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# STRENGTHENING QUALITY CONTROL AND TESTING FACILITIES OF NON-ALCOHOLIC LIQUID FOODS INCLUDING FISH SAUCE AND SOY-BEAN SAUCE

DP/VIE/87/009/11-51

VIET NAM

## Technical report: Consultancy in chemical and instrumental food analysis and laboratory staff training*

Prepared for the Government of Viet Nam
by the United Nations Industrial Development Organization,
acting as executing agency for the United Nations Development Programme

Based on the work of Edward Kaminski,
expert in chemical and instrumental food analysis

Backstopping Officer: A. Ouaouich
Agro-based Industries Branch

United Nations Industrial Development Organization
Vienna

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* This document has not been edited.

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## CONTENTS

**APPENDICES**

**ABSTRACT**

A chemical and instrumental food analysis consultancy visit to Vietnam was undertaken from 1 February to 31 March 1993 as a part of UNIDO's technical assistance project DP/Vie/87/009 13103 at the Department for Quality Control of Goods and Metrology (DTQC), Ministry of Trade, Hanoi.

The objectives of the visit were:

- To assist in the installation and testing of equipment donated by UNIDO to the DTQC as part of the project.
- To conduct training courses in the area:
  - Analysis of pesticides residues in Vietnamese food by using Gas Chromatography techniques
  - Analysis of heavy metal (trace elements) in Vietnamese food by using Atomic Absorption Spectrometer techniques
  - Analysis of mycotoxins in Vietnamese food by using Thin-layer Chromatography
  - Analysis of food additives
  - Conduction of one day seminar on rapid methods for food quality control

The equipment provided by the project was installed:

- Gas chromatograph (GLC) TRACOR Model 9000 by Vietnamese experts from Hanoi University
- Atomic Absorption spectrometer (AAS) by UNIDO expert Mr. D. Kordik and Vietnamese experts.

Both items were tested and found to be in working order.

A training course on the objective reviewed above was conducted from 8 February to 25 March 1993.

Prepared lecture notes, methods and review of the literature related to the project were provided to 12 participants who attended the course.

For the Seminar, a manuscript entitled "Fermented soy products manufacture, quality and health aspect" was prepared.

The major outputs of the visit are:

i/ 12 persons trained in chemical and instrumental food analysis

methods

ii/ 12 persons trained in Gas Chromatography techniques

iii/ 12 persons trained in mycotoxin analysis by Thin Layer Chromatography

iv/ 12 persons trained in methods of trace elements (heavy metals) analysis by AAS techniques

v/ 12 persons trained in food additives analysis (colour additives)

vi/ The laboratory staff has now the capability to evaluate Vietnamese food for contamination of pesticide residues, trace elements, mycotoxins and food additives

vii/ At the end of the course, all participants obtained "Certificate of Attendance" for having satisfactorily completed a training course on "Rapid and Instrumental Food Analysis Methods and Spectrophotometric Techniques".

These certificates were signed by Prof.Dr. Phan Duc Thang - NPD of the Project and by the Expert of UNIDO - Prof.Dr. Edward Kaminski. Copy of the Certificate was given in Annex 5.

## INTRODUCTION

1. A two month consultancy visit in chemical and instrumental food analysis was undertaken at the Department of Quality Control of Goods and Metrology (DTQC), Ministry of Trade, Hanoi, Vietnam. This visit was part of the United Nations Industrial Development Organization's (UNIDO) technical assistance project DP/VIE/87/009.

The purpose of this technical assistance project is to enable it to implement more effective quality control programme by strengthening the analytical expertise in assessing the quality and safety of foods as well as upgrading the extension and advisory services.

2. The consultancy was undertaken by EDWARD KAMINSKI, professor Dr. of the Food Technology Institute of the University of Agriculture, Poznan, Poland.

The specific objectives of the mission were to:

- Assist in the installation of new equipment and test its performance.
- Conduct training of DTQC staff in the use and operation of equipment for chemical and instrumental food analysis.
- Prepare a training manual on methods for the analysis of food contaminants: pesticide by Gas Liquid Chromatography (GLC), trace elements (heavy metals by Atomic Absorption Spectrometer (AAS) and mycotoxins by Thin Layer Chromatography (TLC).
- Conduct a training workshop using the above manual.
- Present papers for a seminar on "Food Quality Control".
- Prepare a terminal report.

3. The consultancy and training workshop took place at the Chemical and Instrumental Laboratories and Training Room of the DTQC, Hanoi, Vietnam.

- The job description is given in Annex 1.
- A plan of the activities at the DTQC is given in Annex 2.
- A list of the senior counterpart staff is given in Annex 3
- A list of the participants and observers of the course of instrumental and chemical analysis is given in Annex 4.

- A list of the people met is given in Annex 4A.
- A list of the equipment used in the chemical and instrumental analysis is given in Appendix 1.

# I. TRAINING COURSE ON THE ANALYSIS OF PESTICIDE RESIDUES IN FOOD

The first week after arriving at DTQC, the consultant took time to get acquainted with the staff members, laboratories, and checked the list of equipments and chemicals provided by UNIDO.

The training course on the analysis of pesticide by Gas Liquid Chromatography (GLC) and Thin Layer Chromatography (TLC) was held from 9 to 20 February 1993. It was attended by 12 participants. A list of participants and observers is given in Annex 4. The course consisted of lecturers which were held in the morning from 9.00 to 10.00 or from 9.00 to 11.00 and laboratory work from 10.00 or 11.00 to 12.00 and 13.30 to 16.30 hours. The lecture notes and laboratory schedules which were prepared in Poland were provided for the participants of the course, and are not included in this report. The methods applied during the course are given in Annexes 6 and 7.

## A. Safety in the laboratory procedures

In order to avoid severe risks associated with handling of pesticides, mycotoxins and chemical reagents the participants and the staff were informed on the correct use of apparatuses and basic laboratory skills. The FAO/WHO Codex Guidelines on Good Practice in Pesticide Residue Analysis were distributed to members of the DTQC staff and participants; they are not included in this report.

## B. Analysis of pesticide residue by gas chromatography (GLC)

At present there are several hundred pesticides in common use for the control of insects, weeds and diseases of plants. The list of pesticide used in Vietnam is given in Appendix 2. During the training course the organo-chloride pesticide and its residues were studied together with the problems associated with its long-term persistence and high poisonousness. An essential knowledge of the organo-chlorine pesticide was given during the lectures.

The low concentration of the pesticides in plants, water, food

products and living organisms requires special methods of analysis. During the course two techniques have been used: Gas Liquid Chromatography (GLC) and Thin Layer Chromatography (TLC). Since the participants had very little knowledge about GLC techniques, basic information concerning Gas Chromatography techniques was given during the lectures and training sessions.

Gas Chromatography is today the most widely used instrument technique for analysis of pesticides with very high sensitivity and high separation efficiency of chemical substances.

Emphasis was put on the practical aspects related to the selection and operation of the various types of detectors (Electron Capture Detector ECD, Flame Ionization Detector FID, Thermal Conductivity Detector TCD).

For organo-chlorine pesticide analysis ECD is commonly used. The Electron Capture Detector cell contains a radioactive isotope (63 Ni) emitting high-energy electrons. Those electrons are partly reduced with collisions of carrier gas and molecules of sample pesticides.

Uncaptured electrons are collected periodically by applying short-term voltage pulses to cell electrodes. During the training course attention was paid to potential hazard associated with ECD handling. The influent gas stream from the ECD must always be vented by a fume hood to prevent possible contamination of the laboratory with radioactive material. A description of ECD, FID and TCD detectors is given in Appendix 3.

The next very important part of the GLC is the column. "The column is the heart of the Gas Chromatograph". Participants were acquainted with packed columns and capillary columns. The most important characteristics of the column for multi-residue pesticide analysis were given during the course: efficiency of the column, sensitivity, retention, compound elution, support material and stationary phase materials for packed column.

For pesticide residues the column with stationary phase such as SE-30; SE-52; OV-17; OV-210; SP-1500; SP-2250; were recommended. Attention was paid to the stability of the column used. A liquid phase loading of 5-10% on support material (Chromosorb W) is commonly employed in pesticide analysis. The liquid phase is

coated onto small particles of support material. The bonding in the coating is not strong and can be disturbed by injection of the polar solvent or by prolonged heating.

The efficiency of some packed columns and retention ratios of 54 pesticides is presented in Appendix 4. The tables presented proved very useful, particularly for tentative peak identifications in the chromatograms of the pesticide multi-residue samples. Each stationary phase or combination thereof has its own elution pattern or "fingerprint" for the compounds of a given mixture. Appendix 5 shows a chromatogram of 8 organo-chlorine pesticide mixtures obtained on 10% SP-1000 on supelcoport during the training course on GLC TRACOR Model 9000.

In gas chromatography laboratories well equipped for environmental studies capillary column are now being used. Appendix 6 shows chromatograms of pesticide separation on a capillary column with a diameter 0.2 - 0.32 mm and 25 - 50 m long and a megabore column as replacement for the packed column. The megabore column has a diameter of 0.53 mm, is 15 - 30 m long and made from fused silica tubing. A technical reason for considering the megabore column is that the stationary phase is surface-bonded and cross-linked to the silica surface and can neither be rinsed out by the solvent nor leaked by high temperature. Megabore columns compared with packed columns are "long life" columns.

## C. Analysis of pesticides on Thin Layer Chromatography (TLC)

The participant were made acquainted with the theoretical and practical use of Thin Layer Chromatography for pesticide analysis.

A growing use of TLC for pesticide analysis can be seen from reports published in previous years. The article on "The updated techniques for quantitative Thin Layer Chromatography of organo-chlorine pesticides" issued by J.Sherma (see point E.4.) was distributed to the participants of the course and could be used as a guide for pesticides analysis using TLC techniques. This article is not included in this report.

At the training course silica gel, aluminum and glass plates were used for the separation of Lindane, DDT, DDE, and Eldrin. As

a mobile phase n-heptan was used. For the detection of spots 1% diphenylamine in ethanol followed by irradiation with UV light (366 nm) was used.

Other mobile phase and chromogenic reagents could also be used. Thin Layer Chromatography methods and techniques are rapid and give results which can be reported semiquantitatively.

According to Practical Analytical Manual, use of these techniques should detect at least 25 μg standards of aldrin, dielrin, eldrin, TDE, DDE and DDT.

According to J.Sherma, commercially precoated silica gel Thin Layer plates are almost exclusively used. According to the Pesticide Analytical Manual the silica gel has been discontinued for routine Thin Layer Chromatography of chlorinated pesticide by aluminum oxide producing more compact spots.

The advantages of Thin Layer Chromatography are speed, low cost, a more simple sample preparation than for GLC technique. Disadvantage of TLC is that in some countries there may be problems caused by high humidity. This fact was observed during the analysis of chloro-organopesticide at the DTQC laboratory in Hanoi during the training course when humidity was very high. To prevent this effect the Thin-layer plate should be activated before spotting and spots application should be in room with reduced humidity.

A diagram of water absorption of the silica gel plates is shown in Appendix 7.

## D. Sample preparation

Sample preparation for any analytical determination must be performed in such a way as to avoid any changes that will be reflected in the analytical results. A sample must be representative of the whole population, otherwise the analytical result will be inaccurate. During the course the participants were made acquainted with sample preparation, sample extraction using different solvents; clean up technique used liquid-liquid partitioning and sorption of extracts to florisil column and selective elution of pesticide used organic solvents. For sample concentra-

tion a rotary Kuderna Danish evaporator supplied by UNIDO was used.

Pesticide Analytical Manual, Vol. 1, edited by the US Dept. of Health and Human Services Food and Drug Admin. in 1990 was distributed to the participants. This manual is not included in this report.

During the training course the participants prepared following samples: rice, green beans, and tap water. Using Gas Chromatograph techniques no pesticide residues were detected in the examined samples. Using internal standards (Lindan) to the samples a good reproducibility of the methods was achieved.

Simplified methods of sample preparation, used in Poland for organo-chlorine determination of pesticide residues by GLC techniques were used during the training course (the Annexes 6,7).

The residue limits of pesticide in agricultural products and foods as well as the acceptable daily intake was presented during the lectures.

The Reprints from the report "Pesticide Residues in Food" issued in 1989 by WHO/FAO were distributed to the participants of the course and are not included in this report.

## E. Recommended literature

1. Gunter Zweig - Pesticide and Plant Growth Regulators Vol.1, II. III, IV, V, VI, VII, VIII, IX, X, XI. Academic Press 1980

2. Moye A. M. - Analysis of Pesticide Residues A. Willey Interscience Publication New York 1981

3. Gas Chromatography - Short training course National Sci. Inst. Training Center Varian

4. Zweig B., Sherma J. - Handbook of Chromatography. Vol. I, II CRC Press 1992

## II. TRAINING COURSE ON THE ANALYSIS OF TRACE ELEMENTS - HEAVY METALS BY ATOMIC ABSORPTION SPECTROSCOPY (AAS) IN FOOD AND FOOD PRODUCTS

The training course on the analysis of trace elements - heavy metals - was held from 22-26 February and continued simultaneously with other topics until the end of the mission.

The course consisted of lectures and laboratory training from 9.00 to 12.00 and 13.30 to 16.30 hours.

Lecture notes and prepared methods were distributed to the 12 participants.

### A. Explanation

The term "contaminants" refer to undesirable materials which have been incorporated inadvertently to food before, during or after processing.

From the food analyst's viewpoint the term "trace element" refers to inorganic elements, mostly metals, which may be present in foods, usually in amounts below 50 mg/kg and have some toxicological or nutritional significance.

The nutritive elements: Co, Cu, Fe, I, Mn, Mg, Zn

The non-nutritive elements: As, Sb, Cd, F, Pb, Hg - toxic elements

### B. Toxic elements

Mercury - Is a very toxic substances as a trace element in food and could accumulate in human and animal bodies mostly in the liver and in the hair.

The most contaminated products are fish and fish products.

The source of contamination are fungicides used for plant protection during cultivation and fungicide formulation to prepare the seeds before sowing. In fungicide formulation mercury is used in N, N-Bis/methyl-Hg/-p-toluolsulphonamid form.

In fish mercury is present in the most toxic form as methyl mercury $(CH_3)_2Hg$.

**Lead** - As a toxic substance it has a limit in foods 1-2.0 mg/kg for fish and 10 mg/kg for shellfish.
The limit for foods especially prepared for infants or young children is 0.2 mg/kg. Small amount of lead occurs in many foods naturally.
Lead is a main source of contamination in industrial countries, where lead is used in petrol as an additive and old water pipes made from lead are still in use. Contamination may also occur from the use of lead alloys or compounds for processing materials, including solders, glazes, enamels, wrapping materials and piping or from pesticides.

**Cadmium** - Is a relatively toxic metal, which is liable to cause soon after ingestion acute gastritis with vomiting and diarrhoea. Cadmium contamination in food arises mainly from the soil (70%) and from the air (30%). In water cadmium contamination may occur from superphosphate fertilizers. The majority of products contain less than 1.2 mg/kg. Higher levels were found in fish pastes, mushrooms and kidneys of pigs. Cadmium can occur in rice and soybeans.

**Arsenic** - The general limit for arsenic in food is 1 mg/kg. Formerly much of the arsenic contamination found in food resulted from the use of impure sulfuric acid in the manufacture of food ingredients, such as sugars (glucose), citric, tartaric and phosphoric acids and their salts.

## C. Methods of trace element analysis in food

There are six different instrumental techniques for the determination of trace elements in food. The use of atomic spectroscopy in the analysis of foods and beverages are now common in various laboratories. Most of the methods are based on atomic absorption spectrometry. In some research laboratories, flame atomic emission spectrometry, emission spectrography and inductively coupled plasma optical emission spectrometry are also used.

D. Sample preparation

In most laboratories sample preparation is the most labor-intensive part of the analytical procedure.
There are two classical methods of sample preparation - destruction of organic matter:

1. Dry ashing
2. Wet oxidation (wet ashing)

Dry ashing is provided at 150-450°C according to the particular elements in muffle furnace by adding an ash acid (magnesium oxide nitrate or sulfuric acid).
Silica or platinum basins are preferred.
A detailed prescription is given in the methods.

Wet oxidation, wet decomposition of organic matter is provided with the various acids: sulfuric, nitric, perchloric and with aqua regia. Aqua regia is a mixture of nitric and hydrochloric acids. Aqua regia oxidizes many materials more efficiently than hydrochloric or nitric acid alone.
A prescription for the destruction of organic matter with different acids, their advantages and disadvantages (according to FAO Manuals of Food Quality Control) was distributed to the participants of the course and is not included in this report.

A number of reports have been issued on sample preparation procedures. Woo and Ryoo (ACC 1.) compared $HNO_3/H_2SO_4$ and aqua regia, and dry ashing methods for the determination of Ca, Cu, Fe, K, Mg, Mn, Na, and Zn in high-fat fish tissue. The authors show that for the determination of Ca, Zn and Mg the aqua regia and dry ashing methods gave superior results compared to the $HNO_3/H_2SO_4$. The three methods gave similar results for the other elements.

The aqua regia digestion method was recommended by the authors for safety, rapidity, simplicity, and many other considerations.

Wet digestion with $HNO_3/HClO_4$ is preferred by many laboratory workers and has been used in the analysis of coffee, soybean, fish, for Cd and Pb.

Some authors suggested that aqua regia cannot be used for fats of foods which are predominantly fat.

A mixture of $H_2SO_4/HNO_3$ was often used for the digestion of foods either with $V_2O_5$ as catalyst for samples of low lipid of carbohydrate content, or with 50% $H_2O_2$ for those of high carbohydrates content.

In conclusion, many authors agreed that the wet ashing procedure appears to be more popular in many laboratories.

For the determination of mercury in food wet digestion under reflux, cooled with water is required. Mercury is easily lost by volatilization during dry ashing. It is recommended therefore, to use a special digestion glass set apparatus according to AOAC Official Methods of Analysis (the Annex 8). Mercury is determined by the AAS (cold vapor technique).

During the course all the participants were made acquainted with all theoretical and practical aspects of trace elements - heavy metals determination. The practical work in the laboratory was consisting of the preparation of reagents, plotting of standard curves, dry ashing and wet ashing processing. During the course mercury, cadmium and lead in rice, fish sauce, and fish were determined. Small amount of cadmium was detected in canned sardines and a small amount of mercury in fish sauce.

The methods applied during the course for determination of Hg, Pb and Cd are given in Annexes 8 and 9.

The plotted standard curves for Hg, Pb and Cd obtained during the course are given in the same Annexes, together with the methods.

E. Recommended literature

1. Annual Reports on Analytical Atomic Spectroscopy Vol. 14
The Royal Society of Chemistry
Editor: M.S. Cresser - University of Aberdeen 1984.

2. Manuals of Food Quality Control
Additives, contaminants, techniques
FAO, Rome 1979.

3. AOAC - Official Methods of Analysis 1990.

4. David Glick - Methods of Biochemical Analysis Vol. V.
Contamination Trace Element Analysis and its Control.
Interscience Publisher Inc. New York 1957.

## III. TRAINING COURSE ON MYCOTOXIN ANALYSIS BY THIN LAYER CHROMATOGRAPHY (TLC) IN FOOD AND FEEDSTUFFS

The training course on mycotoxin analysis was held from 1 to 13 March and continued simultaneously with other topics until the end of the mission. The course consisted of lectures and laboratory training from 9.00 to 12.00 and 13.30 to 16.30 hours.

Lecture notes and methods for mycotoxin analysis were prepared in Poland. At the DTQC office documents were photocopied and distributed to the 12 participants of the course.

Mycotoxin contamination in human foods and animal feeds is now a global problem in view of health aspects and economical consideration. Mycotoxin are a large group of toxic chemical compounds produced by certain strains of a number of fungi species when they grow under favourable conditions on a wide variety of different substrates.

The list of mycotoxins formed by different fungi and association with foodstuffs and their typical effects on humans and animals is given in Appendix 8.

The first symptoms to manifest in humans are poor appetite, weakness, vomiting, headache and fever. According to epidemiologic studies there is evidence that consumption of aflatoxin-containing foods is associated with cancer of the human liver. In addition to aflatoxin more remotely related carcinogenic compounds are sterigmatocystins and aspertoxin which have been associated with Aspergillus verticolor, A. nidulans, A. parasiticus, A. amstelodami, A. regulosus and other.

Another toxigenic group within the genus Aspergillus, A. ochraceus is also widely distributed in food products; Ochratoxin A, produced by A. ochraceus is the major toxic compound. When TLC plates are viewed under UV light, ochratoxin A shows a potent greenish fluorescence. Ochratoxin induces acute nephrasis and liver damage.

A wide variety of mycotoxins was detected in the present of suitable strains. Golinski reviewed 155 toxic fungal secondary metabolites with their structure, physicochemical data, colour and fluoresces in UV, toxicity and their origin. This article was

provided to the DTQC staff and other participants and is given in Appendix 9.

The most potent of the group of mycotoxins are:
Aflatoxins (B1, B2, G1, G2, M1, M2), ochratoxins, zearalenone, sterigmatocytein, citrinin, penicillic acid, T-2 toxin and rubratoxin.

Aflatoxin are probably the most widely known mycotoxins and may occur at any stage of crop production and be distributed in foods and feeds during processing.

Penicillium mycotoxins. Species of the genus Penicillium produce more than 25 toxic metabolites. The penicillium mycotoxins include penicillic acid, citrinin, citreoviridin, and cyclopiazonic acid - yellowed rice toxins. Two toxic metabolites are produced by Penicillium rubrum - rubratoxins A and B. According to some authors, the rubratoxins may potentiate the activity of more dangerous compounds than the Aflatoxins.

Species of genus Fusarium produce very toxic two metabolites;
F-2 toxin - Zearalenone produced by Fusarium graminerum or F.roseum, F. moniliforme.
T-2 toxin - diacetoxyscirpenol isolated from Fusarium tricinctum.

A wide variety of commodities have been shown to yield aflatoxins in presence of suitable strains of A. flavus, A. parasiticus and others. The list includes: maize, rice, cotton seeds, tapioca, wheat, barley, beans, cassava, cocoa beans, hazel nuts, copra, millet, peas, groundnuts (peanuts), peanut butter, peanut milk, raisins, sezame, soybean and soybean milk, soy sauce.

In tropical countries climatic conditions and primitive cultural practices provide favourable conditions for contamination of foodstuffs by fungi. Groundnuts present a special problem since the fruit, after fertilization, penetrates into the soil, where it is contaminated by various fungi, and the damage occurs before the crop is dry.

In Appendix 8 a picture from the scanning electron microscope of Aspergillus and Penicillium species is presented.

A basic knowledge concerning mycotoxins was given to the participants of the course during the lectures.

During the training course a rapid method for detection of multiple forms of mycotoxins using GLC techniques was introduced to all participants. The details of the method are given in Annex 10.

A. The principle of the method

Mycotoxins are extracted from the sample by chloroform acidified with phosphoric acid. After filtration through a filter with anhydrous natrium sulphate the chloroform extract was evaporated using rotary evaporator to dryness. Residues were dissolved with 2 ml chloroform and spotted on the silica gel plate G-60. Impurities (fat) from the spots on TLC plate were removed by "clean up" with ethyl ether or chloroform. Separation of mycotoxins could be performed using developing solvents.
The Rf value of 11 separated mycotoxins is presented in Annex 11.

During the course the participants were instructed in sample preparation, methods of purification extract, spotting techniques on TLC plate, and evaluation of TLC chromatogram in UV light. The samples of standards for 11 mycotoxins were brought from the Food Technology Institute in Poland.

During the laboratory training course the participants analyzed samples of rice, maize, groundnuts and soy-sauce. In all analyzed samples mycotoxins were detected.

In some samples, especially in soy-sauce a lot of fluorescent spots were detected which were compared with aflatoxins, ochratoxin and sterigmatocystin.

Annex 10 provides the rapid method for multiple form of mycotoxin determination by TLC techniques. The method was distributed to all participants.
In Annex 12 procedures for sample preparation and techniques clean up on the silica gel column according to AOAC Official Methods of Analysis for aflatoxins determination in peanuts are given.

Recommendations were given to the participants for practical purposes:
1. For determination of mycotoxins the silica gel type 60 glass

plates or aluminum sheets should be used.

Before spotting the TLC plate must be activated at 110°C for a minimum of 1 hour.

The relative humidity in the room where has to be spotting procedure provided should not be higher than 60%.

2. For the "clean up" procedure by ethyl ether or chloroform TLC plates after spotting were recommended.

3. The developed TLC plate avoid to UV light for the minimum time needed for visualization.

**Recommended literature**

1. Ciegler A., Kadis S. and Ajl S.J. 1971. Microbial toxins. Vol.VI. Fungal toxins. Academic Press, Inc., New York.
2. Enomoto M. and Saito M. 1972. Carcinogens produced by fungi. Annu.Rev.Microbiol.,26:279-306.
3. Goldblatt L.A. (ed.) 1969. Aflatoxin: scientific background, control and implications. Academic Press, Inc., New York.
4. Graham H.D. (ed.) 1968. The safety of foods. AVI Publishing Co., Inc.Westport, Conn.
5. Betina V. 1989. Mycotoxins - chemical, biological and environmental aspects. Elsevier, Amsterdam.
6. Chelkowski J. (ed.) 1991. Cereal grain. Mycotoxins, fungi and quality in drying and storage. Elsevier, Amsterdam.

## IV. TRAINING COURSE ON THE ANALYSIS OF FOOD ADDITIVES

The training course on the analysis of food additives was held from 15 to 25 March 1993.

The course consisted of lectures and laboratory training, a 12 participants attended the course.

The details of the course's content and schedule are provided in Annex 13.

Lecture notes and laboratory schedule were prepared in Poland and were multiplied at DTQC and distributed to the participants.

### A. Colouring matter in food

From a modern technology point of view colour additives are essential because colour has always been associated with the quality of foods. Colour is added to food either because the food has no natural colour, or lost or altered its natural colour as a result of processing or storage.

The list of natural and synthetic colours recommended tc use as additives to foods are given in Annex 13.

During the training course more attention was paid to synthetic colourants and lakes, their solubility in different solvents, stability health aspects and relation to the regulatory international legislative status.

Among of large number of synthetic colours produced, only a few are permitted to be used as food colourants. In table (Annex 14) the chronological history of synthetic colours deleted and currently permitted in USA are given .

In Annex 15 synthetic colouring substances permitted for use in foods in Vietnam are given .

According to the consultant's knowledge the dye Nr. 16185 Amaranth should be deleted from the Vietnamese list of food additives.

Indeed, laboratory tests on rats showed that Amaranth caused a change in the parents genes which subsequently led to defects in the next generations. As a resultAmaranth was deleted in the USA in 1976 and in Poland in 1985.

The large quantities of colouring matters which are permit-

ted are used in the manufacture of carbonated non-alcoholic beverages, candy, dessert, powders, ice cream, bakery goods, and cereals. In some countries a lot of colouring matters are used in the manufacture of "pet food" for domestic animals.

During the course the participants were instructed and acquainted with TLC techniques, separation and identification of colouring matter according to Rf values and spectrometric peaks. Amaranth and Erythrosine were analysed .

The plotted curves obtained on the spectrophotometer using N/10 HCl solvent and water were compared with catalogue spectras.

The extraction method of the colouring matter from the food separation by TLC techniques and identification of colour according to Rf values and spectrometric peaks was distributed to the participants of the course and is given in Appendix 10.

B. Chemical preservatives

The participants of the course were made acquainted with food preservation by use of chemical preservatives.

The national legislations relating to the utilization of chemical preservatives vary from one country to the other. However, common preservatives are used in all countries. The list of preservatives permitted for use in food within the European Economic Community (EC) is presented in Appendix 11, table 1. Admissibility of the foremost preservatives in some countries are given in table 2 of Appendix 11.

The acceptable daily intake of preservatives is given in Appendix 12, table 1.

The action of some preservatives on microorganisms is presented in table 2, Appendix 12.

Recommended levels of preservatives to be added to food products are presented in Annex 16.

The data given in the Annexes are in general in conformity with the regulatory status of FAO/WHO's Codex Alimentarius Commission.

During the course the participants were made acquainted with preservation of food by acetic and lactic acids - products of

natural fermented food. The inhibitory effect of acetic and lactic acid on microorganisms was presented in detail during the lectures.

Copies of literature and lecture notes were distributed to the participants.

## GENERAL REMARKS AND RECOMMENDATIONS

i. The laboratory facilities at the DTQC provided by UNIDO (chemicals and equipments) admitted to carry out food control in the fields of pesticide residues, control of trace elements, mycotoxins and food additives.

ii. The laboratory staff possesses a basic knowledge for chemical and instrumental analysis of food control, and should increase their qualifications through laboratory practice in in well established laboratories locally and abroad.

iii. The staff members of the DTQC should participate in international symposiums and conferences related to food analysis and food control.

iv. A periodical professional international journal and books related to food analysis and food control should be acquired and a related library should be established.

v. National food control infrastructure needs to be strengthened by international organizations such as UNIDO, FAO, WHO on preventive action programmes of food decontamination and detoxification. Priority should be given to establish network for the control of mycotoxins, organochlorine pesticides and trace elements.

vi. Practical recommendations concerning chemical and instrumental methods of pesticides, trace elements, mycotoxins and food additives were given in each training course.

**Acknowledgements**

I am very grateful to all those who assisted during this visit and who showed much hospitality, both at the DTQC and the UNIDO Field Office.

ANNEX 1

April 1990

Project in the Socialist Republic of Viet Nam

## JOB DESCRIPTION

DP|VIE|87|009|11-51|J. 1.1.05

| | |
|---|---|
| Post title | Chemical and Instrumental Food Analysis Consultant |
| Duration | 4 m\|m |
| Date required | As soon as possible |
| Duty station | Hanoi, Viet Nam (with travel within the country) |
| Purpose of project | To upgrade the capabilities of the Department of Quality Control and Metrology (DTQC) of the Ministry of Domestic Trade, to enable it to develop and implement more effective quality control programmes through appropriate chemical, physical, microanalytical and microbiological testing of foods for quality and safety, as well as to strenghten its extension and advisory services to DTQC branch laboratories and the QC network of the Ministry of Domestic Trade. |
| Duties | The consultant, working under the general guidance of the National Project Director and the CTA and in close co-operation with the project administration and the Government authority concerned, will specifically be expected to carry out the following duties:<br>- Assist in the installation of new equipment and test its performance;<br>- Conduct training of DTQC staff in the use and operation of equipment for chemical analysis of food;<br>- Prepare a training manual on methods for the analysis of food composition (N, fat, sugar, etc.) and chemical contaminants in food;<br>- Conduct two in-country training workshops using above manual.<br>The consultant will also be expected to take part in the preparation of the terminal report, which sets out the findings of the mission and recommendations to the Government on further action which might be taken. ..../ |

Applications and communications regarding this Job Description should be sent to:

Project Personnel Recruitment Section, Industrial Operations Division
UNIDO, VIENNA INTERNATIONAL CENTRE, P.O. Box 300, Vienna, Austria

ıalifications

High-level senior chemist, with large experience in the analysis of food composition as well as chemical contaminants as heavy metals and aflatoxins, and food additives and preservatives. He|she should also be experienced in the use of AAS, UV-vis spectrophotometer, gas chromatograph, HPLC and TLC equipment, as well as in the analysis of fish sauce, soy sauce, soybeans, peanuts, cashew nuts, fruits and vegetables, spices, canned meat and fish.

nguage

English, Vietnamese

ckground information

The Department of Quality Control and Metrology (DTQC) was created in 1960 as a Department under the Ministry of Domestic Trade. Government Resolution No.28|HDBT dated 6 April 1983 made DTQC a legal body and gave it the responsibility to test and inspect, and to control the quality of foods in the domestic market, from selling, buying, storage and transport. Among the major food items under its responsibility there are fish sauce, soybean sauce and other fermented sauces. The others are milk, tea, bread, biscuits, confectionery, aquatic products, vegetable oils, solar salt, sugar, canned foods, peas, fruits (dry and fresh), vegetables and spices, cooked meats and alcoholic beverages.

DTQC performs the following functions in relation to its responsibility of ensuring the quality of foods in the market:

1. It takes samples, inspects and tests foods from various markets within the country and has the legal mandate to withdraw any product which it finds defective.

2. It exercises professional supervision over the quality control laboratories of trading companies, collective firms and trade services up to the district level, and provides advice, education and training to its personnel.

3. It acts as the official Government arbiter for quality problems that arise between buyers and sellers.

4. It provides the official Government certificate for the quality of food product samples.

5. More recently, with the Government's new open policy in trade, DTQC has been used by domestic trading companies to also inspect the quality of foods for export markets.

DTQC is headed by a Director and Vice Director. The food testing and quality control functions are implemented by the Agriculture and Food Products Section, which is headed by a Food Engineer and a Chief of Laboratories. The Section has 13 analysts to test and inspect the food quality in 18 provinces in the northern part of the country. Two branch laboratories in Da Nang and Ho Chi Minh City test and inspect food in the central and southern regions of the country, respectively. These branches are also under the Director of DTQC.

DTQC monitors the quality of food in the market through regular inspection and sampling, conducted weekly, monthly or annually, depending on the volume of the food sold and its likelihood of contamination. Sampling is also increased following reports of adulteration and complaints by consumers.

Fish sauce and soy sauce are among the most important of the commodities inspected. Other products analyzed frequently are soybeans, peanuts, groundnuts, fresh fruits and vegetables, confectionery, fruit juice, spices, vegetable oil, margarines and canned meat and fish.

DTQC analyses about 2,000 food samples in the laboratory yearly, which represents 30% of the total number of samples which it must evaluate according to its estimate. The low level of analysis is reportedly due to the slow time consuming methods it uses, and to the lack of chemicals.

The procedures used by DTQC for food testing are taken from the Vietnamese standards, formulated by the General Department for Standardization, Metrology and Quality Control (GDSMQC). Results of analyses are also evaluated against these standards. In the absence of official standards DTQC makes its own standards, which eventually become official standards of the Ministry of Domestic Trade, or it adopts internationl specifications as those from Hungary and Bulgaria.

The test methods used at DTQC are not up-to-date and/or do not cover quality indices presently considered important in the evaluation of food quality. Considerable upgrading in this area is necessary in order for DTQC to fulfill its mandate and responsibilities in the quality control of food products in Viet Nam.

DTQC was created by the Government in 1960 and was given a legal mandate to control the quality of foods in the domestic trade in 1983. Due to the Government's new open policy in trade, it was considered essential to strengthen DTQC, to enable it to ensure the distribution of only good quality products in the domestic market and to make collective firms and cooperatives more competitive. The establishment of DTQC is thus part of the Government's strategy to stimulate the development of the consumer market by ensuring quality, preventing unfair trade practices and protecting consumer health.

It is feasible to upgrade the quality of food products by instituting R&D and quality control procedures in food manufacturing plants. However, even after the institution of such procedures, it would still be necessary to verify product quality in the market, through actual testing and inspection. In as much as the latter activity is DTQC's responsibility the upgrading of its facilities would still be imperative.

The entire process of upgrading of laboratories' facilities will be implemented through the training of other laboratories' staff in Hanoi, according to established standards/testing procedures. DTQC trained staff will prepare training materials and provide on-the-job training for the staff of other institutions. The Government will upgrade the facilities of other laboratories in the network by its own resources.

Thus, no alternative project strategy is envisioned other than that of upgrading DTQC's capabilities, to accomplish the development objective of this project.

The equipment requested is that relevant to the test methods and quality control objectives for the types of food under the responsibility of DTQC. This is important to achieve the project objectives.

With the facilities which will be provided by the project, DTQC should also be able to continuously conduct the R&D necessary to understand and prevent the most important quality problems that cause product rejection, or the R&D needed to produce the quality specifications required by the market.

Additional duties of the international expert:

1. A training manual covering the following topics:

   1.1 Principles of Pesticide Analysis by Gas Chromatography (Lecture)

   1.2 Analysis of pesticides in foods (Hands-on)

   a. Sample preparation
   b. Gas chromatographic separation and analysis

   1.3 Principles of Heavy Metal Analysis by Atomic Absorption Spectrophotometry (Lecture)

   1.4 Analysis of Mercury, Lead, Arsenic and/or Tin (Hands-on)

   a. Sample preparation for soy sauce, fish sauce, and/or fruit juices
   b. AAS analysis

   1.5 Food Analysis Applications of Thin Layer Chromatograph (Lecture)

   a. Preparation of TLC plates for aflatoxin analysis
   b. Preparation of samples
   c. Analysis of food samples for aflatoxin

   1.6 Analysis of Soybeans or Corn for Aflatoxin (Hands-on)

   The manual will be used for 7 week hands-on training course on the actual analysis of pesticides, heavy metals and aflatoxin in Vietnamese foods and food crops.

2. Two 45-minute papers for a seminar on "Food Quality Control" on the following topics:

   2.1 The Food Control System and Methods For Detecting Counterfeit Food in Poland. (counterfeit means imitation and/or adulterated food)

   2.2 Sources and Control of Lead, Cadmium and others types of Heavy Metal Contamination of Foods.

Please see the following training course design for objectives, participants and schedules.

## Design and Schedule of the In-Country Training Program
## Project DP/VIE/87/009

The following covers the content and new schedule of the in-country training program. The contents of this program has been readjusted to fit the shorter duration of the mission of Experts. This mission has been reduced from 4 m/m to 2 m/m to save on cost.

The contents do not deviate from the original project design. However the Experts will participate only in the Training of Trainors Workshop and in a 3 to 4 days seminar in Hanoi on "Food Quality Control". The Training of the QC network will take place without the Experts.

### I. Objectives

1. To train six (6) trainors on the new methods of food testing introduced by the Project.

2. To strengthen manpower capability at DTQC to use the new test methods.

3. To train fifteen (15) food inspectors of the QC network on those aspects of the new testing technologies that will be carried out in the field.

### II. Activities

A. Training of Trainors Workshop – 7 weeks
B. Seminar on "Food Quality Control" – 3 to 4 days
C. Training of the QC Network – 4 weeks

### III. Participants

A. Training of Trainors Workshop

- Lecturers and Trainors

  - International expert in chemistry (1)
  - International expert in microbiology (1)
  - National expert (1)
  - CTA (1)

- Trainees

  6 – future trainors from DTQC: (2) in chemistry, (2) in microbiology and (2) in microanalytical and physical and sensory evaluation.

  20 – invited participants for the lecture portions of the three areas of training: (4) in chemistry, (8) in microbiology and (8) in microanalytical and physical and sensory evaluation.

**ANNEX 2**

**PLAN OF ACTIVITY**
**AT THE DEPARTMENT OF QUALITY CONTROL AND METROLOGY (DTQC)**
**OF THE MINISTRY OF DOMESTIC TRADE OF VIETNAM**
**From 1 FEBRUARY to 29 MARCH 1993**

**TRAINING COURSE ON THE ANALYSIS OF PESTICIDE RESIDUES**

**From 1 February to 18 February 1993**

( L = Lecture; P = Laboratory Practical )

1 February, Monday

Arriving to Hanoi and first visit at the DTQC

2 February, Tuesday

Meeting with:

- Prof.Dr. Phan Duc Thang - Director of DTQC
- Dr. Le Xuan Dich - Deputy Director of DTQC
- Pharm.Sp. Phan Quoc Dong - Chief of Quality Control Laboratory
- Ing. Ngo Dinh Co - Secretary of Project and Head of Quality Control and Food staff section

Briefing of the UNDP office Hanoi:

- Dr. M.J. Meixner - UNDO Country Director
- Ms Anja Latacz - Program officer

3 February, Thursday

Acquainted with laboratory staff and equipments.
Assisting to Gas Chromatography installation.

4 February, Thursday

Checking the list of chemicals and accessory products delivered by UNIDO

5 February, Friday

Preparation the list of reagents and accessories needed to pesticide analysis.
Analysis pesticide standards by Gas Chromatograph (P)

6 February, Saturday

Preparation materials for the lectures

8 February, Monday

9.00 Opening ceremony of the training course at DTQC
Prof.Dr Phan Duc Tang - Director of DTQC - Welcome
Technical Session

9.30 Dr Alicia O. Lustre - Director Food Development Center Manila - Philippines
- The importance of food control on pesticides, trace element and mycotoxins

10.00 Dr Edward Kaminski, Prof. of Food Chemistry and Analysis - Food Technology Institute - The University of Agriculture, Poznan, Poland
- Pesticide formulation, classes of pesticides
- Hazardous organochlorine insecticides
- Hazardous herbicides
- Hazardous fungicides

ANNEX 2

**TRAINING COURSE ON THE USE OF GAS CHROMATOGRAPHY FOR THE ANALYSIS OF ORGANOCHLORINE PESTICIDES IN VIETNAMESE FOOD**

Focus of training

a/ Analytical methods - GC and TLC

b/ Instrument

c/ Extraction of pesticide residues in food samples - rice, water, vegetables

Pesticides to be analysed - Lindane, DDT, DDE, TDE, Aldrin, Meptachlor, Endrin

9 February, Tuesday

- Gas Chromatography columns (l)
- Testing Gas Chromatography (P)

10 February, Wednesday

- Gas Chromatography Detectors ECD, FID, TCD (L)
- Analysis of Lindane (P)

11 February, Thursday

- Pesticide determination by Thin Layer Chromatography (L)
- Preparation of reagents for analysis of pesticide (P)

12 February, Friday

- Assisting in an installation and testing AAS with Dr Kordik (P)
- Preparation for lecture next week

13 February, Saturday

- Pesticide determination by Thin Layer Chromatography (L)
- Preparation of development reagents for Chromatography (P)

15 February, Monday

- Analysis of residues pesticide in Rice, Water and Soybean by GLC and TLC - Chromatography (P)
- Assisting in AAS testing (P)

16 February, Tuesday

- Pesticide determination sample preparation (P)
- Florisil column preparation clean up the sample (P)

17 February, Wednesday

- Pesticide analysis, training laboratory staff (P)
- Using Rotary evaporator for sample concentration (P)

18 February, Thursday

- Pesticide determination in rice grain, extract analysis by GLC and TLC (P)

19 February, Friday

- Pesticide determination in vegetable, sample preparation, extraction, clean up on the florisil column (P)

20 February, Saturday

- Pesticide analysis in vegetable, by GLC and TLC (P)

**ANNEX 2**

**TRAINING COURSE ON THE ANALYSIS OF HEAVY METAL CONTAMINANTS IN VIETNAMESE FOOD BY ATOMIC ABSORPTION SPECTROMETER (AAS)**
**19 February to 28 February 1993**

Focus of training:

a/ Analytical methods - AAS

b/ Instrument - AAS, Continuous vapor system

c/ Foods - Fish sauce, soy sauce, vinegar, mineral water, beer, wine, Fish canned foods

d/ heavy metals - Pb, Cd. As, Hg

22 February, Monday

- Nutritional and toxicological significance of trace elements in food chain (L)
- Introduction to the analysis trace elements by AAS (L)
- Acquainting laboratory staff with Atomic Absorption Spectrometer (P)

23 February, Tuesday

- Determination of Mercury (L)
- Destruction of organic matter (P)

24 February, Wednesday

- Determination of Lead (L)
- Destruction of organic matter (P)

25 February, Thursday

- Determination of Cadmium (L)
- Sample preparation (P)

26 February, Friday

- Microwave sample preparation system for AAS (L)

27 February, Saturday

- Preparation materials for lectures and practical work with mycotoxins.

## ANNEX 2

## TRAINING COURSE ON THE ANALYSIS OF MYCOTOXINS BY THIN LAYER CHROMATOGRAPHY

Focus of training:

a/ Analytical method - TLC

b/ Instrument - UV spectrophotometer

c/ Items to be analysed - mycotoxin
- non-permitted food colours as amaranth
- permitted food additives as sorbic and benzoic acids

d/ Foods to be tested
- Rice, corn, peanuts and soy sauce for aflatoxin
- Beverages and candies for amaranth
- Beverages for sorbic acid and benzoic acid. Results to be compared with permitted levels.

