



# Analysis of Organochlorine Pesticide Residues in Fruits and Vegetables

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# Background : What is Pesticide Residue?

Codex Alimentarius: -

- "Pesticide residue" means any specified substance in food, agricultural commodities, or animal feed resulting from the use of pesticide.
- The term includes any derivatives of a pesticide, such as conversion products, metabolites, reaction products, and impurities considered to be of toxicological significance.





# Ordinance

Public Health and Municipal Services Ordinance  
(Chapter 132)

➤ Food and Environmental Hygiene Department

公眾衛生及市政條例 (第132章)

➤ 食物環境衛生署



香港特別行政區政府  
食物環境衛生署



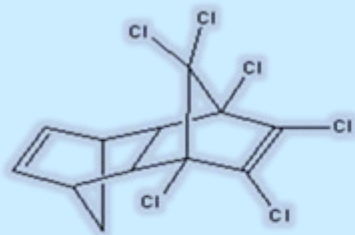
# Organochlorine (OC) Pesticides

➤ 3 main types -

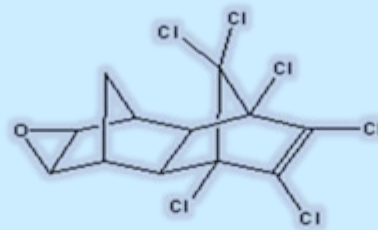
- **DDT & metabolites**  
e.g. DDT, DDD, DDE
- **Benzene derivatives**  
e.g. Hexachlorobenzene (HCB) ,  
pentachloronitrobenze (PCNB)
- **Cyclodienes**  
e.g. aldrin, endrin, chlordane, heptachlor



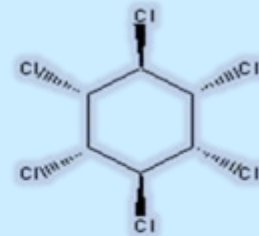
# Examples of OC Pesticides



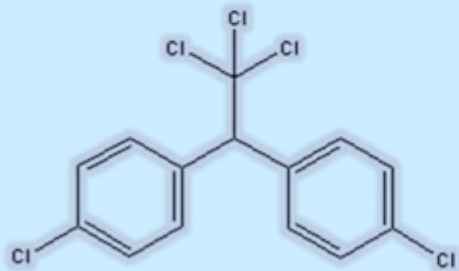
**Aldrin**



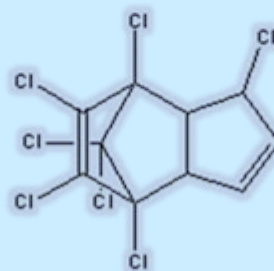
**Endrin**



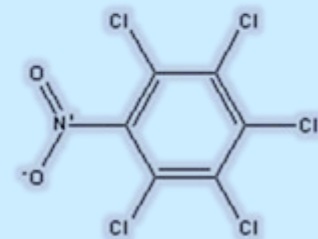
**Lindane**



**DDT**



**Heptachlor**



**PCNB**



# Testing Scope

20 OC pesticides: -

- Aldrin, dieldrin, endrin
- *Trans*-chlordane, *cis*-chlordane, oxychlordane
- o,p'-DDT, p,p'-DDT, p,p'-DDD, p,p'-DDE
- Heptachlor, heptachlor-epoxide
- Hexachlorobenzene (HCB)
- $\alpha$ -Hexachlorocyclohexane,  $\beta$ -hexachlorocyclohexane,  $\delta$ -hexachlorocyclohexane ( $\alpha$ ,  $\beta$ ,  $\delta$  - BHC)
- Lindane
- Quintozene (PCNB), pentachloroaniline (PCA) & methyl-pentachlorophenyl sulphide (MPCPs)



# Sample Type

- Fruits and vegetables
- In 2007, GL analysed around 380 fruit and vegetable samples for OC pesticides.





# Testing methods



## International Reference:

- Analysis of Pesticide Residues: Recommended Methods, Codex Stan 229
- Pesticide Analytical Manual, FDA
- Official Methods of AOAC INTERNATIONAL, 970.52
- Organochlorine pesticides by GC, 8081B, USEPA
- 水果和蔬菜中**500種農藥及相關化學品**殘留的測定-氣相色譜-質譜法, 中華人民共和國國家標準 **GB/T 19648-2006**
- Analytical Methods for Residual Compositional Substances of Agricultural Chemicals, Feed Additives and Veterinary Drugs in Food, Japan official method



