の習得

INFORMATION ON
SPECIALLY OFFERED TRAINING COURSE

MONITORING FOR ENVIRONMENTAL CONTAMINANTS

1. Course Title

Monitoring for Environmental Contaminants

2. Background

Contamination of environment and agricultural products caused by environmental chemicals including pesticide residues is an issue shared by both developed and developing countries. In order to assess the safety of the environment and agricultural products, the improvement of safety assessment and monitoring technologies for environmental chemicals requires immediate attention. (The group training course in "Pesticide Utilization and Safety" will be replaced after completion of the 20-year cooperation period.)

3. Purpose

This course is designed in a way that leading technical officers and researchers in developing countries can gain knowledge and techniques for safety assessment and monitoring environmental chemicals.

4. Course Object

Through this training program, participants are expected:

- (1) to learn about safety assessment technology for environmental contaminants and their impact on human and the environment.
- (2) to learn about the principles and techniques used for monitoring environmental chemicals detected in environmental resources and foods.
- (3) to gain analytical techniques and skills in using instruments, immunoassay, and bioassay methods.

5. Cooperation period

The year of 1999~2003 (Total: 5 years)

6. Duration of the course

February 8, 1999~September 5, 1999

7. No. of Participants

8

8. Training Institutions

Faculty of Agriculture, Kobe University

Hyogo Prefectural Agricultural Research Center

Hyoto Prefectural Environmental Research Center

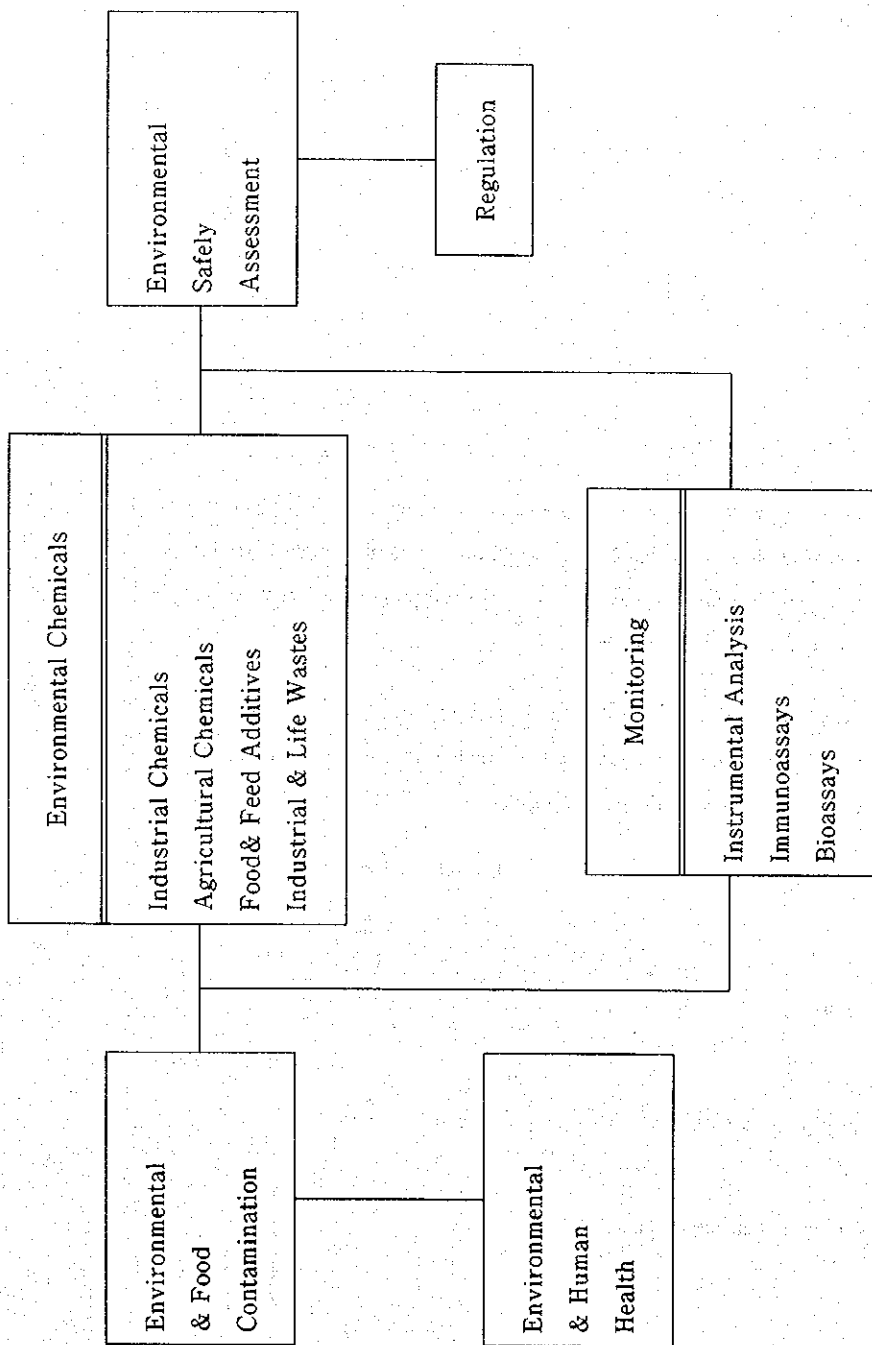
Kobe Quarantine Station, Ministry of Health

Sumika Chemical Analysis Service, LTD

Nissei Sangyo Co., Ltd.

2. Course Contents and Objectives

Course Contents	Lecture	Practice	Observation	Objectives
Safety assesment of environmental chemicals for human & ecosystems	<input type="radio"/>		<input type="radio"/>	To learn about assessment technology for safety assurance and regulation
Principles & techniques for monitoring environmental chemicals	<input type="radio"/>		<input type="radio"/>	To learn about principles and techniques for monitoring
Analysis of environmental chemicals in agricultural products and environmental resources		<input type="radio"/>		To gain analytical techniques & skills in using analytical instruments, immunoassay and bioassays





คณะเกษตร
มหาวิทยาลัยเกษตรศาสตร์

50 ถนนพหลโยธิน จตุจักร กรุงเทพฯ 10900
โทร. 5796131, 5790588, 5796119 โทรสาร 5796152, 9428537

FACULTY OF AGRICULTURE, KASETSART UNIVERSITY
50 PHAHONYOTHIN ROAD, CHATUCHAK, BANGKOK 10900 TEL. 5796131, 5790588, 5796119 FAX 5796152, 9428537

11 November, 1998.

Prof. Dr. Hideo Ohkawa
Japan International Cooperation Agency (JICA)
Hyogo International Center
4-5-10, Ichinotani-cho
Suma-ku
Kobe 654-0076
Japan

Dear Prof. Dr. Hideo Ohkawa :

Firstly, I am indeed very happy to received your post card, reprints and acknowledgment letter and thank you very much for these. May I compliment you on the excellent seminar you have done for us, I would like to personally thank you for the copies of transparencies that you gave me because it is very useful for my work.

Hopefully, Kasetsart University and Kobe University will be able to have the academic and scientific co-operation to promote educational, academic exchange and co-operation in the field of agricultural science between the two Universities.

I hope, and look forward to seeing you again in Thailand.

Sincerely yours,

Prof. Dr. Supamard Panichsakpatana
Dean