1 March, Monday
- Global significance of mycotoxins and their biological effects (L)
- Sample collection for mycotoxin analysis (P)
- Market visit to collect samples

2 March, Tuesday
- Mycotoxin producing fungi (L)
- Reagent preparation for extraction (P)

3 March, Wednesday
- Chemistry and physicochemical properties of secondary metabolites (mycotoxins) produced by fungi (L)
- Standards preparation of mycotoxins (P)

4 March, Thursday
- Mycotoxins analysis using Thin Layer Chromatography (L)
- Preparation of TLC plates (P)

5 March, Friday
- Sample preparation for mycotoxins determination in whole rice grain (P)

6 March, Saturday

- Documentation and data calculation from TLC analysis (P)

8 March, Monday

- Sample preparation for aflatoxin determination in corn grain (Maize) (P)

9 March, Tuesday

- Sample preparation for aflatoxin analysis in fermented products - soy sauce (P)

10 March, Wednesday

- Sample preparation for aflatoxin analysis by TLC in peanuts (P)

11 March, Thursday

- Possibilities of reduction of mycotoxins present in cereal grains and other foods (L)
- Documentation from data analysis (P)

12 March, Friday

- Toxins from fish and other marine organisms (L)
- Brine Shrimp (Artemia salina L.) Larvae as screening system for fungal toxins (L + P)

13 March, Saturday

- Detoxification of cassava products - Enzymatic hydrolysis of Linamarin (L)

15 March, Monday

- Colour additives, natural and synthetic (L)
- Sample collection (P)

16 March, Tuesday

- Analysis of water-soluble and fat-soluble colours by Thin Layer Chromatography (L)
- Preparation of TLC plates (P)

17 March, Wednesday

- Identification of the separated colours from food (P) Rf and Px values

18 March, Thursday
- Extraction of the colours from food (P)
- Separation by TLC (P)

19 March, Friday
- Separation of red colours - Amaranth (FDA Red No 2) determination (P)

20 March, Saturday
- Conformation of the identity of dyes using spectrophotometer (P)

22 March, Monday

Principle of food preservation (L)
- Preservation by use of high temperature
- Preservation by use of low temperature
- Preservation by drying
- Preservation by Food Additives

23 March, Tuesday
- Permitted chemical preservatives in food chain manufacture (L)
- Determination of benzoic and sorbic acids by TLC techniques (L)

## ANNEX 2

### SEMINAR ON FOOD QUALITY CONTROL AND FINAL ACTIVITIES

24 March, Wednesday

- Seminar on Food Quality Control. Lecture presentation on the following topics:
  - The food control system in Poland
  - Rapid methods for food analysis
  - Fermented soy products, manufacture, quality and health aspects

25 March, Thursday

- Participation at the seminar

26 March, Friday

- Preparation of final report and recommendations

27 March, Saturday

- Preparation of final report and recommendations

29 March, Monday

- Departure to Vienna

31 March, Wednesday

- Debriefing in UNIDO, Vienna

## ANNEX 3

## LIST OF SENIOR CONTERPART STAFF

1. Prof. Dr. PHAN DUC THANG, Director of DTQC - Ministry of Trade, National Project Director of Project VIE 97/009 - liquid foods in Vietnam

2. Dr. LE XUAN DICH, Vice director of DTQC - Ministry of Trade

3. Eng. NGO DINH CO, Head of Division of Food Control of DTQC Ministry of Trade

4. Pharm. PHAN QUOC DONG, Head of Microbiological Analysis Lab. of DTQC - Ministry of Trade

5. Dr. PHAM HUNG VIET, Head of Technical Chemistry Dept. Assistant to Dean of Chemical Faculty for Scientific Research and International Relation of University of Hanoi.
   Contrator of UNIDO contract No. 92/217/UK of coupling microcomputer with Gas Chromatograph Tremetics Model 9000.
   Head of group installing AAS equipment at DTQC

6. Ms. Sc. TRAN QUANG VINH, Head of Electronic Section Central Workshop, University of Hanoi

7. Chemist LE DANG DOANH, Gas Chromatographer for pesticide residues analysis

8. Chemist TRAN QUANG TOAN, Gas Chromatographer for enviromental and occupational analysis

ANNEX 4

# THE LIST OF PARTICIPANTS AND OBSERVERS IN THE INSTRUMENTAL AND CHEMICAL ANALYSIS COURSE

| | NAME | OFFICE |
|---|---|---|
| 1. | NGO MINH HUE | Analyst of DTQC |
| 2. | NGUYEN QUANG TUAN | as above |
| 3. | TRINH HONG THUY | as above |
| 4. | HOANG HOA VINH | as above |
| 5. | LY THI DUNG | as above |
| 6. | NGUYEN THI DUNG | as above |
| 7. | NGUYEN THI TINH | as above |
| 8. | NGUYEN THI NGHIA | as above |
| 9. | DANG THAI BINH | as above |
| 10. | PHAN NGOC THU | as above |
| 11. | NGUYEN HONG LOAN | as above |
| 12. | NGO PHUONG HOA | as above |
| 13. | NGUYEN VAN ANH | as above |
| 14. | THAI THI TUAT | as above |
| 15. | VU LAN PHUONG | as above |
| 16. | PHAM THI VAN | as above |
| 17. | DANG LUC | Center Branch of DTQC |
| 18. | NGUYEN LIEN HUONG | South Branch of DTQC |

Hanoi, Feb. 1993

Prof. Dr. PHAN DUC THANG
NPD of DP/VIE/87/009 project

# ANNEX 4A

List of People Met

1. Department of Quality Control of Goods and Metrology, Ministry of Trade.

Prof. Dr. PHAN DUC THANG, Director, DTQC

Dr, LE XUAN DICH, Deputy Dircter, DTQC

Mr. NGO DINH CO, Secrectary of Project and Head of Quality Control of Agriculture and Foodstuff Section.

Mr. PHAN QUOC DONG, Chief of Quality Control Laboratory.

Mrs. HUYNH LE TAM, Food Microbiolgist

Mr. NGUYEN QUANG TUAN, Chemist

Mr. BUI SI DOANH, Chemist - Pesticide Residue Laboratory.

Mr. TRAN QUANG VINH, Lecturer of Radio Physisc, Head of Electronic Section of The Vietnam - Holland Project VH - 16

Dr. Sc. PHAM HUNG VIET, Head of Technical Chemistry Department, Assistant to Dean of Chemical Faculty for Scientific Research and International Relations.

Mr. DO PHUC QUAN, Chemist - Faculty of Chemistry
Dept. Technical Chemistry
Hanoi Universty

2. UNIDO Field Office, Hanoi.

Dr. M. J. Meixner, Country Director.

Dr. Alicia O. Lustre, CTA of DP/VIE/87/009 Project
Director Food Development Center - Manila - Philippines

Miss. A. C. Latacz, Programe Officer.

ANNEX 5

MINISTRY OF TRADE

Department of Quality Control of goods and Metrology

DTQC

Is hereby awarded this

# CERTIFICATE OF ATTENDANCE

For having satisfactorily completed a training course on

## RAPID AND INSTRUMENTAL METHODS OF FOOD ANALYSIS AND SPECTROPHOTOMETRIC TECHNIQUES

February 6 to March 26, 1993

Given at DTQC - Hanoi - Vietnam

This day of March 1993

Prof. Dr. EDWARD KAMINSKI
*Food Analysis - UNIDO Expert*

Prof. Dr. PHAN DUC THANG
NPD,
DP/VIE/87/009 Project

ANNEX 6

# ANALYSIS OF PESTICIDE RESIDUE

## Source

Rapid method used in Plant protection Institute in Poznan

## Reagents

1. Acetone
2. Dicnlorometan
3. Acetonitril
4. Hexane
5. Sodium sulfate
6. Sodium chloride - saturated aqueous solution
7. Florosil PR grade

## Apparatus

1. Florosil column
2. Rotary evaporator Kuderna Danish concentrator
3. Separator funnels
4. High speed blender
5. Gas chromatograph with Electron Capture Detector (ECD)
6. Column
   HP - 17 (10 m x 0.53 mm x 2.0 um)
   Parameters of column operation 160 - 240 C
   5 C/min

ANNEX 6

# DETERMINATION OF ORGANOCHLORINE PESTICIDE IN RICE

1. 10 g sample of rice grind in high-speed blender and extract with acetone-water solvent (2:1) 1 h
   Filter through filter paper. Wash resideu with 2 x 25 ml solvent.

2. Transfer aceton extract to 1 L separator containing 400 ml $H_2O$ and 50 ml saturated NaCl solution and 100 ml dichloromethan. Shake vigorously 2 min. Reextract aqueos solution using 100 ml solvent. Combined extract pass through anhydrous $Na_2SO_4$ and evaporate completly using rotary evaporator.

3. Disolve residue with 50 ml acetonitril and transfer to 500 ml separator. add 50 ml hexan and extract (solvent by solvent not very vigorously to prevent formation of emulsion). After layer separated drain acetonitrile into second separator.
   Hexane layer reextract with 50 ml acetonitril.

4. Combined acetonitril solvents and add 250 ml $H_2O$ + 50 ml saturated NaCl and extract twice with (2 x 75 ml) hexane. Hexane layer pass through anhydrous $Na_2SO_4$ and evaporat using rotary evaporator.

5. Disolve dry residue in 10 ml hexane

6. Florisil clean-up
   Add 10 g activated Florisil to 300 x 20 mm chromatogic tube and add 1 g anhydrous $Na_2SO_4$ to the top and mark tube 1 cm above $Na_2SO_4$ . Add 20 - 25 ml solvent (hexane) and wash column. Solvent level must not go below mark.
   Transfer by Paster pipet 2 to 5 ml sample to column.
   Add 150 ml solvents hexane dichlomethan (8 + 2) and elute (60 - 90 drops/min)

7. Transfer cluant to rotary evaporator and evaporate to dryness
   Disolve residue with 2 ml hexane.

8. Concetrate use for GLC and TLC

## ANNEX 7

## DETERMINATION OF ORGANOCHLORINE PESTICIDES IN GREEN BEANS

1. 50 g sample of green beans + 100 ml aceton mixed into high speed blendor for 3 min, supernatant filtrate. Vegetable pulp extract again with 100 ml aceton in blendor. Decant aceton supernatants through filter and combine with first extract. Rinse blendor jar and filter with the pulp with 3 x 20 ml aceton.

2. Transfer aceton extract to 1 liter separator containing 400 ml $H_2O$ and 5 saturated NaCl solution and 100 ml dichloromethan. Shake vigorously 2 min. Re-extract aqueous residue twice using 50 ml dichloromethan.
   Combined extracts pass through anhydrous $Na_2SO_4$ . Dichloromethan extracts completly evaporate using rotary evaporation.
   Disolve dry residue in 10 ml hexane.

3. 10 g activated Florosil put to the column, on the top put 1 g anhydrous $Na_2SO_4$ .
   Prewet column with 40-50 ml hexane.
   Transfer 5-10 ml hexane extract to the column and eluate column using solvent 150 ml hexan + dichloromethan (8 + 2) at about 60-90 drops/min

4. Transfer eluant to rotary evaporator and evaporate to dryness. Disolve residue in 2 ml hexane

5. Hexane extract use for GLCH and for TLC

ANNEX 8

# DETERMINATION OF TOTAL MERCURY IN FOOD

## Source

Trace elements determination in foods - K. Ludwicki - Motody oznaczanica substancji obcych wzywnosci. Wyd. metodyczne PZH. 1990 Warsawa

## Principle

The sample is digested with mixture nitric acid andsulphuric acid under reflex to prevent loss of mercury.
The mercury is reduced to the element with hydrohylamine and the vapor swept into the cell of an atomic spectrophotometer. The reading is compered with standards treated similarity.

## Apparatus

Atomic absorption Spectrophotometer

## Reagents

- Nitric acid, Sp. gr 1.42
- Sulphuric acid Sp. gr 1.84
- Nitric acid 1% soln.
- Reducing soln. - Mix 25 ml $H_2SO_4$ with 150 ml $H_2O$ dissolve 25 g $SnCl_2$, 7.5 g hydroxylamine sulfate and 2.5 g NaCl. Dissolves all ingredients and dil. to 250 ml (solvent is lasting 2 month)
- Vadium pentoxide $V_2O_5$
- Mercury std soln.
  Solv. A- Dissolve 67 mg $HgCl_2$ in 100 ml 5% $H_2SO_4$. Stock solution conc. 500 ug/ml
  Solv. B- Dilute 1ml solv. A in 500 ml $H_2O$ (conc. 0.5 µg/ml)
  Solv. C- Dilute 10 ml solv. B in 100 ml $H_2O$ . Prepare fresh daily Standard concent. 0.05 µg/ml

- Diluting soln. - To 1 lit vol. flask contg 300-500 ml $H_2O$ add 58 ml $HNO_3$ and 67 ml $H_2SO_4$. Dil. to vol. with $H_2O$

## Determination

Weigh 5-10 g sample into digestion flask. Add 4-5 glass beads, 20 ml $H_2O$ in case of dry products and 10 ml $H_2O$ to wet products, 1 ml $H_2SO_4$ conc. and 20 ml $HNO_3$ conc. and swirl until ahomogenous tarry fluid is obtained. Add 20-40 mg $V_2O_5$ and let stand for 2h or overnight.

Conect condenser (with $H_2O$ circulating thru it) and apply gentle heat ca 20 min. Remove heat and let stand 15 min. Add 9 ml conc. $H_2SO_4$ thru condenser. Turn of $H_2O$ circulating thru condenser and boil vigorously 30 min until start boiling. Cool carefully add 10 ml 1% sol. $HNO_3$ thru condenser. Wash condenser with $H_2O$. Filtr digested samole thru the filter. wash filter with 1% $HNO_3$ dilute sample to 100 ml with $H_2O$.

## Preparation reagent blank

Carry out blank determination using a similar reagents through all

ANNEX 8

stages of the procedure.

Preparation standard curve

| Standard sol. "C" ml | µug Hg |
|---|---|
| 0 | 0 |
| 0.25 | 0.0125 |
| 0.50 | 0.0250 |
| 1.00 | 0.050 |
| 2.00 | 0.100 |
| 5.00 | 0.250 |
| 10.00 | 0.500 |

Add 2.5 ml reducing soln. and dillute with $H_2O$ to 50 ml.

Sample preparation

Use 25 ml digested sample add 2.5 ml reducing soln. dillute with $H_2O$ to 50 ml,

Prepare AAS to optimum conditions. Plot standard curve and mesure the samples and blank solution.

Notice

Acc. observation done during the course - The use of diluting solution to samples and standards instead water better resolts could be obtained on AAS mesurtements.

# ANNEX 8

## MERCURY DETERMINATION IN FISH
(acc. AOAC-Official method of Analysis 1990)

### Reagents

a- Reducing solution
Mix 50 ml $H_2SO_4$ with ca 300 ml $H_2O$. Cool to room temperature and dissolve 15 g NaCl, 15 g hydroxylamin sulfate, and 25 g $SnCl_2$ in solution. Dil. to 500 ml.

b- Diluting soln.
To 1 L vol. flask contg 300-500 ml $H_2O$, add 58 ml $HNO_3$ and 67 ml $H_2SO_4$. Dil. to vol with $H_2O$.

c- Mercury std solns
(1) Stock soln. - 1000 µg/ml. Dissolve 0.1354 $HgCl_2$ in 100.0 ml $H_2O$
(2) Working solns. - 1 µg/ml. Dil. 1 ml stock soln to 1 L with 1N $H_2SO_4$. Prep. fresh daily

### Sample preparation and digestion

Weigh 5.0 g (wet wt) thoroly mixed fish sample into digestion flask. Rinse neck of flask with <5 ml $H_2O$, if necessary. Add ca 20 boiling stones, 10-20 mg $V_2O_5$, and 20 ml $H_2SO_4$ - $HNO_3$ (1+1). Quikly connect flask to condenser, and swirl to mix. Circulate cold $H_2O$ thru condenser during digetion. Apply sufficient heat to produce low initial boil (ca 6 min) and finish digetion with strong boil (ca 10 min). Swirl flask intermittently during digetion. No solid material should be apparent except for globules of fat after ca 4 min.

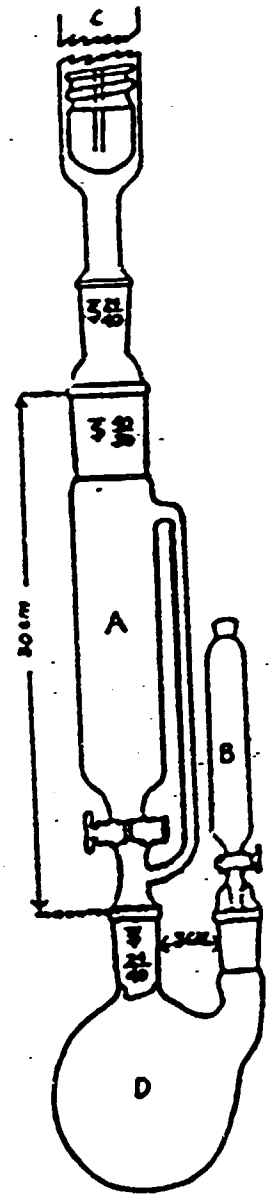
Remove flask from heat and wash condenser with 15 ml $H_2O$. Add 2 drops 30% $H_2O_2$ thru condenser and wash into flask with 15 ml $H_2O$. Cool digested fish soln to room temp. by placing flask, still connected to condenser, in beaker of $H_2O$. Disconnect flask, rinse ground joint with $H_2O$, and quant. transfer digest to 100 ml vol. flask. Ignore solidified fat; it does not interfere. Carefully rinse digestion flask with several portions $H_2O$ and dil. to vol. with rinse $H_2O$.

Pipet 25 ml soln into original digetion flask and add ca 75 ml dilg soln.

Curry out blank determination using a similar reagents through all stages of the procedure.

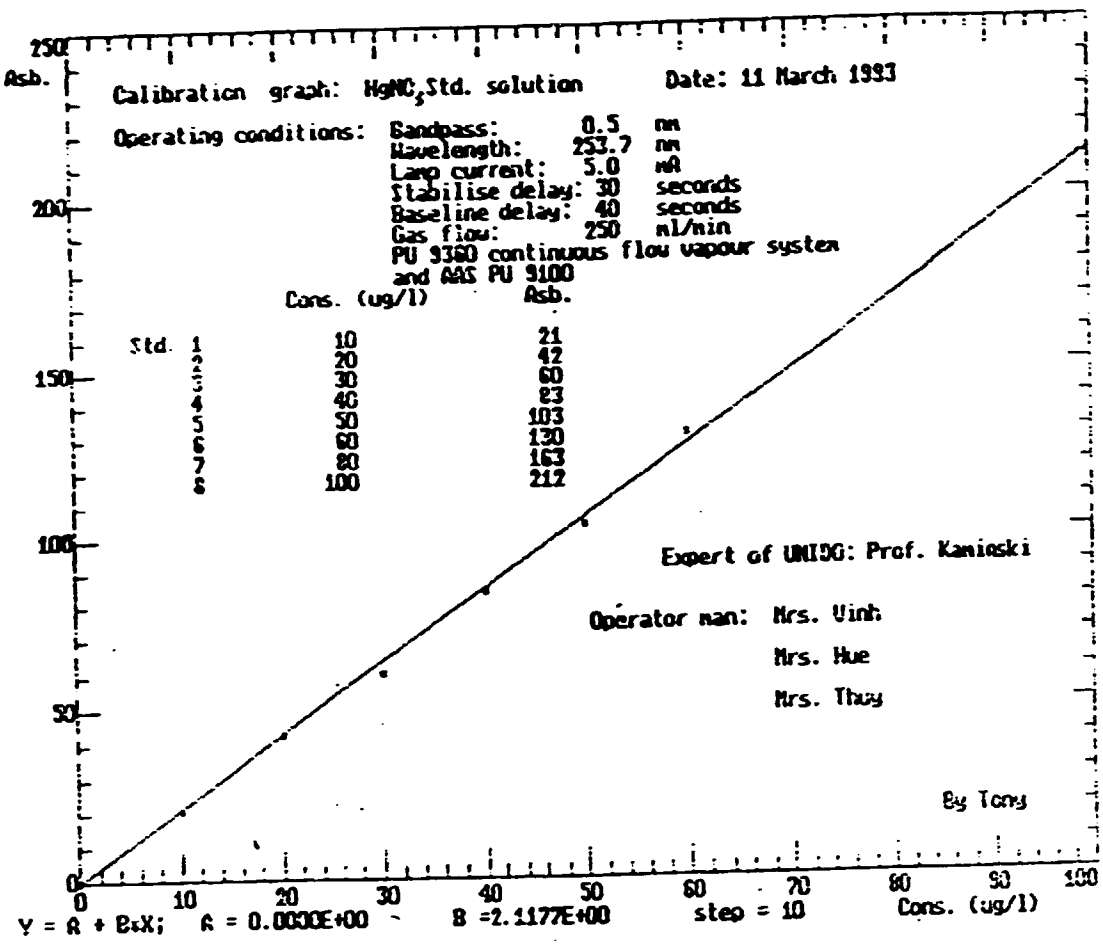
Prepare reagent blank and standard curve by adding 0, 0.2, 0.4, 0.6, 0.8 and 1.0 Mg Hg to series of digestion flasks. To each flask add 100 ml diluting soln. Finaly add reducing soln to standards and samples and aerate in AAS

ANNEX 8




Special Digestion Apparatus for mercury residues

# ANNEX 8


Asb.
Calibration graph: HgNC$_5$Std. solution
Date: 11 March 1993
Operating conditions:
Bandpass: 0.5 nm
Wavelength: 253.7 nm
Lamp current: 5.0 mA
Stabilise delay: 30 seconds
Baseline delay: 40 seconds
Gas flow: 250 ml/min
PU 9360 continuous flow vapour system
and AAS PU 9100
Cons. (ug/l)
Asb.
Std. 1 10 21
2 20 42
3 30 60
4 40 83
5 50 103
6 80 130
7 80 163
8 100 212
Expert of UNIDO: Prof. Kamiaski
Operator nam: Mrs. Vinh
Mrs. Hue
Mrs. Thuy
By Tony
0
50
100
150
200
250
0 10 20 30 40 50 60 70 80 90 100
Cons. (ug/l)
Y = A + BxX; A = 0.0000E+00 B =2.1177E+00 step = 10

# ANNEX 9

Source: Manuals of Food Quality Control, FAO, Rome, 1979

## LEAD & CADMIUM ATOMIC ABSORPTION SPECTROSCOPY METHOD

### PRINCIPLE

Lead and cadmium are extracted from a solution of the ash of the sample 0.5 M with respect to HCl by diethylammonium diethylcarbodithioate in methyl isobutyl ketone. Standards are treated in the same way and both sample and standard extracts are sprayed in the flame of an atomic absorption spectrophotometer.

### APPARATUS

Before use, all items of glassware and silica dishes should be immersed in 5 per cent v/v hydrochloric acid (reagent grade) for several hours, and then rinsed with double-distilled water.

1. Lipped silica dishes volume approx. 30 ml.

2. Graduated 5-ml and 1-ml pipettes.

3. 25-ml volumetric flasks with plastic stoppers.

4. Atomic absorption spectrophotometer.

The operating conditions for lead and cadmium, using an Atomspek H1170, (Hilger & Watts) are shown below:

| | Lead | Cadmium |
|---|---|---|
| Lamp current (mA) | 6 | 5 |
| Wavelength (nm) | 217.0 | 228.9 |
| Slit width (μm) | 100 | 45 |
| Burner height (mm) | 9 | 9 |
| Acetylene (litre $_1$ min$^{-1}$ at 5 psig) | 0.75 | 0.75 |
| Air (litres min$^{-1}$ at 30 psig) | 2 | 2 |
| Scale expansion | 1.0 | 0 |

5. Recorder (e.g. Servoscribe Flat-bed).

### REAGENTS

1. Water: Double-distilled, using silica-sheathed elements.

2. Nitric acid (S.G. 1.42): Low-in-lead quality.

3. Hydrochloric acid (S.G. 1.17): Low-in-lead quality.

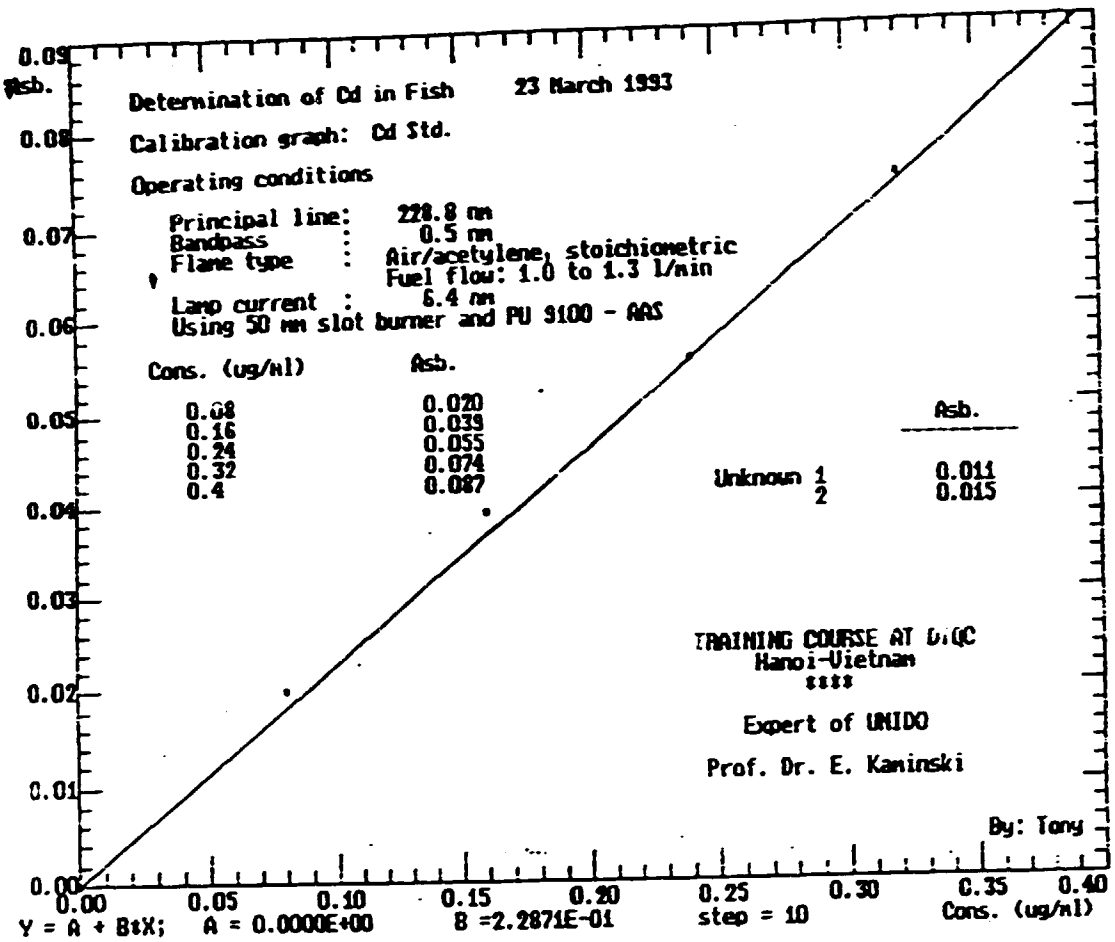
4. Diethylammonium diethylcarbodithioate (DDCD): A 1% w/v solution in methyl isobutyl ketone. The solution may be kept for several weeks without deterioration.

5. Methyl isobutyl ketone (MIBK) solvent: A saturated solution of water in MIBK.

6. Ascorbic acid: A freshly prepared, 10% w/v aqueous solution.

7. Standard solution of lead and cadmium: A 2% w/v solution of hydrochloric acid containing 10 mg/kg of lead and 2 mg/kg of cadmium.

# ANNEX 8


Asb.
Determination of Cd in Fish 23 March 1993
Calibration graph: Cd Std.
Operating conditions
Principal line: 228.8 nm
Bandpass : 0.5 nm
Flame type : Air/acetylene, stoichiometric
Fuel flow: 1.0 to 1.3 l/min
Lamp current : 6.4 mA
Using 50 mm slot burner and PU 9100 - AAS
Cons. (ug/ml) Asb.
0.08 0.020
0.16 0.039
0.24 0.055
0.32 0.074
0.4 0.087
Asb.
Unknown 1 0.011
2 0.015
TRAINING COURSE AT DIQC
Hanoi-Vietnam
Expert of UNIDO
Prof. Dr. E. Kaminski
By: Tony
0.00 0.05 0.10 0.15 0.20 0.25 0.30 0.35 0.40
Cons. (ug/ml)
Y = A + BtX; A = 0.0000E+00 B =2.2871E-01 step = 10

# ANNEX 9

METHOD

The Dry Ash (see Note 1) 10 g of homogenised sample (weighed to nearest 0.01g) at 450-500°C in a silica dish until the ash is grey or white. Moisten with diluted nitric acid/water (1 + 9) and briefly re-ash if necessary. Treat the ash with 5 ml of water followed by 5 ml of hydrochloric acid and evaporate to dryness on a steambath. Add 1.0 ml of hydrochloric acid and 3-5 ml of water, stirring with a glass rod, filter through a small No. 4 paper into a 25-ml volumetric flask and make up to the mark with dish and rod rinsings. Prepare aqueous standards in 25-ml calibrated flasks using 0, 0.10, 0.20, 0.50 and 1.00 ml of the standard lead/cadmium solution. To each flask add 1.0 ml of hydrochloric acid and make up to the mark with water (see Note 2).

To the blank, standards and samples add 0.5 ml of ascorbic acid solution and invert to mix the contents. Then, without delay, add 1.50 ml of DDCD solution, stopper and shake the flasks for 30 seconds. When the phases have separated, tap the flasks on the bench to remove solvent globules caught on the sides of the flasks and aspirate the organic layers into the spectrophotometer first for cadmium and then for lead. Spray MIBK solvent before and after each sample or standard in order to establish the baseline. Construct calibration graphs of the responses to the aqueous standards and hence determine the cadmium and lead contents in each sample.

Note 1. Char milk powders, cereals and dehydrated foods over a Bunsen burner before ashing. A dish larger than the 30-ml size is preferable for these materials.

Note 2. The samples and standards may be left at this stage for several days, but once the extraction process has been started, the analysis should be completed as soon as possible.

Note 3. See the determination of lead by dithizone for precautions to be taken in ashing.

DISCUSSION

Extraction of lead and cadmium from 0.5 N HCl by DDCD in MIBK is a convenient alternative to the procedure given under the dithizone method. If an atomic absorption spectrophotometer is not available, the lead and cadmium may be extracted with DDCD/MIBK, using a somewhat larger volume and repeating the extraction with fresh DDCD to ensure complete extraction. The organic phase must then be evaporated without loss by spattering and digested in the minimum amount of acid and the determination completed with dithizone. Calcium, magnesium and phosphate are unlikely to cause difficulties of precipitation in 0.5 N HCl.

Of the elements likely to be present in food digests, only iron, copper and zinc could interfere in the determination using AAS. These elements are usually masked by forming their cyanide complexes at pH 8.5. In the AAS method given it has been found necessary to eliminate completely the interference from iron only by reducing with ascorbic acid any Fe (III) to Fe (II) prior to the extraction step. Three series of calibrations for 0.1 µg of cadmium and 0.5 µg of lead were carried out by Snodin (1973).

a) standard calibrations

b) calibrations in the presence of 50 µg each of iron (III), copper and zinc

c) calibrations in the presence of 100 µg each of iron (III), copper and zinc.

Lead is subject to slightly more interference than cadmium though the effect causes no more than a 15 per cent decrease in response even in calibration (c). Moreover, since such excessive amounts of iron, copper and zinc as used in (c) are unlikely to be present in foods, any interference by these metals should be within experimental error.

Experiments have indicated that the lead-DDCD complex is stable for several hours,

ANNEX 9

whilst that of cadmium is subject to slight decomposition (judged by a decrease of 10 to 20 per cent in the instrument response). For this reason cadmium is best determined first and lead determination completed within three hours of extraction as recommended by Brook. Presley and Kaplan (1967). Nix and Goodwin (1970) showed that the sodium diethylcarbodithioate complexes of copper, iron, cobalt, nickel, chromium, lead, zinc, but not of manganese were stable for at least 400 minutes.

Note - In the paper referred to earlier by Roschnik, the use of xylene instead of MIBK is recommended and it is stated that some products such as juices and beverages need not be digested. The present author experienced some difficulty with the flame conditions but the method worked well using dinitrogen oxide/acetylene.

Useful papers include the following:

- AMC Report Analyst 79 1954 397-402 and 84 1959 127-134.
- Collaborative study on lead in evaporated milk by atomic absorption, spectroscopy and anodic stripping voltammetry, Fiorino J.A. et al JAOAC 56 (5) 1973 1246-1251.
- Lead in foods by AAS, Roschnik, R.K., Analyst 98 1973 596-604.
- Proposed OIV method for lead in wine by AAS Amati, Minguzzi, A. & Rastelli, R. (1976).
- Release of lead and cadmium from kitchenware, Beckmann, I. & Sark, M. (1974).
- Anodic stripping voltammetry Zink E.W. & Askew, L.R. (1975) - Food Product Development December 1975 49-59.
- Extraction of iodides of lead, cadmium and copper into MIBK , Tsutzumi, C. Koizumi, H. Yoshikawa, S. Japan Analyst 25 (3) 150-154, 1976.

# RAPID METHOD FOR THE DETECTION OF MULTIPLE MYCOTOXINS

## Source

Die Nahrung 29 (1985) 3,229-240.

This method could be for the simultaneous of aflatoxins (B1, B2, G1, G2), ochratoxin A, zearalenone, sterigmatocystin, citrinin, penicillic acid, T-2 toxin and rubratoxin B, determination.

## Reagents

Reagents for extraction:

1. Chloroform
2. Phosphoric acid 0.2 M

Development system:

1. Toluene + ethyl acetate + 90% formic acid (6 + 3 + 1)
2. Chloroform + propanol-2 + ethyl acetate (95 + 5 + 5)
3. Aceton + n-hexane (2 +3)
4. Benzene + methanol + acetic acid (90 + 16 + 8)

Chromatogenic reagents

1. Ammonium concentrate
2. Aluminum chloride ($AlCl_3$)-20% solution(w/v) in ethanol
3. $H_2SO_4$ - 20% solution (v/v) in ethanol
   Sprayed plates could be heated 15 min at 110°C
4. Alcoholic sodium bicarbonate soln. - Disolve 6 g $NaHCO_3$ in 100 ml $H_2O$ and add 20 ml ethanol

## Equipments

1. Food blender or shaker
2. Funnels
3. Elenmeyer flasks
4. Filter paper Whatman Nr 1 and Nr 4
5. Separatory funnels
6. Rotary evaporator (Kuderna-Danish concentrator)
7. Chromatographic tank
8. Pipets spotting for 1 µl and 5 µl
9. Ultraviolet light source 365 nm
10. TLC plates Silica gel 60

## Preparation of the plate

Both home-made and commercially-made (precoated) TLC plates should be activated before use. Put the plate in an oven at 110°C for a minimum of one hour. Plates can be activated and stored in air-tight cabinet until ready to use,Sharper separations are usually obtained when using activated plates.

## Preparation and Extraction of sample

Weigh 25 g prepd sample into 500 ml erlenmeyer flask. Add 125 ml chloroform and 12,5 ml 0.2M phosphoric acid. Shake 30 min or leave sample in solvent over night. Filter through Whatman Nr 4 filter covered with ca 2g anhydrous sodium sulphate. Filtrate evaporate using

ANNEX 10

rotary concentrator to drynees. Disolve residue in 2 ml chloroform. Concentrate extract to 1 ml and spot 5, 10 and 15 µl on TLC plate.
On other plate spot 5 µl and 10 µl of mycotoxins standards

Clean-up

When extracts contain a fat develop the plate (clean-up) in chloroform or in anhydrous ethyl ether in an unequilibrated tank and then dry the plate. Redevelop the plate in the same direction in toluen-ethyl acetate-formic acid (solvent 1) or other solvents.
View the plate under UV light (365 nm) and the prsence or absence of spot originated from the sample extracts.
Examine pattern from sample for fluorescent spots having Rf values close to those of standards and with similar appearance. Compare fluorescence intensities of spots from sample with those of standards spots.
For better visualization the spots developed chromatoplates should be exposed with chromogenic reagents increases the flurescence intensity.

ANNEX 11

Table 1
The Rf values of 11 mycotoxins for the different developing solvents (aluminium sheets MERCK were used as TLC plates)

| Toxin | The Rf values of 11 mycotoxins in different solvents | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 1[1] | 2 | 3 | 4 | 5 | 6 | 7[2] | 8 | 9 | 10[2] | 11 | 12[3] | 13 |
| Sterigmatocystin | 0.65 | 0.84 | 0.85 | 0.64 | 0.79 | 0.66 | 0.48 | 0.41 | 0.74 | 0.68 | 1.00 | 1.00 | 0.66 |
| Zearalenone (F2) | 0.20 | 0.56 | 0.77 | 0.63 | 0.45 | 0.36 | 0.36 | 0.38 | 0.52 | 0.57 | 0.89 | 0.90 | 0.60 |
| Ochratoxin A (OA) | 0.00 | 0.01 | 0.02 | 0.02 | 0.06 | 0.08 | 0.33 | 0.04 | 0.00 | 0.56 | 0.47 | 0.79 | 0.46 |
| Penicillic acid | 0.06 | 0.19 | 0.39 | 0.25 | 0.21 | 0.14 | 0.24 | 0.25 | 0.20 | 0.48 | 0.75 | 0.81 | 0.33 |
| T-2 toxin | 0.16 | 0.32 | 0.53 | 0.22 | 0.33 | 0.22 | 0.33 | 0.26 | 0.21 | 0.58 | 1.00 | 1.00 | 0.30 |
| Citrinin | 0.00 | 0.11[3] | 0.07 | 0.02 | 0.12[3] | 0.00 | 0.13[3] | 0.03 | 0.09 | 0.28[3] | 0.17[3] | 0.27[3] | 0.41[3] |
| Citrinin[4] | 0.55 | 0.83 | 0.80 | 0.54 | 0.83 | 0.64 | 0.56 | 0.59 | 0.83 | 0.68 | 1.00 | 1.00 | 0.59 |
| Aflatoxin $B_1$ | 0.16 | 0.39 | 0.53 | 0.05 | 0.39 | 0.20 | 0.25 | 0.17 | 0.28 | 0.50 | 1.00 | 1.00 | 0.19 |
| Aflatoxin $B_2$ | 0.13 | 0.31 | 0.48 | 0.05 | 0.31 | 0.16 | 0.22 | 0.16 | 0.23 | 0.46 | 1.00 | 1.00 | 0.16 |
| Aflatoxin $G_1$ | 0.10 | 0.27 | 0.43 | 0.04 | 0.28 | 0.15 | 0.19 | 0.13 | 0.20 | 0.41 | 1.00 | 1.00 | 0.14 |
| Aflatoxin $G_2$ | 0.07 | 0.23 | 0.39 | 0.03 | 0.25 | 0.13 | 0.16 | 0.12 | 0.17 | 0.39 | 1.00 | 1.00 | 0.09 |
| Rubratoxin B | 0.31 | 0.35 | 0.30 | 0.38 | 0.38 | 0.33 | | | 0.00 | 0.00 | | | 0.34 |

[1] Number of solvent see methods C 1—13
[2] Two fronts of developing solvents were observed on TLC plates, thin bands of F-2 in solvent No 7, OA, F-2 and T-2 in No 10 and OA in No 12 were observed close to the second one front
[3] Tailing
[4] Aluminium sheets with $H_2SO_4$ (see Methods B-5).

Table 2
Colour of fluorescence of 11 mycotoxins before and after treatment with different spraying reagents

| Toxin | Colour in UV-360 nm (without spraying) | Colour of fluorescence in UV-360 nm light | | | | |
|---|---|---|---|---|---|---|
| | | $AlCl_3$ (D-1)[4] | $H_2SO_4$ (D-2) | $Ce(SO_4)_2$ (D-3) | p-anisaldehyde (D-4) | DNP (D-5) |
| Sterigmatocystin | red | orange | brick-red<br>red-brown[1,3] | beige<br>brown[1] | red<br>brown[1] | red<br>dark-brown[1] |
| Zearalenone | gray-blue | dark-blue | gray-blue<br>brown[1,3] | light-beige[1] | brown[1] | light gray<br>beige[1] |
| Ochratoxin A | greenish-blue<br>dark-blue[2] | dark-blue | greenish-blue<br>dark-blue[2] | greenish-blue<br>dissappeared | greenish-blue | greenish-blue |
| Penicillic acid | dark-blue[2] | dark-blue[2] | pink-violet[2] | pink-violet[1] | brown[1] | pink-violet[1] |
| T-2 toxin | | | light-greenish-blue[1,3]<br>yellow-beige | beige[1,3] | greenish-blue[1,3] | beige[1,3] |
| Citrinin | yellow<br>disappeared[2] | yellow<br>disappeared[2] | yellow<br>greenish-yellow[1]<br>partially dis-appeared[2] | | yellow-beige[1] | yellow |
| Aflatoxin $B_1$ | dark-blue | dark-blue | yellow-beige<br>dark-blue[2] | beige<br>disappeared[1] | light-beige | yellow-beige |
| Aflatoxin $B_2$ | dark-blue | dark-blue | yellow-beige<br>dark-blue[2] | beige<br>disappeared[1] | light-beige | yellow-beige |
| Aflatoxin $G_1$ | greenish-blue | greenish-blue | beige<br>greenish-blue[2] | light-beige<br>disappeared[1] | light-beige | gray |
| Aflatoxin $G_2$ | greenish-blue | greenish-blue | beige<br>greenish-blue[2] | light-beige<br>disappeared[1] | light-beige | gray |
| Rubratoxin B | blue or<br>yellow | blue | pink-violet[1,3]<br>pink-beige | light-beige[1] | brown[1] | light-beige[1] |

[1] after 10—20 min heating at 110 °C; [2] after ammoniation; [3] spots visible in day light; [4] see Methods D

# ANNEX 12

## METHOD FOR ANALYSIS OF AFLATOXINS IN PEANUTS AND PEANUT PRODUCTS

### Source

AOAC Official Methods of Analytica (1990)

### Preparation of sample

Grind raw and roasted peanuts to reduce particles by milling.

### Extraction

Weigh 50 g pred. sample into 500 ml glass erlenmeyer. Add 25 ml $H_2O$, 25 g diatomaceous earth (celite 5u5) and 250 ml $CHCl_3$ , and secure stoper with masking tope. Shake 30 min on wrist action shaker and filter through fluted paper. Collect first 50 ml portion $CHCl_3$ , filtrate and clean up through the column.

### COLUMN PREPARATION FOR CLEAN-UP

Extract from peanut meal and peanut products need clean-up using silicagel column.

Source AOAC method 1990

### Column chromatography

Place ball of glass wood in bottom of 22 x 300 mm chromatogic tube and add 5 g anhydrous $Na_2SO_4$ to give base for silica gel. Add $CHCl_3$ until tibe is ca 1/2 full; then add 10 g silica gel. Wash sides of tube with ca 20 ml $CHCl_3$ and stir to disperse silica gel, when rate is of setting slow, drain some $CHCl_3$ to aid setting , leaving 5-7 cm above silica gel. Slowly add 15 g anhydrous $Na_2SO_4$. Drain $CHCl_3$ to top of $Na_2SO_4$ .

Add 50 ml sample extract to column elute at max. flow rate with 150 ml hexane followed by 150 ml anhydrous ether, and discard. Elute aflatoxins with 150 ml methyl-alcohol-chloroform (3 + 9 + 7).

Collect this fraction and concentrate using rotary evaporator nearly to dryness. Use this fractin for TLC analysis

ANNEX 13

# RECOMMENDED COLOR ADDITIVES FOR FOOD USE

## NATURAL COLORS
## UNCERTIFIED COLORANTS

1. Beta-Carotene

Color-orange-yellow
Commercial forms; liquid suspension - 30% in vegetable oil. Semi-solid suspension - 24% in hydrogenated vegetable oil. Beadlet-water dispersible - 10% and 2.4% Emulsion, beverage type - 3.6%. The colorent is used at levels ranging from 2-50 ppm as pure color to shade margarine, shortening, butter, cheese, baked goods, confections, juices and beverages.

Nutritional value: 1g of beta-carotene = 1,666,666 USP units of Vitamin A.

2. Beta-Apo-8' carotenal

Color - more orange than beta-carotene. Commercial forms: as a dry powder; as 1-1.5% vegetable oil solutions, as 20% suspensions in vegetable oil or as 10% dry beadlets, colloidal dispersions of colorant in a matrix of getaltin, vegetable oil, sugar, starch. Use levels range within 1-20 ppm as pure color. Nutritional value: 1g of the colorant = 1,200,000 IE

3. Canthaxanthin

Color - orange - red.
Commercially available as a water dispersible, dry beadlet composed of 10% colorant, gelatin, vegetable oil, sugar, starch Used levels range within 5-60 ppm as pure color. The colorant is useful in coloring tomato products such as tomato soup, spaghetti sauce, and pizza sauce, French dressings, fruit drinks, sausage products, and leaked goods.

Canthaxantin has no vitamin A activity.

4. Annatto Extract /Bixin/

Color - butter - yellow.
Commercial forms; as a dry powder, oil solutions and suspensis alkaline aqueous solutions. All containing 1-15% active color calculated as Bixin.
It is used in foods such as butter, margarine, cooking oils, salad dressings, cereals, ice cream and spices at levels of 0.5 10 ppm as pure color.

ANNEX 13

5. Cochineal extract (carmine)

Color: red
Cochineal extract is the concentrated solution from an aqueous - alcoholic extract of cochineal/insect coccus cacti/. The active coloring matter is carminic acid, a total solids content of about 6%.
Commercially available carmine mostly as an aluminium or calcium-aluminium lake on an aluminium hydroxide substrate; carmine obtained 50% or more carminic acid. Carmine is useful for producing pink shades in some protein products, candy, confections.

Use levels are within the range 25-1000 ppm.

6. Riboflavin (Vitamin $B_2$)

Color - yellow-orangish
Commercial form: as a dry powder, water soluble. Is useful for coloring some beverages, dessert powders, ice cream, candy. No restriction to use.

7. Caramel

Color dark brown to black
Commercial form - liquid or solid form.
A major use for caramel is in soft drinks, particularly colas drink. It is also may be used to standardize the hue of blended whiskys and beer. Other uses: coloring of baked goods, syrops, candies and pharmaceuticals. Is good stability in most products. Use levels are 1000-5000 p

8. Saffron/Cl Natural Yellow 6/

Is also known as crocus, It is a reddish brown or golden yellow powder. Active form of saffron is crocin and crocetin It is very stable colorant soluble in water. Is useful for coloring bakery goods, snack foods.
Use levels are within the range 1-260 ppm.

9. Paprika and paprika oleoresin

Paprika as a dry powder is the deep red.
Paprika oleoresin is obtained by extracting paprika with solvent such as ethyl alcohol, ethylene dichloride, hexane.
Paprika oleosesin is brown-red.
Paprika and its oleoresin is useful for coloring some kinds of products.
Use levels are within the range 0.2-100 ppm.

10. Dehydrated red beets

It is a dark red powder. The active ingredient is Betanin.
Beet powder is used in ice cream, desserts, candy gelatin, yogurt in concentrations of 0.1 to 1% of the final product.

11. Vegetable and fruit juices. The concentrated or unconcentrated liquid expressed from mature varieties of fresh, edible vegetables and fruits.