# Testing methods



## International Reference:

- Guidelines on the Use of MS for Identification, confirmation & Quantitative Determination of Residues, Codex CAC/GL 56-2005
- Analysis of Pesticides: Guidelines on Good Laboratory Practice in Residue Analysis, Codex CAC/GL40-1993, Rev.1 – 2003
- Commission Decision, 2002/657/EC, implementing Council Directive 96/23/EC concerning the performance of analytical methods & the interpretation of results
- Method Validation & Quality Control Procedures for Pesticide Residues Analysis in Food & Feed, SANCO/2007/3131, EU
- Food Standards Agency Information Bulletin on Methods of Analysis & Sampling for Foodstuffs, Food Standards Agency, No. 42, Jan. 2004



# International Reference

Website:

- [www.codexalimentarius.net](http://www.codexalimentarius.net)
- [www.cfsan.fda.gov](http://www.cfsan.fda.gov)
- <http://ec.europa.eu>
- [www.food.gov.uk](http://www.food.gov.uk)
- [www.epa.gov](http://www.epa.gov)





# Testing methods (GL)

- Classification of food commodities
- Sample preparation
- Extraction
- Sample cleanup
- Analysis
- Data interpretation





# Classification of Food Commodities

## Codex Alimentarius

- Vol. 2, Section 2: Pesticides Residues in Food, Codex Classification of Foods & Animal Feeds.
- Vol. 2A, Section 2.1: Analysis of Pesticides Residues: Portion of the Commodities to Which Codex MRLS Apply & Which is Analysed.

Classification	Example	Analytical portion
Leafy vegetables	spinach, lettuce	whole commodities
Root & tuber vegetables	carrots, beets	whole commodities after removing tops
Citrus fruits	orange, lemon	whole commodities
Pome fruits	apple, pear	whole commodities after removing stems
Stone fruits	peach, cherries	whole commodities after removing stems & stones but the residue calculated & expressed on the whole commodity without stem



# Sample Preparation

- Comminute and homogenize the sample with a blender.
- Weigh 50 – 100 g sample into a centrifuge bottle.



# Extraction

- Add 

500 $\mu\text{g}$ PCB209 (surrogate)
200 mL ethyl acetate
~ 200 g anhydrous $\text{Na}_2\text{SO}_4$

 to the centrifuge bottle.
- Homogenize for ~5 min.



# Extraction

- Centrifuge at 2000 rpm for 10 min.
- Filter the supernatant solution through anhydrous  $\text{Na}_2\text{SO}_4$  into a measuring cylinder.
- Record the volume of the extract collected (V).





# Sample Cleanup

- Gel permeation chromatography (GPC)
  - Remove interfering compounds of high molecular weight e.g. lipids, waxes, proteins, natural resin.
  
- Solid phase extraction (SPE)
  - Remove interfering compounds of different polarity. e.g. aliphatic, aromatics & N-containing compounds
  
- Sulphuric acid treatment
  - Remove complex organic sample matrix.
  - Also destroy 6 OC pesticides: aldrin, endrin, dieldrin, PCA, MPCPs & heptachlor epoxide.

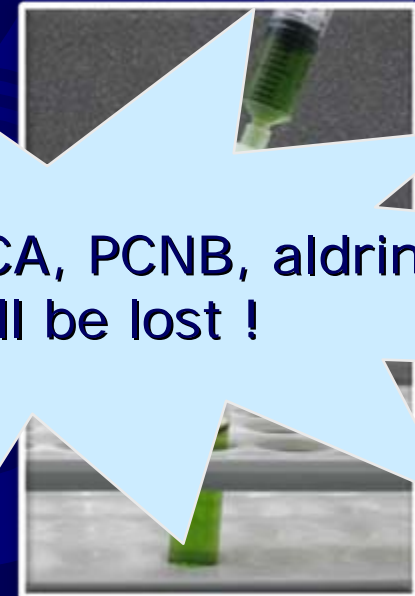


# Gel Permeation Chromatograph (GPC)

- Transfer the sample extract quantitatively to a flat bottom flask.
- Concentrate to just dryness using a rotary evaporator.
- Reconstitute the residue with 10 mL  $\text{C}_6\text{H}_{12}$ : $\text{CH}_2\text{Cl}_2$  (1:1) & add 2g anhydrous  $\text{Na}_2\text{SO}_4$ .
- Centrifuge at 2000 rpm for 10 min.
- Filter the supernatant solution thro. a Puradisc disposable filter for GPC analysis.



**HCB, BHC, PCA, PCNB, aldrin  
etc. will be lost !**





# Gel Permeation Chromatograph (GPC)

## ➤ GPC conditions: -

- ❑ GPC column : 60cm x 2.5cm id glass column packed with 60g 200-400 mesh Bio-beads SX-3 resin in  $\text{C}_6\text{H}_{12}$ : $\text{CH}_2\text{Cl}_2$  (1:1)
  - ❑ Loop vol. : 5 mL
  - ❑ Eluent :  $\text{C}_6\text{H}_{12}$ : $\text{CH}_2\text{Cl}_2$  (1:1)
  - ❑ Flow rate : 5 mL/min.
  - ❑ Dump time : 30 min.
  - ❑ Collect time : 20 min.
- Collect the GPC extract & evaporate to just dryness.
- Reconstitute with 0.5 mL petroleum ether.





# Solid Phase Extraction (SPE)

- SPE column : Florisil 500mg, conditioned with 10 mL 15% EtOH in petroleum ether.
- Quantitatively transfer the GPC extract on top of the SPE column.
- Elute with 25 mL 15% diethyl ether in petroleum ether.
- Concentrate the eluate to just dryness under a gentle stream of  $N_2$ .
- Reconstitute the residue in 1 mL isooctane.

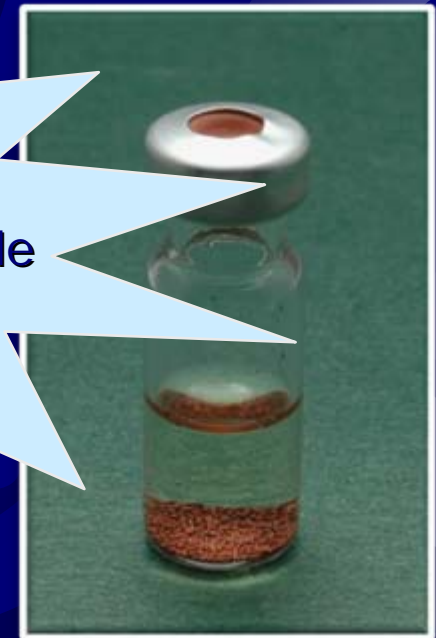
**Elution rate**





# Sulphuric Acid Treatment

- Add 3 mL conc.  $\text{H}_2\text{SO}_4$  to the reconstitute extract (in isooctane).
- Shake vigorously using a vortex mixer.
- Allow the two layers to separate & collect the upper organic layer.
- Add ~0.2 g Cu powder & allow to stand overnight.



**Aldrin, endrin, dieldrin,  
PCA, MPCPs & heptachlor epoxide  
will be destroyed !**



# Gas Chromatographic Analysis

## ➤ Quantitation

- Analyzed by GC/ $\mu$ -ECD.

## ➤ Confirmation

- Dual-column analysis.  
(e.g. DB-5, DB-1701)
- GC/MS.





# Gas Chromatographic Analysis

## ➤ Injector port is critical !

- Use inert deactivated liner.
- Keep the injector port clean.
- Injection temperature not too high.
- Degradation of some pesticides:

e.g. endrin → endrin aldehyde & endrin ketone

DDT → DDD or DDE



# Quantitation : GC/ $\mu$ -ECD

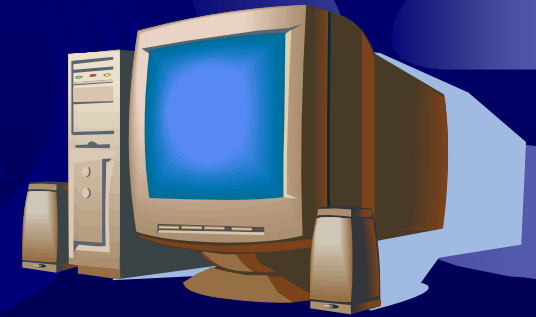
## ➤ GC conditions: -

- Column : DB-1701, 30m, 0.25-mm i.d., 0.25- $\mu$ m film thickness  
DB-5, 30m, 0.25-mm i.d., 0.25- $\mu$ m film thickness

- Oven temp. :