ANNEX 13

## The Certified Color-Additives

The certified color additives are all synthetically produced.

| | Common Name | FDA Name | Color Index |
|---|---|---|---|
| 1. | Erythrosine | FD+C Red No. 3 | 45430 |
| 2. | Indigotine | FD+C Blue No. 2 | 73015 |
| 3. | Tartrazine | FD+C Yellow No. 5 | 19140 |
| 4. | Fast Green FCF | FD+C Green No. 3 | 42053 |
| 5. | Sunset yellow FCF | FD+C Yellow No. 6 | 15985 |
| 6. | Brilliant Blue FCF | FD+C Blue No. 1 | 42090 |
| 7. | Citrus Red No. 2 (Only can used to color the skins of oranges) | Citrus Red No. 2 | 12156 |
| 8. | Orange B | Orange B | 19235 |
| 9. | Allura Red AC | FD+C Red No. 40 | 16035 |

The certified color additives are available in forms:

| | |
|---|---|
| Powder ..... | 88-93%, pure dye |
| Granular ..... | 88-93%, pure dye |
| Aqueous Liquid ..... | 1-6% pure dye |
| Non-aqueous Liquid ..... | 1-8% pure dye |
| Paste ..... | 4-10% pure dye |

### Use of Color Additives

The range and maximum of color used in the various categories of processed foods

ANNEX 14

# CHRONOLOGICAL HISTORY OF SYNTHETIC COLORS IN UNITED STATES

| Year Listed for Food Use | Common Name | FDA Name | Color Index Number | Year De-listed | Currently Permitted in Food |
|---|---|---|---|---|---|
| 1907 | Ponceau 3R | FD&C Red No.1 | 16155 | 1961 | No |
| 1907 | Amaranth | FD&C Red No.2 | 16185 | 1976 | No |
| 1907 | Erythrosine | FD&C Red No.3 | 45430 | - | Yes |
| 1907 | Orange 1 | FD&C Orange No.1 | 14600 | 1956 | No |
| 1907 | Naphthol Yellow S | FD&C Yellow No.1 | 10316 | 1959 | No |
| 1907 | Light Green SF Yellowish | FD&C Green No.2 | 42095 | 1966 | No |
| 1907 | Indigotine | FD&C Blue No.2 | 73015 | - | Yes |
| 1916 | Tartrazine | FD&C Yellow No.5 | 19140 | - | Yes |
| 1918 | Sudan 1 | - | 12055 | 1918 | No |
| 1918 | Butter Yellow | - | | 1918 | No |
| 1918 | Yellow AB | FD&C Yellow No.3 | 11380 | 1959 | No |
| 1918 | Yellow OB | FD&C Yellow No.4 | 11390 | 1959 | No |
| 1922 | Guinea Green B | FD&C Green No.1 | 42085 | 1966 | No |
| 1927 | Fast Green FCF | FD&C Green No.3 | 42053 | - | Yes |
| 1929 | Ponceau SX | FD&C Red No.4 | 14700 | 1976 | No |
| 1929 | Sunset Yellow FCF | FD&C Yellow No.6 | 15985 | - | Yes |
| 1929 | Brilliant Blue FCF | FD&C Blue No.1 | 42090 | - | Yes |
| 1939 | Naphthol Yellow S potassium salt | FD&C Yellow No.2 | 10316 | 1959 | No |
| 1939 | Orange SS | FD&C Orange No.2 | 12100 | 1956 | No |
| 1939 | Oil Red XO | FD&C Red No. 32 | 12140 | 1956 | No |
| 1950 | Benzyl Violet 4B | FD&C Violet No.1 | 42640 | 1973 | No |
| 1959 | Citrus Red No.2 | Citrus Red No.2 | 12156 | - | Yes |
| 1966 | Orange B | Orange B | 19235 | - | Yes |
| 1971 | Allura Red AC | FD&C Red No.40 | 16035 | - | Yes |

# ANNEX 15

## SYNTHETIC COLORING MATTERS USING IN FOOD PROCESSING promulgated in Resolution No 505 - April 4, 1992 MINISTRY OF HEALTH IN VIETNAM

| Comon name | Colour index No | Chemical name |
|---|---|---|
| Tartazine | 19140 | Trisodium salt of 5 - Hydroxy - 1 - p - sulfophenyl - 4 - (p - sulfophenylazo) pyrazob - 3 - carboxylic acid |
| Sunset yellow FCF | 15985 | Disodium salt of 1 - (4 - sulfophenylazo) - 2 - Naphthol - 6 - sulfonic acid |
| Indigocarmine | 73015 | Disodium salt of indigocarmine - 5 - 5' disulfonic acid and Indigo 5:7 |
| Brilliant blue FCF | 42090 | Disodium salt of 4 - (4 - N - Ethyl - sulphobena - amino) - phenyl - (2 - sulph - niumphenyl) - methylene benzyl) - delta 2;5 cyc -hexadieni - mine |
| Erythrosine | 45430 | Disodium salt or potassium of 2:4:5 :7 tetraiodo - fluorescein |
| Amaranth | 16185 | Trisodium salt of I -(4 - sulphona - phthylazo) hydroxylnaphthalen - 3;6 - disulphonic acid |
| Ponceau | 16255 | Trisodium of I sulpho - I - naphthy - lazo - 2 - naphthol - 6:8 - disul- nic - acid |
| Cármoisine | 14720 | Disodium of 2 - sulpho I - naphthy - laze - I - naphthol - 4 - sulphor acid |

ANNEX 16

## Recommended Levels of Preservatives to be added to Food Products

### Preservatives most frequently used

| Name of Preservatives | Types of food products | Maximum levels to be used |
|---|---|---|
| Benzoic acid or Sodium Benzoate | Fruit products, Vegetable products | 0.1 g/100 g products |
| | Drinks: Carbonated and non-carbonated beverages | from 0.05 to 0.1% |
| Ethyl and propyl Parabens | Fruit and vegetable products, margarine Processed meat and fish, confectionery | 0.1g/100 g products (from 0.05 to 0.1%) |
| Sorbates | Vegetable products | 0.15 g/100 g (0.05 to 0.15%) |
| | Fruit products: Jellies, jams, preserves | 0.1 g/100 g. (0.05 to 0.1%) |
| | Fruit juices Soft drinks | 0.1g/100 g (0.025 to 0.1%) |
| | Dairy ptoducts: Processed cheese | 0.05 to 0.07% |
| | For surface treatment | 0.1 - 0.4 g/dm2 |
| | Fish products | 01 g/100 g |
| | Margarine, and other fats | 0.1 g/100 g |
| | Wines | 0.2 g/liter |
| | Bakery goods | 0.1 - 0.2% relative to the flour |
| | Confectionery (fillings for chocolate and pralines) | 0.05 to 0.2% |
| Propionates | Dairy products: Processed cheese | 0.2 - 0.5% |
| | Surface treatment of hard cheese | 5 - 10% solution of sodium or calcium propionate |
| | Bakery products | 0.15 - 0.3% relative to flour. |
| | Miscellaneous uses | 0.2 - 0.4% |

ANNEX 16

| | | |
|---|---|---|
| Sulfites | Fruit products:<br>fruit pulps and<br>fruit purees<br>Drinks: fruit juices,<br>wines<br>Starch syrup<br>Starch<br>Gelatin | 0.2 g/100 g products<br>0.01-0.2%<br>0.03 - 0.06%<br>0.15-0.20 g/liter<br>0.04g/100 g.<br>0.01g/100 g.<br>0.075 g/100 g |
| Nitrates /$NaNO_3$/ | Meat and meat products | 0.05 g/100 g products |
| Nitrites $NaNO_2$ | Meat and meat products | 0.02 g/100 produc<br>0.008-0.016% |
| Acetic Acid and Acetates | Fish products | 1 to 5% solutions of acetic acid |
| | Meat products | 3 to 3.6% acetic acid solution |
| | Vegetable products | 0.5 to 3% acetic acid solution |
| | Bakery goods | 0.2 to 0.14% of sodium diacetat |

Recommended Literature

1. Lueck E., Antimicrobial Food Additives
Springer-Verlag Berlin, Heidelberg
New York (1980)

2. Frazier W.C., Westhoff D.C., Food Microbiology.
McGraw Hill Book Company (1958)

# APPENDIX 1

## LIST OF EQUIPMENTS PROVIDED BY UNIDO, USED DURING THE TRAINING PARTICIPANTS AT THE COURSE ORGANIZED BY DTQC IN HANOI

1. Gas Chromatograph "TRACOR" Model 9000
2. Single beam Atomic Absorbtion Spectrometer (AAS) PU 9100X04
3. Single beam Spectrophotometer 200 - 1000 nm
4. Rotary evaporator, Heidolph Model VV 2000
5. Balance
6. Ultraviolet Cabinet

# APPENDIX 2

## PESTICIDES USE IN VIETNAM

1. Aluminium phosphide (gastoxin)
2. Bacillus thuringiensis
3. Buprofezin (Applaud) 10 WP
4. Cartap (Padan) 95 SP
5. Alpha cypermetarin (Fastac) 5 EC
6. Carbaryl (Sevin) 85 WP
7. Carbonfuran (Furadan, Curaterr) 3 G
8. Cypernathrin (Sherpa, Cynbush, Polytrin) 10 EC, 25EC
9. Cypernathrin + Profenofos (Polytrin) 440-SWC
10. Diazinon (Basudin, Neocidil) 50 EC
11. Dicofol (Kelthane) 20 EC
12. Dicrotophos ( Bidrin) 50 EC
13. Dichlorvos (DDVP, Nuvan) 50 EC
14. Decanethrin (Deltanethrin, Decis) 2,5 EC
15. Dimethoate (Bi Rogor, Roxion, Fostion) 50 EC
16. Endosufan (Thidron) 30 EC
17. Esfenvalerate (Sumi-alpha) 5 EC
18. Ethofenprox (Trebon) 10 EC
19. Ethoprophos (Mocap) 6 EC, 10 G, 20 EC
20. Fenithro thion (Sunithion) 50 EC
21. Fenobucarb (Bassa, BPMC, Hopcrin) 10 EC
22. Fenpropathrin (Danitol, Rody, Moethrin) 10 EC
23. Fervalerate (Salfodin) 10 EC, 20 EC

## APPENDIX 2

24. Fenthion (Lebaycid, Baycid) 50 EC
25. Isoprocarb (Mipcin) 20 EC, 25 EC, 50 WP
26. Lindane (Gamma-BHC, Gamma-HCH) 99,5 WP
27. Methamidophos (Filitox, Monitor) 60 SC
28. Methidathion (Supracide) 40 EC
29. Methyl Bromide 98 %
30. Monocrotophos (Azodrin, Nuvacron) 50 SCW
31. Methyl parathion (Parathion, Wofatox, Metaphos) 50 EC
32. Naled (Dibrom, Fliból, Bromchlorphos) 50 EC, 96 EC
33. Phoselone (Zolone) 35 EC
34. Phosphamidon (Dimecron, Apertidon) 50 SCW
35. Teflubenzuron (Nomolt) 5 EC
36. Trichlorfon 50 EC, 80 WP
37. Rotenone
38. Benomyl (Fundazol, Benlate) 50 EC
39. Calcium polysulphide
40. Copper oxychloride
41. Copper sulphate
42. Captafol (Difolatan, Folcid) 80 WP
43. Captan (Captane) 75 WP
44. Diniconazol (Sumi-8) 12,5 WP
45. Edifenphos (Hinosan) 40 EC, 50 EC
46. Fosetyl - Al (Aliette) 80 WP
47. Hexaconazol (Anvil) 5 EC
48. Iprofenphos (Kitazin) 50 EC
49. Iprodione (Rovral) 50 WP
50. Iprothiolane (Fuji-one) 40 EC
51. Kasugamycin + Rabcide (Kasei) 21,2 WP
52. Kasuran WP (2 % Kasugamycin + 45 % Copper oxychloride)
53. Kasuran WP (5 % Kasugamycin + 45 % Copper oxychloride)
54. Mancozeb - 80-WP
55. Monceren (Pencycuron) 25 WP
56. Ridomil - MZ (Metalaxyl + Mancozeb) 72 WP
57. Sulphur 95 D
58. Thiram 85 WP

## APPENDIX 2

59. Tilt (Propiconazole) 250 EC
60. Topsin - M 50 WP, 70 WP
61. Validamycin (Validecin) 3 SC
62. Triadimefol (Bayleton) 25 EC
63. Zincopper WP (20 % Zineb + 30 % Copper oxychloride)
64. Zineb 80 WP

Thuốc trừ cỏ : Wild grass - weeds herbicydes

65. Ametryn (Gesapex) 50 WP, 80 WP
66. Benthiocarb (Saturn) 10 G, 20 WP, 50 EC
67. Butachlor (Lambest) 5 G, 10 EC
68. 2,4 D 60-EC, 72-EC, 80-WP, 96-WP
69. Dalapon (Dalapôn) 80 WP
70. Diuron 80 WP
71. MCPA (Agroxone) 80 WP
72. Pretilachlor (Sofit) 30 EC
73. Pyrazosulfuron ethyl (NC 311) 25 EC, 10 EC
74. Simazin (Gesatop) 80 WP
75. Atrazin (Gesaprin) 80 WP

Thuốc trừ chuột :

76. Brodifecoum (Klerat) 0,05 %
77. Phosphue kẽm (Fokebe) 20 %

Chú thích những từ viết tắt trong danh mục :

EC : dạng thuốc hóe sữe hay nhủ dầu
WP : " bột thấm nước hay bột hòa nước
SP : " bột tan trong nước
G hay GR : dạng thuốc hạt
SWD ( hoặc SCW, SL, SC) : dung dịch tan trong nước
D : dạng thuốc bột ./.

APPENDIX 3

# Electron Capture Detector (ECD)

**WARNING**

**THE EFFLUENT GAS STREAM FROM THE DETECTOR MUST BE VENTED TO A FUME HOOD TO PREVENT POSSIBLE CONTAMINATION OF THE LABORATORY WITH RADIOACTIVE MATERIAL.**

**SPECIFIC CLEANING PROCEDURES ARE PROVIDED IN CHAPTER 8, *PREVENTIVE MAINTENANCE.***

## Requirements for USA Owners

**WARNING**

**DETECTOR VENTING MUST BE IN CONFORMANCE WITH THE LATEST REVISION OF TITLE 10, CODE OF FEDERAL REGULATIONS, PART 20 (INCLUDING APPENDIX B).**

**THIS DETECTOR IS SOLD UNDER GENERAL LICENSE: OWNERS MAY NOT OPEN THE DETECTOR CELL OR USE SOLVENTS TO CLEAN IT. ADDITIONAL INFORMATION IS AVAILABLE IN THE PUBLICATION INFORMATION FOR GENERAL LICENSEES, PUB. NO. 43-5953-1798(D).**

**OWNERS OF THIS DETECTOR MUST PERFORM A RADIOACTIVE LEAK TEST (WIPE TEST) AT LEAST EVERY SIX MONTHS. THE PROCEDURE IS DESCRIBED IN CHAPTER 8, *PREVENTIVE MAINTENANCE.***

**WARNING**

**IN THE EXTREMELY UNLIKELY EVENT THAT BOTH THE OVEN AND THE ECD HEATED ZONE SHOULD GO INTO THERMAL RUNAWAY (MAXIMUM, UNCONTROLLED HEATING IN EXCESS OF 400°C) AT THE SAME TIME, AND THAT THE ECD REMAINS EXPOSED TO THIS CONDITION FOR MORE THAN 12 HOURS, THE FOLLOWING MUST BE DONE:**

- **AFTER TURNING OFF MAIN POWER AND ALLOWING THE INSTRUMENT TO COOL, CAP ECD INLET AND EXHAUST VENT OPENINGS. WEAR DISPOSABLE PLASTIC GLOVES AND OBSERVE NORMAL SAFETY PRECAUTIONS.**
- **RETURN THE CELL FOR EXCHANGE, FOLLOWING DIRECTIONS INCLUDED WITH THE FORM GENERAL LICENSE CERTIFICATION (HP PUB. NO. 43-5954-7621).**

**IT IS UNLIKELY, EVEN IN THIS VERY UNUSUAL SITUATION, THAT RADIOACTIVE MATERIAL WILL ESCAPE THE CELL. PERMANENT DAMAGE TO THE $^{63}$Ni PLATING WITHIN THE CELL IS POSSIBLE, HOWEVER, SO THE CELL MUST BE RETURNED FOR EXCHANGE.**

APPENDIX 3

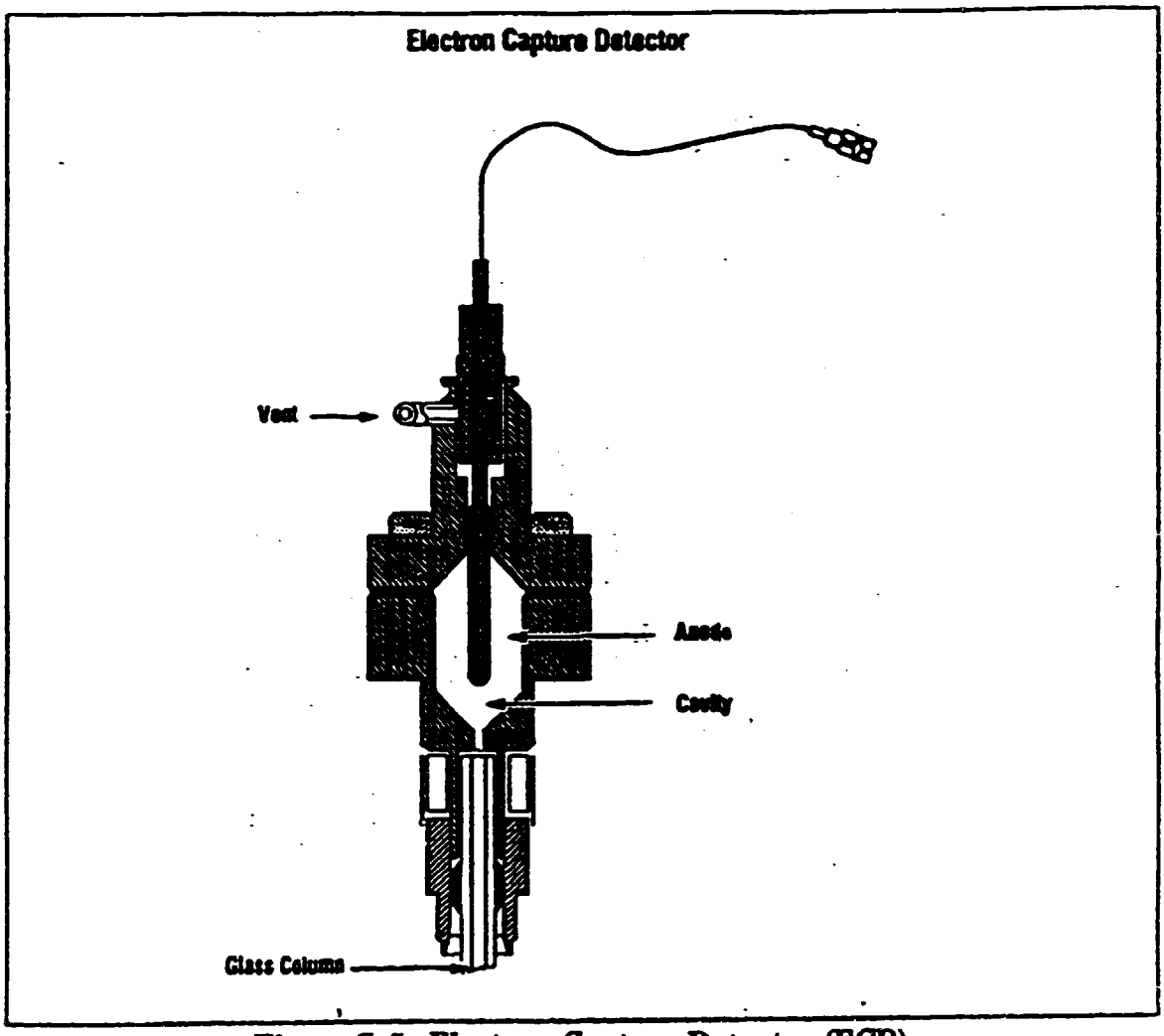



**Figure 7–5. Electron Capture Detector (ECD)**

The electron capture detector (ECD) cell contains $^{63}Ni$, a radioactive isotope emitting high-energy electrons (β-particles). These undergo repeated collisions with carrier gas molecules, producing about 100 secondary electrons for each initial β-particle.

Further collisions reduce energy of these electrons into the thermal range. These low energy electrons are then captured by suitable sample molecules, thus reducing total electron population within the cell.

Uncaptured electrons are collected periodically by applying short-term voltage pulses to cell electrodes. This cell current is measured and compared to a reference current, and the pulse interval is then adjusted to maintain constant cell current.

# Flame Ionization Detector (FID)

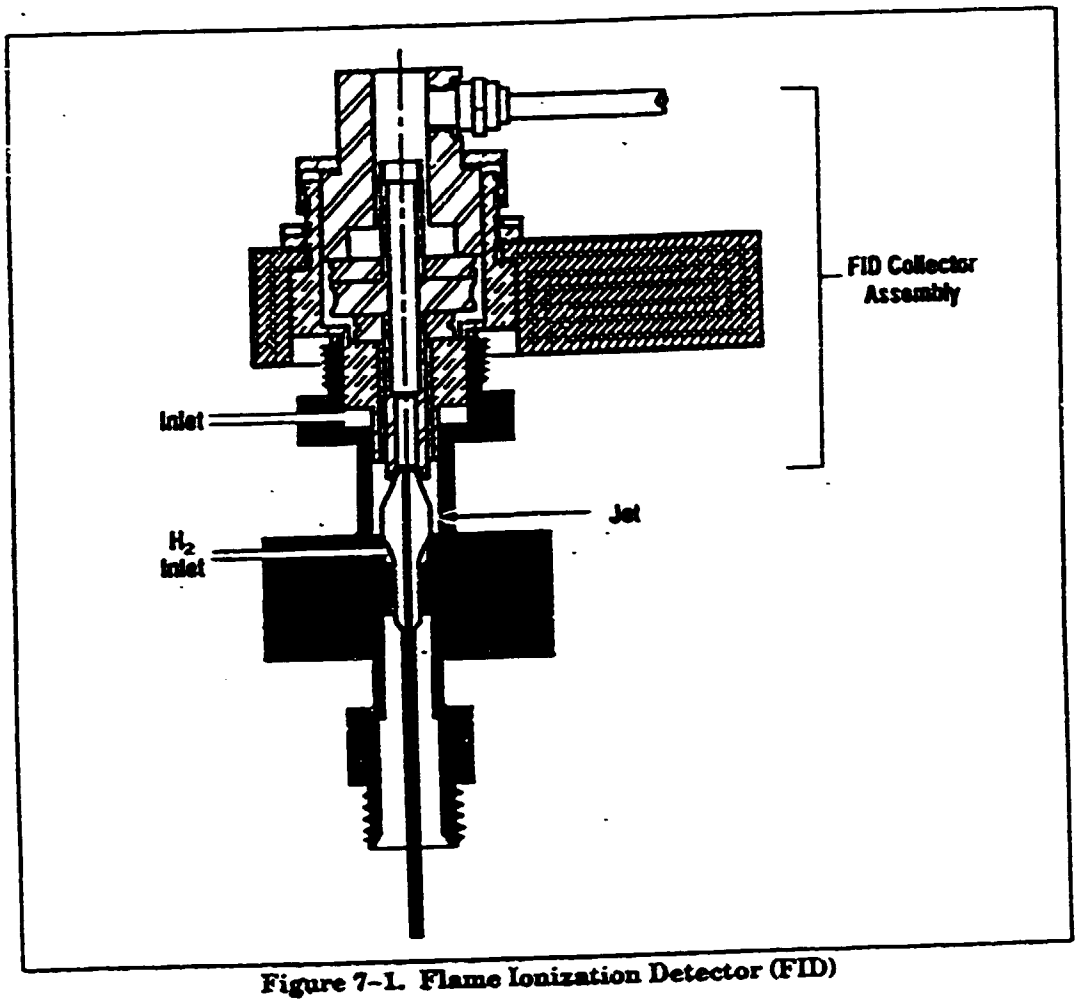



**Figure 7-1. Flame Ionization Detector (FID)**

The flame ionization detector (FID) responds to compounds that produce ions when burned in a $H_2$-air flame. These include all organic compounds, although a few (e.g., formic acid, acetaldehyde) exhibit poor sensitivity.

## APPENDIX 3

Compounds producing little or no response include:

| | | |
|---|---|---|
| Rare gases | $N_2$ | $N_2$ |
| Nitrogen Oxides | CO | *$CCl_4$ |
| Silicon Halides | $CO_2$ | |
| $H_2O$ | $CS_2$ | |
| $NH_3$ | $O_2$ | |

* Measured at the jet tip.

This selectivity can be advantageous: for example, $H_2O$ or $CS_2$, used as solvent, do not produce large solvent peaks.

The system is linear for most organic compounds, from the minimum detectable limit through concentrations greater than $10^7$ times the minimum detectable limit. Linear range depends on each specific compound; it is directly proportional to sensitivity of the FID toward the given compound.

In general, where sample components of interest are in high concentration, increased air flow may be necessary (up to 650 ml/min). Where components of interest are in low concentration, reduced air flow rates are acceptable (375 to 425 ml/min).

For maximum sensitivity, it is recommended that a standard sample be made containing components of interest in concentrations expected. By experimenting with different carrier, air, and $H_2$ flow rates, the standard is used to determine flow rates giving maximum response.

APPENDIX 3

# Thermal Conductivity Detector (TCD)

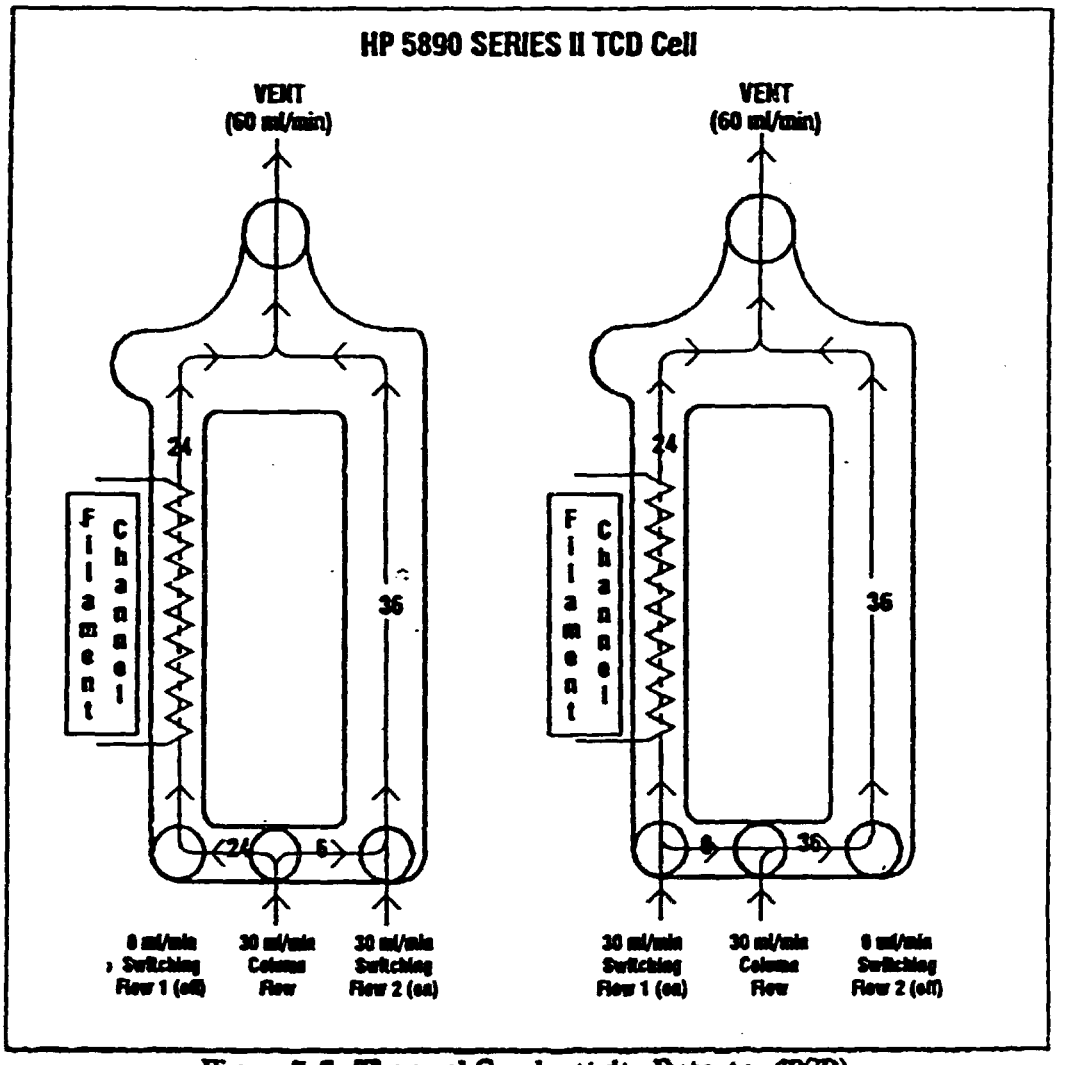



**Figure 7-7. Thermal Conductivity Detector (TCD)**

The Thermal Conductivity Detector (TCD) detects the difference in thermal conductivity between column effluent flow (carrier gas + sample components) and a reference flow of carrier gas alone; it produces voltage proportional to this difference. The voltage then becomes the output signal to the connected chart recording or integrating device.

APPENDIX 3

The TCD uses a single filament to examine alternately relative thermal conductivities of reference versus column effluent gas streams every 200 msec. At this frequency, the detector is insensitive to thermal drift

Factors which influence TCD response include the following:

- Temperature difference between the filament versus the surrounding detector block.
- Flow rate ratio between carrier effluent versus reference gas streams.
- Type of carrier/reference gas used.

**CAUTION**

**The TCD filament can be permanently damaged if gas flow through the detector is interrupted while the filament is operating. Make sure the detector is off whenever changes/adjustments are made affecting gas flows through the detector.**

**Likewise, exposure to $O_2$ can permanently damage the filament. Make sure the entire flow system associated with the TCD is leak-free and that carrier/reference gas sources are uncontaminated before turning on the detector. Do not use Teflon tubing, either as column material or as gas supply lines, because it is permeable to $O_2$.**

Since the TCD responds to any compound whose thermal conductivity is different from that of the carrier gas, $H_2$ and He are most commonly used as carrier gases, with $H_2$ giving somewhat greater sensitivity. However, $H_2$ forms explosive mixtures with air ($O_2$), and some components, particularly unsaturated compounds, may react with $H_2$. He produces almost as much sensitivity as $H_2$ and is free from problems of reactivity with sample components or the filament. $N_2$ or Ar may be used but give lower response for most materials; however, they are useful if $H_2$ or He is being analyzed.

Because of its exceptionally high thermal conductivity and chemical inertness, He is the recommended carrier gas: it gives large thermal conductivity differences with all compounds except $H_2$ (considerations necessary in $H_2$ analyses are discussed later). With He as carrier, the TCD exhibits universal response. For propane, the sensitivity limit is about 400 picograms/ml of He carrier gas.

The TCD exists in two configurations: either its exhaust vent tube exits at the top of the detector or the exhaust vent tube returns to the inside of the oven for connection to an FID or other device.

# APPENDIX 4
## TABLE 1

SE(F)-220

Column Temperature, °C.

| 170 | 174 | 178 | 182 | 186 | 190 | 194 | 198 | 202 | 204 | Compound |
|---|---|---|---|---|---|---|---|---|---|---|
| .43 | .44 | .44 | .45 | .46 | .46 | .46 | .47 | .47 | .48 | Hexachlorobenzene |
| .51 | .51 | .51 | .51 | .52 | .52 | .52 | .52 | .53 | .53 | Dimethyl Phthalate |
| .52 | .53 | .53 | .54 | .54 | .55 | .56 | .56 | .57 | .58 | Terrazene |
| .58 | .59 | .60 | .60 | .61 | .62 | .62 | .63 | .64 | .65 | Chlordane |
| .62 | .62 | .63 | .64 | .64 | .65 | .65 | .66 | .66 | .67 | o-DDE |
| .66 | .66 | .66 | .66 | .66 | .66 | .66 | .66 | .66 | .66 | Perthane |
| .69 | .69 | .69 | .69 | .69 | .69 | .69 | .69 | .69 | .69 | 2,4-D (ME) |
| .69 | .69 | .69 | .69 | .69 | .70 | .70 | .70 | .70 | .70 | ODC |
| .73 | .73 | .73 | .73 | .73 | .73 | .73 | .73 | .72 | .72 | Diethyl Phthalate |
| .75 | .75 | .75 | .75 | .75 | .74 | .74 | .74 | .73 | .73 | Dieldrin |
| .80 | .80 | .80 | .81 | .81 | .81 | .82 | .82 | .83 | .83 | Lindane |
| .86 | .86 | .86 | .86 | .86 | .86 | .86 | .86 | .86 | .86 | PCNB |
| .87 | .87 | .87 | .87 | .87 | .87 | .87 | .88 | .88 | .88 | Heptachlor |
| .87 | .87 | .87 | .86 | .86 | .85 | .85 | .84 | .83 | .82 | 2,4-D (IPE) |
| .97 | .96 | .96 | .96 | .96 | .96 | .96 | .95 | .94 | .94 | o-BHC |
| 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | Aldrin (BI/EREAC) |
| 1.08 | 1.07 | 1.07 | 1.06 | 1.05 | 1.04 | 1.03 | 1.02 | 1.01 | 1.00 | 2,4,5-T (ME) |
| 1.36 | 1.33 | 1.32 | 1.30 | 1.29 | 1.27 | 1.26 | 1.25 | 1.23 | 1.22 | 2,4,5-T (IPE) |
| 1.41 | 1.39 | 1.38 | 1.37 | 1.36 | 1.35 | 1.34 | 1.33 | 1.32 | 1.31 | Ronnel |
| 1.43 | 1.41 | 1.40 | 1.39 | 1.38 | 1.38 | 1.37 | 1.36 | 1.35 | 1.34 | 1-Hydroxychlordene |
| 1.60 | 1.58 | 1.57 | 1.56 | 1.55 | 1.54 | 1.53 | 1.53 | 1.52 | 1.51 | Oxychlordane |
| 1.67 | 1.65 | 1.63 | 1.62 | 1.61 | 1.58 | 1.57 | 1.55 | 1.53 | 1.52 | o,p'-DDE |
| 1.90 | 1.88 | 1.87 | 1.85 | 1.84 | 1.82 | 1.80 | 1.79 | 1.77 | 1.76 | Chlordane, Gamma |
| 1.88 | 1.86 | 1.85 | 1.83 | 1.82 | 1.80 | 1.78 | 1.77 | 1.75 | 1.74 | Trans-Nonachlor |
| 2.02 | 2.00 | 1.98 | 1.97 | 1.95 | 1.93 | 1.91 | 1.89 | 1.87 | 1.85 | Heptachlor Epoxide |
| 2.08 | 2.06 | 2.04 | 2.02 | 2.00 | 1.98 | 1.97 | 1.95 | 1.93 | 1.91 | Chlordane, Alpha |
| 2.16 | 2.15 | 2.12 | 2.09 | 2.06 | 2.04 | 2.01 | 1.98 | 1.95 | 1.92 | Dimethoate |
| 2.24 | 2.21 | 2.18 | 2.15 | 2.12 | 2.10 | 2.07 | 2.04 | 2.01 | 1.98 | p,p'-DDE |
| 2.29 | 2.25 | 2.22 | 2.18 | 2.14 | 2.10 | 2.07 | 2.03 | 1.99 | 1.95 | Dibutyl Phthalate |
| 2.63 | 2.60 | 2.57 | 2.54 | 2.51 | 2.48 | 2.45 | 2.42 | 2.39 | 2.36 | Endosulfan I |
| 2.74 | 2.70 | 2.67 | 2.63 | 2.59 | 2.55 | 2.51 | 2.47 | 2.43 | 2.39 | o,p'-DDD |
| 2.75 | 2.73 | 2.70 | 2.67 | 2.64 | 2.61 | 2.58 | 2.55 | 2.52 | 2.49 | Chlordecone |
| 2.84 | 2.80 | 2.75 | 2.71 | 2.67 | 2.63 | 2.59 | 2.54 | 2.50 | 2.46 | DCPA |
| 2.92 | 2.87 | 2.83 | 2.79 | 2.74 | 2.70 | 2.65 | 2.61 | 2.57 | 2.52 | o,p'-DDT |
| 3.10 | 3.05 | 3.00 | 2.94 | 2.89 | 2.84 | 2.79 | 2.74 | 2.69 | 2.64 | DDA (ME) |
| 3.17 | 3.12 | 3.07 | 3.02 | 2.97 | 2.92 | 2.87 | 2.82 | 2.76 | 2.71 | M. Parathion |
| 3.30 | 3.24 | 3.18 | 3.12 | 3.06 | 3.01 | 2.95 | 2.89 | 2.83 | 2.77 | Malathion |
| 3.21 | 3.17 | 3.13 | 3.09 | 3.04 | 3.00 | 2.96 | 2.92 | 2.88 | 2.84 | Dicofol |
| 3.82 | 3.77 | 3.72 | 3.67 | 3.61 | 3.56 | 3.50 | 3.45 | 3.39 | 3.34 | Endrin |
| 4.06 | 4.01 | 3.95 | 3.89 | 3.84 | 3.78 | 3.73 | 3.66 | 3.62 | 3.57 | Mirex |
| 4.10 | 4.05 | 3.96 | 3.89 | 3.82 | 3.75 | 3.68 | 3.61 | 3.53 | 3.46 | p,p'-DDD |
| 4.24 | 4.17 | 4.09 | 4.02 | 3.94 | 3.87 | 3.78 | 3.71 | 3.63 | 3.56 | E. Parathion |
| 4.47 | 4.38 | 4.31 | 4.23 | 4.15 | 4.07 | 3.98 | 3.90 | 3.82 | 3.74 | p,p'-DDT |
| 4.98 | 4.91 | 4.83 | 4.75 | 4.67 | 4.59 | 4.51 | 4.43 | 4.35 | 4.27 | Endosulfan II |
| 5.29 | 5.19 | 5.09 | 4.99 | 4.89 | 4.78 | 4.68 | 4.58 | 4.48 | 4.37 | Carbophenothion |
| 6.00 | 5.89 | 5.76 | 5.64 | 5.51 | 5.37 | 5.25 | 5.12 | 4.99 | 4.86 | Ethion |
| 7.4 | 7.2 | 7.0 | 6.8 | 6.7 | 6.5 | 6.3 | 6.1 | 5.96 | 5.76 | Methoxychlor |
| 13.2 | 13.0 | 12.7 | 12.4 | 12.1 | 11.8 | 11.5 | 11.2 | 11.0 | 10.7 | Ronnel Ketone "153" |
| 13.5 | 13.1 | 12.6 | 12.2 | 11.8 | 11.4 | 11.0 | 10.6 | 10.1 | 9.7 | Dioctyl Phthalate |
| 21.4 | 20.8 | 20.1 | 19.5 | 18.8 | 18.2 | 17.5 | 16.9 | 16.2 | 15.6 | Diphenyl Phthalate |
| 21.1 | 20.6 | 20.0 | 19.5 | 18.9 | 18.4 | 17.9 | 17.3 | 16.7 | 16.2 | Tetradifon |

170 174 178 182 186 190 194 198 202 204

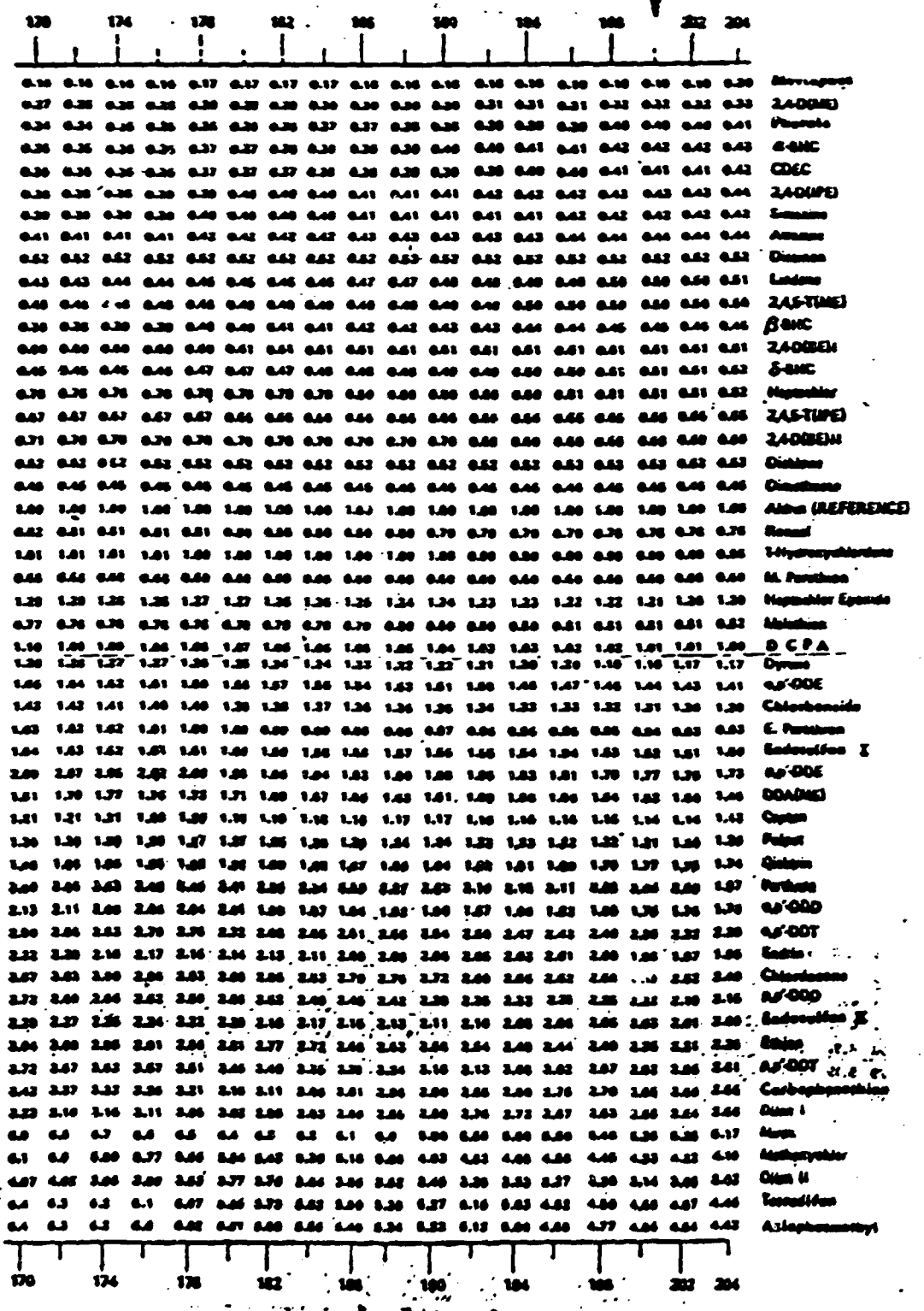
Retention ratios, relative to dieldrin, of 47 compounds at temperature from 170 to 204°C; support of gas Chrom Q, 80/100 mesh; electron capture detector; ⁶³Ni source; all absolute retentions measured from injection point. Arrow indicated optimum column operating temperature with carrier flow at 50 ml per min.

The efficiency of OV;DC;SE packed columns and retention ratios of 54 pestic des.

APPENDIX 4

TABLE 2

# 10% DC-200
## Column Temperature, °C

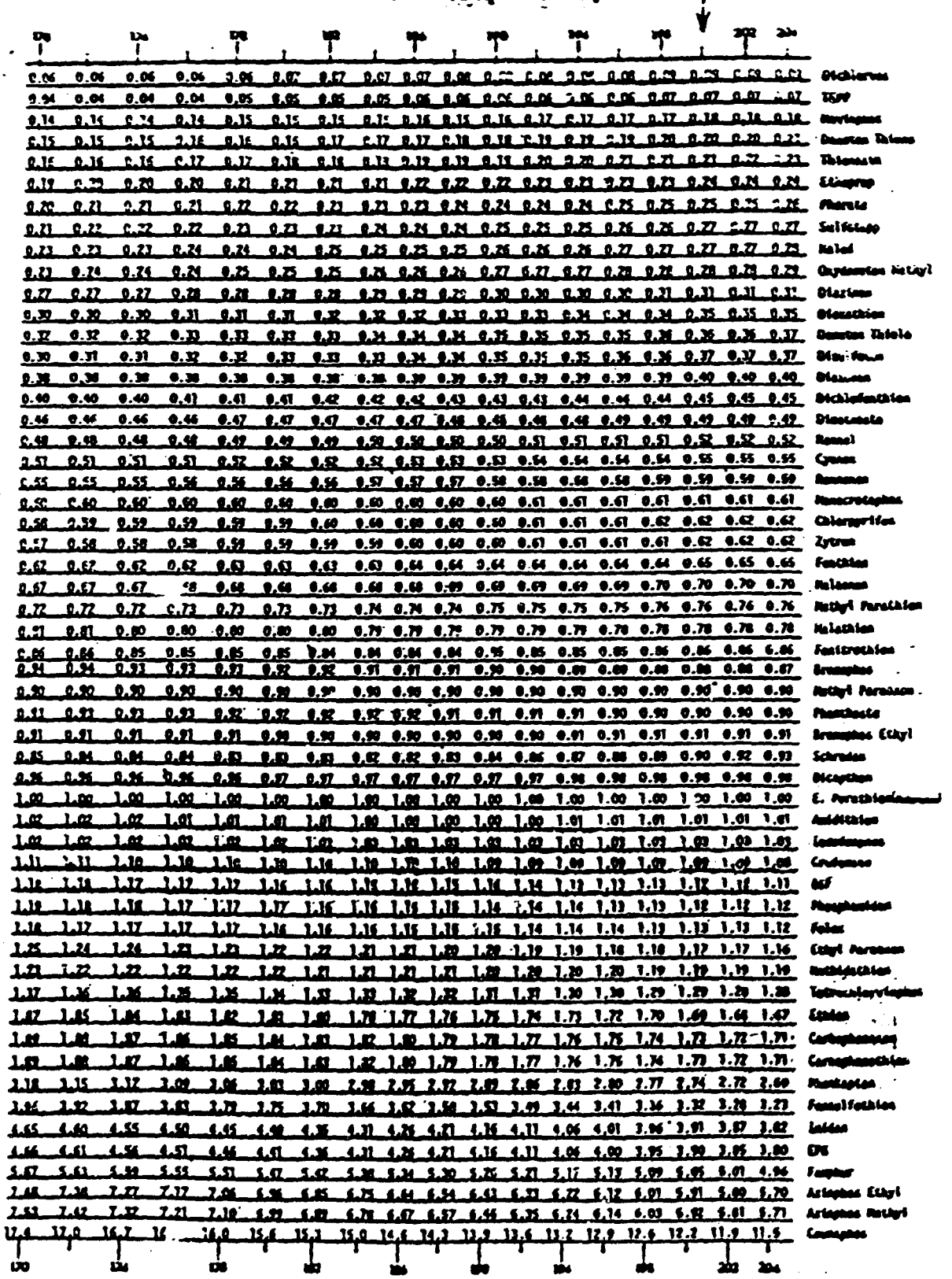



Retention ratios, relative to aldrin, of 48 pesticides on a column of 10% DC-200 at temperatures from 170 to 204°C; support of Chromosorb W, A.W., 80/100 mesh; electron capture detector, tritium source, coastal plain; all standard retentions measured from injection point. Arrow indicates optimum column operating temperature with carrier flow at 120 ml per minute.