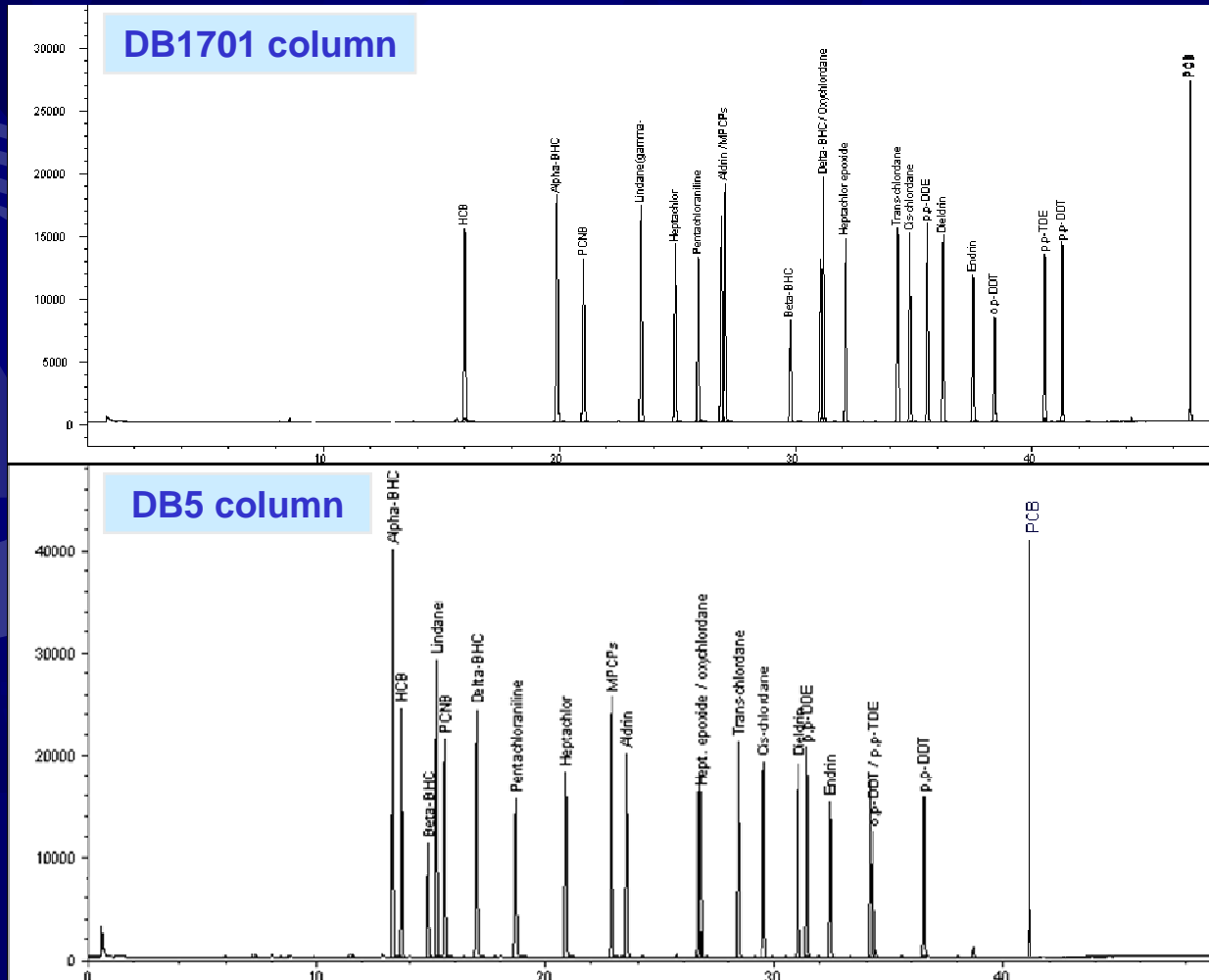
100 °C (2 min)  $\xrightarrow{10\text{ }^\circ\text{C/min}}$  165 °C (10 min)  $\xrightarrow{3\text{ }^\circ\text{C/min}}$  230 °C  $\xrightarrow{15\text{ }^\circ\text{C/min}}$  280 °C (10 min)

- Injection mode : splitless
- Injection vol. : 1  $\mu$ L
- Injector temp.: 210 °C.
- Detector temp.: 300 °C
- Carrier gas flow: 1 mL/min. nitrogen





# GC/ $\mu$ -ECD Analysis (Dual Columns)





# Quantitation

## ➤ Corrected weight

$$W_o = \text{weight of sample used} \times \underbrace{\frac{V}{200}}_{\text{Extraction}} \times \underbrace{\frac{5}{10}}_{\text{GPC cleanup}}$$

where

$V$  = volume of sample solution collected in the extraction step





# Confirmation : GC/MS

## ➤ SIM mode

- Selected ion monitoring.
- Monitor 3 ions for each pesticide.
- Calculate the relative ion intensity (% intensity relative to the most abundant ion).

## ➤ SCAN mode

- Supplementary information
- Spectrum matches with the reference spectrum (e.g. NIST or reference material).



# GC/MS Conditions

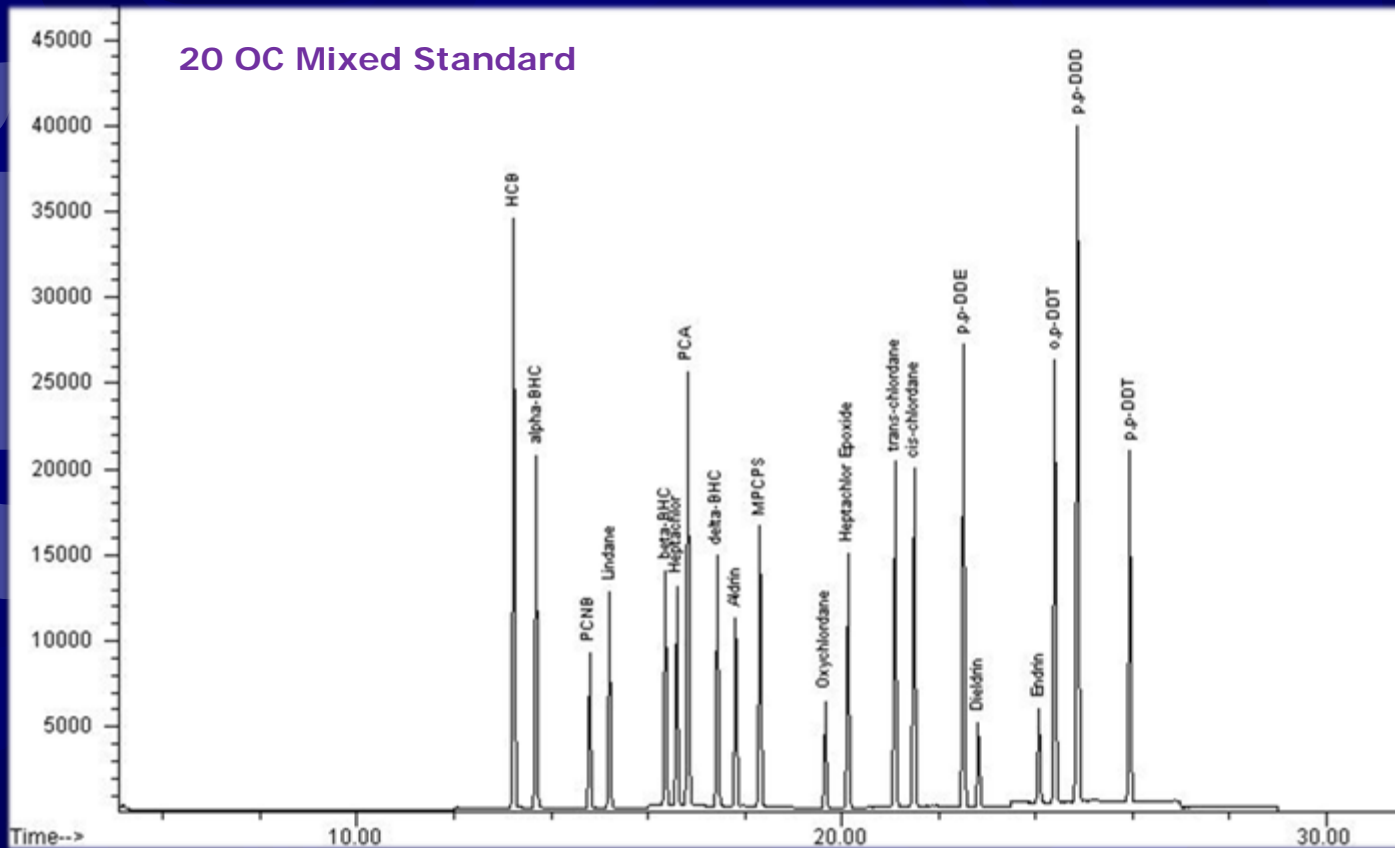
- Column : DB-35MS, 30m, 0.25-mm i.d., 0.25- $\mu$ m film thickness
- Oven temp. :

100 °C (2 min)  $\xrightarrow{15\text{ }^{\circ}\text{C}/\text{min}}$  160 °C  $\xrightarrow{5\text{ }^{\circ}\text{C}/\text{min}}$  230 °C (10 min)  $\xrightarrow{\text{Post run}}$  300 °C (5 min)

- Injection mode : splitless
- Injection vol. : 1  $\mu$ L
- Injector temp.: 200 °C
- Carrier gas flow: 1 mL/min He
- Ion source : 230 °C
- Scan range : m/z 45 – 400 (scan mode) or m/z of 3 monitoring ions for each pesticide (SIM mode)