# APPENDIX 4

## TABLE 3

## 4% SE-30/6% OV-210
## Column Temperature, °C.



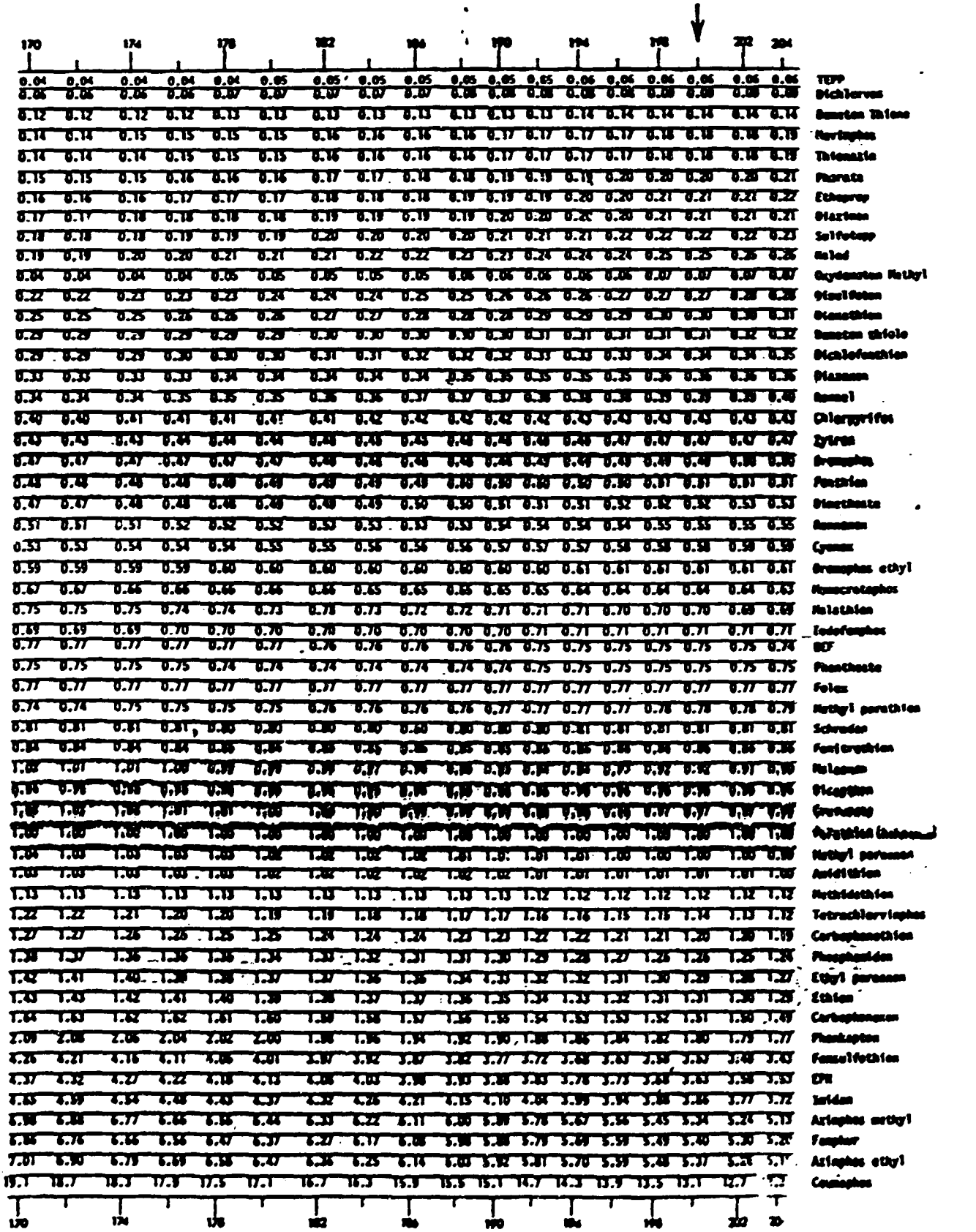

Retention ratios, relative to parathion, of 56 organophosphorous pesticides on a column of 4% SE-30/6% OV-210 at temperatures from 170 to 206°C; support of Gas Chrom-Q, 80/100 mesh; flame photometric detector, 5260 A° filter; all absolute retentions measured from injection point. Arrow indicates optimum operating temperature with carrier flow set at 75 ml per minute.

# APPENDIX 4

## TABLE 4

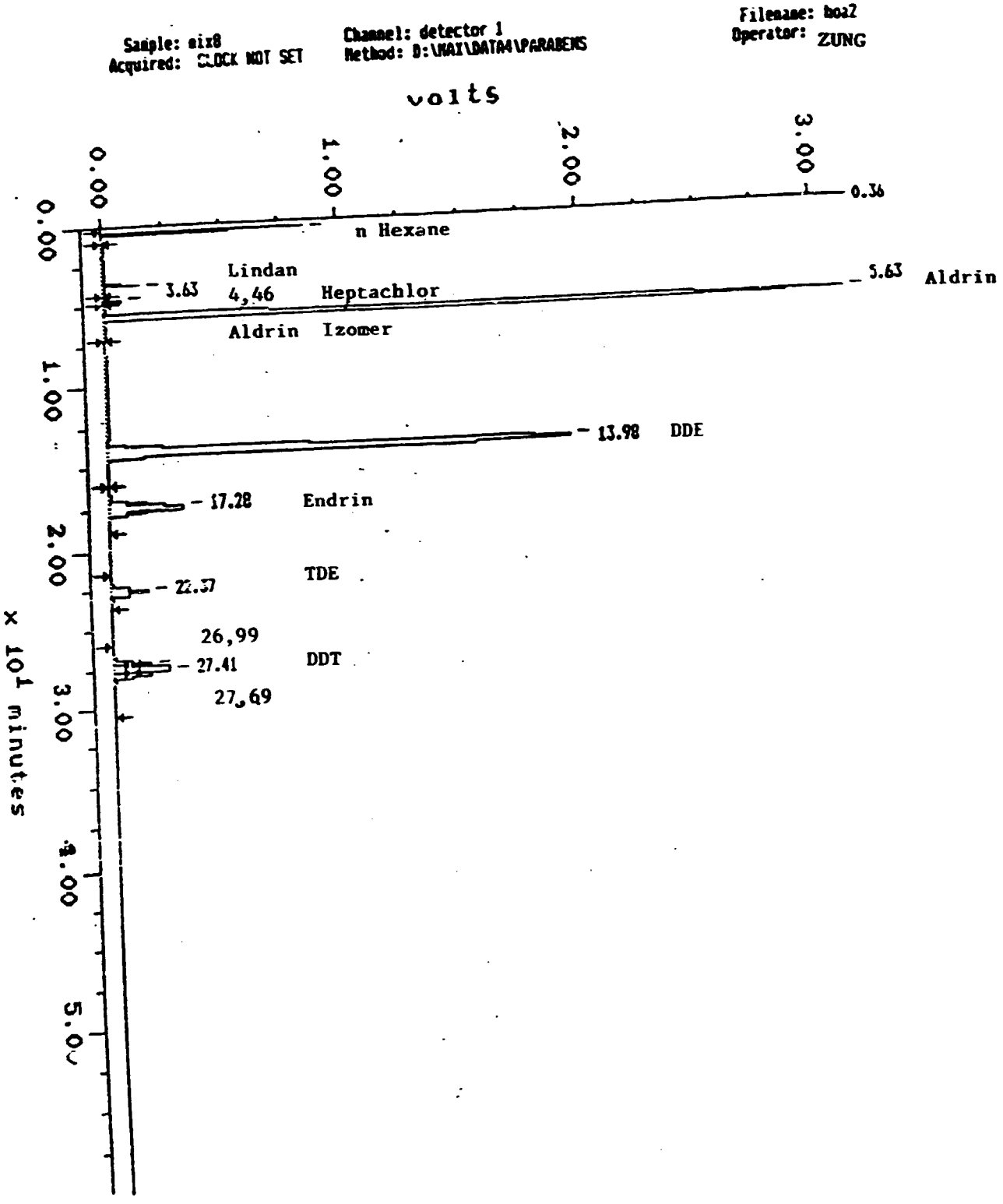
## 10 % OV-210

### Column Temperature , °C.




Retention ratios, relative to ethyl parathion, of 54 organophosphorous pesticides on a column of 10% OV-210 at temperatures from 170 to 204°C; support of Gas Chrom Q, 100/120 mesh; flame photometric detector, S260 Å filter; all absolute retentions measured from injection point. Arrow indicates optimum column operating temperature with carrier flow at 70 ml per minute.

APPENDIX 5




Chromatogram of 8 organochlorine pesticide mixtures separated on the 10% SP-1000 on Supelcoport column.

# APPENDIX 5

# PEAK INTERGRATION REPORT

Printed: 20-MAR-1993 11:48:37

SAMPLE: air8 Type: STND

81 in Method: ZONG METHOD 1 Instrument: Instrument 1

Acquired: CLOCK NOT SET Filename: hoa2

Rate: 2.0 points/sec Index: Disk

Duration: 60.000 minutes

Operator: ZONG

Inject: 2 µl.

DETECTOR: detector 1

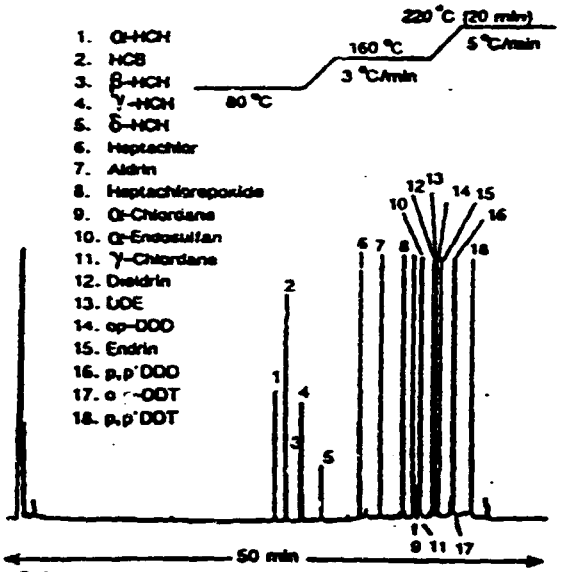
| PK# | ID# | Peak Start (minutes) | Peak End (minutes) | Retention (minutes) | Type | Peak Area (microvolt-sec) | Peak Height (microvolt) | Area Percent | Hght Percent | Component Name |
|---|---|---|---|---|---|---|---|---|---|---|
| 1 | | 0.208 | 1.042 | 0.358 | BP | 3010311.6 | 859507.40 | 2.37 | 11.95 | n-Hexane |
| 2 | | 1.042 | 4.350 | 3.625 | PP | 1094274.7 | 156534.93 | 0.86 | 2.18 | Lindan |
| 3 | | 4.350 | 4.792 | 4.458 | PP | 705976.59 | 78390.844 | 0.56 | 1.09 | Heptachlor |
| 4 | | 4.792 | 7.050 | 5.633 | PP | 46625985 | 3125132.6 | 36.68 | 43.47 | Aldrin |
| 5 | | 7.050 | 16.075 | 13.963 | PP | 50498450 | 1953392.2 | 39.73 | 27.17 | DDE |
| 6 | | 16.075 | 18.867 | 17.275 | PB | 9959406.0 | 312777.79 | 7.76 | 4.35 | Endrin+TDE Izomer |
| 7 | | 21.400 | 23.575 | 22.367 | BB | 4075153.8 | 156530.16 | 3.21 | 2.18 | TDE |
| 8 | | 25.000 | 27.183 | 26.992 | BP | 2682079.5 | 156526.58 | 2.11 | 2.18 | |
| 9 | | 27.183 | 27.608 | 27.408 | PP | 5786424.5 | 234653.97 | 4.55 | 3.26 | DDT |
| 10 | | 27.608 | 30.525 | 27.692 | PB | 2763070.9 | 156471.74 | 2.17 | 2.18 | |
| TOTAL | | | | | | 1.27102E8 | 7189918.3 | | | |

Chromatograms of organochlorine pesticides separated on capillary column.

APPENDIX 6

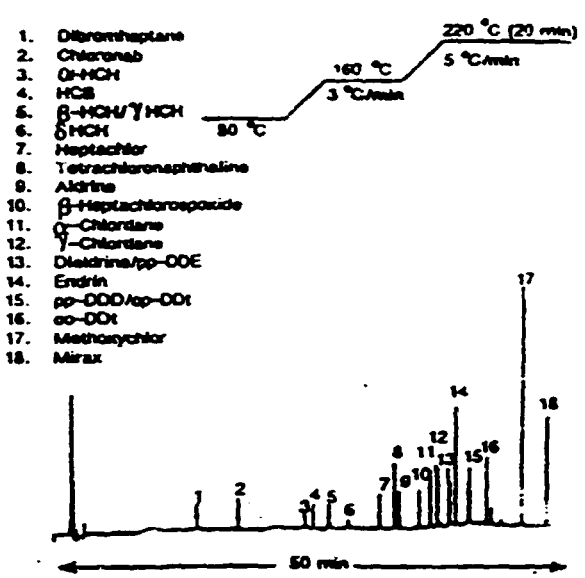
# ENVIRONMENTAL

## Pesticides



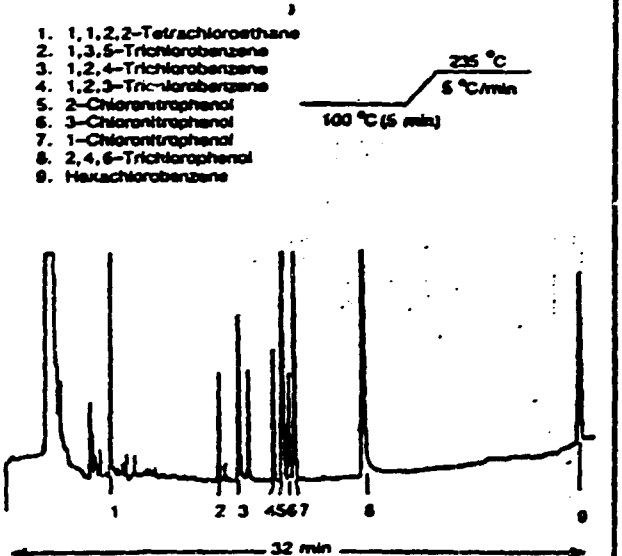

Column: HP-101 (Methyl Silicone Fluid)
50 m x 0.2 mm x 0.2 µm film
(HP Part No. 19091Y-105)
Carrier: $H_2$, 57 cm/min, 40 psi
Oven: Temperature program listed above
Injection: 2 µL, split 50:1
Detector: ECD

## Pesticides



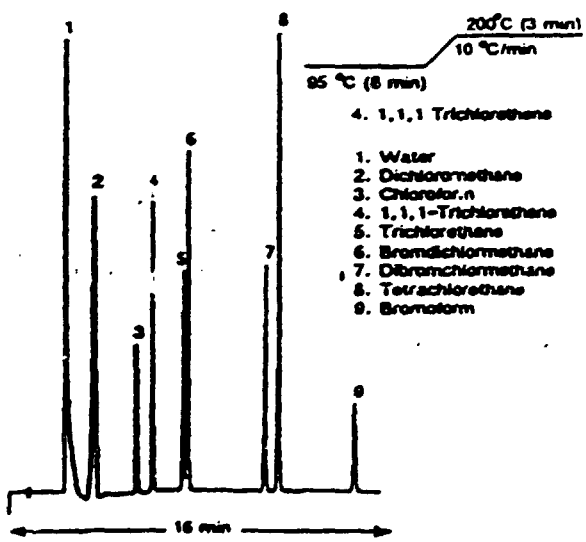

Column: HP-101 (Methyl Silicone Fluid)
50 m x 0.2 mm x 0.2 µm film
(HP Part No. 19091Y-105)
Carrier: $H_2$, 57 cm/min, 40 psi
Oven: Temperature program listed above
Injection: 2 µL, split 50:1
Detector: ECD

## Chlorinated Hydrocarbons




Column: Ultra 2 (Cross-Linked 5% Phenyl)
25 m x 0.2 mm x 0.33 µm film
(HP Part No. 19091B-102)
Carrier: Helium 25 cm/min
Oven: Temperature program listed above
Injection: On-column, 1 µL of 25 to 150 pg/µL in Toluene
Detector: ECD

## Halogenated Hydrocarbons in Water




4. 1,1,1 Trichlorethane

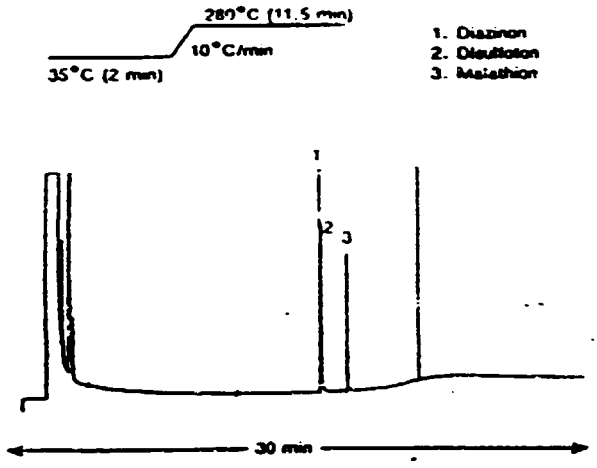
1. Water
2. Dichloromethane
3. Chloroform
4. 1,1,1-Trichlorethane
5. Trichlorethane
6. Bromdichlormethane
7. Dibromchlormethane
8. Tetrachlorethane
9. Bromoform

Column: HP-5 (Cross-Linked 5% Phenyl)
50 m x 0.32 mm x µm film
(HP Part No. 1905[illegible]-215)
Carrier: Helium 17 cm/sec
Oven: Temperature program listed above
Injection: 1 µL, 20 ug/liter
Detector: ECD

APPENDIX 6

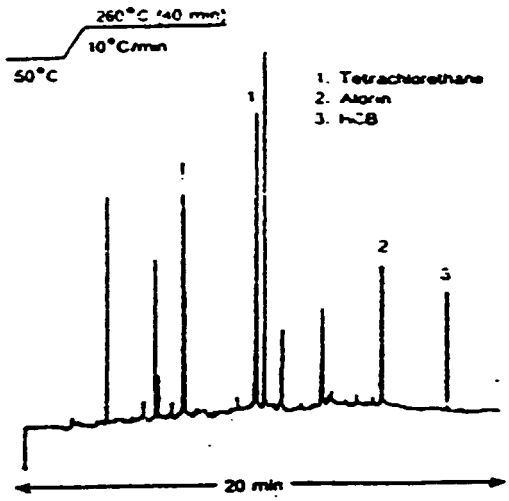
# ENVIRONMENTAL

## Pesticides



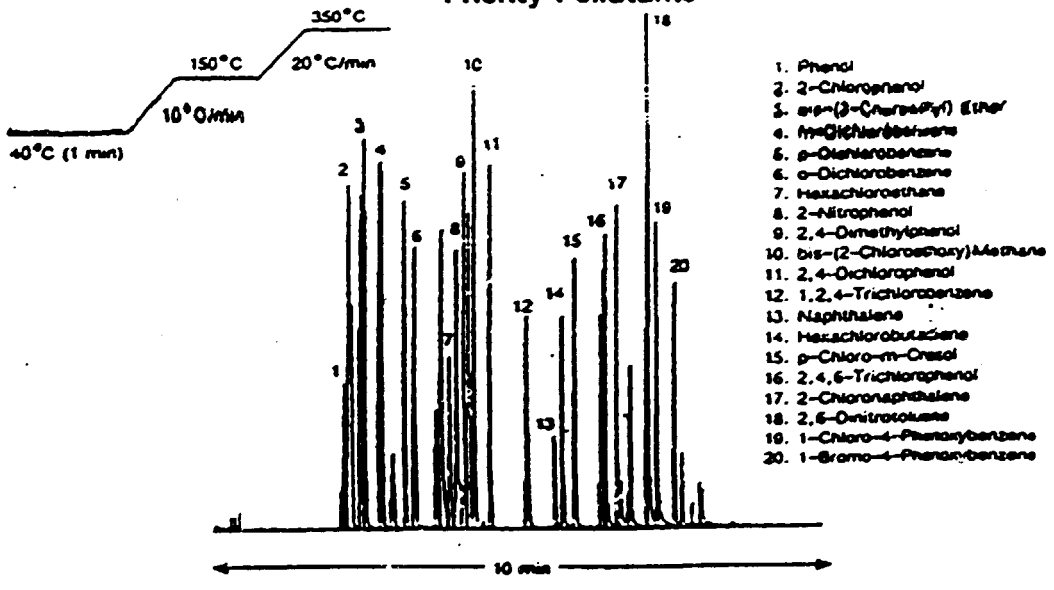

Column: Ultra 2 (Cross-Linked 5% Phenyl Silicone)
25 m x 0.32 mm x 0.17 µm film
(HP Part No. 19091B-012)
Carrier: µ(He)=21 cm/sec
Injection: On-column
Detector: FID

## Pesticides in Rape Seed Oil




Column: HP-101 (Methyl Silicone Fluid)
25 m x 0.2 mm x 0.2 µm film
(HP Part No. 19091Y-102)
Carrier: $N_2$ 10 psi
Injection: 1 µL 1-3 ppm each component
Detector: ECD

## Priority Pollutants



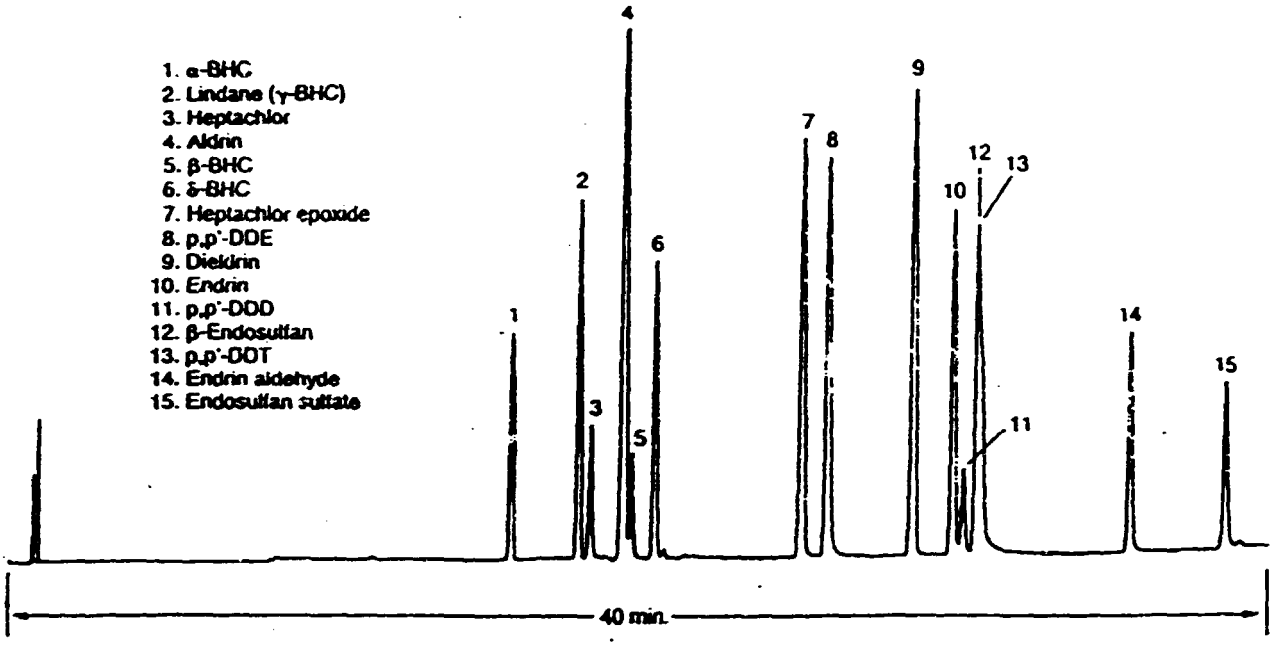

Column: HP-5 (Cross-Linked 5% Phenyl Silicone)
10 m x 0.2 mm x 0.34 µm film
(HP Part No. 19091S-003)
Carrier: Helium, 30 psi
Injection: [illegible]
Detector: Mass Selective

# APPENDIX 6

## EPA METHOD 608 - ORGANOCHLORINE PESTICIDES

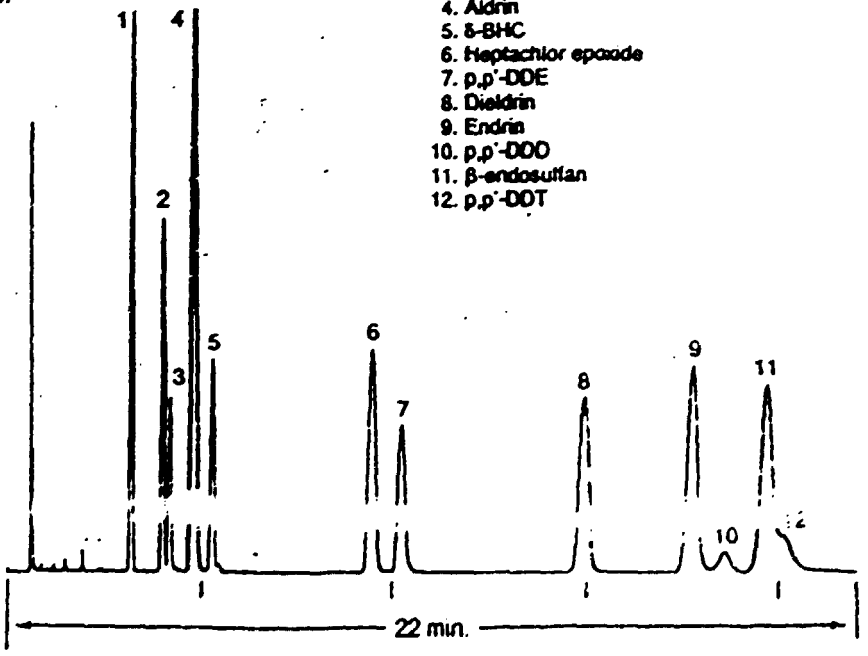
Column: DB-210
15m X 0.53mm I.D.
Film Thickness: 1.0 micron
Carrier: Helium at 11 ml/min.
Oven: 100°C to 220°C at 3°/min.
Injection: Direct, 1µl of approx. 600pg of each pesticide in iso-octane
Detector: Varian 3400 E.C.D.

1. α-BHC
2. Lindane (γ-BHC)
3. Heptachlor
4. Aldrin
5. β-BHC
6. δ-BHC
7. Heptachlor epoxide
8. p,p'-DDE
9. Dieldrin
10. Endrin
11. p,p'-DDD
12. β-Endosulfan
13. p,p'-DDT
14. Endrin aldehyde
15. Endosulfan sulfate




Column: DB-210
30m X 0.53mm I.D.
Film Thickness: 1.0 micron
Carrier: Helium at 10 ml/min.
Oven: Isothermal at 170°C
Injection: Direct, 1µl of approx. 600 pg/µl of each pesticide in iso-octane
Detector: Varian 3400 E.C.D.

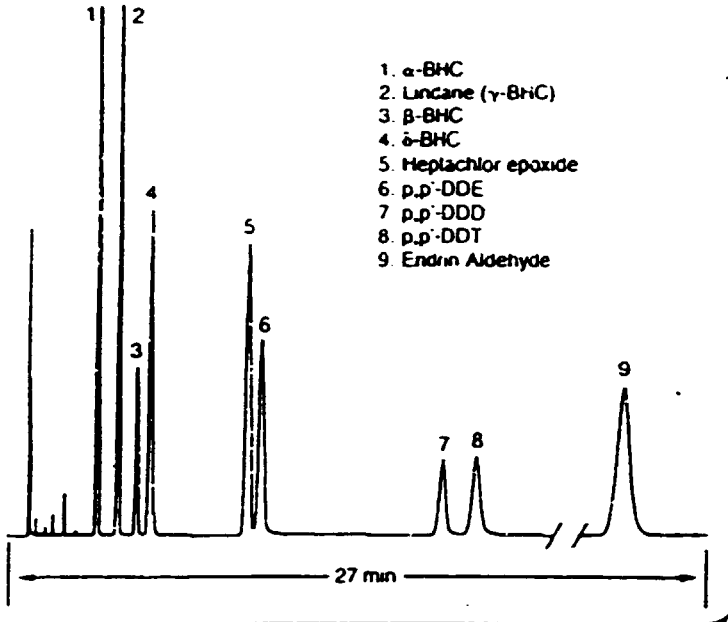
1. α-BHC
2. Lindane (γ-BHC)
3. Heptachlor
4. Aldrin
5. δ-BHC
6. Heptachlor epoxide
7. p,p'-DDE
8. Dieldrin
9. Endrin
10. p,p'-DDD
11. β-endosulfan
12. p,p'-DDT



APPENDIX 6

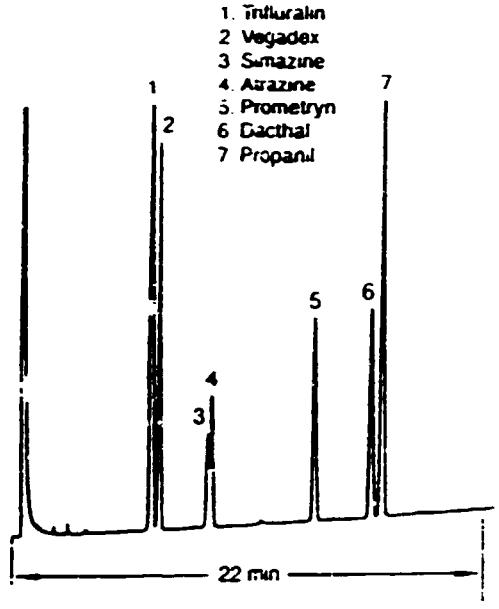
## EPA METHOD 608 - ORGANOCHLORINE PESTICIDES

| | |
|---|---|
| Column: | DB-210<br>30m X 0.53mm I.D |
| Film Thickness: | 1.0 micron |
| Carrier: | Helium at 10 ml/min. |
| Oven: | Isothermal at 180°C |
| Injection: | Direct, 1µl of approx. 600pg of each pesticide in i-octane |
| Detector: | Varian 3400 E.C.D |



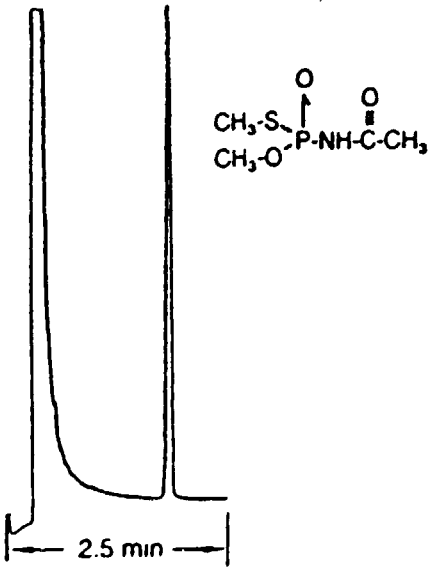

## HERBICIDE

| | |
|---|---|
| Column | DB-1301<br>30m X 0.53mm I.D.<br>1.0 micron film thickness |
| Carrier: | Helium @ 11 mL/min |
| Oven: | 160°C/1 min<br>3°C min to 225°C |
| Injection: | 1µL direct injection |
| Detector. | F.I.D. |




## ACEPHATE

| | |
|---|---|
| Column: | DB-1<br>15m X 0.53mm I.D. |
| Carrier. | Helium at 15 mL/min |
| Oven: | Isothermal 200°C |
| Injection: | 0.1µL of 1µg/µL Hot on-column (150°C injection port temp.) |
| Detector: | F.I.D. |





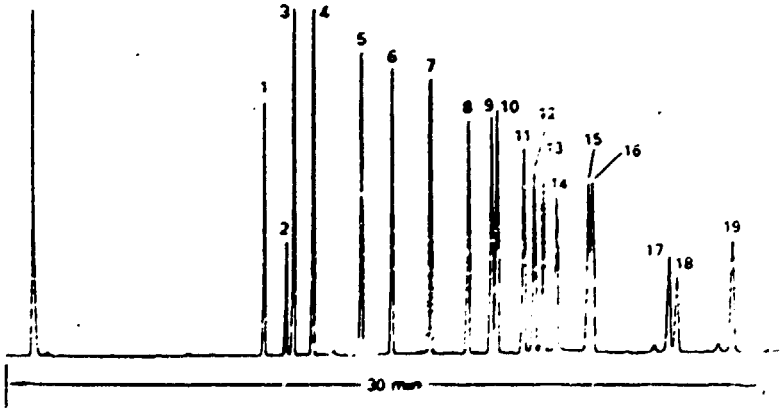


## EPA METHOD 608 – PESTICIDES

200 pg/component

| | |
|---|---|
| Column | DB-5<br>30m X 0.53mm I.D. |
| Carrier | Helium @ 6 mL/min |
| Oven: | 150°C to 225°C @ 5°/min, 225°C to 260°C @ 2°/min |
| Injection: | 1µL Direct flash vaporization |
| Detector: | Varian 3400 E.C.D.<br>Nitrogen make-up gas @ 30 mL/min |

1. α-BHC
2. β-BHC
3. γ-BHC
4. δ-BHC
5. Heptachlor
6. Aldrin
7. Heptachlor epoxide
8. Endosulfan I
9. 4, 4'-DDE
10. Dieldrin
11. Endrin
12. Endosulfan II
13. 4, 4'-DDD
14. Endrin aldehyde
15. Endosulfan sulfate
16. 4, 4'-DDT
17. Hexabromobenzene
18. 4, 4'-Methoxychlor
19. Dibutyl chlorendate



## APPENDIX 6 A

### CONSIDERATIONS FOR PACKED COLUMN OPERATION

Either $N_2$ or Ar containing 5 or 10% $CH_4$, may be used as carrier gas. $N_2$ yields somewhat higher sensitivity, but it is accompanied by higher noise; minimum detectable limit is about the same. $N_2$ sometimes produces a negative solvent peak. $Ar/CH_4$ gives greater dynamic range.

The carrier gas must be dry and $O_2$-free. Moisture and and $O_2$ traps are strongly recommended for highest sensitivity. Because plastic tubing is permeable to many gases, use copper tubing for all connections.

Total flow into the detector must be at least 20 ml/min to prevent peak tailing.

### CONSIDERATIONS FOR CAPILLARY COLUMN OPERATION

$H_2$ carrier gas (with $N_2$ makeup gas) gives best column performance. $Ar/CH_4$ as makeup gas may also be used. For most purposes, 60 ml/min of makeup gas is satisfactory. For very fast runs this can be increased to 100 ml/min to sharpen peaks, but some sensitivity will be lost since the ECD is a concentration-dependent detector.

$H_2$ or He carrier gas affords the best column performance with reduced retention times. $Ar/CH_4$ or $N_2$ makeup gas is used in the range of 30–60 ml/min. Since the ECD is a concentration dependent detector, reduced sensitivity is obtained at higher flow rates.

Moisture and $O_2$ traps for carrier gas are essential with capillary/ECD operation.

## Temperature

Some compounds exhibit strong dependence of response to detector temperature. The effect may be either positive or negative. Different detector temperatures may be tried, always remaining above the oven temperature, to determine the effect on sensitivity. Generally a detector temperature between 250–300°C is satisfactory for most applications.

## Background Level

If the ECD system becomes contaminated, whether from impurities in the carrier (or makeup) gas, or from column or septum bleed, a significant fraction of detector dynamic range may be lost. In addition, the output signal becomes noisy.

To check background level, allow ample time for components from previous analyses to be flushed from the system, and then make a blank run (one with no sample injected).

# APPENDIX 6A

SOLVENT EFFECT (see Ref.Man Vol.I/Sect 10.16)

This effect requires vaporised sample components to be retained by a region of stationary phase and absorbed solvent at the head of the column.

In this region,the front of the sample "plug" undergoes stronger retention than does the rear.Solvent saturated stationary phase acts as a barrier to sample components thereby reducing their bandwidths.

(Refs. Grob,K and Grob,Jr.,K., Journal of Chromatography,p53(1974) and Grob,K. and Grob,g.,Chromatographia,5,p3(1972))

To use this effect oven temperatures must be low enough for the solvent to remain at the head of the column for a sufficenty long period.

A good guide is to have the column 10 to30 degrees centigrade below the solvent boiling point.

| SOLVENT | BOILING POINT (Deg C) | INIT. OVEN TEMP (Deg C) |
|---|---|---|
| DIETHYL ETHER | 35 | 10 to 25 |
| n-PENTANE | 36 | 10 to 25 |
| METHYLENE CHLORIDE | 40 | 10 to 30 |
| CARBON DISULPHIDE | 46 | 10 to 35 |
| *CHLOROFORM | 61 | 25 to 50 |
| *METHANOL | 65 | 35 to 55 |
| n-HEXANE | 69 | 40 to 60 |
| *ETHYL ACETATE | 77 | 45 to 65 |
| ACETONITRILE | 82 | 50 to 70 |
| n-HEPTANE | 98 | 70 to 90 |
| i-OCTANE | 99 | 70 to 90 |
| TOLUENE | 111 | 80 to 100 |

*SHOULD ONLY BE USED WITH CROSS-LINKED STATIONARY PHASES.

APPENDIX 6 A

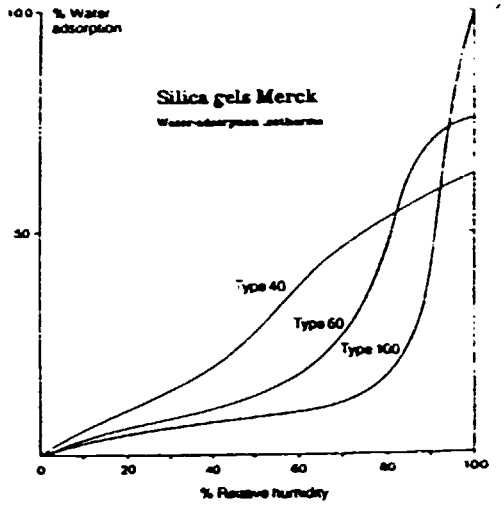
# Physical Constants*

| SOLVENT | BOILING PT (°C) | SPECIFIC GRAVITY | HEAT OF VAPOR-IZATION (kcal) | CONSTANT B |
|---|---|---|---|---|
| Dioxide | 111 | 1.034 | 98 | 0.195 |
| Pentane | 37 | 0.626 | 85 | 0.200 |
| Hexane | 71 | 0.659 | 85 | 0.200 |
| Heptane | 98 | 0.680 | 74 | 0.200 |
| Benzene | 80 | 0.879 | 93 | 0.195 |
| Toluene | 111 | 0.867 | 86 | 0.195 |
| Xylene | 137/140 | 0.860 | 82 | 0.195 |
| Methylene chloride | 40 | 1.325 | 41 | 0.200 |
| Chloroform | 61 | 1.480 | 59 | 0.200 |
| Carbon tetrachloride | 77 | 1.594 | 45 | 0.200 |
| Acethylene chloride | 85 | 1.256 | 78 | 0.200 |
| Dichloroethylene | 55 | 1.250 | 41 | 0.200 |
| Trichloroethylene | 87 | 1.462 | 57 | 0.200 |
| Acetylene tetrachloride | 147 | 1.602 | 53 | 0.200 |
| Tetrachloroethylene | 121 | 1.624 | 50 | 0.200 |
| Pentachloroethane | 159 | 1.700 | 45 | 0.200 |
| Methyl alcohol | 65 | 0.792 | 267 | 0.160 |
| Ethyl alcohol | 78 | 0.791 | 205 | 0.160 |
| Propyl alcohol | 97 | 0.804 | 165 | 0.160 |
| Isopropyl alcohol | 82 | 0.785 | 160 | 0.158 |
| Butyl alcohol | 118 | 0.810 | 120 | 0.160 |
| Iso-butyl alcohol | 108 | 0.803 | 120 | 0.160 |
| Iso-amyl alcohol | 132 | 0.800 | 120 | 0.160 |
| Diethylether | 35 | 0.714 | 90 | 0.195 |
| Acetone | 56 | 0.791 | 125 | 0.195 |
| Cyclohexane | 81 | 0.779 | - | 0.200 |
| Nitrobenzene | 211 | 1.204 | - | 0.190 |
| Ethyl acetate | 77 | 0.900 | 102 | 0.190 |
| Methylethyl Ketone | 80 | 0.810 | 113 | 0.206 |
| N-Amyl Alcohol | 137 | 0.814 | 142 | 0.158 |
| Tert. Butanol | 83 | 0.787 | 141 | 0.154 |
| Chlorobenzene | 132 | 1.110 | 90 | 0.202 |
| 1.2 Dichloroethane | 83 | 1.235 | 126 | 0.201 |
| Dipropylether | 68 | 0.726 | 76 | 0.207 |
| 1,1,1, Trichloroethane | 74 | 1.346 | 76 | 0.205 |
| 1,1,2,2, Tetrachloroethane | 131 | 1.603 | 60 | 0.198 |

*Reference Handbook of Chemistry & Physics, 65th Edition, 1984-85, CRC Press.

APPENDIX 7

In the following diagram the water adsorption of the Silica gel types 40, 60 and 100 after drying at 200° C (3 h) are represented in percents by weight at increasing relative humidity.




The respective higher activity of the silica gel with the respective smaller pore diameter but larger surface applies exactly only for relative humidities of 0% and of 20%. At very high relative humidity of 80% a complete reversal of the levels of activities is detectable, occasioned by the percentually higher water loading of the silica gel with the respectively smaller pore diameter. In the region of 40% and 60% relative humidity, overlappings of the activity conditions occur, induced by the interaction of partially or completely hydratized silanol groups.

Between the relative humidities of 0% to 80% the activity span is

in the case of Silica gel 40, large
in the case of Silica gel 60, medium
in the case of Silica gel 100, small.

All three silica gel types, Silica gel 40, 60 and 100, are markedly distinct from one another in their pore structures; they hence also differ in respect of their binding tendencies to polar and non-polar substance groups. Each of these

The diagram of water absorption of the silica gel plates.