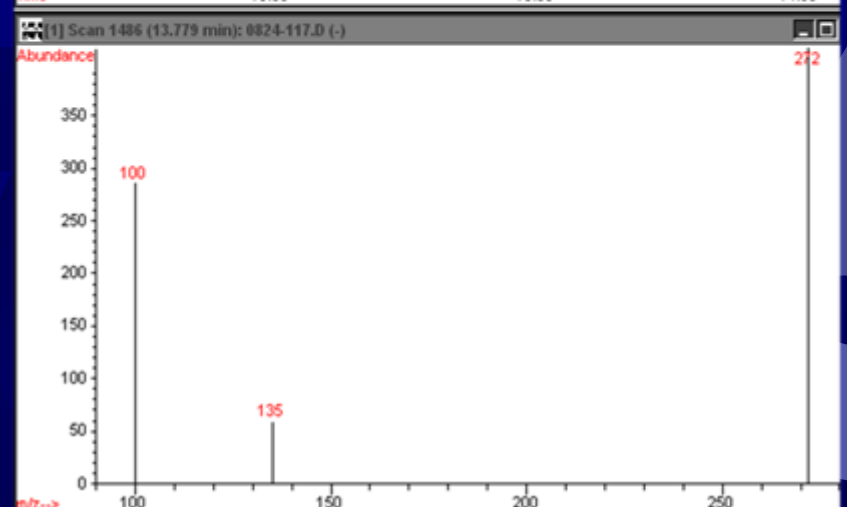
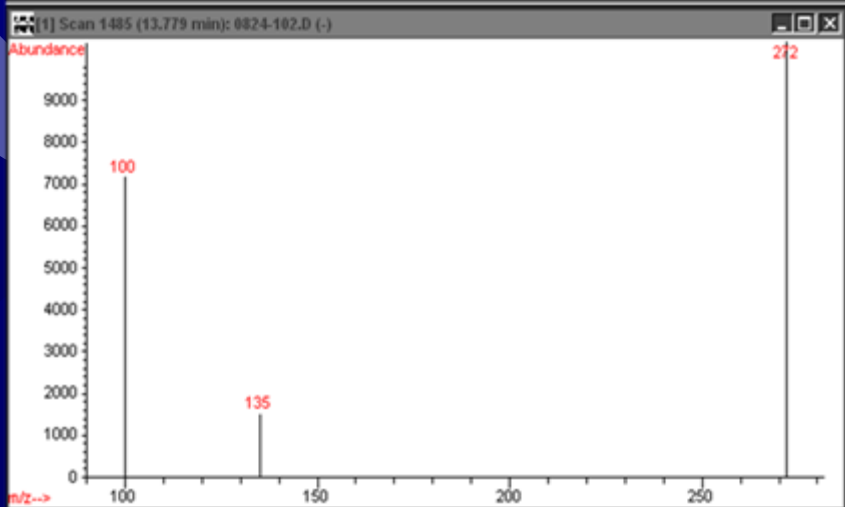
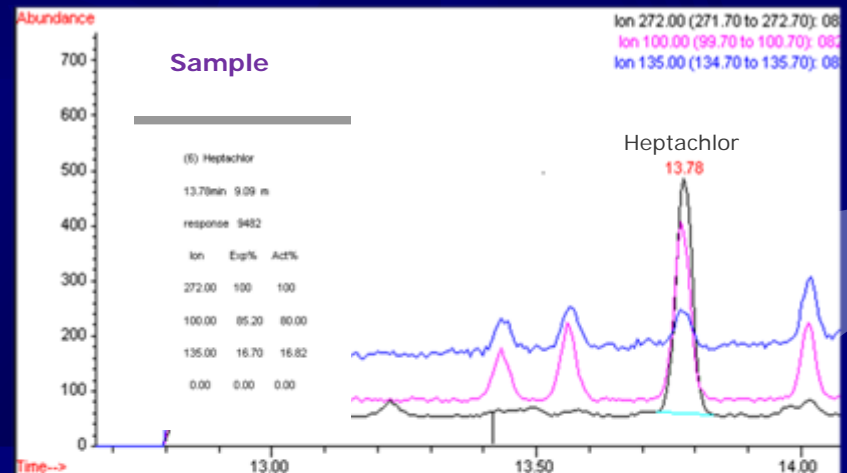
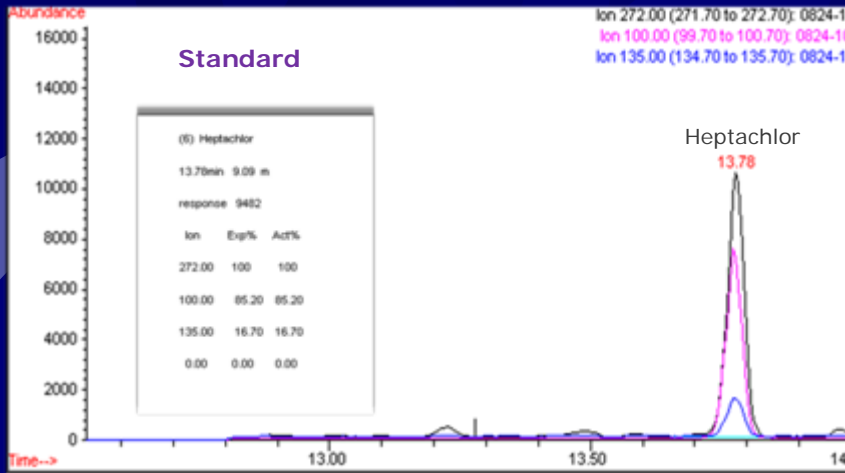


# GC/MS : Chromatogram (SIM)





# GC/MS : Chromatogram (SIM)



# Tolerance for Relative Ion Intensity

## ➤ Relative ion intensities of heptachlor

m/z	Relative ion ratio(%) [standard]	Relative ion ratio (%) [sample]	Deviation (%)
272	100	100	-
100	85.2	80	6.1
135	16.7	16.82	0.7

## ➤ Recommended tolerance for relative ion intensities (2002/657/EC)

Relative Intensity (%)	GC/MS (EI)
> 50%	± 10%
> 20% to 50%	± 15%
> 10% to 20%	± 20%
≤ 10%	± 50%

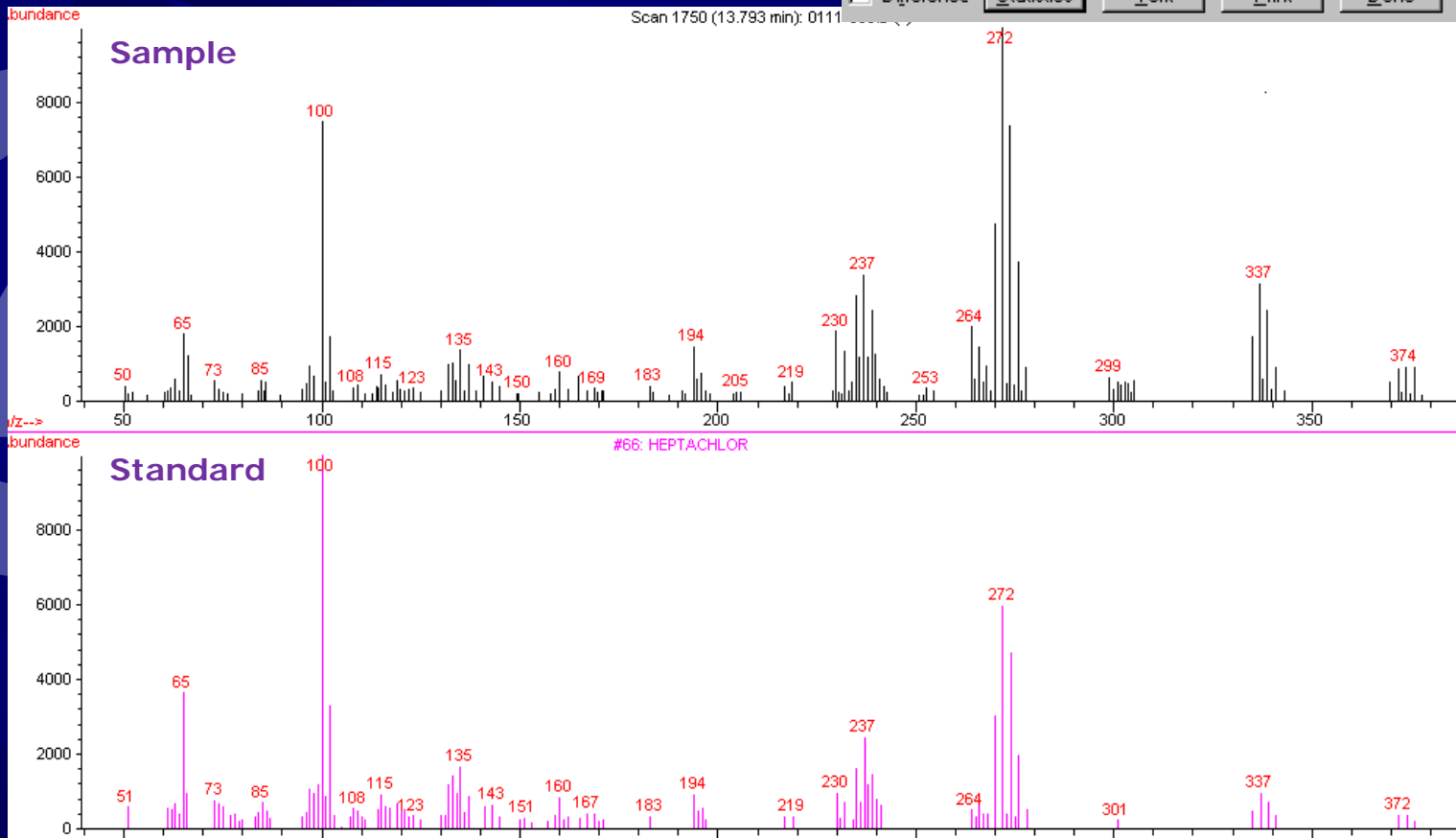


# MS Spectra (SCAN)

PBM Search Results: R:\MISC\MS LIBRARY\WIST98.L

Name	Ref No.	MW	Qual
1. Heptachlor	#118685	370	99
2. Heptachlor	#118684	370	99
3. Heptachlor	#43874	370	91

Difference              





# Reporting Limits

➤ Quantitation of individual OC pesticide:

0.005 mg/kg



# Certified Reference Material

<http://irmm.jrc.ec.europa.eu>

Organochlorine Pesticides  
in Animal Feed

BCR-115

(from Community Bureau of Reference)



EUROPEAN COMMISSION  
JOINT RESEARCH CENTRE  
Institute for Reference Materials and Measurements

*irmm*  
Institute for Reference Materials and Measurements

**CERTIFIED REFERENCE MATERIAL**  
**BCR<sup>®</sup> - 115**

CERTIFICATE OF ANALYSIS

Compound	Mass fraction (based on dry mass)		Number of accepted sets of results p
	Certified value <sup>1)</sup> [mg/kg]	Uncertainty <sup>2)</sup> [mg/kg]	
HCB	0.0194	0.0014	7
β-HCH	0.0234	0.0026	7
γ-HCH	0.0218	0.0020	6
Heptachlor	0.0190	0.0015	7
γ-Chlordane	0.048	0.006	5
α-Endosulfan	0.046	0.004	8
Dieldrin	0.0181	0.0023	8
Endrin	0.046	0.006	7
o,p'-DDT	0.046	0.006	6
p,p'-DDE	0.047	0.004	4