## APPENDIX 8

Mycotoxins formed by different fungi and their association with foodstuffs

| Toxin | Organism | Foodstuffs affected | Some toxic effects |
|---|---|---|---|
| 1. Aflatoxins* | *Aspergillus flavus* *A. parasiticus* | Groundnuts and products, rice, maize, other nuts and seeds, cottonseeds, cocoanut, wheat, tree nuts, milk, cheese | Liver and kidney carcinoma, bileduct proliferation, fatty infiltration of liver of animals |
| 2. Sterigmatocystin* | *A. versicolor* | Cereals | Hepatoma in rats |
| 3. Ochratoxins* | *A. ochraceus* | Cereals | Liver and kidney pathology of rats |
| 4. Aspergillic acid | *A. flavus* | Cereals | Antimicrobial and toxic to mice |
| 5. Kojic acid* | *A. flavus* and spp. of *Aspergillus* | Cereals | Antimicrobial and mammalian toxicity |
| 6. beta-nitropropanoic acid | *A. flavus* | Cereals | Toxic to man and animals |
| 7. Tremorgenic toxin | *A. flavus* | Maize and other foodstuffs | Sustained trembling in mice |
| 8. Luteoskyrin | *Penicillium islandicum* | Rice | Liver toxicity Hepatoma |
| 9. Rugulosin | *P. rugulosum* | Rice | Nephrosis and liver damage |
| 10. Chlorine-containing peptide | *P. islandicum* | Rice | Hepatotoxin hepatoma of animals |
| 11. Islanditoxin | *P. islandicum* | Rice | |

* Mycotoxins detected as natural contaminants

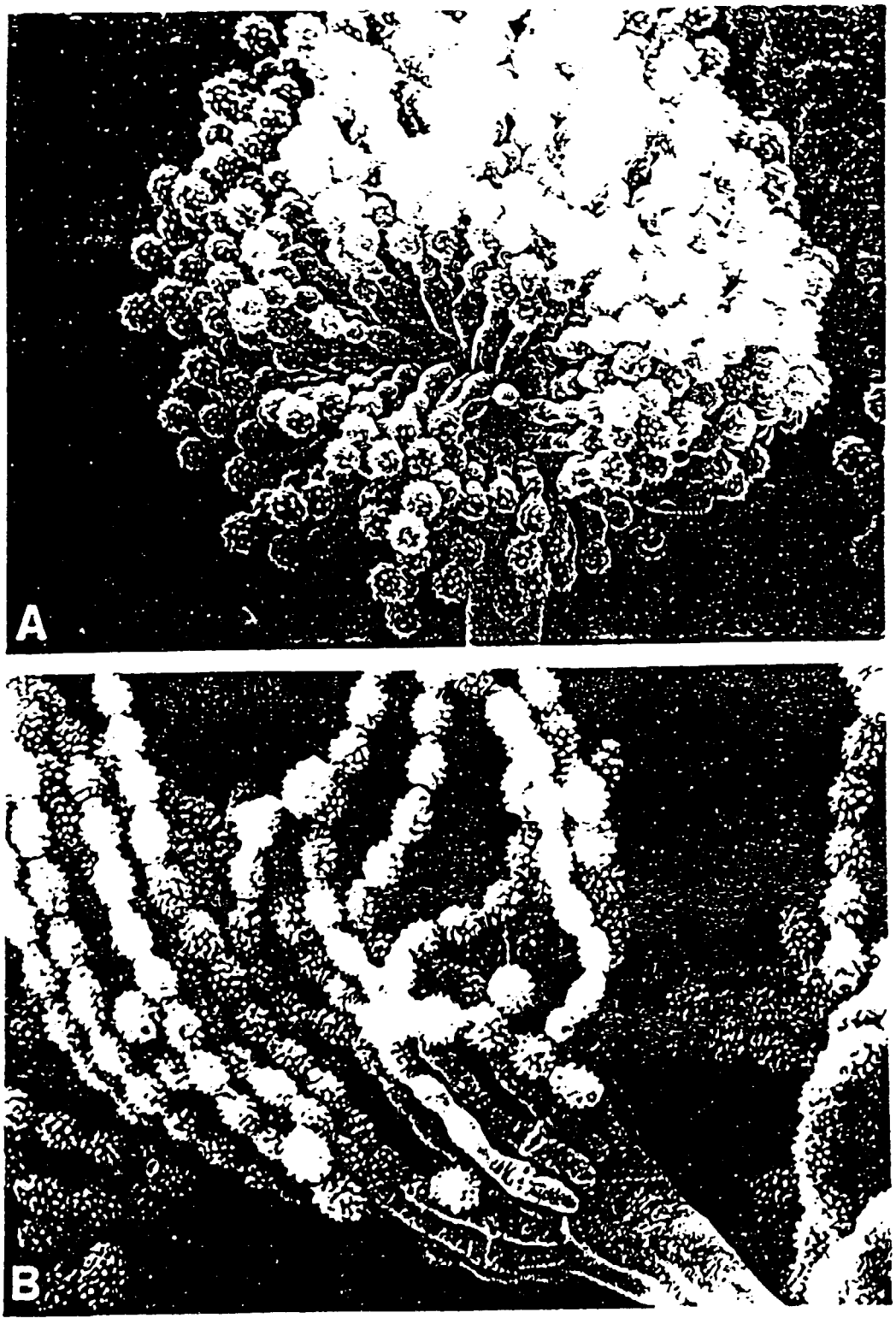
APPENDIX 8

| Toxin | Organism | Foodstuffs affected | Some toxic effects |
|---|---|---|---|
| 12. Citrinin* | P. citrinum | Rice | Automicrobial nephropathy of animals |
| 13. Citreoviridin | P. citreo-viride and other Penicillium spp. | Rice | Mammalian paralysis |
| 14. Rubratoxins | P. rubrum | Maize | Fatty infiltration of liver of rats |
| 15. Patulin* | P. expansum<br>A. clavatus | Apple Rice Feeds | Antimicrobial phytotoxic, carcinogenic in rats |
| 16. Penicillic acid* | P. puberulum<br>P. cyclopium | Maize | Antimicrobial carcinogenic in rats |
| 17. Cyclopiazonic acid | P. cyclopium | Food and foodstuff | Convulsions in rats. Severe lesions in spleen and kidney |
| 18. Psoralens | Sclerotinia Sclerotiorum | Celery plant | Toxic to man and animals dermatitis |
| 19. Stachybotrys toxin | Stachybotrys atra<br>S. alternans | Straw | Farm and other animals and man. Stachybotryotoxicosis (Dermal Toxicity) |
| 20. ATA toxin | Fusarium sporotrichioides | Oats, Wheats, Barley | Alimentary toxic Aleukia of animals and man (blood dyserasia) |
| 21. Diacetoxy-scirpenol* | F. scirpi<br>F. trioinotum | Wheat, Oats, Rye, Maize | Skin necrosis and eye damage in rats |
| 22. T-2 toxin* | F. trioinotum<br>F. nivale | Cereals, Maize, Fescue grass | Necrosis of the epidermal tissue of rats "Fescue Food of Cattle" |
| 23. Nivalenol*<br>Deoxynivalenol* | F. nivale | Rice | Inhibition of DNA synthesis |

APPENDIX B

| Toxin | Organism | Foodstuffs affected | Some toxic effects |
|---|---|---|---|
| 24. Fusarenone | F. nivale | Rice and cereals | Inhibition of protein synthesis in mice |
| 25. Butenolide | F. nivale | Maize, Fescue, Cereals, Hay | Fescue foot in cattle and tail necrosis |
| 26. Zearalenone* | F. graminearum | Maize, Hay, Barley, Feed | Hyper-estrogenic in animals |
| 27. Sporidesmin* | Pithomyces chartarum | Pasture | Facial eczema of animals |
| 28. Rhizoctonia toxin | Rhizoctonia leguminicola | Hay, Red Clover | Slobbering in cattle and horses |
| 29. Ergot | Claviceps spp. | | Gangrene |

APPENDIX 8




Scanning electron microphotos of two specias A-Aspergillus and B-Penicillium.

# APPENDIX 9

## SECONDARY METABOLITES (MYCOTOXINS) PRODUCED BY FUNGI COLONIZING CEREAL GRAIN IN STORAGE — STRUCTURE AND PROPERTIES

Piotr Goliński

Secondary metabolites, which are formed by fungi and naturally occur in food and feed products are well known in the literature. However information about toxic secondary metabolites called mycotoxins was relatively obscure until the discovery of aflatoxins. Since early sixties, growing interest in mycotoxins and mycotoxicoses caused by them, increased the number of publications in this field, raised issues of food hygiene and today represents a public health problem.

Toxic fungal secondary metabolites are relatively small molecules (MW lower than 700), formed by different fungi. They are often genotypically specific for group of species, species or subspecies of fungi belonging to the same genus, but the same compound can also be formed by fungi belonging to different genera.

Taking into consideration the fact that the majority of secondary metabolites are synthetized on the base of small molecules (acetate, pyruvate, malonate etc) and only a few types of reactions (condensation, methylation, reduction-oxidation and halogenation) are involved in their biosynthesis, we can conclude that diversity of mycotoxins molecular structures are spectacular. On the other hand, groups of fungal metabolites (e.g. roquefortines) with identical molecular formula and chemical structure - being stereoisomers only - are known.

Fungi of genera *Aspergillus* and *Penicillium*, the most potent mycotoxin producers, are well known saprophytes and are recognized as grains deteriorating microorganisms which cause storage problems in grains, feeds, foodstuffs and feedstuffs. Up to now several their metabolites only (e.g. aflatoxins, ochratoxin, penicillic acid, citrinin) were recognized as natural contaminants of cereals and two of them - aflatoxin $B_1$ in maize and ochratoxin A in small cereal grains - were reported as frequently occurring toxic contaminants. Other compounds were formed by fungi of genus *Aspergillus* and/or *Penicillium* under laboratory conditions and can be considered rather as potential contaminants.

Metabolites of *Aspergillus* and *Penicillium* elicit various physiological and toxicological responses in animals, bacteria, moulds and plants. However taking into consideration fact that they are mostly grain contaminants, their toxicity to animals is most important in comparison to antibacterial or phytotoxic activity.

This chapter will list toxic metabolites with their structure, physicochemical data (MW, structure of crystals, m.p., UV, fluorescence), toxicity and fungal source. In addition, several other compounds with unknown or not reported toxicity were included on the base of their chemical structure resemblance to toxic metabolites.

The chapter was prepared similarly to the way it was done in the former publication R.F. Vesonder and P. Golinski "*Fusarium* metabolites" *In*: J. Chelkowski (Ed.), 1989, *Fusarium*: Mycotoxins, Taxonomy and Pathogenicity, Elsevier, Amsterdam - Oxford - New York - Tokyo. It was rather impossible to divide chapter into two parts devoted to metabolites of *Aspergillus* and *Penicillium* separately because 95 compounds are formed by fungi of genus *Aspergillus*, and 40 by fungi of genus *Penicillium* while 21 by fungi belonging to both genera *Aspergillus* and *Penicillium*.

Metabolites are listed in alphabetical order with exceptions only when groups of compounds exhibit similar - presented on the same drawing - chemical structure.

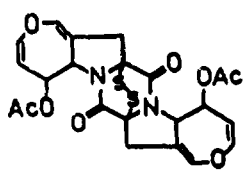
Cited in this chapter literature represents key references only and preparation of this chapter was greatly aided by use of General References - several reviews and books:


1. V. Betina, 1989, Mycotoxins - Chemical, Biological and Environmental Aspects, Elsevier, Amsterdam - Oxford - New York
2. A. Ciegler, S. Kadis and S.J. Ajl (Eds), 1971, Microbial Toxins, vol.6, Academic Press, New York
3. A. Ciegler and R.F. Vesonder, *In*: A.I. Laskin and H.A. Lechevalier (Eds), 1987, CRC Handbook of Microbiology, vol. 8, CRC Press, Boca Raton, Fl, pp 19-126
4. R.J. Cole and R.H. Cox (Eds), 1981, Handbook of Toxic Fungal Metabolites, Academic Press, New York
5. I.F.H. Purchase (Ed.), 1974, Mycotoxins, Elsevier Scientific Publishing Company, Amsterdam - Oxford - New York
6. J.V. Rodricks (Ed.), 1976, Mycotoxins and Other Fungal Related Food Problems, Advances in Chemistry, Series 149, American Chemical Society, Washington D.C.
7. W.B. Turner, 1971, Fungal Metabolites, Academic Press, New York
8. W.B. Turner and D.C. Aldridge, 1983, Fungal metabolites II, Academic Press, New York
9. T.D. Wyllie and L.G. Morehouse, 1977, Mycotoxic Fungi, Mycotoxins, Mycotoxicoses, vols. 1,2,3, Marcel Dekker, New York.


In this chapter the following abbreviations were used:

MW - molecular weight
m.p. - melting point
d - decomposition
aq - aqueous
$\lambda_{max}$ nm (lg $\varepsilon$) - maximum (nm) of UV/VIS absorption with lg of molecular absorption coefficient
sh - shoulder
$\lambda^{Ex}_{max}$ - maximum (nm) of fluorescence excitation
$\lambda^{Em}_{max}$ - maximum (nm) of fluorescence emission
$LD_{50}$ - sublethal dose
$LD_{100}$ - lethal dose

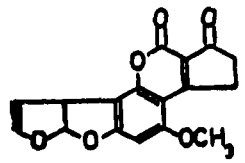
1. ACETYLARANOTIN (LL-S888α), $C_{20}H_{20}O_8N_2S_2$, MW 504, yellow tetragonal bipyramidal crystals, m.p. 215-230°C (d)(from ethanol), $\lambda^{MeOH}_{max}$ nm (lg $\varepsilon$): end absorption with shoulders 270 (3.355) and 225 (4.009). Toxicity: antiviral activity. Fungal source: *Aspergillus terreus* (NRRL 3319).



Reference: [114]

15-ACETOXYVERRUCULOGEN see 55

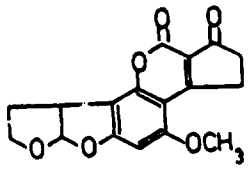
2. AFLATOXIN $B_1$, $C_{17}H_{12}O_6$, MW 312, m.p. 268-269°C (d) (from chloroform), $\lambda^{EtOH}_{max}$ nm (lg $\varepsilon$): 223 (4.408), 265 (4.127) and 362(4.338); bright blue fluorescence under UV 360 nm light $\lambda^{Ex}_{max}$ 332 nm; $\lambda^{Em}_{max}$ 425 nm. Toxicity: highly toxic, acute and chronic toxicity, carcinogenic, hepatotoxic and teratogenic to most animals. Fungal source: *Aspergillus flavus* Link, *A. parasiticus* Speare.



References: [8,27,85,94]

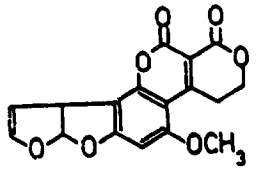
3. AFLATOXIN $B_2$, $C_{17}H_{14}O_6$, MW 314, m.p. 286-289°C (d) (from chloroform-pentane), $\lambda^{MeOH}_{max}$ nm (lg $\varepsilon$): 220 (4.311), 240 (4.187 sh), 265 (4.104) and 363 (4.380); bright blue fluorescence under UV 360 nm

light, $\lambda^{Ex}_{max}$ 365 nm, $\lambda^{Em}_{max}$ 425 nm. Toxicity: less toxic than aflatoxin $B_1$ however the toxic properties of both aflatoxins were similar. Fungal source: *Aspergillus flavus* Link, *A. parasiticus* Speare.



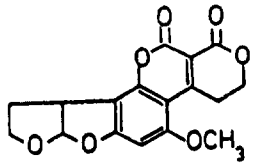
References: [37,110]

4. AFLATOXIN $G_1$. $C_{17}H_{12}O_7$. MW 328, m.p. 244-246°C (d)(from chloroform), $\lambda^{EtOH}_{max}$ nm (lg ε): 243 (4.061), 257 (3.996), 264 (4.000) and 362(4.207); blue-green fluorescence under UV 360 nm light: $\lambda^{Ex}_{max}$ 365 nm; $\lambda^{Em}_{max}$ 450 nm. Toxicity: in between aflatoxins $B_1$ and $B_2$. $LD_{50}$ in ducklings was 0.78 mg/kg. Fungal source: *Aspergillus flavus* Link, *A. parasiticus* Speare.



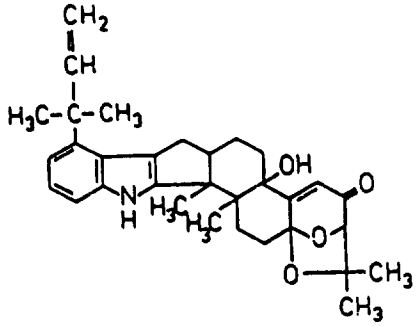
References: [9,28,34,110]

5. AFLATOXIN $G_2$. $C_{17}H_{14}O_7$. MW 328, m.p. 230°C (from ethyl acetate), $\lambda_{max}$ nm (lg ε): 217 (4.447), 245 (4.111), 265 (4.049) and 365 (4.286); blue-green fluorescence under UV 360 nm light: $\lambda^{Ex}_{max}$ 365 nm, $\lambda^{Em}_{max}$ 450 nm. Toxicity: less toxic than aflatoxin $G_1$. $LD_{50}$ in duckling was 3.45 mg/kg. Fungal source: *Aspergillus flavus* Link, *A. parasiticus* Speare.



References: [9,28,34,110]

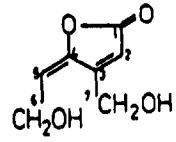
6. AFLATREM (α,α-Dimethylallyl paspalinine). $C_{32}H_{39}O_4N$.



MW 501, colorless needles, m.p. 222-224°C, $\lambda^{EtOH}_{max}$ nm (lg ε): 231 (4.443), 282 (3.957) and 292 (3.895 sh). Toxicity: tremorgenic to mice, guinea pigs and rats when dosed i.p. or orally at level 1 mg of compound per animal. Fungal source: *Aspergillus flavus*.
References: [69,177]

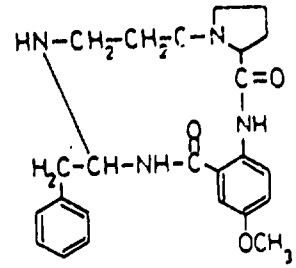
AGROCLAVINE see 45

7. ASCLADIOL. $C_7H_8O_4$. MW 156, m.p.65-66°C (from chloroform--acetone), $\lambda^{EtOH}_{max}$ 271 nm (lg ε not reported). Toxicity: less toxic than patulin. Fungal source: *Aspergillus clavatus*.



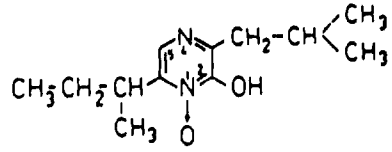
Reference: [159]

8. ASPERCOLORIN. $C_{25}H_{28}O_5N_4$. MW 464, $\lambda^{EtOH}_{max}$ nm (lg ε): 210 (4.448), 226 (4.277), 260 (4.134) and 315 (3.620). Toxicity: not reported. Fungal source: *Aspergillus versicolor*.
Reference: [10]



9. ASPERGILLIC ACID ([3-sec--Butyl-6-isobutyl-2-hydroxy--2(1H)-pyrazinone]). $C_{12}H_{20}O_2N_2$. MW 224, yellow rods, m.p. 97-99°C (from methanol), $\lambda^{EtOH}_{max}$ nm (lg ε) 328 (3.929) and 235 (4.021) Toxicity: $LD_{100}$ in mice was 150 mg/kg (i.p.) - acutely toxic to mice, antibiotic activity. Fungal source: *Aspergillus flavus* Link.



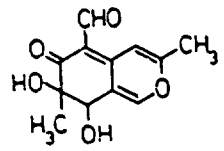


References: [60,65]

ASPERTOXIN see 115

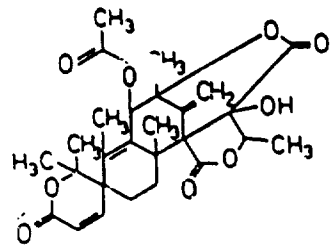
10. AUSTDIOL ([(7R,8S)-8-Hydro--7,8-dihydroxy-3,7-dimethyl--6-oxo-6H-2-benzopyran-5--carbaldehyde]) $C_{12}H_{12}O_5$ MW 236, yellow needles, m.p

255°C (d) (from chloroform), $\lambda_{max}^{EtOH}$ nm (lg ε): 256 (4.179) and 376 (4.380). Toxicity: gastrointestinal toxicoses in ducklings. Fungal source: *Aspergillus ustus* (Bainier) Thom and Church C.S.I.R., 1128; NRRL 5856.



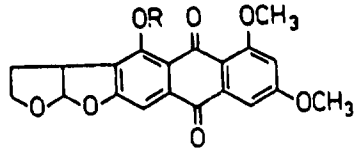
References: [154,170]

11. AUSTIN. $C_{27}H_{32}O_9$, MW 500, m.p. 298-300°C. $\lambda_{max}^{EtOH}$ nm (lg ε): 243 (4.076). Toxicity: $LD_{50}$ 250-375 mg/kg (orally) to day-old cockerels. Fungal source: *Aspergillus ustus*.



Reference: [38]

12. AVERSIN. $C_{20}H_{16}O_7$ (structure 1), MW 368, slender golden needles, m.p. 217°C. $\lambda_{max}$ nm (lg ε): 224 (4.565), 251 (4.127), 285 (4.526), 313 (3.949), 363 (3.695) and 440 (3.894). Toxicity: not reported. Fungal source: *Aspergillus versicolor* (Vuill.) Tiraboschi.



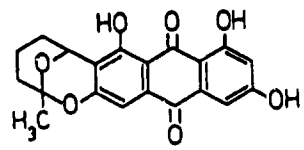
1: R=H
2: R=$CH_3$

Reference: [24]

13. O-METHYLAVERSIN (Tri-O-methylversicolorin B). $C_{21}H_{18}O_7$ (structure 2 above), MW 382, fine golden-yellow needles, m.p. 216-217°C. $\lambda_{max}^{EtOH}$ nm (lg ε): 222 (4.529), 285 (4.593), 348 (3.672) and 407 (3.623). Toxicity: not reported. Fungal source: *Aspergillus versicolor* (Vuill.) Tiraboschi.

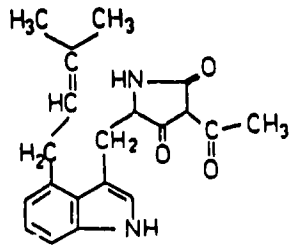
References: [24,87]

14. AVERUFIN. $C_{20}H_{16}O_7$, MW 368, bright orange-red lathe, m.p. 280-282°C (d)(from acetone), $\lambda_{max}^{EtOH}$ nm (lg ε): 223 (4.519), 256 (4.217 sh), 265 (4.267), 286 (4.394 sh), 294 (4.489), 319 (4.097) and 454 (4.021). Toxicity: not reported. Fungal source: *Aspergillus versicolor*, *A. flavus*, *A. parasiticus*, *A. ustus*.



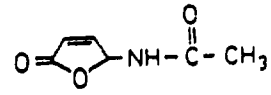
Reference: [61]

15. BISSECODEHYDROCYCLOPIAZONIC ACID. $C_{20}H_{22}O_3N_2$, MW 338, m.p. 168-169°C, $\lambda_{max}$ nm (lg ε): 225 (4.550), 276 (4.280 sh) and 296(4.070 sh). Toxicity: weak, much lower than cyclopiazonic acid. Fungal source: *Penicillium cyclopium* Westling, *Aspergillus versicolor* (Vuill.) Tiraboschi.



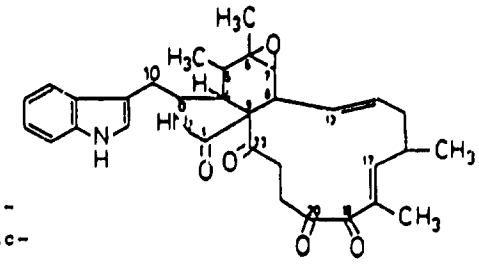
References: [86,126]

16. BUTENOLIDE (4-Acetamido-4-hydroxy-2-butenoicacid γ-lactone). $C_6H_7O_3N$, MW 141, m.p. 115°C (from ethyl acetate), $\lambda_{max}^{MeOH}$ nm (lg ε): 202 (4.053). Toxicity: toxic to cattle - "fescue foot" agent. Fungal source: *Aspergillus terreus*, produced also by *Fusarium sporotrichioides*.



Reference: [129]

17. CHAETOGLOBOSIN C (6,7-Epoxy--10-(indol-3-yl)-5,6,16,18-tetramethyl-[13]cytochalas-13,17-diene-19,20,23-trione). $C_{32}H_{36}O_5N_2$, MW 528, colorless leaflets, m.p. 260--263°C (from methanol), $\lambda_{max}^{EtOH}$ nm (lg ε): 222 (4.560), 273 (3.826), 281 (3.826) and 291 (3.756). Toxicity: crude extract highly toxic, cytotoxic to He-La cells. Fungal source: *Penicillium aurantio-virens* (identified as *P. echinulatum*), produced also by *Chaetomium*.



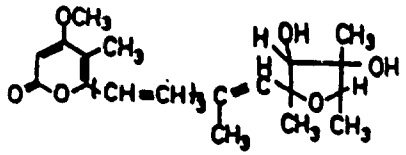
References: [125,150]

CHAETOMIDIN see 93

8-CHLORORUGULOVASINE A see 108

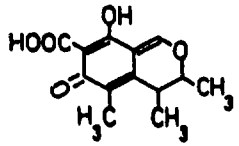
8-CHLORORUGULOVASINE B see 109

18. CITREOVIRIDIN. $C_{23}H_{30}O_6$, MW 402, yellow crystals, m.p. 107-111°C (from methanol), $\lambda_{max}^{EtOH}$ nm (lg ε): 388 (4.681), 294 (4.433), 286 (4.391 sh), 234 (4.009) and 204 (4.230), bright yellow fluorescence under UV 360 nm light. Toxicity: rat and mice neurotoxic agent; paralysis, respiratory failure, a dose 20 mg/kg (s.c.) was lethal to mice. Fungal source: *Penicillium toxicarium* Miyake, *P. ochrosalmoneum* Udagawa.



References: [140,166]

19. CITRININ [(3R-trans)-4,6-Dihydro-8-hydroxy-3,4,5-trimethyl-6-oxo-3H-2-benzopyran-7-carboxylic acid]. $C_{13}H_{14}O_5$, MW 250, lemon-yellow crystals, m.p. 175°C (d) (from ethanol), $\lambda_{max}^{EtOH}$ nm (lg ε): 222 (4.348), 253 (3.919) and 319 (3.673); yellow fluorescence under UV 360 nm light, $\lambda_{max}^{Em}$ 504 nm. Toxicity: antibiotic and nephrotoxic properties. $LD_{50}$ in rats was 67 mg/kg (s.c.) and in mice 35 mg/kg. Fungal source: *Penicillium citrinum* Thom, *P. citreo-viride*, *P. expansum*, *P. jensenii*, *P. viridicatum*, *Aspergillus terreus* Thom, *A. niveus*, *A. candidus* (both identified as *A. carneus*).

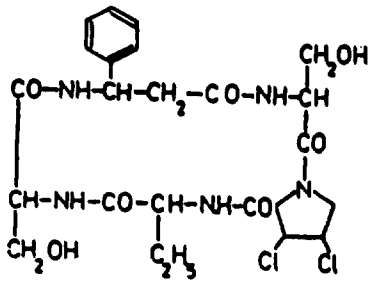


References: [4,21,22,139]

CRYPTOCHINULIN A see 33

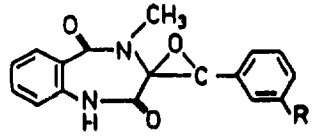
CRYPTOCHINULIN G see 34

20. CYCLOCHLOROTINE. $C_{24}H_{31}O_7N_3Cl_2$, MW 571, white needles, m.p. 255°C (d) (from methanol),



$\lambda_{max}$ nm (lg ε): 257 (2.362). Toxicity: toxic to mice, $LD_{50}$ in mg/kg was: 0.3, 0.5 and 6.6 i.p., s.c. and p.o. respectively.
Fungal source: *Penicillium islandicum* Sopp.
References: [67,93,162]

21. CYCLOPENIN (trans-3,10-Epoxycyclo[anthranoyl-N-methylphenylalanyl]). $C_{17}H_{14}O_3N_2$ (structure 1), MW 294, m.p. 207°C (from ethyl acetate), $\lambda_{max}^{MeOH}$ nm (lg ε): 211 (4.571) and 290 (3.314). Toxicity: antibiotic properties against *Micrococcus pyogenes* var. *aureus* and *Escherichia coli*. Fungal source: *Penicillium cyclopium* Westling, *P. viridicatum* Westling.

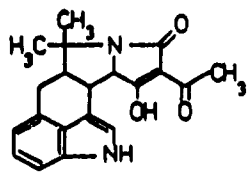


1: R = H
2: R = OH

Reference: [20]

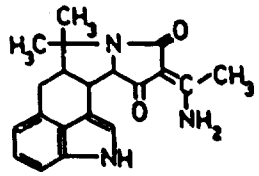
22. CYCLOPENOL (trans-3,10-Epoxycyclo[anthranoyl-N-methyl-15-hydroxylphenylalanyl]). $C_{17}H_{14}O_4N_2$ (structure 2 above), MW 310, prisms, m.p. 215°C (d) (from ethyl acetate-benzene), $\lambda_{max}^{MeOH}$ nm (lg ε): 258 (3.573). Toxicity: not reported. Fungal source: *Penicillium cyclopium* Westling (NRRL 3557), *P. viridicatum* Westling.
References: [18,107,115]

23. CYCLOPIAZONIC ACID. $C_{20}H_{20}O_3N_2$, MW 336, m.p. 245-246°C, $\lambda_{max}$ nm (lg ε): 225 (4.600), 253 (4.220), 275 (4.280 sh), 284 (4.310) and 292 (4.240 sh). Toxicity: lethal to ducklings and rats. Rats are very sensitive to the compound - $LD_{50}$ (in males) 2.3 mg/kg (i.p.) and 36 mg/kg; 63 mg/kg in males and females respectively (orally). Fungal source: *Penicillium cyclopium* Westling (NRRL 3523), *P. puberulum*, *Aspergillus versicolor* (Vuill.) Tiraboschi, *A. flavus*.



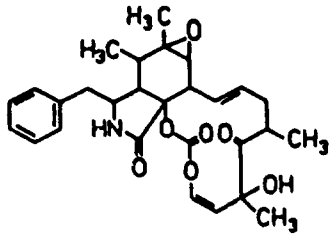
References: [86,126]

24. CYCLOPIAZONIC ACID IMINE. $C_{20}H_{21}O_2N_3$. MW 335, m.p. 277-278°C, $\lambda_{max}$ nm (lg ε): 224 (4.519), 244 (4.037 sh), 275 (4.160 sh), 286 (4.320 sh) and 293 (4.350). Toxicity: weak, much lower than cyclopiazonic acid. Fungal source: *Penicillium cyclopium* Westling, *Aspergillus versicolor* (Vuill.) Tirabo-schi.



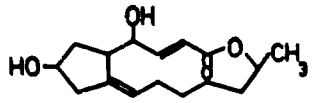
References: [86,126]

25. CYTOCHALASIN E (6,7-Epoxy--10-phenyl-5,6,16,18-tetra-methyl-21,23-dioxa-[13]-cy-tochalas-13,19-diene-17,22--dione). $C_{28}H_{33}O_7N$, MW 495, m.p. 206-208°C (d), $\lambda_{max}$ nm: 240-300 weak absorption. Toxicity: necrosis of kidney, spleen and liver, $LD_{50}$ rat 2.6 mg/kg (i.p.), 9.1. mg/kg (orally); mice 4.60 mg/kg (i.p.); guinea pig 1.5 mg/kg (i.p.). Fungal source: *Aspergillus clavatus*.
References: [29,71]



DECHLOROGRISEOFULVIN see 68

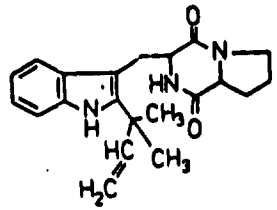
26. DECUMBIN. $C_{16}H_{24}O_4$, MW 280, m.p. 203°C (from acetone), $\lambda_{max}^{EtOH}$ nm (lg ε): 215 (4.049). Toxicity: toxic to rats, $LD_{50}$ was 275 mg/kg, in day--old cockerels $LD_{50}$ was 62 mg/kg. Antibiotic to *Candida albicans*. Fungal source: *Penicillium brefeldianum*, *P. decumbens* Thom, *P. cyaneum*.



References: [13,82]

DEHYDROGLIOTOXIN see 66

27. DEOXYBREVIANAMIDE E (L-Pro-lyl-2-(1',1'-dimethylallyl)--L-tryptophyldiketopipera-zine). $C_{21}H_{25}O_2N_3$, MW 351, $\lambda_{max}^{EtOH}$ nm (lg ε): 225 (4.509), 275 (3.845 sh), 283 (3.908) and 291 (3.845). Toxicity: tremorgenic properties not reported. Fungal source: *Aspergillus ustus* (Bainier) Thom and Church (C.S.I.R. 1128; NRRL 5856), *Penicillium italicum* Wehmer.



References: [14,154]

DEOXYNORTRYPTOQUIVALINE see 128

DEOXYNORTRYPTOQUIVALONE see 129

DEOXYTRYPTOQUIVALINE see 130

DIHYDRODEMETHYLSTERIGMATO-CYSTIN see 119

DIHYDRO-O-METHYLSTERIGMATO-CYSTIN see 120

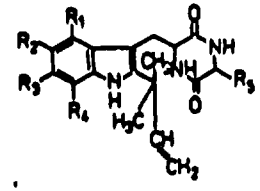
DIHYDROSTERIGMATOCYSTIN see 121

DIMETHOXYSTERIGMATOCYSTIN see 116

DIMETHYLNIDURUFIN see 82

## ECHINULINS: (28-32) AND (33-40)

28. ECHINULIN (3([2-(1,1-Dime-thylallyl)-5,7-bis(3-methyl--2-butenyl) indol-3-yl] me-thyl)-6-methyl-2,5-piperazi-nedione). $C_{29}H_{39}O_2N_3$ (struc-ture 1), MW 461, needles, m.p. 242-243°C, $\lambda_{max}^{EtOH}$ nm (lg ε): 230 (4.600), 279 (3.980) and 286 (3.960). Toxicity: tremorgenic pro-perties not reported. Fungal source: *Aspergillus amstelodami*, *A. chevalieri* (Mangin) Thom and Church, *A. echinulatus*.



1: $R_1$=H, $R_2$=$R_4$=$\begin{matrix}CH_3\\CH_3\end{matrix}$>C=CH-$CH_2$-, $R_3$=H, $R_5$=$CH_3$

2: $R_1$=H, $R_2$=$\begin{matrix}CH_3\\CH_3\end{matrix}$>C=CH-$CH_2$-, $R_3$=$R_4$=H, $R_5$=$CH_3$

3: $R_1$=H, $R_2$=H, $R_3$=H, $R_4$=H, $R_5$=$CH_3$

4: $R_1$=H, $R_2$=H, $R_3$=$\begin{matrix}CH_3\\CH_3\end{matrix}$>C=CH-$CH_2$-, $R_4$=H, $R_5$=$CH_3$

5: $R_1$=H, $R_2$=H, $R_3$=H, $R_4$=H, $R_5$=$CH_3$

References: [36,59,157]

29. ISOECHINULIN A. $C_{24}H_{29}O_2N_3$ (structure 2 above), MW 391, $\lambda_{max}^{EtOH}$ nm (lg ε): 227 (4.498),

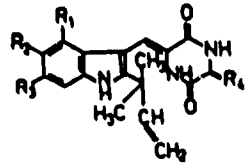
289 (3.949) and 341 (3.973). Toxicity: inhibits growth of silkworm larvae. Fungal source: *Aspergillus ruber*. Reference: [123]

30. NEOECHINULIN A. $C_{19}H_{21}O_2N_3$ (structure 3 above), MW 323, ivory crystals, m.p. 264--265°C, $\lambda_{max}^{EtOH}$ nm (lg ε): 229 (4.367), 286 (4.068), 292 (4.017) and 338 (3.978). Toxicity: tremorgenic properties not reported. Fungal source: *Aspergillus ruber*, *A. amstelodami*. Reference: [106]

31. NEOECHINULIN D. $C_{24}H_{29}O_2N_3$ (structure 4 above), MW 391, ivory crystals, m.p. 225--235°C, $\lambda_{max}^{EtOH}$ nm (lg ε): 231 (4.339), 285 (4.037), 296 (4.017) and 345 (4.037). Toxicity: unknown, tremorgenic properties not reported. Fungal source: *Aspergillus amstelodami*, *A. ruber*. Reference: [106]

32. PREECHINULIN [(+)-1-Allenyl (1,1-dimethylallyl) tryptophan anhydride]. $C_{19}H_{23}O_2N_3$ (structure 5 above), MW 325, fine white needles, m.p. 294-297°C, $\lambda_{max}^{EtOH}$ nm (lg ε): 225 (4.512), 283 (3.889) and 291 (3.845). Toxicity: unknown, tremorgenic properties not reported. Fungal source: *Aspergillus amstelodami*, *A. chevalieri*. References: [89,157]

33. CRYPTOECHINULIN A. $C_{24}H_{27}O_2N_3$ (structure 1), MW 389, yellowish crystals, m.p. 190-192°C, $\lambda_{max}^{EtOH}$ nm (lg ε): 231 (4.568), 273 (4.382), 283 (4.330 sh) and 380 (4.146). Toxicity: unknown, tremorgenic properties not reported. Fungal source: *Aspergillus amstelodami*.



1: $R_1$=H, $R_2$=H, $R_3$= $\begin{matrix}CH_3\\CH_3\end{matrix}$>C=CH-$CH_2$-, $R_4$=$CH_3$

2: $R_1$=$R_2$= $\begin{matrix}CH_3\\CH_3\end{matrix}$>C=CH-$CH_2$-, $R_3$=H, $R_4$=$CH_3$

3: $R_1$=H, $R_2$= $\begin{matrix}CH_3\\CH_3\end{matrix}$>C=CH-$CH_2$-, $R_3$=H, $R_4$=$CH_3$

4: $R_1$=H, $R_2$= $\begin{matrix}CH_3\\CH_3\end{matrix}$>C(O)-CH-$CH_2$-, $R_3$=H, $R_4$=$CH_3$

5: $R_1$=H, $R_2$=H, $R_3$= $\begin{matrix}CH_3\\CH_3\end{matrix}$>C=CH-$CH_2$-, $R_4$=O

6: $R_1$=H, $R_2$=H, $R_3$=H, $R_4$=$CH_3$

7: $R_1$=H, $R_2$=H, $R_3$= $\begin{matrix}CH_3\\CH_3\end{matrix}$>C=CH-$CH_2$-, $R_4$=$CH_3$

8: $R_1$=H, $R_2$=H, $R_3$=H, $R_4$=O

Reference: [32]

34. CRYPTOECHINULIN G. $C_{29}H_{35}O_2N_3$ (structure 2 above), MW 457. Toxicity: unknown, tremorgenic properties not reported. Fungal source: *Aspergillus ruber*. Reference: [70]

35. ISOECHINULIN B. $C_{24}H_{27}O_2N_3$ (structure 3 above), MW 389, $\lambda_{max}^{EtOH}$nm (lg ε): 228 (4.446), 272 (4.272), 285 (4.220 sh) and 370 (4.017). Toxicity: unknown, tremorgenic properties not reported. Fungal source: *Aspergillus ruber*. Reference: [124]

36. ISOECHINULIN C. $C_{24}H_{27}O_3N_3$ (structure 4 above), MW 405, $\lambda_{max}^{MeOH}$nm (lg ε): 229 (4.427), 272 (4.255), 286 (4.199 sh) and 371 (3.987). Toxicity: unknown, tremorgenic properties not reported. Fungal source: *Aspergillus ruber*. Reference: [124]

37. NEOECHINULIN. $C_{23}H_{25}O_3N_3$ (structure 5 above), MW 391, $\lambda_{max}^{EtOH}$nm (lg ε): 231 (4.509), 287 (4.117) and 420 (3.987). Toxicity: unknown, tremorgenic properties not reported. Fungal source: *Aspergillus amstelodami* (Mangin) Thom and Church. Reference: [106]

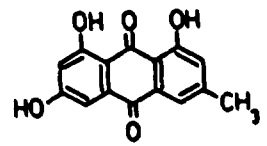
38. NEOECHINULIN B. $C_{19}H_{19}O_2N_3$ (structure 6 above), MW 321, yellow crystals, m.p. 234--236°C, $\lambda_{max}^{EtOH}$ nm (lg ε): 228 (4.449), 273 (4.279), 284 (4.258) and 374 (4.017). Toxicity: unknown, tremorgenic properties not reported. Fungal source: *Aspergillus amstelodami*. Reference: [106]

39. NEOECHINULIN C. $C_{24}H_{27}O_2N_3$ (structure 7 above), MW 389, yellow crystals, m.p. 205--207°C, $\lambda_{max}^{EtOH}$ nm (lg ε): 231 (4.539), 275 (4.348), 290 (4.270) and 380 (4.079). Toxicity: unknown, tremorgenic properties not reported. Fungal source: *Aspergillus amstelodami*. Reference: [106]

40. NEOECHINULIN E. $C_{18}H_{17}O_3N_3$ (structure 8 above), MW 323, orange-red crystals, m.p. 275°C, $\lambda_{max}^{EtOH}$ nm (lg ε): 228 (4.569), 281 (3.959) and 410 (3.908). Toxicity: unknown, tremorgenic properties not reported. Fungal source: *Aspergillus amstelodami*. Reference: [106]

41. EMODIN (1,3,8-Trihydroxy-6--methyl-antrequinone). $C_{15}H_{10}O_5$, MW 270, m.p. 256--257°C (from chloroform), $\lambda_{max}^{EtOH}$nm (lg ε): 223 (4.549), 250 (4.260), 267 (4.270), 290 (4.338) and 442 (4.097). Toxicity: toxic to cockerels, signs of anorexia, diarrhea and epidermal irritation around the cloaca.

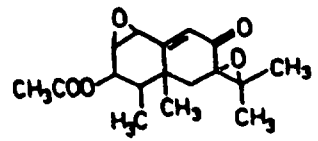
Fungal source: *Penicillium islandicum* Sopp, *P. clavariae-formis*, *P. brunneum* Udagawa, *P. avellaneum* (Thom et Turesson), *Aspergillus ochraceus*, *A. ventii* Wehmer.



References: [17,174]

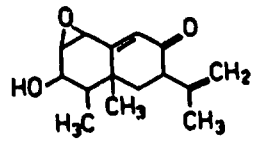
ENDOTHIANIN see 123

42. EREMOPORTIN A. $C_{17}H_{22}O_3$. MW 306, crystals, m.p. 159–161°C, $\lambda_{max}$ nm (lg ε): 246 (4.121). Nontoxic. Fungal source: *Penicillium roqueforti* (NRRL 849).

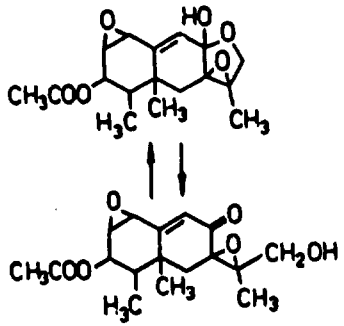


References: [116,117]

43. EREMOPORTIN B. $C_{15}H_{20}O_3$. MW 248, crystals, m.p. 121–123°C. Nontoxic. Fungal source: *Penicillium roqueforti* (NRRL 849).
Reference: [116]



44. EREMOPORTIN C. $C_{17}H_{22}O_6$. MW 322, crystals, m.p. 122–126°C (from ethyl ether), $\lambda_{max}$ nm (lg ε): 248 (4.229). Toxicity: not reported. Fungal source: *Penicillium roqueforti* (NRRL 849).



Reference: [117]

ERGOCORNINE see 46

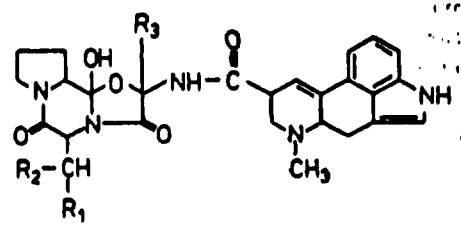
ERGOCRISTINE see 47

ERGOCRYPTINE see 48

ERGOMETRINE see 50

ERGOTAMINE see 49

ERGOT ALKALOIDS: (45–50)
Group of compounds toxic to man, sheep and cattle – vasoconstriction, uterus contraction. Fungal source: *Aspergillus fumigatus* (well known as metabolites of *Claviceps purpurea* present in ergot sclerots).



1 : $R_1 = R_2 = CH_3$, $R_3 = -CH(CH_3)_2$

2 : $R_1 = H$, $R_2 = C_6H_5$, $R_3 = -CH(CH_3)_2$

3 : $R_1 = H$, $R_2 = R_3 = -CH(CH_3)_2$

4 : $R_1 = H$, $R_2 = C_6H_5$, $R_3 = H$

References: [83,158]

45. AGROCLAVINE. $C_{16}H_{20}N_2$. MW 240, m.p. 198–203°C (d), $\lambda_{max}$ nm (lg ε): 225 (4.470), 284 (3.866) and 293 (3.81); $\lambda^{Ex}_{max}$ 280 nm, $\lambda^{Em}_{max}$ 350 nm (water) and $\lambda^{Ex}_{max}$ 285 nm, $\lambda^{Em}_{max}$ 345 nm (EtOH).

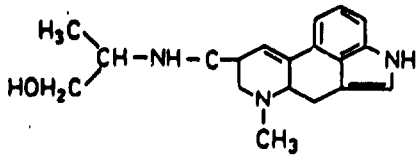
46. ERGOCORNINE. $C_{31}H_{39}O_5N_5$ (structure 1 above), MW 561, m.p. 182–184°C, $\lambda^{Ex}_{max}$ 320 nm, $\lambda^{Em}_{max}$ 435 nm (water) and $\lambda^{Ex}_{max}$ 330 nm, $\lambda^{Em}_{max}$ 420 nm (EtOH).

47. ERGOCRISTINE. $C_{35}H_{39}O_5N_5$ (structure 2 above), MW 609, m.p. 155–157°C, $\lambda^{Ex}_{max}$ 320 nm, $\lambda^{Em}_{max}$ 435 nm (water) and $\lambda^{Ex}_{max}$ 325 nm, $\lambda^{Em}_{max}$ 415 nm (EtOH).

48. ERGOCRYPTINE. $C_{32}H_{41}O_5N_5$ (structure 3 above), MW 575, m.p. 212°C (d), $\lambda_{max}$ nm (lg ε): 312(3.930); $\lambda^{Ex}_{max}$ 320 nm, $\lambda^{Em}_{max}$ 435 nm (water) and $\lambda^{Ex}_{max}$ 325 nm, $\lambda^{Em}_{max}$ 420 nm (EtOH).

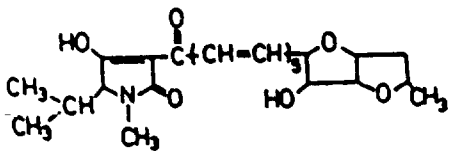
49. ERGOTAMINE. $C_{33}H_{35}O_5N_5$ (structure 4 above), MW 581, m.p. 212–214°C (d), $\lambda_{max}$ nm (lg ε): 318 (3.860), $\lambda^{Ex}_{max}$ 320 nm, $\lambda^{Em}_{max}$ 425 nm (water), $\lambda^{Ex}_{max}$ 325 nm, $\lambda^{Em}_{max}$ 415 nm (EtOH).

50. ERGOMETRINE. $C_{19}H_{23}O_2N_3$. MW 325, m.p. 162–163°C (d), $\lambda^{Ex}_{max}$ 320 nm, $\lambda^{Em}_{max}$ 435 nm (water) and $\lambda^{Ex}_{max}$ 325 nm, $\lambda^{Em}_{max}$ 415 nm (EtOH).



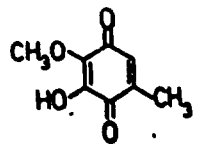
51. ERYTHROSKYRIN. $C_{26}H_{33}O_6N$, MW 455, orange-red crystals, m.p. 130–133°C (from ethanol), $\lambda^{EtOH}_{max}$ nm (lg ε): 409 (4.449) and 260 (3.949); $\lambda^{0.1N\ NaOH}_{max}$ nm (lg ε): 392

(4.778) and 260 (4.140). Toxicity: $LD_{50}$ 60 mg/kg (i.p.) in 20 g mice. Fungal source: *Penicillium islandicum* Sopp (NRRL 1036).



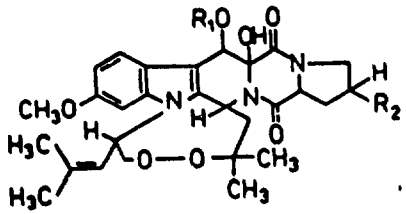
References: [148,167]

52. FUMIGATIN (3-Hydroxy-4-methoxy-2,5-toluquinone). $C_8H_8O_4$, MW 168, maroon needles, m.p. 116°C, UV properties strongly depend solvent polarity $\lambda_{max}^{MeOH}$ nm: 223, 268 and 320 (lg ε not reported), $\lambda_{max}^{CHCl_3}$ nm: 285 and 430 (lg ε not reported). Toxicity: strong antibiotic activity against gram-positive and gram-negative bacteria. Fungal source: *Aspergillus fumigatus* Fresenius.



References: [7,131,179]

53. FUMITREMORGIN A. $C_{32}H_{41}O_7N_3$ (structure 1), MW 579, colorless prisms, m.p. 206–209°C, $\lambda_{max}^{EtOH}$ nm (lg ε): 226 (4.501), 277 (3.724) and 296 (3.690). Toxicity: tremorgenic to mice, dose 5 mg (i.p.) caused 70% mortality and tremors. Fungal source: *Aspergillus fumigatus* Fres., *A. caespitosus*.



1: $R_1$ = $CH_3$>$CH$-$CH$=$CH_2$-, $R_2$ = H

2: $R_1$ = H, $R_2$ = H

3: $R_1$ = H, $R_2$ = $CH_3COO$-

References: [184,186]

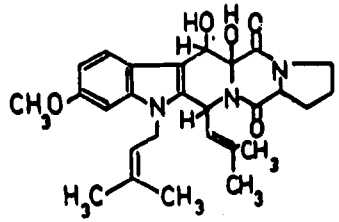
54. VERRUCULOGEN. $C_{27}H_{33}O_7N_3$ (structure 2 above), MW 511, colorless plates, m.p. 233–235°C (d), $\lambda_{max}^{EtOH}$ nm (lg ε): 226 (4.677), 277 (4.041) and 295 (3.989). Toxicity: tremorgenic to mice at dose 0.39 mg/kg (i.p.); and to day-old cockerels, 0.33 mg/kg (i.p.), $LD_{50}$ to mice, 2.4 mg/kg (i.p.); 126.7 mg/kg (orally), to day-old cockerels, 15.2 mg/kg (i.p.); (i.p.); 265.5(orally). Fungal source: *Penicillium verruculosum* Peyronel (ATCC 24640; NRRL 5881), *P. paraherquei*, *P. piscarium* Westling, *P. janthinellum*, *Aspergillus caespitosus* (NRRL 1929), *A. fumigatus*.

Reference: [45]

55. 15-ACETOXYVERRUCULOGEN. $C_{29}H_{33}O_9N_3$ (structure 3 above), MW 569, m.p. 217–218°C (d), $\lambda_{max}^{MeOH}$ nm 227 and 295 (lg ε not reported). Toxicity: tremorgenic but less active than verruculogen. Fungal source: *Penicillium verruculosum*.

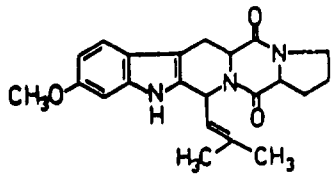
Reference: [54]

56. FUMITREMORGIN B. $C_{27}H_{33}O_5N_3$, MW 479, colorless needles, m.p. 211–212°C (from methanol), $\lambda_{max}^{EtOH}$ nm (lg ε): 228 (4.561), 278 (3.875) and 297 (3.924). Toxicity: tremorgenic activity at dose 1 mg/mouse (i.p.), sustained tremors at 5 mg/mouse (i.p.). Fungal source: *Aspergillus fumigatus* Fres. *A. caespitosus* (NRRL 1929).



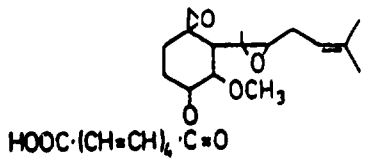
References: [58,142,184,185]

57. FUMITREMORGIN C (BM-Q). $C_{22}H_{25}O_3N_3$, MW 379, m.p. 125–130°C, $\lambda_{max}^{MeOH}$ nm: 224, 272 and 294 (lg ε not reported). Toxicity: tremorgenic to day-old cockerels 25 mg/kg (orally). Fungal source: *Aspergillus fumigatus* Fres.



Reference: [53]

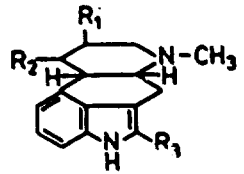
58. FUMAGILLIN. $C_{26}H_{34}O_7$, MW 458, light-yellow crystals, m.p. 145–147°C (from methanol aq). Toxicity: $LD_{50}$ in mice was 800 mg/kg (s.c.), compound with weak antifungal and antibacterial activity. Fungal source: *Aspergillus fumigatus*.



Reference: [66]

## FUMIGACLAVINES (59-69)

59. FUMIGACLAVINE A (9-Acetoxy--6,8-dimethylergoline).
$C_{18}H_{22}O_2N_2$ (structure 1), MW 298, colorless needles, m.p. 84-85°C (from methanol aq), $\lambda_{max}$ nm: 225, 275, 282 and 293 (lg ε not reported).
Toxicity: not reported.
Fungal source: *Aspergillus fumigatus* Fres.



1: $R_1$ = ---$CH_3$, $R_2$ = —OAc, $R_3$ = H
2: $R_1$ = —$CH_3$, $R_2$ = ---OAc, $R_3$ = H
3: $R_1$ = ---$CH_3$, $R_2$ = —OH, $R_3$ = H
4: $R_1$ = —$CH_3$, $R_2$ = ---OH, $R_3$ = H
5: $R_1$ = ---$CH_3$, $R_2$ = —OAc, $R_3$ = $CH_2$=CH-C($CH_3$)-$CH_3$

Reference: [149]

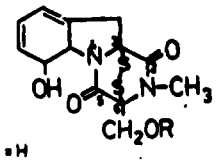
60. ROQUEFORTINE A (Isofumigaclavine A; 9α-Acetoxy-6,8-β-/-dimethylergoline).
$C_{18}H_{22}O_2N_2$ (structure 2 above), MW 298, m.p. 190--193°C (from benzene), $\lambda^{MeOH}_{max}$ nm (lg ε): 226 (4.666), 277 (3.778 sh), 283 (3.813) and 293 (3.748). Toxicity: pharmacological actions e.g. muscle relaxant, antidepressant in mice. $LD_{50}$ was 340 mg/kg (i.p.). Fungal source: *Penicillium roqueforti* Thom.
References: [128,143]

61. FUMIGACLAVINE B (6,8-Dimethylergolin-9-ol). $C_{16}H_{20}ON_2$ (structure 3 above), MW 256, needles, m.p. 244-245°C (from methanol aq), at 260°C the melt solidified and remelted 265-267°C, $\lambda_{max}$ nm (lg ε): 225 (4.490), 275 (3.792), 282 (3.820) and 293 (3.716). Toxicity: not reported. Fungal source: *Aspergillus fumigatus* Fres.
Reference: [149]

62. ROQUEFORTINE B (Isofumigaclavine B; 6,8β-Dimethylergolin-9α-ol). $C_{16}H_{20}ON_2$ (structure 4 above), MW 256, colorless needles, m.p. 222--223°C (from methanol), $\lambda^{EtOH}_{max}$ nm (lg ε): 225 (4.400), 277 (3.633 sh), 283 (3.663) and 293 (3.580). Toxicity: $LD_{50}$ to mice was 1000 mg/kg (i.p.). Fungal source: *Penicillium roqueforti*.
References: [128,143]

63. FUMIGACLAVINE C (2-Dimethylallyl-9-acetoxy-6,8-dimethylergoline). $C_{23}H_{30}O_2N_2$ (structure 5 above), MW 366, prisms, m.p. 191-193°C (from ethanol), needles m.p. 198°C (from methanol $\lambda^{MeOH}_{max}$ nm (lg ε): 225 (4.540), 227 (4.009), 283 (4.041) and 292 (3.978). Toxicity: histopathological changes of hepatic perenchymal cells, $LD_{50}$ to day-old cockerels was 150 mg/kg (orally). Fungal source: *Aspergillus fumigatus* Fres.
References: [2,53,182]

64. GLIOTOXIN. $C_{13}H_{14}O_4N_2S_2$ (structure 1), MW 326, m.p. 221°C (d), $\lambda^{EtOH}_{max}$ nm (lg ε): 216 (3.462) and 272 (3.799). Toxicity: strong antibiotic activity, toxic to laboratory animals. $LD_{50}$ was 45 mg/kg rabbit; 50 mg/kg mice and 50-65 mg/kg rats. Fungal source: *Penicillium terlikowskii*, *P. obscurum*, *Aspergillus terreus*, *A. chevalieri*, *A. fumigatus*.

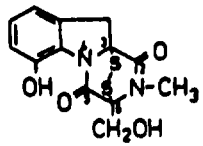


1: R = H
2: R = Ac

References: [79,97]

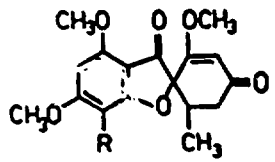
65. GLIOTOXIN ACETATE.
$C_{15}H_{16}O_5N_2S_2$ (structure 2 above), MW 368, m.p. 162--163°C; 170-172°C, $\lambda_{max}$ nm (lg ε): 268 (3.799). Toxicity: not reported. Fungal source: *Penicillium terlikowskii*.
References: [98,163]

66. DEHYDROGLIOTOXIN.
$C_{13}H_{12}O_4N_2S_2$, MW 324, m.p. 188-189°C, $\lambda^{EtOH}_{max}$ nm (lg ε): 214 (4.338), 272 (3.724) and 300 (3.663). Toxicity: antibiotic activity against *Bacillus subtilis* similar to those of gliotoxin. Fungal source: *Penicillium terlikowskii*.



References: [105,138]

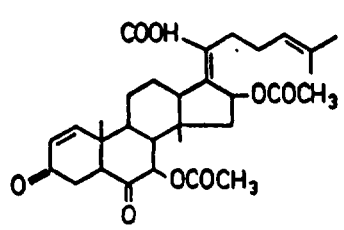
67. GRISEOFULVIN ([7-chloro-4,6--dimethoxycoumaran-3-one-2--spiro-1'-(2-methoxy-6'--methylcyclohex-2'-en-3'--one)]). $C_{17}H_{17}O_6Cl$ (structure 1), MW 352, m.p. 220--221°C, $\lambda^{MeOH}_{max}$ nm (lg ε): 324 (3.716), 291 (4.338), 252 (4.097) and 236 (4.328).
Toxicity: $LD_{50}$ in rats was 500 mg/kg (i.v.); antifungal activity. Fungal source: *Penicillium griseofulvum*, *P. patulum*, *P. albidum*, *P. urticae*.
References: [73,76]



1: R = Cl
2: R = H

68. DECHLOROGRISEOFULVIN ([4,6--Dimethoxycoumaran-3-one-2--spiro-1'-(3'-methoxy-6--methylcyclohex-2'-en-4'--one)]). $C_{17}H_{18}O_6$ (structure 2 above), MW 318, m.p. 179--181°C, $\lambda_{max}^{MeOH}$ nm (lg ε): 322 (3.613), 290 (4.389), 253 (4.179) and 233 (4.330). Toxicity: antifungal activity. Fungal source: *Penicillium janczewskii* Zal, *P. griseofulvum* Dierckx, *P. urticae*. References: [42,113]

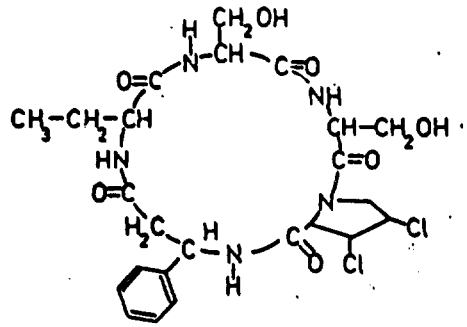
69. HELVOLIC ACID. $C_{33}H_{44}O_8$. MW 568, m.p. 208-212°C (d) (from methanol aq), $\lambda_{max}^{EtOH}$ nm (lg ε): 231 (4.238) and 322 (2.000). Toxicity: antibiotic activity with gram-positive bacteria. Fungal source: *Aspergillus fumigatus*. References: [3,33]



4-HYDROXYMELLEIN see 79

4-HYDROXYOCHRATOXIN A see 92

70. ISLANDIOTOXIN. $C_{24}H_{31}O_7N_5Cl_2$, MW 572, colorless needles, m.p. 250-251°C (d) (from methanol), m.p. 254°C (from acetone), $\lambda_{max}^{H_2O}$ 250 nm, lg ε 2.462. Toxicity: $LD_{50}$ in mice was 3,6 mg/kg (s.c.) - degeneration of liver. Fungal source: *Penicillium islandicum* Sopp.



References: [108,109]

ISORCHINULIN A see 29

ISOECHINULIN B see 35

ISOECHINULIN C see 36

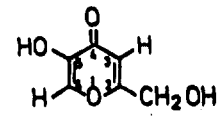
ISOFUMIGACLAVINE A see 60

ISOFUMIGACLAVINE B see 62

ISOOSPORIN see 93

ISOPATULIN see 95

71. KOJIC ACID (2-Hydroxymethyl--5-hydroxy-2-γ-pyrone). $C_6H_6O_4$, MW 142, white prismatic needles, m.p. 152--154°C, $\lambda_{max}$ nm (lg ε): 270 (3.893) (in methanolic HCl); 268 (3.907) (in $H_2O$) and 315 (3.817) (in 0,1 M NaOH). Toxicity: $LD_{50}$ in 17 g mice was 30 mg/mouse (i.p.); convulsant metabolite. Fungal source: *Aspergillus oryzae*, *A. flavus*, *A. glaucus*, *A. tamarii*, *Penicillium citrinum* and several other *Aspergillus* and *Penicillium* strains.

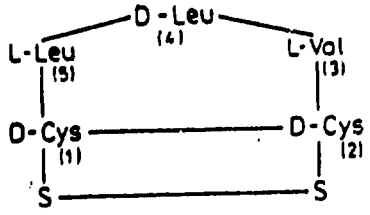


References: [118,181]

LL-S88α see 1

LUTEOSKYRIN see 123

72. MALFORMIN $A_1$. $C_{23}H_{39}O_5N_5S_2$, MW 529, [two D-cysteine (1,2), one L-valine (3), one D-leucine (4) and one L-isoleucine aminoacids residues] (see structure below). Toxicity: $LD_{50}$ for mice was 3,1 mg/kg (i.p.); phytotoxic and antibiotic properties. Fungal source: *Aspergillus niger* van Tieghem, *A. ficuum*, *A. awamori*, *A. phoenicis*.




References: [56,161]

73. MALFORMIN $A_2$. $C_{22}H_{37}O_5N_5S_2$, MW 515. Toxicity: not reported. Fungal source: *Aspergillus niger*. References: [56,161]

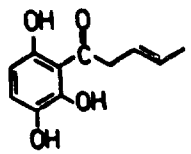
74. MALFORMIN $B_1$. $C_{23}H_{39}O_5N_5S_2$, MW 515. Fungal source and toxicity similar to those of malformin $A_1$. References: [56,161]

75. MALFORMIN $B_2$. $C_{22}H_{37}O_5N_5S_2$, MW 515, [two D-cysteine (1,2), two L-valine (3,5) and D-leucine (4) aminoacids residues] (see structure

above). Toxicity and fungal source similar to those of malformin $A_1$.
References: [56,161]

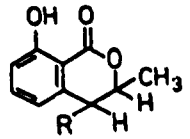
76. MALFORMIN C. $C_{23}H_{39}O_5N_5S_2$, MW 529, [two D-cysteine (1,2), one L-valine (3) and two L-leucine (4,5) amino-acids residues] (see structure above). Toxicity: antibacterial and phytotoxic activity, toxic to rats.
Fungal source: *Aspergillus niger*.
References: [5,101]

77. MALTORYZINE [6-(9-Penthyl)--1,4,5-trihydroxybenzene]. $C_{11}H_{12}O_4$, MW 208, pale brownish-yellow needles, m.p. 69°C (d), $\lambda_{max}$ nm (lg ε): 220 (4.100), 280 (3.114 sh) and 320 (2.097). Toxicity: toxic to mice and dairy cattle, $LD_{50}$ in mice was 3 mg/kg (i.p.) with signs of muscular narcotism. Fungal source: *Aspergillus oryzae* var. microsporus.



Reference: [93]

78. MELLEIN, (Ochracin, [(-)3,4--Dihydro-8-hydroxy-3-methyl-isocoumarin]). $C_{10}H_{10}O_3$ (structure 1), MW 178, m.p. 54-55°C, $\lambda^{EtOH}_{max}$ nm (lg ε): 212 (4.301), 246 (3.813) and 314 (3.613); blue fluorescence under UV 360 nm light.
Toxicity: not reported.
Fungal source: *Aspergillus melleus* Yukawa, *A. ochraceus* Wilh.



1: R = H
2: R = OH

Reference: [19]

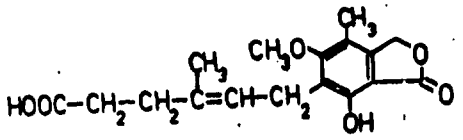
79. 4-HYDROXYMELLEIN (3-Methyl--4,8-dihydroxy-3,4-dihydro-isocoumarin). $C_{10}H_{10}O_4$ (structure 2 above), MW 194, m.p. 131-132°C, $\lambda^{MeOH}_{max}$ nm (lg ε): 247 (3.724) and 315 (3.623), blue fluorescence under UV 360 nm light.
Toxicity: not reported.
Fungal source: *Aspergillus ochraceus* Wilh (NRRL 3174).
References: [44,141]

5-METHOXYSTERIGMATOCYSTIN see 117

O-METHYLAVERSIN see 13

O-METHYLSTERIGMATOCYSTIN see 118

80. MYCOPHENOLIC ACID [6-(4--Hydroxy-6-methoxy-7-methyl--3-oxo-5-phthalanyl)-4--methyl-4-hexenoic acid]. $C_{17}H_{20}O_6$, MW 320, m.p. 141°C (from ethanol aq), $\lambda^{MeOH}_{max}$ nm: 217, 249 and 304 (lg ε not reported). Toxicity: antibacterial and antiviral activity; toxic to laboratory animals, $LD_{50}$ in mice was 2000 mg/kg (orally) and 500 mg/kg (i.v.); $LD_{50}$ in rats was 700 mg/kg (orally) and 450 mg/kg (i.v.).
Fungal source: *Penicillium brevi-compactum* Dierckx, *P. stoloniferum* Thom, *P. urti-cae*.



References: [68, 72]

NEOECHINULIN see 37

NEOECHINULIN A see 30

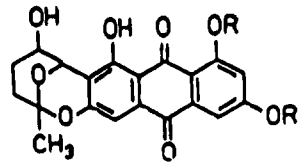
NEOECHINULIN B see 38

NEOECHINULIN C see 39

NEOECHINULIN D see 31

NEOECHINULIN E see 40

81. NIDURUFIN. $C_{20}H_{16}O_8$ (structure 1), MW 384, m.p. 188°C, $\lambda^{EtOH}_{max}$ nm (lg ε): 223 (4.525), 253 (4.185), 264 (4.279), 291 (4.483), 319 (4.029) and 450 (4.000). Toxicity: not reported. Fungal source: *Aspergillus nidulans*.



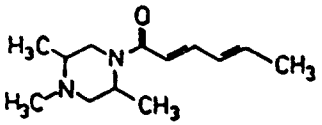
1: R = H
2: R = $CH_3$

Reference: [11]

82. DIMETHYLNIDURUFIN. $C_{22}H_{20}O_8$ (structure 2 above), MW 412, m.p. 211-213°C, $\lambda^{EtOH}_{max}$ nm (lg ε): 224 (4.683), 251 (4.279), 288 (4.490), 314 (3.931) and 444 (3.944).
Toxicity: not reported.
Fungal source: *Aspergillus versicolor*.
Reference: [100]

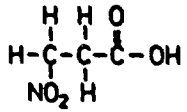
83. NIGRAGILLIN (N-Methyl-trans--2,5-dimethyl-N'-sorbylpipe-razine). $C_{13}H_{22}ON_2$, MW 222, m.p. 129-132°C, $\lambda^{MeOH}_{max}$ nm (lg ε): 262 (4.418). Toxicity: $LD_{50}$ 250 mg/kg (orally) to day-old cockerels. Toxic to silkworms at the compound concentration in

diet over 10 mg/kg. Fungal source: *Aspergillus niger*, *A. phoenicis*.



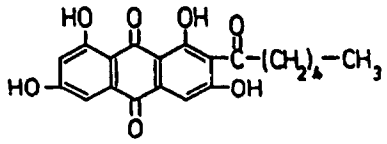
References: [31,96]

84. β-NITROPROPIONIC ACID. $C_3H_5O_4N$, MW 119, m.p. 65--67°C, $\lambda_{max}$ nm (lg ε): 273 (1.373). Toxicity: a dose over 150 mg/kg (i.v.) was toxic to mice and caused death within 24 h. Fungal source: *Aspergillus oryzae*, *A. flavus*, *A. wentii*, *Penicillium atrovenetum* G.Smith.



References: [26,139,179]

85. NORSOLORINIC ACID (2-n-Hexanoyl-1,3,6,8-tetrahydroxyantraquinone). $C_{20}H_{18}O_7$, MW 370, red prisms, m.p. 256--257°C (d) (from acetone), $\lambda_{max}^{EtOH}$ nm (lg ε): 234 (4.374), 265 (4.221), 283 (4.239), 297 (4.298 sh), 313 (4.376) and 465 (3.863). Toxicity: not reported. Fungal source: *Aspergillus versicolor* (Vuill.) Tiraboschi.



References: [77,102]

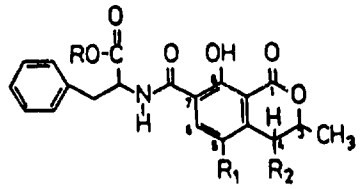
NORTRYPTOQUIVALINE see 131

NORTRYPTOQUIVALONE see 132

OCHRACIN see 78

## OCHRATOXINS: (86-92)

86. OCHRATOXIN A (7-Carboxy-5--chloro-8-hydroxy-3,4-dihydro-3R-methylisocoumarin--7-L-β-phenylalanine). $C_{20}H_{18}O_6NCl$ (structure 1), MW 403, m.p. 94-96°C (from xylene), $\lambda_{max}^{EtOH}$ nm (lg ε): 213 (4.566) and 332 (3.806), $\lambda_{max}^{Ex}$ 340 nm, $\lambda_{max}^{Em}$ 500 nm, green fluorescence under UV 360 nm light. Toxicity: compound caused tubular necrosis of kidney and mild liver degeneration, nephrotoxic agent to pigs, dogs and human being. $LD_{50}$ in rats was 22 mg/kg (orally) and in trout was 3.0 mg/kg (i.p.). The compound is hydrolysed by proteolitic enzymes to nontoxic isocoumaric acid called ochratoxin α. Fungal source: *Aspergillus ochraceus* Wilh. (NRRL 3174), *A. sulphureus* (NRRL 4077), *A. melleus* (NRRL 3519, 3520), *Penicillium viridicatum* (ATCC 18411).



1: R=H, $R_1$=Cl, $R_2$=H
2: R=H, $R_1$=H, $R_2$=H
3: R=$C_2H_5$, $R_1$=Cl, $R_2$=H
4: R=H, $R_1$=Cl, $R_2$=OH
5: R=$CH_3$, $R_1$=Cl, $R_2$=H
6: R=$C_2H_5$, $R_1$=H, $R_2$=H
7: R=$CH_3$, $R_1$=H, $R_2$=H

References: [62,74,111,112]

87. OCHRATOXIN B (7-Carboxy-8--hydroxy-3,4-dihydro-3R-methylisocumarin-7-L-β-phenylalanine). $C_{20}H_{19}O_6N$ (structure 2 above), MW 369, m.p. 221°C (from methanol), $\lambda_{max}^{EtOH}$ nm (lg ε): 218 (4.571) and 318 (3.839), blue fluorescence under UV 360 nm light. Toxicity: less toxic than ochratoxin A, $LD_{50}$ in day-old chicks was 54 mg/kg (orally). Fungal source: *Aspergillus ochraceus* Wilh. (NRRL 3174).
References: [39,111,112]

88. OCHRATOXIN C (Ochratoxin A ethyl ester). $C_{22}H_{22}O_6NCl$ (structure 3 above), MW 431, $\lambda_{max}$ nm (lg ε): 214 (4.477), 333 (3.045) and 378 (3.312), light green fluorescence under UV 360 nm light. Toxicity: similar to ochratoxin A, $LD_{50}$ in day-old duckling was 135-170 μg per bird. Fungal source: *Aspergillus ochraceus* Wilh.
References: [111,151]

89. OCHRATOXIN A METHYL ESTER. $C_{21}H_{20}O_6NCl$ (structure 5 above), MW 417, $\lambda_{max}$ nm (lg ε): 213 (4.515), 331 (3.613) and 378 (3.290), light blue fluorescence under UV 360 nm light. Toxicity: similar to those of ochratoxin C. Fungal source: *Aspergillus ochraceus* Wilh.
Reference: [151]

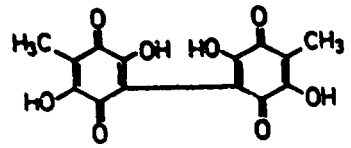
90. OCHRATOXIN B ETHYL ESTER. $C_{22}H_{23}O_6N$ (structure 6 above), MW 397, m.p. 102--103°C (from ether), $\lambda_{max}$ nm (lg ε): 218 (4.505), 318 (3.716) and 364 (3.097), light blue fluorescence under UV 360 nm light. Toxicity: low toxicity. Fungal source: *Aspergillus ochraceus* Wilh.
Reference: [151]

91. OCHRATOXIN B METHYL ESTER. $C_{21}H_{21}O_6N$ (structure 7 above), MW 383.5, m.p. 134--135°C (from benzene). UV data similar to those of ochratoxin B methyl ester. Toxicity: nontoxic. Fungal

source: *Aspergillus* ochraceus Wilh.
Reference: [131]

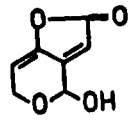
92. 4-HYDROXYOCHRATOXIN A. $C_{20}H_{18}O_7NCl$ (structure 4 above), MW 419, colorless crystals, m.p. 216-218°C (from benzene), $\lambda_{max}^{EtOH}$ nm (lg ε): 213 (4.512) and 334 (3.806), green fluorescence under UV 360 nm light. Toxicity: not reported. Fungal source: *Penicillium viridicatum* Westling (ATCC 18411).
Reference: [91]

93. OOSPOREIN (Isoosporin; chaetomidin; 3,3',6,6'-Tetrahydroxy-2,2'-dimethyl-5,5'-bi-p-benzoquinone). $C_{14}H_{10}O_8$, MW 306, bronze plates, m.p. 290-295°C (from methanol aq), $\lambda_{max}^{MeOH}$ nm (lg ε): 208 (4.523) and 291 (4.362). Toxicity: $LD_{50}$ 6.12 mg/kg (orally) to day-old cockerels. Fungal source: *Penicillium phoeniceum*, *P. rubrum*.



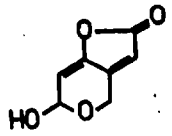
Reference: [48]

94. PATULIN (4-Hydroxy-4-H-furo-[3,2-c]pyran-2(6H)-one). $C_7H_6O_4$, MW 154, m.p. 110-111°C (from benzene), $\lambda_{max}^{MeOH}$ nm (lg ε): 269 (4.107). Toxicity: antibiotic and toxic agent; carcinogenic to laboratory animals, $LD_{50}$ in mice and rats was 15-35 mg/kg (i.v.) and 5 mg/kg (i.p.). Fungal source: *Aspergillus clavatus*, *A. giganteus*, *A. terreus*, *Penicillium expansum*, *P. urticae* and several other *Penicillium* spp.



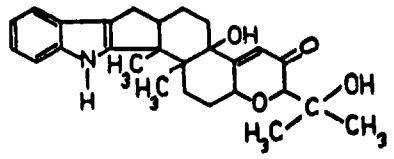
References: [80,180]

95. ISOPATULIN (5-Hydroxy-4H-furo-[3,2-c]pyran-2(6H)-one). $C_7H_6O_4$, MW 154, m.p. 88-90°C, $\lambda_{max}^{MeOH}$ 269 nm (lg ε not reported). Toxicity: not reported. Fungal source: *Penicillium urticae* (NRRL 2159A).



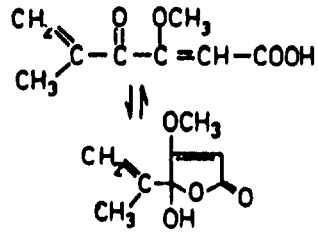
Reference: [144]

96. PAXILLINE. $C_{27}H_{33}O_4N$, MW 435, clear cubes, m.p. 252°C (from acetone), $\lambda_{max}^{MeOH}$ nm (lg ε): 230 (4.618) and 281 (3.903). Toxicity: tremorgenic to day-old cockerels at dosage 100 mg/kg (orally) and mice at dosage 35 mg/kg (i.p.). Fungal source: *Penicillium paxilli* Bainier.



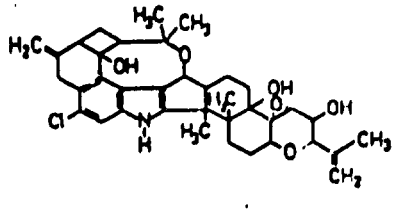
Reference: [47]

97. PENICILLIC ACID (3-Methoxy-5-methyl-4-oxo-2,5-hexadienoic acid or 2-keto-β-methoxy-δ-methylene-Δα-hexenoic acid). $C_8H_{10}O_4$, MW 170, m.p. 86-87°C (from petroleum ether); m.p. 58-64°C (from water), $\lambda_{max}^{MeOH}$ nm (lg ε): 221 (4.021). Toxicity: antibiotic, antiviral, antitumor and cytotoxic activity. $LD_{50}$ in mice was 110 mg/kg (s.c.) and 250 mg/kg (i.v.). Fungal source: *Aspergillus ochraceus*, *A. sulphureus*, *A. melleus* and several other of genus *Aspergillus*, *Penicillium fluidum*, *P. puberulum*, *P. cyclopium* and many other of genus *Penicillium*.



References: [16,168,175]

98. PENITREM A. $C_{37}H_{44}O_6NCl$, MW 633, fine needles, m.p. 237-239°C (d)(from ethanol aq), $\lambda_{max}^{MeOH}$ nm (lg ε): 233 (4.568) and 295 (4.064). Toxicity: dose 250 mg/kg (i.p.) in mice caused perceptible tremors; $LD_{50}$ in mice was 1 mg/kg (i.p.). Fungal source: *Penicillium cyclopium*, *P. crustosum*, *P. palitans* (NRRL 3468), *P. puberulum*, *P. spinulosum*.



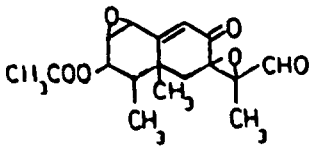
References: [40,88,178]

99. PENITREM B. $C_{37}H_{45}O_5N$, MW 583, fine needles, m.p. 185-195°C (from ethanol aq), $\lambda_{max}^{MeOH}$ nm (lg ε): 227 (4.585),

286 (4.117) and 297 (4.041). Toxicity: dose 1.3 mg/kg (i.p.) in mice caused perceptible tremors; $LD_{50}$ in mice was 9.84 mg/kg (i.p.). Fungal source: *Penicillium palitans* (NRRL 3468).
Reference: [167]

PREECHINULIN see 32

100. PR TOXIN. $C_{17}H_{20}O_6$, MW 320, m.p. 135-137°C. $\lambda_{max}^{EtOH}$ nm 349 (4.184), dark-blue fluorescence under UV 360 nm light. $\lambda_{max}^{Ex}$ 300 nm, $\lambda_{max}^{Em}$ 360 nm. Toxicity: congestion and edema of lung, kidney and brain, degenerative changes in liver and kidney with hemorrhage in latter stage. $LD_{50}$ in mice was 5.8 mg/kg (i.p.) and $LD_{50}$ in rats was 115 mg/kg (orally) and 11 mg/kg (i.p.). Fungal source: *Penicillium roqueforti* (NRRL 849).

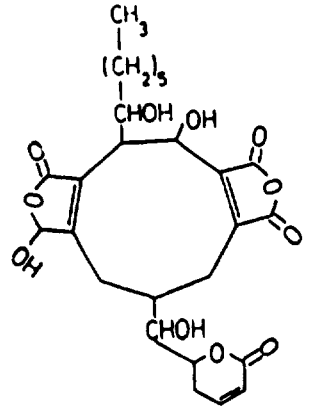


References: [172,173]

ROQUEFORTINE A see 60

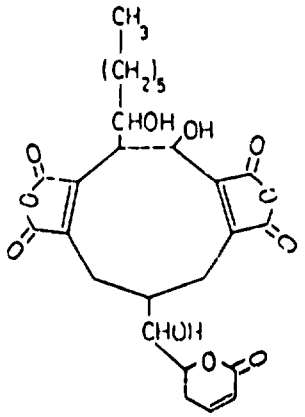
ROQUEFORTINE B see 62

101. RUBRATOXIN A. $C_{26}H_{32}O_{11}$, MW 520, needles, m.p. 204-206°C (d)(from ethyl acetate), $\lambda_{max}^{EtOH}$ nm (lg ε): 204 (4.304), inflection at 225 and 252 (3.646 sh). Toxicity: hepatotoxic to swine, cattle and laboratory animals. $LD_{50}$ in mice was 6.3 μg/kg (i.p.). Fungal source: *Penicillium purpurogenum* Stoll, *P. rubrum* (NRRL A-11 785).



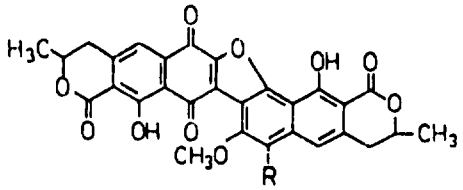
References: [119,120,121]

102. RUBRATOXIN B. $C_{26}H_{30}O_{11}$, MW 518, m.p. 168-170°C (d) (from benzene-ethyl acetate), $\lambda_{max}^{CH_3CN}$ nm (lg ε): 251 (3.987). Toxicity: hepatotoxic to swine, cattle and laboratory animals. $LD_{50}$ in mice was 3.0 mg/kg (i.p.). Fungal source: *Penicillium rubrum* Stoll (NRRL 3290), *P. purpurogenum* Stoll.



References: [119,120,121]

103. RUBROSULPHIN. $C_{29}H_{20}O_{11}$ (structure 1), MW 528, red plates, m.p. >310°C (d) (from chloroform-petroleum ether), $\lambda_{max}^{CHCl_3}$ nm 280, 357 and 415 (lg ε not reported). Toxicity: not reported. Fungal source: *Aspergillus sulphureus*, *A. melleus*, *Penicillium viridicatum*.



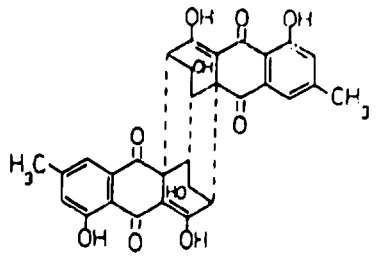
1: R = H
2: R = OH

Reference: [64]

104. VIOPURPURIN. $C_{29}H_{20}O_{11}$ (structure 2 above), MW 544, purple-black beads, m.p. >310°C (d)(from chloroform-petroleum ether), $\lambda_{max}^{CHCl_3}$ nm (lg ε): 274 (4.571), 282 (4.588), 377 (3.949) and 500 (3.477). Toxicity: not reported. Fungal source: *Aspergillus sulphureus*, *A. melleus*, *Penicillium viridicatum*
Reference: [64]

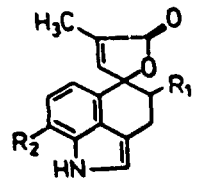
RUGULOSINS: (105-109)

105. RUGULOSIN (2,2',4,4',5,5'-hexahydroxy-2,2',3,3'-tetrahydro-7,7'-dimethyl-1,1'-bianthraquinone). $C_{30}H_{22}O_{10}$, MW 542, fine yellow crystals, m.p. 290°C (d) (from methanol), $\lambda_{max}^{MeOH}$ nm (lg ε): 240 (4.439), 273 (4.290), 302 (4.079), 324 (4.140), 345 (4.146), 391 (4.342) and 402 (4.322). Toxicity: antibiotic and hepatotoxic activity: $LD_{50}$ in mice was 83 mg/kg (i.p.)



and $LD_{50}$ in rats was 44 mg/kg (i.p.). Fungal source: *Penicillium rugulosum* Thom, *P. variabile*, *P. brunneum*, *P. lardum* Thom, *P. wortmanni* Klöcker.
References: [132,160,165]

106. RUGULOVASINE A. $C_{16}H_{16}O_2N_2$ (structure 3), MW 268, m.p. 138°C (d), $\lambda_{max}^{EtOH}$ nm (lg ε): 224 (4.369), 277 (3.699), 288(3.778) and 295 (3.778). Toxicity: hypotensive action on chloralose-urethanized cats, the minimum effective dose was 0.2-0.5 mg/kg. Fungal source: *Penicillium islandicum* Sopp, *P. concavo-rugulosum*, *P. verrucosum* Dierckx.



1: $R_1$ = ----$NHCH_3$, $R_2$=Cl
2: $R_1$ = ═$NHCH_3$, $R_2$=Cl
3: $R_1$ = ----$NHCH_3$, $R_2$=H
4: $R_1$ = ═$NHCH_3$, $R_2$=H

References: [2,51]

107. RUGULOVASINE B. $C_{16}H_{16}O_2N_2$ (structure 4 above), MW 268, colorless resinous oil, $\lambda_{max}^{EtOH}$ nm (lg ε): 224 (4.161), 277 (3.681), 288 (3.732) and 295 (3.176). Toxicity: similar as rugulovasine A, minimum effective dose was 0.025-0.05 mg/kg. Fungal source: *Penicillium islandicum* Sopp, *P. concavo-rugulosum*.
References: [2,52,102]

108. 8-CHLORORUGULOVASINE A. $C_{16}H_{15}O_2N_2Cl$ (structure 1 above), MW 302, $\lambda_{max}^{MeOH}$ nm (lg ε): 225 (4.330), 280 (3.663 sh), 292 (3.716) and 298 (3.732). Toxicity: $LD_{50}$ 75-125 mg/kg to day-old cockerels (orally). Fungal source: *Penicillium islandicum* Sopp.
References: [51,52]

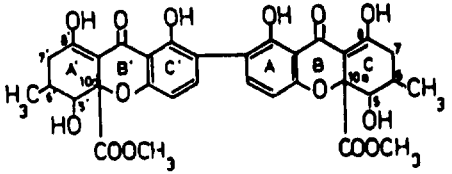
109. 8-CHLORORUGULOVASINE B. $C_{16}H_{15}O_2N_2Cl$ (structure 2 above), MW 302, $\lambda_{max}^{MeOH}$ nm (lg ε): 224 (4.350), 278 (3.672), 288 (3.708) and 295 (3.732). Toxicity: $LD_{50}$ 75-125 mg/kg to day-old cockerels (orally). Fungal source: *Penicillium islandicum* Sopp.
References: [51,52]

## SECALONIC ACIDS: (110-112)

Family of six isomers of xanthone dimers (linked at the 2,2' positions) with molecular formula $C_{32}H_{30}O_{14}$ and molecular weight 638. The compounds differ stereochemically and the following functional groups: OH (positions 5,5'), $CH_3$ (positions 6,6') and $CH_3COO$ (positions 10a, 10a') exhibit different α or β stereochemical configuration (see table below):

| | 5 | 5' | 6 | 6' | 10a | 10a' |
|---|---|---|---|---|---|---|
| A | α | β | β | α | α | β |
| B(E) | β | α | β | α | α | β |
| C | α | β | α | α | β | β |
| D | β | α | α | β | β | α |
| E(B) | see secalonic acid B | | | | | |
| F | β | β | α | β | β | α |
| G | α | α | β | α | α | β |

Since the only three compounds of this group were isolated from *Aspergillus* or *Penicillium* fungal culture, only secalonic acids A, D and F are described below.



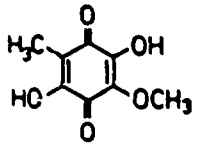
110. SECALONIC ACID A. $C_{32}H_{30}O_{14}$, MW 638, yellow crystals, m.p. 246-248°C (from chloroform), m.p. 243°C (from acetone), $\lambda_{max}^{EtOH}$ nm (lg ε): 252 (4.226) and 346 (4.491). Toxicity: paw edema of rats, $LD_{100}$ in mice was 100 mg/kg (i.p.). Fungal source: *Aspergillus ochraceus*.
Reference: [183]

111. SECALONIC ACID D. $C_{32}H_{30}O_{14}$, MW 638, pale yellow crystals, m.p. 253-255°C (from chloroform), $\lambda_{max}^{EtOH}$nm (lg ε): 248 (4.260) and 337 (4.500). Toxicity: $LD_{50}$ in mice was 42 mg/kg (i.p.). Fungal source: *Penicillium oxalicum*, *Aspergillus aculeatus* Iizuka.
References: [152,153,183]

112. SECALONIC ACID F. $C_{32}H_{30}O_{14}$, MW 638, yellow needles, m.p. 218-221°C (from benzene-cyclohexene), $\lambda_{max}^{EtOH}$ nm (lg ε): 236 (4.284), 263 (4.238) and 388 (4.568). Toxicity: antibiotic activity against *Bacillus megaterium*; toxicity to vertebrate not reported. Fungal source: *Aspergillus aculeatus* Iizuka.
Reference: [6]

113. SPINULOSIN (Substance "Z"; 3,6-Dihydroxy-3-methoxy-1,4-toluquinone). $C_8H_8O_5$, MW 184, purple-black microcrystals, m.p. 201°C (from sublimation), $\lambda_{max}^{CHCl_3}$ nm: 294 and 460 (lg ε: not reported). Toxicity: antibiotic activity. Fungal source: *Aspergillus fumigatus* Fre-

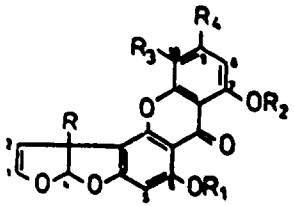
seniua. *Penicillium spinulosum* Thom. *P. cinerascens*.



References: [7,15,130,131]

**STERIGMATOCYSTINS: (114-118)**

114. STERIGMATOCYSTIN. $C_{18}H_{12}O_6$ (structure 1), MW 324, pale yellow crystals, m.p. 246°C (d), $\lambda_{max}^{EtOH}$ nm (lg ε): 208 (4.279), 235 (4.389), 249 (4.439) and 327 (4.117); orange-red fluorescence under UV 360 nm light. Toxicity: hepatic damage in primates; in rats kidney, liver damage and renal necrosis. $LD_{50}$ in rats was 166 mg/kg (orally) and 60 mg/kg(i.p.). Fungal source: *Aspergillus versicolor* (Vuill.) Tiraboschi, *A. nidulans*, *A. aurantio-brunneus* (Atkins, Hindson and Russel).
References: [23,84,133]



1: R = H, $R_1$ = $CH_3$, $R_2$ = H, $R_3$ = H, $R_4$ = H
2: R = OH, $R_1$ = $CH_3$, $R_2$ = $CH_3$, $R_3$ = H, $R_4$ = H
3: R = H, $R_1$ = $CH_3$, $R_2$ = $CH_3$, $R_3$ = H, $R_4$ = H
4: R = H, $R_1$ = $CH_3$, $R_2$ = H, $R_3$ = $OCH_3$, $R_4$ = H
5: R = H, $R_1$ = H, $R_2$ = H, $R_3$ = H, $R_4$ = H
6: R = H, $R_1$ = $CH_3$, $R_2$ = H, $R_3$ = $CH_3O$, $R_4$ = $CH_3O$

115. ASPERTOXIN (3-Hydroxy-6,7-dimethoxydifuroxanthone). $C_{19}H_{14}O_7$ (structure 2 above). MW 354, m.p. 240--280°C (d) (from dioxane/water), $\lambda_{max}^{MeOH}$ nm (lg ε): 241 (4.530) and 310(4.083); blue fluorescence under UV 360 nm light. Toxicity: $LD_{50}$ in chicken embryo was 0,7 μg/embryo, with beak malformation and loss of muscle tone. Fungal source: *Aspergillus flavus* Link.
References: [136,171]

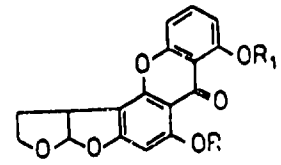
116. DIMETHOXYSTERIGMATOCYSTIN (6,9,10-Trimethoxy-7-hydroxydifuroxanthone). $C_{20}H_{16}O_8$ (structure 6 above), MW 384, pale yellow needles, m.p. 233-234°C (from acetone), $\lambda_{max}^{EtOH}$ nm (lg ε): 233 (4.435), 284 (4.531), 275 (3.886 sh) and 330 (4.283). Toxicity: not reported. Fungal source: *Aspergillus versicolor*.
Reference: [70]

117. 5-METHOXYSTERIGMATOCYSTIN (7-Hydroxy-6,10-dimethoxydifuroxanthone). $C_{19}H_{14}O_7$ (structure 4 above), pale yellow needles, m.p. 223°C (d). Toxicity: not reported. Fungal source: *Aspergillus versicolor* (Vuill.) Tiraboschi.
Reference: [23]

118. O-METHYLSTERIGMATOCYSTIN (6,7-Dimethoxydifuroxanthone). $C_{19}H_{14}O_6$ (structure 3 above), MW 338, faintly yellow slender rods, m.p. 265-267°C (from methanol), $\lambda_{max}^{MeOH}$ nm (lg ε): 236 (4.610) and 310 (4.217); blue fluorescence under UV 360 nm light. Toxicity: not reported. Fungal source: *Aspergillus flavus* Link.
References: [25,43,135]

**DIHYDROSTERIGMATOCYSTINS: (119-121)**

119. DIHYDRODEMETHYLSTERIGMATOCYSTIN (1,2-Dihydro-6,7-dihydroxydifuroxanthone). $C_{17}H_{12}O_6$ (structure 1), MW 312, pale yellow needles, m.p. 202°C (d) (from acetone), $\lambda_{max}^{EtOH}$ nm (lg ε): 233 (4.428), 250 (4.520), 259 (4.481) and 335 (4.288). Toxicity: not reported. Fungal source: *Aspergillus versicolor* (Vuill.) Tiraboschi.