1) The certified value is the unweighted mean of the means of p sets of results. These sets of results were provided by different laboratories using GC-ECD under different conditions and with different sample preparation methods. The certified value is traceable to determination by GC-ECD.

2) The uncertainty is taken as the half-width of the 95 % confidence interval of the mean value defined in 1).

This certificate is valid for one year after purchase.  
Sales date:  
The minimum amount of sample to be used is 1 g.

**NOTE**  
This material has been certified by BCR (Community Bureau of Reference, the former reference materials programme of the European Commission). The certificate has been revised under the responsibility of IRMM.

Brussels, November 1995  
Last revision: April 2007

Signed:   
Prof. Dr. Hendrik Emons  
Unit for Reference Materials  
EC-JRC-IRMM  
Retieseweg 111  
2440 Geel, Belgium



# Proficiency Test

## ➤ FAPAS

Proficiency Test	Start Date	Matrix	Analytes
0560	18/6/2008	Hydrogenated vegetable oil	OC Pesticides & PCBs
0562	19/8/2008	Hydrogenated vegetable oil	OC Pesticides & PCBs
1934	15/09/2008	Apricot Purée	Pesticides
1984	15/09/2008	Lettuce Purée	Pesticides
1985	13/10/2008	Apple Purée	Pesticides

<http://www.fapas.com>



# Looking Forward

- **Extend the scope of OC pesticides**  
e.g. dicofol, endosulfan, chlorothalonil, mirex, chlorfenapyr, vinclozolin etc.
- **Extend to other types of pesticides**  
e.g. pyrethroids such as cypermethrin, permethrin, bifenthrin, cyfluthrin, cyhalothrin, deltamethrin, fenpropathrin, fenvalerate.
- **Extend to other food matrix**  
e.g. cereal, tea, aquatic products, processed food etc.



# Contact Us

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The End



Thank You