1: R = H, $R_1$ = H
2: R = $CH_3$, $R_1$ = $CH_3$
3: R = $CH_3$, $R_1$ = H

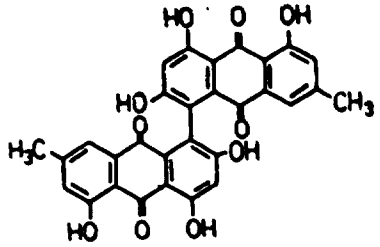
Reference: [81]

120. DIHYDRO-O-METHYLSTERIGMATOCYSTIN (1,2-Dihydro-6-methoxy-7-hydroxydifuroxanthone). $C_{19}H_{16}O_6$ (structure 2 above), MW 340, colorless rods, m.p. 282-283°C (from methanol); colorless needles, m.p. 281-282°C (from chloroform-methanol), $\lambda_{max}$ nm (lg ε): 203 (4.420), 237 (4.590) and 311 (4.283); blue fluorescence under UV 360 nm light. Toxicity: toxic in cell culture. Fungal source: *Aspergillus flavus*.
References: [43,57]

121. DIHYDROSTERIGMATOCYSTIN (1,2-Dihydro-6-methoxy-7-hydroxydifuroxanthone). $C_{18}H_{14}O_6$ (structure 3 above), MW 326, pale yellow needles, m.p. 230°C (d), $\lambda_{max}^{EtOH}$ nm (lg ε): 233 (4.441), 247 (4.508) and 325 (4.220); red-orange fluorescence under UV 360

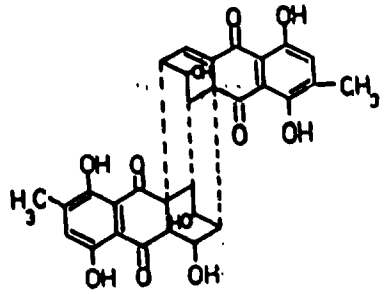
nm light. Toxicity: inhibited mitosis of kidney ephithelial cells. Fungal source: *Aspergillus versicolor* (Vuill.) Tiraboschi.
References: [57,81]

122. SKYRIN (Endothianin; 1,1'-Bisemodin). $C_{30}H_{18}O_{10}$, MW 538, yellow plates, m.p. >380°C (from acetone). Fungal source: *Penicillium islandicum* Sopp (NRRL 1036 and 1175), *P. rugulosum*, *P. wortmanni*, *P. tardum*, *P. brunneum*.



References: [90,160]

123. LUTEOSKYRIN (2,2',4,4',5,-5',8,8'-Octahydroxy-2,2',-3,3'-tetrahydro-7,7'-dimethyl-1,1'-biantraquinone). $C_{30}H_{22}O_{12}$, MW 574, yellow crystals, m.p. 287°C (d) (from acetone-hexane). $\lambda_{max}$ nm (lg ε): 245 (4.318), 275 (4.328) and 430 (4.460) (in ethanol) and 350 (3.903), 429 (4.453) and 446 (4.458) (in acetone). Toxicity: $LD_{50}$ in mice was 40.8 mg/kg (i.p.), 147.0 mg/kg (s.c.), 221 mg/kg (orally) and 6.65 mg/kg (i.v.). Acute toxicity (with hepatic lesions) of the compound to mice, rats, rabbits and monkeys. Fungal source: *Penicillium islandicum* Sopp (NRRL 1036).

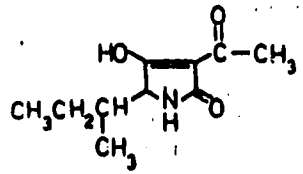


References: [132,146,147, 164]

SM-Q see 57

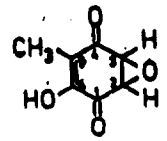
SUBSTANCE Z see 113

124. TENUAZONIC ACID (3-Acetyl-5-*sec*-butyltetranic acid). $C_{10}H_{15}O_3N$, MW 197, $\lambda_{max}^{EtOH}$ nm (lg ε): 217 (3.720) and 277 (4.130), spectroscopic properties of the compound depend solvent polarity and pH. Toxicity: tenuazonic acid acts as a phytotoxin, inhibits growth of cells by inhibition of protein and nucleic acid biosynthesis. Antiviral, antitumor activity inhibition of protein synthesis by tenuazonic acid was reported. The role of the compound in the ethiology of hematologic disorder named onyalai is not elucidated. Fungal source: *Aspergillus* spp., also produced by *Alternaria* spp.



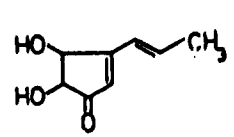
References: [155,169]

125. TERREIC ACID (5,6-Epoxy-2-hydroxytoluquinone). $C_7H_6O_4$, MW 154, yellow plates, m.p. 127°C (from benzene), $\lambda_{max}^{EtOH}$ nm (lg ε): 214(4.029) and 316 (3.881). Toxicity: antibiotic and antifungal properties, $LD_{50}$ in mice was 70-120 mg/kg (i.v.). Fungal source: *Aspergillus terreus* Thom.



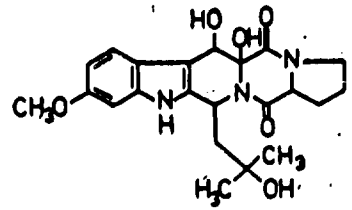
Reference: [145]

126. TERREIN (5,6-Dihydroxy-4-(1-propenyl)cyclopent-4-en-7-one). $C_8H_{10}O_3$, MW 154, colorless needles, m.p. 127°C (from acetone), $\lambda_{max}^{EtOH}$ nm (lg ε): 275 (4.415) and 342 (2.114). Toxicity: not reported. Fungal source: *Aspergillus terreus* (IMI 44339), *A. fischeri* Wehmer, *A. versicolor*, *Penicillium raistrickii*.



References: [12,63,76]

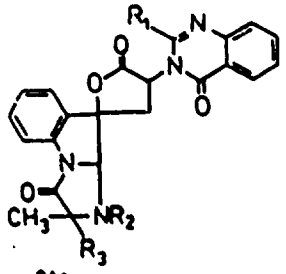
127. TR-2. $C_{22}H_{27}O_6N_3$, MW 429, m.p. 150-152°C, $\lambda_{max}^{EtOH}$ nm (lg ε): 224 (4.373), 268 (3.834) and 294 (3.877). Toxicity: tremorgenic to day-old cockerels dosed orally at level 12.5 mg/kg. Fungal source: *Penicillium verruculosum* Peyronel, *Aspergillus fumigatus* Fres.



References: [46,50,53]

## TRYPTOQUIVALINES (128-142)

128. DEOXYNORTRYPTOQUIVALINE.
$C_{28}H_{28}O_6N_4$ (structure 1), MW 516, colorless prisms, m.p. 158-160°C (from diethyl ether), $\lambda_{max}^{EtOH}$ nm (lg ε): 228 (4.642), 233 (4.603 sh), 254 (4.193 sh), 268 (4.068), 278(4.021 sh), 305 (3.613) and 317(3.519). Toxicity: unknown, tremorgenic properties not reported. Fungal source: *Aspergillus clavatus*.
Reference: [30]



1: $R_1$ = $(CH_3)_2CH-CH(OAc)-$ ; $R_2$ = H ; $R_3$ = H
2: $R_1$ = $(CH_3)_2CH-C(=O)-$ ; $R_2$ = H ; $R_3$ = H
3: $R_1$ = $(CH_3)_2CH-CH(OAc)-$ ; $R_2$ = H ; $R_3$ = $CH_3$
4: $R_1$ = $(CH_3)_2CH-CH(OAc)-$ ; $R_2$ = OH ; $R_3$ = H
5: $R_1$ = $(CH_3)_2CH-C(=O)-$ ; $R_2$ = OH ; $R_3$ = H
6: $R_1$ = $(CH_3)_2CH-CH(OAc)-$ ; $R_2$ = OH ; $R_3$ = $CH_3$
7: $R_1$ = ----H ; $R_2$ = OH ; $R_3$ = H
8: $R_1$ = —H ; $R_2$ = H ; $R_3$ = H
9: $R_1$ = ----H ; $R_2$ = OH ; $R_3$ = $CH_3$
10: $R_1$ = —H ; $R_2$ = OH ; $R_3$ = H
11: $R_1$ = $(CH_3)_2CH-C(=O)-$ ; $R_2$ = H ; $R_3$ = $CH_3$
12: $R_1$ = ----H ; $R_2$ = H ; $R_3$ = ----H
13: $R_1$ = ——H ; $R_2$ = OH ; $R_3$ = $CH_3$
14: $R_1$ = $(CH_3)_2CH-CH(OAc)-$ ; $R_2$ = OH ; $R_3$ = ----H
15: $R_1$ = $(CH_3)_2CH-C(=O)-$ ; $R_2$ = H ; $R_3$ = ----$CH_3$

129. DEOXYNORTRYPTOQUIVALONE.
$C_{26}H_{24}O_5N_4$ (structure 2 above), MW 472, fine white needles, m.p. 192-193°C (from diethyl ether), $\lambda_{max}^{EtOH}$ nm (lg ε): 232 (4.311), 288 (3.966) and 320 (3.796 sh). Toxicity: unknown, tremorgenic properties not reported. Fungal source: *Aspergillus clavatus*.
Reference: [30]

130. DEOXYTRYPTOQUIVALINE.
$C_{29}H_{30}O_6N_4$ (structure 3 above), MW 530, white needleless, m.p. 150-152°C (from dichloromethane-hexane), $\lambda_{max}^{EtOH}$nm (lg ε): 227(4.648), 232 (4.622 sh), 252 (4.267 sh), 267 (4.079 sh), 278 (4.013 sh), 304 (3.519) and 318 (3.431 sh). Toxicity: tremorgenic activity not reported. Fungal source: *Aspergillus clavatus*.
Reference: [30]

131. TRYPTOQUIVALINE D (Nortryptoquivaline). $C_{28}H_{28}O_7N_4$ (structure 4 above), MW 532, colorless prisms, m.p. 256-258°C (from dichloromethane-hexane), $\lambda_{max}^{EtOH}$ nm (lg ε): 228 (4.639), 233 (4.623 sh), 254 (4.372 sh), 267 (4.076 sh), 279(4.090), 306 (3.653) and 319(3.544). Toxicity: tremorgenic properties not demonstrated. Fungal source: *Aspergillus clavatus* (NRRL 5890), *A. fumigatus*.
References: [30,41]

132. TRYPTOQUIVALINE B (Nortryptoquivalone, Tryptoquivalone). $C_{26}H_{24}O_6N_4$ (structure 5 above), MW 488, colorless prisms, m.p. 208-209°C (from dichloromethane-hexane), $\lambda_{max}^{EtOH}$ nm (lg ε): 228 (4.496 sh), 232 (4.502), 253 (4.228 sh), 278 (3.881), 289 (3.813), 304 (3.732) and 316 (3.653 sh). Toxicity: tremorgenic properties, induced persistent tremors in rats. Fungal source: *Aspergillus clavatus* (NRRL 5890).
References: [30,41]

133. TRYPTOQUIVALINE A and C.
$C_{29}H_{30}O_7N_4$ (structure 6 above), MW 546, colorless prisms, m.p. 155-157°C, $\lambda_{max}^{EtOH}$ nm (lg ε): 228 (4.623), 232 (4.604 sh), 252 (4.290 sh), 268(4.037), 279 (3.978), 307 (3.568) and 319 (3.477). Toxicity: tremorgenic to humans and rats. Fungal source: *Aspergillus clavatus* (NRRL 5890)
References: [30,41]

134. TRYPTOQUIVALINE E.
$C_{22}H_{18}O_5N_4$ (structure 7 above), MW 418, colorless feathers, m.p. 257°C (d), $\lambda_{max}^{MeOH}$nm (lg ε): 226(4.509), 232 (4.474 sh), 254 (4.204 sh), 266 (4.068 sh), 276 (3.924 sh), 291 (3.544 sh), 303 (3.477) and 315 (3.415). Toxicity: tremorgenic properties not demonstrated. Fungal source: *Aspergillus fumigatus*.
References: [187,188]

135. TRYPTOQUIVALINE F.
$C_{22}H_{18}O_4N_4$ (structure 8 above), MW 402, colorless fine needles, m.p. 277°C (d), $\lambda_{max}^{MeOH}$ nm (lg ε): 226 (4.524), 232 (4.494 sh), 255 (4.093 sh), 265 (4.045 sh), 276 (3.908 sh), 290 (3.491 sh), 303 (3.447) and 315 (3.342). Toxicity: tremorgenic properties not demonstrated. Fungal source: *Aspergillus fumigatus*.
Reference: [188]

136. TRYPTOQUIVALINE G.
$C_{23}H_{20}O_5N_4$ (structure 9 above), MW 432, colorless

prisms, m.p. 240-242°C (d), $\lambda_{max}^{MeOH}$ nm (lg ε): 226 (4.535), 232 (4.501 sh), 253 (4.241 sh), 265 (4.072 sh), 275 (3.914 sh), 291 (3.568 sh), 302 (3.477) and 315 (3.398). Toxicity: tremorgenic properties not demonstrated. Fungal source: *Aspergillus fumigatus*.
References: [187,188]

137. TRYPTOQUIVALINE H.
$C_{22}H_{18}O_5N_4$ (structure 10 above), MW 418, colorless fine needles, m.p. 274°C (d), $\lambda_{max}^{MeOH}$ nm (lg ε): 226 (4.520), 232 (4.490 sh), 253 (4.220 sh), 266 (4.053 sh), 276 (3.929 sh), 291 (3.556 sh), 303 (3.491) and 315 (3.398). Toxicity: tremorgenic properties not demonstrated. Fungal source: *Aspergillus fumigatus*.
Reference: [188]

138. TRYPTOQUIVALINE I.
$C_{27}H_{26}O_6N_4$ (structure 11 above), MW 502, colorless leaflets, m.p. 232-236°C (d), $\lambda_{max}^{MeOH}$ nm (lg ε): 235 (4.501), 250 (4.330 sh), 292 (3.982) and 321 (3.785). Toxicity: tremorgenic properties not demonstrated. Fungal source: *Aspergillus fumigatus*.
Reference: [188]

139. TRYPTOQUIVALINE J.
$C_{22}H_{18}O_4N_4$ (structure 12 above), MW 402, colorless fine needles, m.p. 254-258°C (d), $\lambda_{max}^{MeOH}$ nm (lg ε): 226 (4.613), 231 (4.580 sh), 253 (4.210 sh), 264 (4.108 sh), 275 (3.987 sh), 290 (3.623 sh), 302 (3.591) and 310 (3.491). Toxicity: tremorgenic properties not demonstrated. Fungal source: *Aspergillus fumigatus*.
Reference: [188]

140. TRYPTOQUIVALINE L.
$C_{23}H_{20}O_5N_4$ (structure 13 above), MW 432, colorless leaflets, m.p. 265-268°C, $\lambda_{max}^{MeOH}$ nm (lg ε): 216 (4.530 sh), 226 (4.519), 231 (4.489 sh), 252 (4.241 sh), 264 (4.090 sh), 274 (3.924 sh), 290 (3.568 sh), 302 (3.477) and 315 (3.380). Toxicity: tremorgenic properties not reported. Fungal source: *Aspergillus fumigatus*.
Reference: [189]

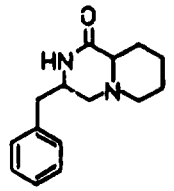
141. TRYPTOQUIVALINE M.
$C_{28}H_{28}O_7N_4$ (structure 14 above), MW 532, colorless plates, m.p. 157-164°C, $\lambda_{max}^{MeOH}$ nm (lg ε): 228(4.515), 232 (4.487), 255 (4.188), 278 (3.968), 305 (3.462) and 317 (3.431). Toxicity: tremorgenic properties not reported. Fungal source: *Aspergillus fumigatus*.
Reference: [189]

142. TRYPTOQUIVALINE N.
$C_{26}H_{24}O_5N_4$ (structure 15 above), MW 472, colorless needles, m.p. 193-197°C, $\lambda_{max}^{MeOH}$ nm (lg ε): 232 (4.509), 251 (4.253 sh), 291(3.934) and 320 (3.785). Toxicity: tremorgenic properties not reported. Fungal source: *Aspergillus fumigatus*.
Reference: [189]

TRYPTOQUIVALONE see 132

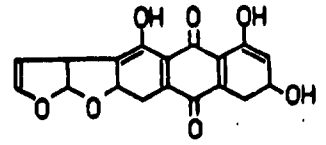
VERRUCULOGEN see 54

143. VERRUCULOTOXIN (3-Toluyl-octahydro-2H-pyrido-[1,2-a]-pyrazine). $C_{15}H_{20}ON_2$, MW 244, m.p. 152°C (from benzene), $\lambda_{max}^{MeOH}$ nm: strong end absorption and weak 240-260 band (lg ε not reported). Toxicity: toxic to cockerels, loss of muscular coordination, $LD_{50}$ in day-old cockerels was 20 mg/kg (orally). Fungal source: *Penicillium verruculosum* Peyronel.



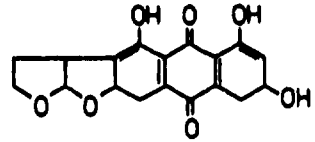
Reference: [49]

144. VERSICOLORIN A. (2,3-Bisfurano-1,6,8-trihydroxyantraquinone). $C_{18}H_{10}O_7$, MW 338, orange-yellow needles, m.p. 287-288°C (d) (from acetone), fine yellow needles: m.p. 287-288°C (d) (from hexane), $\lambda_{max}^{EtOH}$ nm (lg ε): 222 (4.498), 254(4.183), 265 (4.249), 290 (4.424), 321 (4.083) and 453(3.912). Toxicity: not reported. Fungal source: *Aspergillus versicolor*.



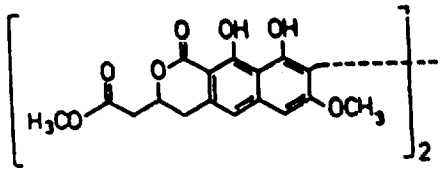
References: [77,102]

145. VERSICOLORIN B (2,3-Bistetrahydrofurano-1,6,8-trihydroxyantraquinone). $C_{18}H_{12}O_7$, MW 340, fine yellow needles, m.p. 298°C (d) (from acetone), $\lambda_{max}^{EtOH}$ nm (lg ε): 223 (4.378), 255 (4.130), 266 (4.290), 291 (4.380), 324 (4.107) and 450 (3.940). Toxicity: not reported. Fungal source: *Aspergillus versicolor* (Vuill.) Tiraboschi.



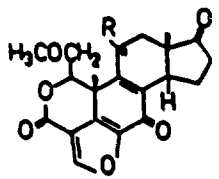
Reference: [77]

380. $\lambda^{Em}_{max}$ nm 430 and 550. Toxicity: antibiotic activity against *Bacillus brevis*, *B. cereus*, *Corynebacterium fascians* and *Streptococcus faecalis*.



References: [103,104]

134. WORTMANNIN (H-1 toxin). $C_{23}H_{24}O_8$ (structure 1). MW 428, white crystals, m.p. 222-223°C, $\lambda^{CHCl_3}_{max}$ (nm): 210, 254 and 292 (lg ε not reported). Toxicity: feed refusal factor in rats, causes hemorrhage in the stomach, intestines, heart and thymus and death. $LD_{50}$ in white female rats was 4 mg/kg (orally). Fungal source: *Penicillium wortmanni* Klocker (the compound is also produced by *Myrothecium roridum* Tode ex Fries and by species of *Fusarium*).



1 : R = $CH_3COO$-

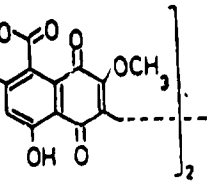
2 : R = H

References: [194-196]

135. 11-DESACETOXYWORTMANNIN. $C_{21}H_{22}O_6$ (structure 2 above), MW 370, white crystals. Toxicity: $LD_{50}$ in rats was 28 mg/kg (orally) and 51.5 mg/kg (i.p.); in Rhesus monkeys a single dose 1-3 mg/kg (s.c.) caused weakness, decreased mobility, incoordination and anorexia; the compound exhibited anti inflamatory activity on acute edema and anti arthritic activity. Fungal source: *Penicillium funiculosum* Thom.

Reference: [197]

156. XANTHOMEGNIN [(-)-3,3'-Bis-[2-methoxy-5-hydroxy-7-(2-hydroxypropyl)-8-carboxyl-1,4-naphtoquinone lactone]). $C_{30}H_{22}O_{12}$, MW 574, orange platelets, m.p. >264°C (d) (from chloroform-benzene). $\lambda^{dioxane}_{max}$ nm (lg ε): 228 (4.389), 289 (3.898) and 389 (3.898). $\lambda^{MeOH}_{max}$ nm (lg ε): 222 (4.415), 264 (4.286) and 380 (3.898). Toxicity: gross alterations, liver discolorations and histologic alterations in liver of mice fed diet containing 448 mg of xanthomegnin per kilogramme of feed. Fungal source: *Aspergillus ochraceus*, *A. melleus*, *A. sulphureus*, *Penicillium viridicatum*, *P. cyclopium*.



References: [33,64,99]

REFERENCES


1. M. Abe, S. Yamatodani, T. Yamano, Y. Kory and S. Yamada, Nippon Nogei Kagaku Kaishi, 41 (1967) 72.
2. M. Abe, S. Ohmomo, T. Ohashi and T. Tabuchi, Agric. Biol. Chem., 33 (1969) 464.
3. N.L. Allinger and J.L. Coke, J. Org. Chem., 26 (1961) 4522.
4. A.M. Ambrose and F. De Rds, J. Pharmacol. Exp. Ther., 88 (1946) 173.
5. R.J. Andereyg, K. Biemann, G. Büchi and M. Cushman, J. Am. Chem. Soc., 98 (1976) 3365.
6. R. Anderson, G. Büchi, B. Kobbe and A.L. Demain, J. Org. Chem., 42 (1977) 352.
7. W.K. Anslow and H. Raistrick, Biochem. J., 32 (1938) 687.
8. T. Asao, G. Büchi, M.M. Abdel-Kader, S.B. Chang, E.L. Wick and G.N. Wogan, J. Am. Chem. Soc., 85 (1963) 1706.
9. T. Asao, G. Büchi, M.M. Abdel-Kader, S.B. Chang, E.L. Wick and G.N. Wogan, J. Am. Chem. Soc., 87 (1965) 882.
10. P.J. Aucamp and C.W. Holzapfel, J. S. Afr. Inst. 22 (1969) 535
11. P.J. Aucamp and C.W. Holzapfel, J. S. Afr. Chem. Inst., 2: (1970) 40.
12. D.H.R. Barton and E. Miller, J. Chem. Soc., (1955) 1028.
13. V. Betina, L. Drobnica, P. Nemec and H. Zemanova, J. Antibiot., 17 (1964) 93.
14. A.J. Birch and J.J. Wright, Tetrahedron, 26 (1970) 2329.
15. J.H. Birkinshaw and H. Raistrick, Philos. Trans. R. Soc London, Ser. B., 220 (1931) 245.
16. J.H. Birkinshaw, A.R. Oxford and H. Raistrick, Biochem. J., 3( (1936) 394.
17. J.H. Birkinshaw, J. Biochem., 59 (1955) 485.
18. J.H. Birkinshaw, M. Luckner, Y.S. Mohammed, K. Mothes and C.E Stickinge, Biochem. J., 89 (1963) 196.
19. J. Blair and G.T. Newbold, Chem. Ind. (London), (1955) 2871.
20. A. Bracken, A. Pocker and H. Raistrick, Biochem. J., 57 (1954 587.
21. J.P. Brown, N.J. Cartwright, A. Robertson and W.B. Whalley, J Chem. Soc., (1949) 859.
22. W.A.C. Brown and G.A. Sim, J. Chem. Soc., (1963) 1050.
23. E. Bullock, J.C. Roberts and J.G. Underwood, J. Chem. Soc. (1962) 4179.
24. E. Bullock, D. Kirdaldy, J.C. Roberts and J.G. Underwood, J Chem. Soc., (1963) 829.
25. H.J. Burkhardt and J. Porgacs, Tetrahedron, 24 (1968) 717.
26. M.T. Bush, O. Touster and J.E. Brockman, J. Biol. Chem., 10 (1951) 685.
27. H.B. Butler, Br. J. Cancer, 18 (1964) 756.
28. W.H. Butler, J. Pathol., 102 (1970) 209.

APPENDIX 10



## Identification of Water-Soluble Coal-Tar Dyes Present in Foodstuffs

The general scheme for identifying coal-tar dyes present in foods normally involves:

1. Preliminary treatment of the food.
2. Extraction of the colour from the prepared solution of the food.
3. Separation of mixed colours if more than one is present.
4. Identification of the separated colours.

Schemes for the extraction and identification of colours from various foods have been described by the APA (*Separation and Identification of Food Colours Permitted by the Colouring Matters in Food Regulations*, 1957, APA, 1960, London), BFMIRA (*Analyst, Lond.*, 1963, 88, 854) and Stanley and Kirk (*J. agric. Fd Chem.*, 1963, 11, 492) and reviewed by Minor (*Lab Pract.*, 1962, 11, 33, 1307).

### 1. Preliminary Treatment of the Food

Assuming that an acidic colour is present, the preliminary treatment involves removing interfering substances and obtaining the dye in acid solution preparatory to boiling with wool.

(*a*) *Non-alcoholic liquids*, e.g. soft drinks: As most of the foods in this group are acidic they can usually be treated directly with wool. Otherwise, acidify slightly with acetic acid or potassium hydrogen sulphate.

(*b*) *Alcoholic liquids*, e.g. wines: Boil to remove the alcohol and acidify (if necessary) as for (*a*).

(*c*) *Soluble foods*, e.g. jams, sweets, icings: Dissolve in 30 ml water and treat as in (*a*).

(*d*) *Starch-based foods*, e.g. cakes, custard powder, golden raising powder: Grind 10 g of sample very thoroughly with 50 ml of 2% ammonia in 70% alcohol, allow to stand for some hours and centrifuge. Pour the separated liquid into a dish and evaporate on the water bath. Take up the residue in 30 ml water containing acid and treat as in (*a*).

(*e*) *Candied fruits*: Treat as in (*d*).

(*f*) *Products with a high fat content*, e.g. sausages, meat and fish pastes: De-fat with light petroleum and obtain the colour in aqueous solution by treatment with hot water (acidify, etc., as usual). Note that oil-soluble colours tend to give coloured solutions in organic solvents.

In difficult cases treatment with warm, 50-90% acetone or alcohol (which precipitates starch) containing 2% ammonia is often helpful. The organic solvent should be removed before acidifying (see (*d*) above).

For sausages, the BFMIRA Report recommends mixing 20 g sample with 14 ml water, 25 ml ethanol and 1 ml 0·88 ammonia. After setting aside for 30 min the liquid is filtered and concentrated. With fish paste, 20 g sample is thoroughly stirred with 6 ml water, 20 ml acetone and 1 drop 0·88 ammonia and after centrifuging and boiling off the acetone the sample is re-extracted and the process repeated. Fat is removed by shaking with light petroleum.



Note that basic colours dye the wool in alkaline solution and therefore require treatment with acid where the use of ammonia is called for above.

### 2. Extraction of the Colour from the Food

*N.B.* The wool should be prepared by boiling pure white knitting wool in dilute sodium or ammonium hydroxide and then in water (cf. Thaler, H. and Sommer, G., *Chem. Abstr.*, 1954, 48, 4714i).

Add a 20-cm strip of wool to about 35 ml of the prepared, slightly acidified solution and boil. Continue boiling, wash the wool under the cold tap, transfer it to a small beaker and boil gently with dilute ammonia. If the colour is stripped by the alkali, the presence of an acidic coal-tar dye is indicated. Remove the wool, make the liquid slightly acid and boil. Add a little wool and continue boiling until all the colour is removed. Extract the dye from the wool again with a small volume of dilute ammonia, filter through a small plug of cotton wool and evaporate to low bulk. This double stripping technique usually gives a purer product, but it is not always necessary.

Heat processing during manufacture and the wool dyeing may both affect the values obtained in the subsequent chromatography and spectrophotometry (Howard, F. J., *J. Assoc. Publ. Analysts*, 1965, 3, 137; BFMIRA, *loc. cit.*). Natural colours may also dye the wool during the first treatment, but the colour is not usually removed by ammonia.

Basic dyes can be separated by making the food alkaline with ammonia, boiling with wool and then stripping with acetic acid. As all the permitted water-soluble colours are acidic, an indication of the presence of a basic dye at this early stage suggests that a non-permitted colour is present. Basic rhodamine dyes were quite frequently used prior to the 1957 Regulations.

The use of synthetic polyamide powder (Silon or nylon) instead of wool avoids the necessity of de-fatting the sample (Davidek, J. and Davidkova, E. (*Analyt. Abstr.*, 1967, 14, 5741). Dyes may also be removed from treated food with organic solvents such as quinoline (Krauze, S. *et al.*, *Analyt. Abstr.*, 1962, 9, 3445) or higher alcohols (BFMIRA, *loc. cit.*).

### 3. Separation and Identification of the Extracted Colour(s)

*General tests.* Although it is always advisable to run chromatograms for the final detection of the colours, much work can often be saved by applying simple qualitative tests to fragments of the dyed wool and comparing the results with those obtained from known dyes. It is useful for instance to apply common reagents (e.g. conc HCl) which may produce characteristic colour changes. Some xanthene dyes are extracted by ether from aqueous solution. It is sometimes possible to detect the presence of mixed colours by spotting on filter paper and adding a few drops of water so that separate zones appear.

*Chromatographic separations.* Colours can be separated using any of the usual paper chromatographic techniques. The APA booklet recommends the use of Whatman CRL 1 paper in large beakers (Hunt, E. C., North, A. A. and Wells, R. A., *Analyst, Lond.*, 1955, 80, 172) for general work and the circular zone plate method of Rutter (*ibid.*, 1950, 75, 37) for the final identification.

*Solvents* (APA, BSI, BFMIRA)

No. 1 1 ml 0·88 ammonia + 99 ml water.

No. 2 2·5%

No. 4 *iso*-butanol (1 vol), ethanol (2 vols), water (1 vol).
No. 5 *n*-butanol (20 vols), water (12 vols), glacial acetic acid (5 vols).
No. 6 *iso*-butanol (3 vols), ethanol (2 vols), water (2 vols); then to 99 ml add 1 ml of 0·88 ammonia.
No. 7 80 g phenol + 20 g water.
No. 8 ethyl methyl ketone (350 vols), acetone (150 vols), water (150 vols), 0·88 ammonia (1 vol).
No. 9 ethyl methyl ketone (7 vols), acetone (3 vols), water (3 vols).
No. 10 ethyl acetate (11 vols), pyridine (5 vols), water (4 vols).
No. 11 Dilute 5 ml of 0·88 ammonia with water to 100 ml and dissolve 2 g trisodium citrate in the solution.
No. 12 water (30 vols), hydrochloric acid, sp.gr. 1·18 (6·5 vols).

*Separation of components of mixed colours.* Paint a horizontal line of the prepared dye solution about 2 cm from the bottom edge of a piece of Whatman No. 1 chromatographic paper and dry. Then elute upwards in a covered beaker using the most effective solvent to effect a separation (solvent No. 5 is often useful for general purposes, but it is advisable to study the $R_F$ values in the table first in relation to the probable colours present). If more than one colour appears to be present, cut out the separate lines of colour, extract with water or aqueous-acetone, evaporate to dryness and re-dissolve in a few drops of water. If the separation is indefinite, repeat the process using different solvents.

## 4. Identification of the Separated Colours

*Paper chromatography.* Cut a piece of Whatman No. 1 chromatographic paper so that it can be rolled into a cylinder which fits inside a litre beaker without touching the sides. Draw a line parallel to the bottom of the paper and about 2 cm away from it. Place a spot of the cone solution of the unknown dye on the line together with a series of spots of aqueous solutions of known dyes of similar colour and dry. Roll the paper into a cylinder and fasten it near the top and below the middle with an office stapling machine so that the vertical edges do not touch. Place the paper centrally in a litre beaker containing a 1 cm layer of selected solvent (Table 3.1) and immediately cover with a clock glass. When the solvent has risen about 10 cm above the baseline, remove the paper, mark the solvent front and compare the $R_F$ values of the unknown spot with those from known dyes on the same paper. Run similar chromatograms using different solvents, chosen for their ability to differentiate between any which have similar $R_F$ values on the first run. The $R_F$ values, of the permitted and non-permitted colours in Table 3.1 (Pearson D. and Walker, R., *J. Assoc. Publ. Analysts*, 1965, 3, 45) mostly represent the position of the bisector (mean of leading and trailing edges). With long streaks both the leading and trailing edges are quoted. Using the same method, Pearson and Chaudhri (*J. Assoc. Publ. Analysts*, 1964, 2, 22) and Pearson (*ibid.*, 1966, 4, 61) have tabulated the $R_F$ values for several other water-soluble colours (mostly ones not permitted to be used in foods in the UK). Many of the spots from non-permitted colours (e.g. rhodamines) show intense fluorescence under ultraviolet light.

Work on colours permitted in the USA using paper chromatography has been described by Tilden (*J. Ass. off. agric. Chem.*, 1952, 35, 423; 1953, 36, 802; 1954, 37, 812) and Stanley and Kirk (*loc. cit.*).

*Thin layer chromatography.* In general TLC gives more rapid and precise results than separations on paper. Dickes (*J. Assoc. Publ. Analysts*, 1965, 3, 49) has



(cf. Chapman, W. B. and Oakland, D., *J. Assoc. Publ. Analysts*, 1968, 6, 124; Perry, A. R. and Woolley, D. G., *ibid.*, 1969, 7, 94):

*Solvents*

A. *iso*-propanol (10 vols), 0·88 ammonia (1 vol), water (1 vol).
B. Saturated potassium nitrate solution.

METHOD

Prepare thin layers (250 μ) of Kieselgel G on 20 × 10 cm glass plates and heat at 160°C for 1½ hr. Cool in a desiccator. Pipette 5 μl of each of the aqueous solutions of the colours at least 2 cm from the edge of the plate. Place in a suitable tank containing solvent. Develop at 20°C for a distance of 10 cm and calculate the $R_F$ values. For spectrophotometric confirmation remove the silica and colour with a suction assembly (Sahasrabudhe, M. R., *J. Ass. off. agric. Chem.*, 1964, 47, 889). Dickes' paper tabulates the $R_F$ values for permitted and non-permitted colours using the two-solvent system. In all cases the unknown spot should be compared with those obtained from known colours.

*Confirmation of the Identity of Dyes using the Spectrophotometer*

Dilute the pure neutral dye solution to a suitable intensity of colour and determine the absorption curve on the spectrophotometer in neutral, acid and alkaline solution and compare the maxima with those obtained with the known dye (Table 3.1). The APA booklet illustrates the absorption curves of all the permitted water-soluble colours, except Black 7984 which has been examined by Pearson (*J. Assoc. Publ. Analysts*, 1967, 5, 37). Each dye was examined in neutral (0·02% ammonium acetate), acid (0·1 N HCl) and alkaline (0·1 N NaOH) solution. Dyeing and stripping sometimes causes some decomposition of colours and this affects the chromatograms and spectrophotometric curves obtained. If the dye appears to be a non-permitted one, it is particularly important to apply chemical tests also.

### Oil-Soluble Colours

Oil Yellow GG and Oil Yellow XP are the only oil-soluble coal tar colours on the permitted list. Oil Yellow GG can be extracted by sodium hydroxide from its ethereal solution, but XP and many of the non-permitted colours cannot. For the isolation of the purified colour, the oil should first be extracted from the food with light petroleum. Then the colour can be removed with fairly concentrated acid, (*AOAC*, 1965, p. 673), or by solvent partition using N,N-dimethylformamide (DMF) and light petroleum. The latter method due to Mark and McKeown (*J. Ass. off. agric. Chem.*, 1958, 41, 817) is described below:

METHOD

Dissolve 10 g of oil in 50 ml light petroleum, filter and transfer to a separator. Extract with three 20 ml portions of DMF, discarding the petrol layer. Extract the combined DMF solution with four 25 ml portions of light petroleum, back-extracting each time with 5 ml DMF. Discard the petrol extracts. Dilute the combined DMF solution with an equal volume of water and extract with one 30 ml and one 10 ml portion of chloroform. Centrifuge and discard the aqueous DMF layer. Wash the combined chloroform solution with water to remove dissolved DMF. Evaporate the chloroform solution to dryness at room tempera-

or spectrophotometric examination may commence at this point). Dissolve the residue in 25 ml DMF, transfer to a separator, add 25 ml water and extract with four 25 ml portions of light petroleum. Discard the aqueous DMF layer, wash the combined light petroleum solution with water to remove the dissolved DMF and evaporate the light petroleum solution under reduced pressure at room temperature.

Oil-soluble colours can be separated by reverse-phase paper chromotography. The papers used are previously soaked in a 10% solution of liquid paraffin in mixed ethers, drained and then dried under vacuum. The paper is spotted with the colour after dissolving in a suitable solvent (e.g. ether, mixed ethers or alcohol). Suitable chromatographic solvents are (1) 70 vols acetone+30 vols water and (2) 60 vols dioxan+40 vols water (cf. Silk, R. S., *J. Ass. off. agric. Chem.*, 1959, 42, 427; Szokolay, A., *Analyt. Abstr.*, 1964, 11, 1099).

Davidek, Pokorny and Janicek (*Analyt. Abstr.*, 1962, 9, 4481) have described methods for the detection and determination of oil-soluble dyes using TLC on alumina.

Oil-soluble colours can also be identified by dissolving the extracted dye in chloroform or acetone and obtaining the absorption curve by means of the spectrophotometer. The absorption peaks for the permitted oil-soluble colours are Oil Yellow GG at 380 mμ and Oil Yellow XP at 405 mμ.

## Natural Colours

Tests for various natural and other colours are given in the AOAC (1965, 10th Edn, p. 673). Pigments of annatto can be separated by TLC (Ramamurthy, M. K. and Bhalerao, V. R., *Analyst, Lond.*, 1964, 89, 740; Francis, B. J., *ibid.*, 1965, 90, 374).

## EMULSIFIERS AND STABILISERS

In 1956 the Food Standards Committee adopted a Report on Emulsifying and Stabilising Agents which they had received from their Preservatives Sub-Committee. Most of the substances covered in the Report do act as emulsifiers and stabilisers, but some of them are used for other properties they possess, e.g. as crumb-softeners or anti-staling agents, or to produce or break foams. The Report added that when emulsifying agents were first used they were few in number and those used were either natural substances, particularly gums or synthetic substances of simple constitution. Progress in chemical synthesis, however, has now made available a large number of new products with characteristics suitable for virtually every requirement. The possible emulsifiers and stabilisers were classified as to available biological data and whether their use in food appeared to



### THE FIRST SCHEDULE

PERMITTED EMULSIFIERS AND PERMITTED STABILISERS

Stearyl tartrate.
Complete glycerol esters.
Partial glycerol esters.
Partial polyglycerol esters.
Propylene glycol esters.
Monostearin sodium sulphoacetate.
Sorbitan esters of fatty acids and their polyoxyethylene derivatives.
Cellulose ethers.
Sodium carboxymethyl cellulose.
Brominated edible vegetable oils.*

* Due to be withdrawn from permitted list on 1st September 1970.

Important provisions are given in Regulations 3 and 4 (cf. p. 444):

*Sale, etc. of food containing emulsifiers or stabilisers*

3.—(1) Subject to the provisions of this regulation, no food shall contain any emulsifier or any stabiliser which is not a permitted emulsifier or a permitted stabiliser.

(2) No flour, intended for sale as such, shall contain any emulsifier or any stabiliser.

(3) No bread shall contain any emulsifier or any stabiliser other than stearyl tartrate or partial glycerol esters.

(4) No person shall sell, consign or deliver, or import into England and Wales, any food which does not comply with this regulation.

(5) This regulation shall not apply in relation to any food containing any emulsifier or any stabiliser:

(*a*) inasmuch as that emulsifier or that stabiliser is naturally present in that food;

(*b*) by reason not of the use of that emulsifier or that stabiliser as an ingredient in that food but only of the use during the preparation of that food of a tin greasing emulsion containing that emulsifier or that stabiliser.

*Sale, etc. of cream and reconstituted cream* (cf. p. 444)

4.—(1) No cream or reconstituted cream shall contain any thickening substance.

(2) No person shall sell, consign or deliver, or import into England and Wales, any cream or reconstituted cream which does not comply with this regulation.

Under 'Interpretation', Regulation 2 includes the following definitions:

'emulsifier' and 'stabiliser' mean respectively any substance, other than a natural food substance, which is capable:

(*a*) in the case of an emulsifier, of aiding the formation of, and
(*b*) in the case of a stabiliser, of maintaining

the uniform dispersion of two or more immiscible substances, but do not in either case include agar, alginic acid, calcium alginate, sodium alginate, carrageen, edible gums, dextrin, pectin, calcium pectate, sodium pectate, calcium chloride, calcium lactate, lecithin, quillaia, proteins (whether hydrolysed or not) and

# APPENDIX 10

## ADDITIVES AND CONTAMINANTS

TABLE 3.1

Rf values[1] and spectrophotometric peaks[2, 3] of various water-soluble colours

| | Solvent number 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | Main spectrophotometric peaks mμ |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **REDS—** | | | | | | | | | | | | | |
| Ponceau MX‡ | 0·06–0·67 | 0·12 | 0·40 | 0·50 | 0·33 | 0·55 | 0·35 | 0·41 | 0·41 | 0·23 | 0·19 | 0·06 | 500 in acid |
| Ponceau 4R | 0·87 | 0·39 | 0·51 | 0·32 | 0·18 | 0·26 | 0·13 | 0·26 | 0·25 | 0·07 | 0·57 | 0·33 | 505 in acid |
| Carmoisine | 0·48 | 0·08 | 0·59 | 0·54 | 0·44 | 0·17 | 0·37 | 0·28 | 0·55 | 0·30 | 0·15 | 0·05 | 515 in acid |
| Amaranth | 0·62 | 0·15 | 0·27 | 0·29 | 0·14 | 0·19 | 0·11 | 0·17 | 0·16 | 0·04 | 0·33 | 0·07 | 520 in acid |
| Red 10B | 0·51 | 0·14 | 0·36 | 0·37 | 0·26 | 0·30 | 0·23 | 0·37 | 0·37 | 0·21 | 0·20 | 0·00, 0·14 | 525 in acid |
| Erythrosine BS | 0·21 | 0·03 | 0·52 | 0·61 | 1·00 | 0·58 | 0·47 | 0·57 | 0·52, 0·36–1·00 | 0·56, 0·20–0·68 | 0·06 | 0·00 | 525 in alkali |
| Red 2G | 0·70 | 0·18 | 0·42 | 0·43 | 0·34 | 0·35 | 0·38 | 0·39 | 0·41 | 0·18 | 0·46 | 0·16 | 530 in acid |
| Red 6B | 0·48 | 0·08 | 0·20 | 0·26 | 0·18 | 0·17 | 0·37 | 0·22 | 0·22 | 0·10 | 0·28 | 0·05 | 515 in acid |
| Red FB | 0·11 | 0·01 | 0·10 | 0·22 | 0·25 | 0·11 | 0·49 | 0·13 | 0·00–0·58 | 0·24, 0·00–0·35 | 0·01 | 0·00 | 385 and 495 in acid |
| Ponceau SX† | 0·67 | 0·10 | 0·45 | 0·48 | 0·39 | 0·30 | 0·41 | 0·39 | 0·51 | 0·26 | 0·32 | 0·07 | 500 in acid |
| Ponceau 3R† | 0·27 | 0·08 | 0·35 | 0·43 | 0·39 | 0·47 | 0·36 | 0·45 | 0·58 | 0·21 | 0·11 | 0·04 | 505 in acid |
| Fast Red E | 0·44 | 0·10 | 0·60 | 0·50 | 0·38 | 0·47 | 0·45 | 0·49 | 0·51 | 0·24 | 0·19 | 0·06 | 505 in acid |
| **ORANGES—** | | | | | | | | | | | | | |
| Orange G | 0·91 | 0·61 | 0·78 | 0·51 | 0·35 | 0·47 | 0·48 | 0·52 | 0·52 | 0·23 | 0·66 | 0·54 | 475 in acid |
| Orange RN | 0·25 | 0·08 | 0·84 | 0·80 | 0·59 | 0·75 | 0·74 | 0·75 | 0·78 | 0·57 | 0·28 | 0·09 | 485 in acid |
| Sunset Yellow FCF | 0·73 | 0·29 | 0·72 | 0·46 | 0·28 | 0·45 | 0·40 | 0·43 | 0·46 | 0·22 | 0·43 | 0·24 | 480 in acid |
| **YELLOWS—** | | | | | | | | | | | | | |
| Tartrazine | 0·91 | 0·32 | 0·41 | 0·28 | 0·12 | 0·17 | 0·09 | 0·20 | 0·25 | 0·04 | 0·70 | 0·33 | 430 in acid |
| Naphthol Yellow S† | 0·73 | 0·36 | 0·76 | 0·50 | 0·44 | 0·54 | 0·17 | 0·68 | 0·73 | 0·44 | 0·40 | 0·57 | 370 in acid |
| Yellow 2G | 0·96 | 0·81 | 1·00 | 0·63 | 0·44 | 0·41 | 0·41 | 0·37 | 0·65 | 0·31 | 0·76 | 0·75 | 400 in acid |
| Yellow RFS† | 0·85 | 0·56 | 0·79 | 0·46 | 0·33 | 0·47 | 0·30 | 0·43 | 0·47 | 0·22 | 0·54 | 0·69 | 510 in acid |
| Yellow RY† | 0·84 | 0·27 | 0·23 | 0·22 | 0·07 | 0·04 | 0·18 | 0·07 | 0·16 | 0·03 | 0·27 | 0·23 | 435 in acid |
| **GREENS, BLUES AND VIOLETS—** | | | | | | | | | | | | | |
| Green S | 0·96 | 0·70 | 1·00 | 0·63 | 0·44 | 0·44 | 0·70 | 0·41 | 0·67 | 0·30 | 0·83 | 0·90 | 635 in acid |
| Blue VRS† | 0·84 | 0·78 | 1·00 | 0·76 | 0·54 | 0·70 | 0·76 | 0·64 | 0·70 | 0·32 | 0·79 | 1·00 | 415 in acid |
| Indigo Carmine | 0·52 | 0·12 | 0·27 | 0·25 | 0·14 | 0·20 | 0·30 | 0·28 | 0·34 | 0·14 | 0·15 | 0·13 | 285 and 615 in acid |
| Violet BNP | 0·83 | 0·00–0·88 | 1·00 | 0·72 | 0·54 | 0·63 | 0·80 | 0·68 | 0·75 | 0·32 | 0·00–0·94 | 0·89 | 590 in neutral |
| **BROWNS, BLACKS—** | | | | | | | | | | | | | |
| Brown FK | 0·08, 0·26 | 0·03 | 0·00–0·78 | 0·39 | 0·18 | 0·39 | 0·36, 0·49, 0·84 | 0·75, 0·57 | 0·77, 0·61 | 0·49, 0·27 | 0·18, 0·03 | 0·00, 0·12 | 455 in acid |
| Chocolate Brown FB | 0·00–1·00 | 0·00–1·00 | 0·00–1·00 | 0·22 | 0·00–0·49 | 0·13 | 0·00–0·50 | 0·15 | 0·15 | 0·00 | 0·00–1·00 | 0·00–1·00* | 470 in acid |
| Chocolate Brown HT | 0·00–1·00 | 0·00–1·00 | 0·00–1·00 | 0·22 | 0·00–0·47 | 0·13 | 0·00–0·50 | 0·15 | 0·15 | 0·00 | 0·00–1·00 | 0·00–1·00* | 465 in acid |
| Black PN | 0·00–0·80 | 0·06 | 0·10 | 0·17 | 0·05 | 0·06 | 0·10 | 0·13 | 0·14 | 0·02 | 0·10 | 0·02 | 570 in acid |
| Black 7984 | 0·00–0·68 | 0·05 | 0·06 | 0·12 | 0·07 | 0·10 | 0·03 | 0·11 | 0·00 | 0·03 | 0·06 | 0·00 | 565 in acid |

* Position of leading edge of streak varies with concentration.

† These colours were withdrawn from the permitted list on 26th June 1967.
‡ Due to be withdrawn from the permitted list in 1971.

References: 1. Pearson, D. and Walker, R. W., *J. Assoc. Publ. Analysts*, 1965, 3, 45.
2. APA, Separation and Identification of Food Colours Permitted by The Colouring Matters in Food Regulations, 1957 (published 1960).
3. Pearson, D., *J. Assoc. Publ. Analysts*, 1967, 5, 37.

APPENDIX 11

Table [1]. List of preservatives permitted for use in food within the European Economic Community (EEC)

Preservatives (in the proper sense of the term)

| | |
|---|---|
| E 200 | Sorbic acid |
| E 201 | Sodium sorbate (sodium salt of sorbic acid) |
| E 202 | Potassium sorbate (potassium salt of sorbic acid) |
| E 203 | Calcium sorbate (calcium salt of sorbic acid) |
| E 210 | Benzoic acid |
| E 211 | Sodium benzoate (sodium salt of benzoic acid) |
| E 212 | Potassium benzoate (potassium salt of benzoic acid) |
| E 213 | Calcium benzoate (calcium salt of benzoic acid) |
| E 214 | Ethyl p-hydroxybenzoate (ethyl ester of p-hydroxybenzoic ac |
| E 215 | Sodium ethyl p-hydroxybenzoate |
| E 216 | Propyl p-hydroxybenzoate (propyl ester of p-hydroxybenzoic acid) |
| E 217 | Sodium propyl p-hydroxybenzoate |
| E 218 | Methyl p-hydroxybenzoate (methyl ester of p-hydroxybenzoic acid) |
| E 219 | Sodium methyl p-hydroxybenzoate |
| E 220 | Sulfur dioxide |
| E 221 | Sodium sulfite |
| E 222 | Sodium bisulfite (acid sodium sulfite) |
| E 223 | Sodium metabisulfite (sodium pyrosulfite or sodium disulfite |
| E 224 | Potassium metabisulfite (potassium pyrosulfite or potassium disulfite) |
| E 226 | Calcium sulfite |
| E 227 | Calcium bisulfite (calcium hydrogen sulfite) |
| E 230 | Biphenyl (diphenyl) |
| E 231 | Orthophenyl phenol |
| E 232 | Sodium orthophenyl phenolate |
| E 233 | Thiabendazoic |
| E 236 | Formic acid |
| E 237 | Sodium formate (sodium salt of formic acid) |
| E 238 | Calcium formate (calcium salt of formic acid) |
| E 239 | Hexamethylene tetramine |

Preserving agents exclusively for surface treatment

| | |
|---|---|
| E 240 | Sodium silicate |
| E 241 | Potassium silicate |
| E 242 | Calcium hydroxide [illegible] |
| E 243 | Paraffin wax |

Substances intended mainly for other purposes but which may have a subsidiary preservative property

| | |
|---|---|
| E 249 | Potassium nitrite |
| E 250 | Sodium nitrite |
| E 251 | Sodium nitrate |
| E 252 | Potassium nitrate |
| E 260 | Acetic acid |
| E 261 | Potassium acetate |
| E 262 | Sodium diacetate |
| E 263 | Calcium acetate |
| E 270 | Lactic acid |

APPENDIX 11

Table 1 (Contd.)

E 280 Propionic acid
E 281 Sodium propionate (sodium salt of propionic acid)
E 282 Calcium propionate (calcium salt of propionic acid)
E 283 Potassium propionate (potassium salt of propionic acid)
E 290 Carbon dioxide

Table 2. Admissibility of the foremost preservatives in some countries

| | sulfur dioxide | propionic acid | sorbic acid | benzoic acid | p-hydroxybenzoic acid ester |
|---|---|---|---|---|---|
| Australia | ++ | + | ++ | ++ | + |
| Austria | ++ | + | ++ | ++ | ++ |
| Belgium | ++ | + | ++ | ++ | + |
| Brazil | ++ | + | ++ | ++ | |
| Canada | ++ | + | ++ | ++ | |
| Denmark | ++ | + | ++ | ++ | + |
| EEC guidelines | ++ | + | ++ | ++ | ++ |
| Finland | ++ | + | ++ | ++ | ++ |
| France | + | (+) | + | (+) | (+) |
| Germany, Dem. Republic | ++ | + | ++ | ++ | ++ |
| Germany, Fed. | ++ | + | ++ | ++ | ++ |
| Italy | ++ | + | ++ | + | + |
| Japan | ++ | + | ++ | ++ | + |
| Netherlands | ++ | + | ++ | ++ | + |
| New Zealand | ++ | + | ++ | ++ | + |
| Norway | ++ | + | ++ | ++ | + |
| South Africa | ++ | + | ++ | ++ | + |
| Soviet Union | ++ | | ++ | ++ | |
| Spain | ++ | + | ++ | ++ | + |
| Sweden | ++ | + | ++ | ++ | + |
| Switzerland | ++ | + | ++ | ++ | ++ |
| UK | ++ | + | + | ++ | ++ |
| USA | ++ | | | | |
| Yugoslavia | ++ | + | ++ | ++ | |

Key to symbols: ++ permitted for many foods

(+) permitted only in exceptional cases

# APPENDIX 12

Table - Acceptable Daily Intake of Preservatives

| | acceptable daily intake (mg/kg body weight per day |
|---|---|
| Acetic acid, including its potassium and sodium salts | no limit |
| Benzoic acid, including its potassium and sodium salts | 0 - 5 |
| Diethyl pyrocarbonate | should not be used; value formerly determine has been deleted |
| Formic acid | 0 - 3 |
| Hexamethylene tetramine | 0 - 0.15 |
| p-Hydroxybenzoic acid butyl ester | not yet determined |
| p-Hydroxybenzoic acid ethyl ester | 0 - 10 |
| p-Hydroxybenzoic acid methyl ester | 0 - 10 |
| p-Hydroxybenzoic acid propyl ester | 0 - 10 |
| Hydrogen peroxide | not determined |
| Lactic acid, including its ammonium, calcium, potassium and sodium salts | no limit |
| Natamycin (pimaricin) | 0 - 0.3 |
| Propionic acid, including its calcium, potassium and sodium salts | no limit |
| Sodium diacetate | 0 - 15 |
| Sodium nitrate and potassium nitrate | 0 - 5 |
| Sodium nitrite and potassium nitrite | 0 - 0.2 (temporary) |
| Sorbic acid, including its calcium, potassium and sodium salts | 0 - 25 |
| Sulfur dioxide, sodium and potassium metabisulfite, sodium sulfite and sodium hydrogen sulfite | 0 - 0.7 |

APPENDIX 12

Table 12: Mode of action of some preservatives on microorganisms

| | Bacteria | Yeasts | Molds |
|---|---|---|---|
| Nitrite | ++ | - | - |
| Sulfite | ++ | + | + |
| Formic acid | + | ++ | ++ |
| Propionic acid | + | ++ | ++ |
| Sorbic acid | + | +++ | +++ |
| Benzoic acid | ++ | +++ | +++ |
| p-Hydroxybenzoic acid esters | ++ | +++ | +++ |
| Diphenyl | - | ++ | ++ |

Key:
- \- ineffective
- \+ slightly effective
- ++ moderately effective
- +++ highly effective

APPENDIX 13

Paper prepared for seminar "Food Quality Control" at the Department of Quality Control and Metrology in Hanoi, March 1993.

# FERMENTED SOY PRODUCTS
## MANUFACURE, QUALITY AND HEALTH ASPECT

Many kinds of soy products are used as a nutrient foods. In the Far East very popular are fermented soybean products such as miso and soy sauce.

## MISO

Miso is a semisolid fermented food made from soybeans, rice or barley and salt. Miso is popular in various countries produced at different types under different names.

*Types*
*Barley miso* (with barley—Japan)
*Chiang* (often with wheat flour—China)
*Denjang* (with rice or barley—Korea)
*Doenjang* (same as *denjang*—Korea)
*Edo miso* (light red-brown colour, low salt, short fermentation—Tokyo, Japan)
*Hishio* (Japan)
*Kinzanji miso* (Japan)
*Kochuman* (with rice and mustard—Korea)
*Kome miso* (with milled rice—Japan)
*Mame miso* (all soybean, no cereal, long fermentation—deep red-brown colour—Japan)
*Mugi-miso* (with barley—Japan)
*Rice miso* (with rice, the major type in Japan)
*Sendai miso* (red-brown colour, high salt content—Japan)
*Shinsu miso* (with rice, light yellow-brown colour, high salt—Japan)
*Soybean paste* (general term for *miso*)
*Soy jam* (with wheat, thick viscous black paste, may be sediment which forms during production of *soy sauce*—China)
*Tao-chieo* (Thailand)
*Taoco* (Indonesia)
*Tao-tjung* (China)
*Tenjan* (same as *denjang*—Korea)
*Tien mien chang* (from wheat, using steamed bread—China)
*White miso* (with rice, low salt, light colour, only 1 week fermentation—Japan)

Miso can be in general classified into three major types on the basis of the raw materials: rice miso made from rice, soybean miso made from soybean and salt, barley miso made from barley. These types are further classified by taste into sweet medium salty and salty groups. Each group is further classified by colour into white, light yellow and red.

Composition of several kinds of miso is presented on table 1.

Table 1
Composition of Several Kinds of Miso

| Variety | Moisture (%) | Protein (%) | Reducing sugar (%) | Fat (%) | Sodium chloride (%) |
|---|---|---|---|---|---|
| White miso | 44 | 8 | 33 | 2 | 5 |
| Edo sweet miso | 46 | 10 | 20 | 4 | 6 |
| Salty light yellow miso | 49 | 11 | 13 | 5 | 12 |
| Salty red miso | 50 | 12 | 14 | 6 | 13 |
| Barley sweet miso | 46 | 11 | 15 | 5 | 11 |
| Barley salty miso | 48 | 12 | 11 | 5 | 12 |
| Soybean miso | 47 | 19 | 2 | 10 | 10 |

The manufacturing process of miso is presented on fig 1.



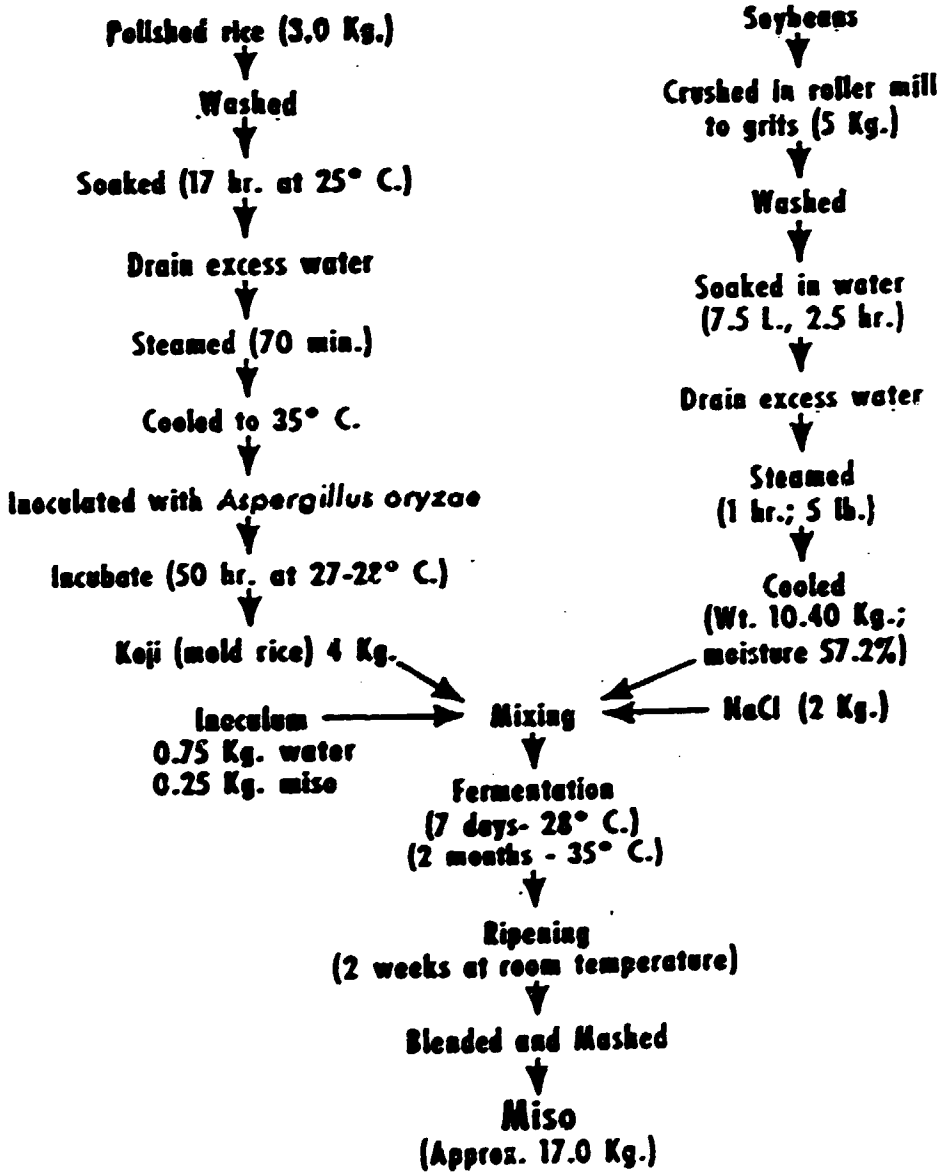



FIG. 1 Flow sheet on production of miso.

Although the manufacturing method differs from variety to variety, the principle is almost always the same. The outline of the process for manufaturing salty rice miso,one of the most principal varieties, consists of cooking soybeans, preparation of Koji, mixing with fermentation is in a vat or tank. After fermentation is blending and packaging including sterilization.

For koji fermentation, milled rice is at first cleaned and washed and then soaked in water at a temperature of 15°C for 15 h or more. The soaked rice is drained off to be steamed in a open cooker for 40-70 min. After coolling the spores of Aspergillus oryzae specially prepared for this purpose are sprayed on the rice and mixed well. The most suitable conditions are 30°C and 96% relative humidity for 15 h mold develops. The completed Koji is taken out 40-48 h after inoculation to be mixed with salt to halt further mold development. Then the cooked soybeans are mixed with salted rice koji, and with the inoculum of culture yeast and lactic acid bacteria until an even mixture is obtained.
This mixture green miso is packed into a fermenting vat or tank usually of 4 tons or more capacity and covered with a plate. Fermentation is at around 30°C for the 1-3 months depending on the variety being made. During this period this mixture is moved from one vat to another at least twice to improve the fermentation. Fermented miso is transfered to a ripening vat or tank held at room temperature. The ripened miso is then blended and mashed. From 1000 Kg of soybeans, 600 Kg rice and 430 Kg of salt could obtained 2700 Kg of miso with 48% water content. The shelf life of miso depends on the variety only 1 week for white miso, 2 weeks for Edo miso, 2 - 3 months for salty ligh yellow miso and 5 months for salty red miso kept unpackaged at temperature below 20°C. Sometimes sorbic acid is added as a preservative at a level less than 1 g/Kg of rice ( ).

In Japan miso is very popular as a national food. Usually is eaten as an ingredient of miso soup, or as a sweet paste with sugar and a variety of foods such as meat, fish, vegetables and fruit.

Recently the technique of miso production has progressed spectacularly. Automated fermentations and continous cookers are used for the industrial production. In 1985 the total production amounted more than 600,000 tons in Japan. Recently miso has often been sold in Japan in plastic film bags, each containing 500-1000 g. Miso is pasteurised to prevent swelling caused by activity of remaining living yeast cells.

SOYSAUCE

Soysauce or shoyu - name for soysauce in Japan, is a brown liquid made from soybeans often with wheat or other ceral crops. It is a very popular liquid pungent seasoning used in Oriental cuisine with almost every dish. The varietyies and uses of soysauce in Japan are given in table 2.

Table 2- Characteristics and uses of various types of soysauces

| Soy sauce | Characteristic | Use |
|---|---|---|
| Regular soy sauce | Strong aroma<br>Exellent flavour<br>Deep reddish brown | Cooking condiment for every foodstuff. Table condiment for every foodstuff |
| Light-coloured soy sauce | Light reddish brown colour | Cooking for keeping the original flavour and colour of the foodstuff itself |
| Tamari soy sauce | Dark brown colour, Rich consistency, Deeper flavour | Japanese rice cracker. Table condiment for raw fish |
| Saushikomi soy sauce | Dark brown colour, Strong taste | Cooking for hot dishes, "Kabayaki of eel" Glaze of "Sushi" |
| Shiro soy sauce | Light yellow to tan colour, sweet. | Cooking for keeping the original colour of foodstuff itself, Japanese noodle soup. |

In Oriental countries the dining table brings out the best flavors. People without a taste for soysauce would not enjoy oriental cooking. Many varieties of soy sauce are produced in all Asian countries types with names used popular in those contries.
In some Asian countries soysauce is conducted by people in small family-operated plants.
In Korea, the homemade soysauce is prepared by a traditional method in which cooked soybeans are smashed and made into small balls, then subjected to natural inoculation of Aspergillus and Rhizopus moulds. This process taking several months in Winter. When Spring comes, this mold-cultured materials are extracted with salt water. The liquid part is boiled and fermented under the sun to make soysauce. The resideu of extraction is mixed with salt and stored to make miso.

Below are leasted types of soysauce produced in Asian countries.

*Types*

*Chau yau* (mainly soybeans, dark colour, sometimes with added sugar—China; Korea)
*Chiang-yu* (with wheat or barley—Mandarin, China)
*Ganjang* (with cayenne pepper—Korea)
*Hao yu* (type of *oyster sauce*—China)
*Huan tou* (China)
*Inyu* (from black soybeans—Taiwan)
*Jan* (with wheat—Korea)
*Jang* (Korea)
*Kanjang* (Korea)
*Kecap* (from black soybeans, with roasted cassava flour—Indonesia)
*Kecap asin* (salty—Indonesia)
*Kecap manis* (sweetened—Indonesia)
*Ketjap* (same as *kecap*—Indonesia)
*Kicap* (Malaysia)
*Koikuchi shoyu* (with 50% wheat, main type in Japan)
*Lak-yau* (thick variety—Canton, China)
*Makjang* (Korea)
*Meju* (Korea)
*Oyster sauce* (oysters cooked in soy sauce to give mild fish flavour—China)
*Pak yau* (China)
*Saishikomi shoyu* (dark brown colour—Japan)
*Sai-yau* (thick—Canton, China)
*San-chau* (thin—Canton, China)
*See-iau* (same as *si-iu*—Thailand)
*Shih-yu* (with wheat—Mandarin, China)
*Shimshiki shoyu* (Japan)
*Shiro shoyu* (yellow-brown colour—Japan)
*Sho* (Japan)
*Shoyu* (main name for soy sauce in Japan)
*Si-iu* (Central Thailand)
*Si-yau* (with wheat—Canton, China)
*Tamari* (with 10% wheat, dark brown colour—China)
*Tao-yu* (Indonesia; China)
*Toyo* (Philippines)
*Usukuchi shoyu* (with wheat, light colour—Japan)

According Japanise Agricultural Standards the production of fermented shoyu is as follow.
Raw material soybeans and wheat are inoculated with Koji mold (Aspergillus oryzae or A. sojae) to make a Koji. Then this Koji is mixed with salt water make mash or maromi. Maromi is fermented with lactobacilli and yeast and then well aged.The flow sheet on production of shoyu is presented in Fig. 2



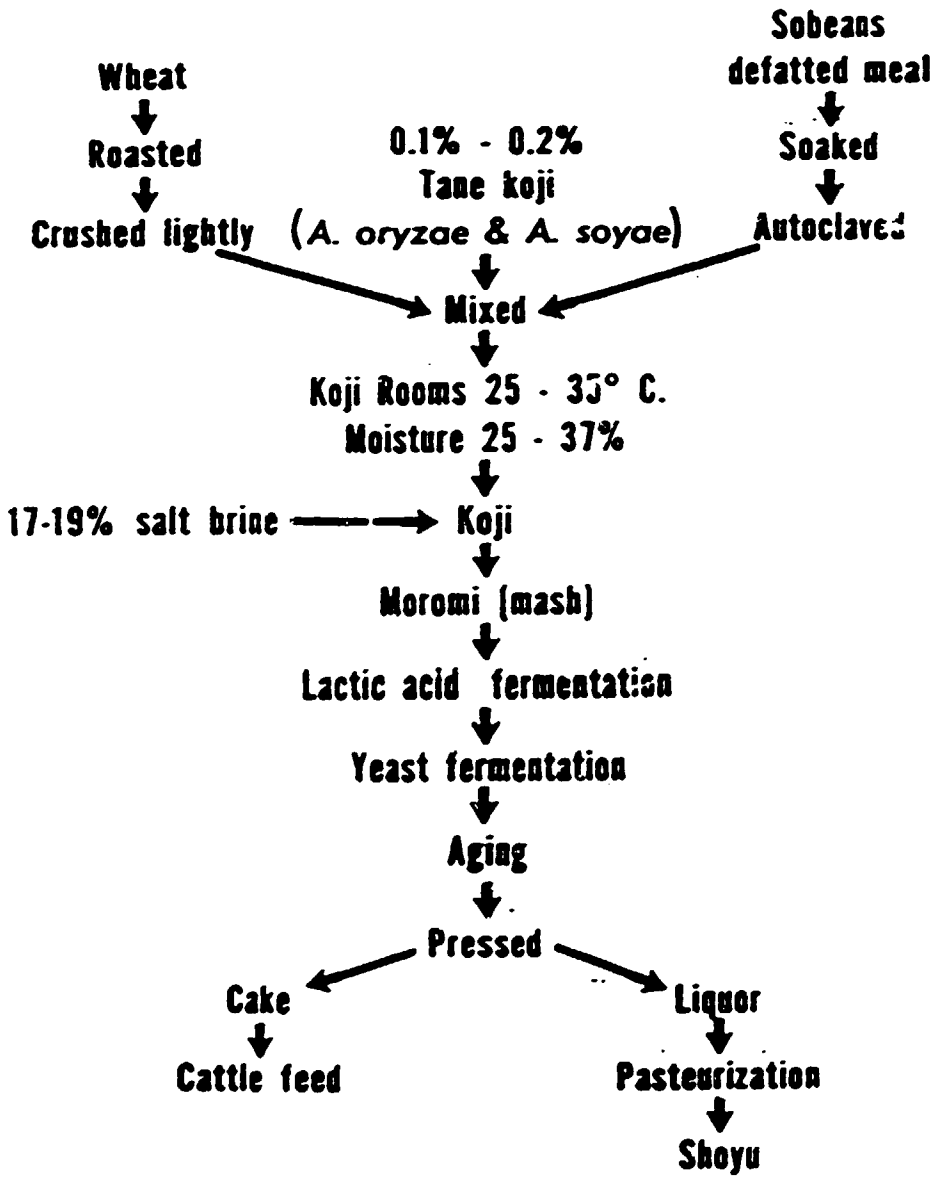



FIG. 2. Flow sheet on production of shoyu.

# MANUFACTURE

## A. KOIKUCHI SHOYU

Japanese-fermented shoyu of the koikuchi type involves five main processes: the treatment of raw materials, the making of koji, the making and aging of mash, pressing, and refining. One example of the preparation of koikuchi shoyu is schematically indicated in Fig. 1.

### *1. Treatment of Raw Materials*

Whole soybeans, or more commonly, defatted soybean grits, are moistened and cooked with steam under pressure. This process greatly influences the digestibility of soybean protein. Details will be provided in a later section. Wheat kernels, the other half of the raw materials, are roasted at 170–180°C for a few minutes, then coarsely crushed into four or five pieces.

### *2. The Making of Koji*

These two materials are inoculated with a small amount of seed mold or pure culture of *A. oryzae* or *A. sojae*. This mixture is spread to a depth of 30–40 cm on a large perforated stainless-steel plate having a rectangular shape that is 5 m in width and 12 m in length, for example, or a doughnut shape with a diameter of 15–30 m. The heat-treated raw materials are aerated for 2–3 days with controlled temperatures and moisture-controlled air, which comes up from the bottom holes through the ingredients to create the proper conditions for mold cultivation and enzyme formation. The temperature of the materials is kept at ~30°C, and the moisture content of the materials, which is 40–43% at the beginning of cultivation, decreases to 25–30% after 2 or 3 days. This allows the mold to grow throughout the mass and provides the enzyme necessary to hydrolyze the protein, starch, and other constituents of the raw materials. This mold-cultured material is called koji.

### *3. The Making and Aging of Mash*

In making mash, the koji is mixed with saline water which has a 22½% salt content and a volume 120–130% that of raw materials. The mash, or "moromi, is transferred to deep fermentation tanks. Approximately 5- to 10-kl wooden kegs or 10- to 20-kl concrete tanks for shoyu fermentation are now being replaced by resin-coated iron tanks of 50–300 kl. The moromi is held for 4–8 months, depending upon its temperature, with occasional agitation with com-

pressed air to mix the dissolving contents uniformly and to promote the microbial growth. During the fermentation period, the enzymes from koji mold hydrolyze most of the protein to amino acids and low-molecular-weight peptides. Approximately 20% of the starch is consumed by the mold during koji cultivation, but almost all of the remaining starch is converted into simple sugars. More than half of this is fermented to lactic acid and alcohol by lactobacilli and yeasts, respectively. The initial pH value drops from 6.5–7.0 to 4.7–4.9. The lactic acid fermentation produced in the beginning stage is gradually replaced by yeast fermentations. Pure-cultured *Pediococcus halophylus* and *Saccharomyces rouxii* are sometimes added to the mash. The salt concentration of mash remains at 17–18% (weight per volume) after 1 or 2 months. The high concentration of mash effectively limits the growth to only a few desirable types of microorganisms.

### 4. *Pressing of Mash*

An aged mash is filtered through cloth under high hydraulic pressure. Usually 12–13 liters of shoyu mash is put on a square sheet of cloth, 100 × 100 cm, which is then folded into a square, 70 × 70 cm. A second, smaller square sheet of cloth, 65 × 65 cm, is placed on top to wrap the mash. Successive layers are added and placed in a wooden box until there are 300–400 sheets of folded cloth containing the mash. These are then pressed for 2–3 days under hydraulic pressure. The pressure is increased in two or three steps, sometimes reaching 100 kg/cm$^2$ in the final stage, making the moisture content of the presscake less than 25%. A diaphragm-type of pressing machine has recently been used for shoyu mash filtration instead of the batch-type hydraulic press, resulting in a presscake with a moisture content of more than 30%. The residue from the pressing of the shoyu mash, or shoyu cake, is used for animal feeds for cows and ducks.

### 5. *Refining*

The liquid part of the mash obtained by pressing is stored in a tank and divided into three layers: the sediment on the bottom, the clear supernatant of the middle layer, and the oil layer floated on top. The middle layer is sometimes further clarified by filtration with Kieselgel as a filter aid in order to get the raw shoyu. After adjusting the salt and nitrogen concentrations to the standard, the clarified raw shoyu is pasteurized at 70–80°C and stored in a semiclosed tank. The clear middle layer is bottled or canned, or sometimes spray dried. The oil layer separated from the heated shoyu consists of free fatty acids, and their ethyl esters derived from the yeast metabolism of soybean and wheat oils, and it is sometimes mixed with paint as a antifreezing agent.

Good quality Kojkuchi soysauce contains 1.5-1.8 g/l total nitrogen, 3-5% reducing sugar, 2-2.5% ethanol, 1-1.5% polyalcohol (glicerol), 1-2% organic acid (predominantly lactic acid) and 17-18 sodium chloride. The detailed composition of fermented soysauce is presented in table 3.
The middle columns gives the component percentages based on the liquid condiment as it is used by the consumer.

Among the components given in table 3, the free amino-acid have been of most important because of their nutrient value and as precursors for characteristic aroma and taste. The free amino-acid usually account for 40-50% of the total soluble nitrogen.

COLOR OF SOYSAUCE

The color of soysauce mostly develops from the nonenzymatic reation during thermal process and aging throu maillard reaction between amino-acids and sugars.
The color of shoyu is a type of melanoidin pigments consisted mainly of two compounds: $C_{27}H_{49}N_3O_{13}$ and $C_{27}H_{45}N_3O_{12}$. The color of shoyu have a dominant wovelengh of 590-620 nm. About 50% of the color of shoyu is formed during fermentation and aging of mash, and the remaining during pasteurisation.

FLAVOR OF SOYSAUCE

The flavor of soysauce is the most important criterium of soysauce quality. The relationship between the organoleptic evaluation of a shoyu and its chemical constituents was very well investigated in Japan. More than 20 Japanese investigators had isolated about 130 flavor compounds from fermented shoyu using Gas Chromatography - mass Spectrometry technique. The flavor components found in shoyu are presented in Annex 1
Up to day were isolated from soysauce following numbers of different volatile compounds.

37 hydrocarbons
30 alcohols
41 esters
15 Aldehydes
4 Acetals
19 Ketones
24 Acids
17 Phenols
16 Furans
4 Lactones
6 Furanones
5 Pyrones
27 Pyrazines
16 Sulfur containing compounds
4 Thiazoles
3 Terpens
8 Miscellanous nitrogen containing compounds

TABLE 3

DETAILED COMPOSITION OF FERMENTED SHOYU- /3/.

| Component | Percent (w/w) of shoyu, "as is" | Percent (w/w) of shoyu, "dry" basis |
|---|---|---|
| Soluble solids (dry matter) | 34.00 | |
| Alcohol | 1.47 | |
| Water (by difference) | 64.53 | |
| Inorganic components | | |
| Sodium | 6.10 | 17.94 |
| Chlorine | 8.82 | 25.94 |
| Calcium | 0.02 | 0.06 |
| Potassium | 0.40 | 1.17 |
| Phosphorus | 0.15 | 0.44 |
| Magnesium | 0.07 | 0.21 |
| Sulfur | 0.06 | 0.17 |
| Iron | 0.002 | 0.006 |
| Manganese | 0.001 | 0.003 |
| Total | 15.60 | 45.94 |
| Organic components | | |
| Polyols | | |
| Glycerol | 1.50 | 4.41 |
| Mannitol | 0.17 | 0.50 |
| Total | 1.67 | 4.91 |
| Ether-soluble compounds | 0.14 | 0.41 |
| Ether-soluble volatile matter | 0.005 | 0.01 |
| Amino acids | | |
| Lysine | 0.56 | 1.65 |
| Histidine | 0.21 | 0.62 |
| Cystine | 0.07 | 0.21 |
| Arginine | 0.22 | 0.65 |
| Aspartic acid | 0.90 | 2.65 |
| Threonine | 0.36 | 1.06 |
| Serine | 0.45 | 1.32 |
| Glutamic acid | 1.92 | 5.65 |
| Proline | 0.59 | 1.74 |
| Glycine | 0.34 | 1.00 |
| Alanine | 0.38 | 1.12 |
| Valine | 0.47 | 1.38 |
| Methionine | 0.12 | 0.35 |
| Isoleucine | 0.41 | 1.21 |
| Leucine | 0.62 | 1.82 |
| Tyrosine | 0.08 | 0.24 |
| Phenylalanine | 0.36 | 1.06 |
| Ornithine | 0.49 | 1.44 |
| Total | 8.55 | 25.17 |

(continued)

TABLE 3 (*Continued*)

| Component | Percent (w/w) of shoyu, "as is" | Percent (w/w) of shoyu, "dry" basis |
|---|---|---|
| Ammonia | 0.30 | 0.88 |
| Organic acids | | |
| Formic | 0.02 | 0.06 |
| Acetic | 0.16 | 0.47 |
| Citric | 0.04 | 0.12 |
| Succinic | 0.05 | 0.15 |
| Lactic | 0.68 | 2.00 |
| Total | 0.95 | 2.80 |
| Sugars | | |
| Monosaccharides | | |
| Mannose | 0.06 | 0.18 |
| Arabinose | 0.08 | 0.24 |
| Galactose | 0.17 | 0.50 |
| Xylose | 0.06 | 0.18 |
| Glucose | 2.05 | 6.03 |
| Unidentified | 0.23 | 0.68 |
| Total | 2.65 | 7.81 |
| Disaccharides | 0.65 | 1.91 |
| Oligosaccharides | — | — |
| Polysaccharides | 1.15 | 3.38 |
| Total sugars (as glucose) | 4.45 | 13.10 |
| Total organic components | 16.1 | 47.3 |
| Solids accounted for | 31.7 | 93.2 |
| With ammonia calculated as amino acids | 32.69 | 96.1 |

For analysis those compounds solvent extraction, steam or vacuum distilation of shoyu was applied to the concentration of volatile flavor constituents. The Gas Chromatogramm of shoyu flavor concentrate is presented on Fig. 3

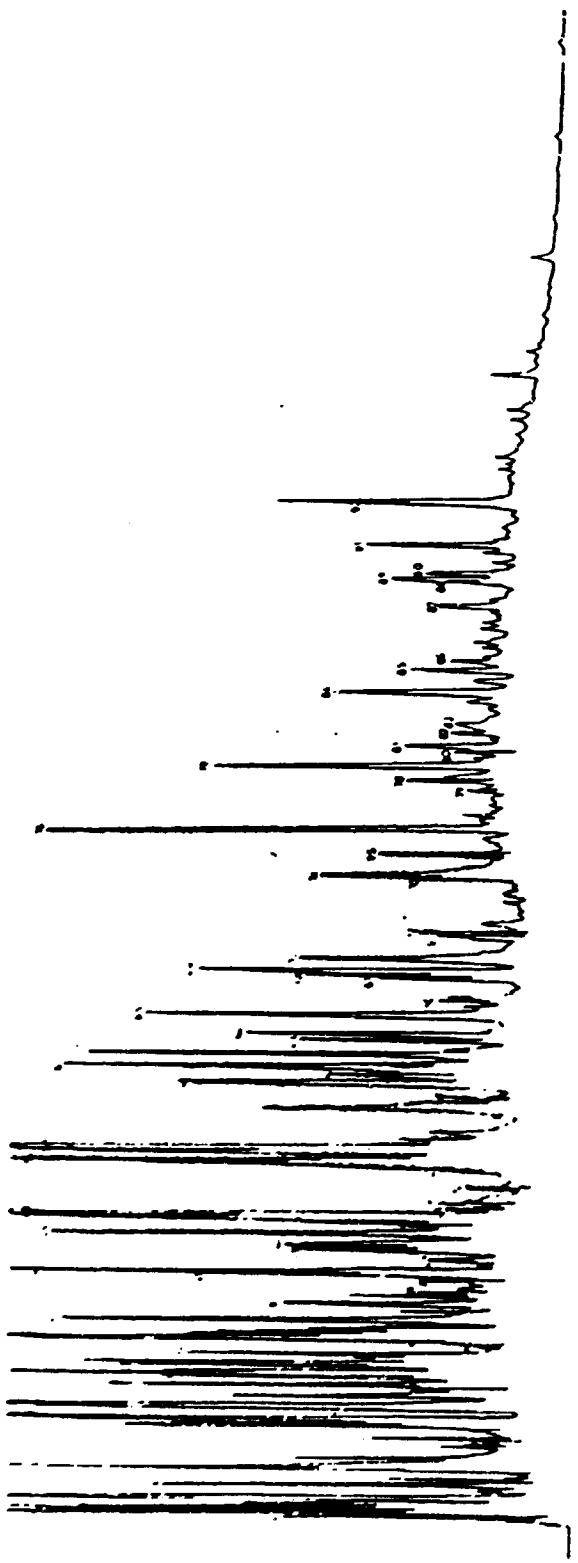


FIG. 3 Gas chromatograph (capillary column) of shoyu flavor concentrate (GCMS). Instrument: RMU-6MG. Column: FFAP, glass, 0.25 mm i.d. × 30 m. Oven temperature: 60–220°C, 2°C/min. He: 0.2 kg/cm², Ionizing voltage: 20 eV. Ion source temperature: 200°C. From Nunomura *et al.* (1980).

Newly pasteurized fermented shoyu has a characteristic pleasent odor, most of which disappears in a short time by natural evaporation. Sasaki and Nunomura ( ) directly analyzed the topnote flavor concentrate of pasteurised shoyu by GLC-MS method. The sample to be analysed was prepared by passing helium gas through the shoyu at 20 C and then trapping the vapor by dry ice-ethanol, liquid nitrogen, and activated carbon, in succession. A total 24 compound were isolated, the main 14 compounds are presented in table 4

Table 4 – QUANTITATIVE ANALYSIS OF HEADSPACE GAS FROM SHOYU*

| Compounds | Concentrations (ppm) ($x$, $n$ = 10) | Coefficient of variation (%) |
|---|---|---|
| Methanol | 9.45 | 4.43 |
| Acetaldehyde | 3.76 | 9.58 |
| Ethanol | 5605.18 | 3.50 |
| Propionaldehyde | 1.70 | 8.52 |
| Acetone | 2.09 | 3.75 |
| Ethyl formate | 1.66 | 3.02 |
| n-Propyl alcohol | 0.82 | 5.64 |
| Isobutyraldehyde | 6.38 | 3.16 |
| Ethyl acetate | 33.41 | 1.83 |
| Isobutyl alcohol | 3.79 | 1.75 |
| n-Butyl alcohol | 0.69 | 10.75 |
| Isovaleraldehyde | 8.17 | 2.88 |
| 2,3-Pentanedione | 0.76 | 8.25 |
| Isoamyl alcohol | 2.36 | 9.38 |

* From Sasaki and Nunomura (1978).

There were investigation between odor components and sensory evaluation. The highest correlation coeficient was found for ethyl acetate (r= 0.551). Ethyl acetate was found to give a kind of freshoess to shoyu. The sum contents of acetoin and isobutiric acid was found to be highly associated with sensory evaluation of shoyu. The odor units of 6 of 16 constituents are presented in table 5

Table 5– ODOR UNITS OF 6 OF 14 CONSTITUENTS OF HEADSPACE GAS FROM SHOYU* 137.

| Compound | Concentration (ppm) | Threshold (ppm in water) | Odor units | Relative odor units (%) |
|---|---|---|---|---|
| Ethanol | 5605.18 | $1.83 \times 10^{-1}$ | 30,629.40 | 33.05 |
| Ethyl acetate | 33.41 | $6.0 \times 10^{-1}$ | 55.68 | 0.06 |
| Isovaleraldehyde | 8.17 | $1.5 \times 10^{-4}$ | 54,466.67 | 58.77 |
| Isobutyraldehyde | 6.38 | $9.0 \times 10^{-4}$ | 7,088.89 | 7.65 |
| Acetaldehyde | 3.76 | $1.5 \times 10^{-2}$ | 250.67 | 0.27 |
| Propionaldehyde | 1.70 | $9.5 \times 10^{-3}$ | 178.95 | 0.19 |

* From Sasaki and Nunomura (1978)

Tanaka ( )in his investigations on flavor evaluation indicated that chemical composition, as a whole conntributed, has preference 46.3%. Among the individual chemical components,preferred rating are alcohols, reducing sugar,color,amino-nitrogen, total nitrogen, glutamic acid and amonium nitrogen. There were no prodominant factors, but fragrance and the aroma of alcohol were the major desirable factors. The major negative factors were the smell of chemically hydrolyzed proteins, an oily smell, a warm brewing smell, a steamed soybean smell, and a moldy smell. The factors related to taste: a good after taste, a pure, a polatable, and a moderate salty taste were the major desirable factors. To sweet, to sour, and abnormal aste were the major negative factors.

## HEALTH ASPECT AND SAFETY PROBLEMS

Certain strains of the common mold Aspergillus flavus able to synthesize highly toxic aflatoxins. Industrial mold mostly belongs to the Aspergillus oryzae, Aspergillus sojae and Aspergillus tamari were tested by Japanese and American scientists for aflatoxins.

Aibara and Miyaki (ACC.3) examined 180 strains used in the preparation of miso. Fluorescence spectra revealed no producer of aflatoxin. Murakami ( ) examined 214 kinds of Aspergillus mold by Thin-layer chromatography for their aflatoxin producing-ability. Thirteen strains gave fluorescent spots on TLC corresponding to aflatoxin. Aflatoxin $B_1$ is the most acutely toxic with on LD 50 in duckling of 0.36 mg/Kg. $M_1$ is almost as active, followed by $G_1$, $B_2$, $M_2$ and $G_2$. Aflatoxin is a potent hepatic carcinogen for ducks, rats, rainbow trout, guina pigs.

According to epidemiologic studies conducted in Africa and Sountheast Asia, there is evidence that consumption of aflatoxin containing food is associated with cancer of the liver in humans.

Manab (3) observed that 49 strains among 212 Koji molds exhibited aflatoxin like fluorescence spot on TLC, but that all of their UV spectro were different from those of aflatoxins.

Hesseltine ( 5 ) studiet 53 cultures in USA. He obtain negative effect. The aflatoxin producing molds belong to Aspergillus group sometimes difficult to classify these molds using only their morphological features. This is especially true in differentiating between Aspergillus sojae and Aspergillus parasiticus.

Some investigators have found that a fairly large number of strains of Aspergillus mold do produce aflatoxin like fluorescent compounds having Rf values on TLC, similar to the aflatoxins, but with different UV max. absorptions. These include seven kind of pyrazine compounds, isocoumarin, lumichrome and unknown compounds.

## MYCOTOXINS OTHER THAN AFFLATOXINS

Except aflatoxins in moldy foods could be contaminated with another mycotoxins.
Golinski (11) described 156 secondary metabolites produced by fungi. In his review was given the structure of molecules, molecular weight, colar of fluorescens in UV light, toxicity and fungal source. Some of the listed substances are more toxic than aflatoxins. The list of 29 mycotoxins originated of molds and their toxic effects is given in Appendix 1

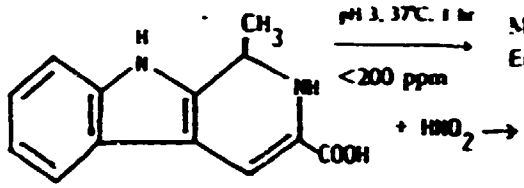
Sasaki ( 3 ) checked the ability of 33 kinds of industrial Aspergillus moid to produce aflatoxin, sterigmatocystin, ochratoxin, patulin cyclopiazonic and penicillic acid. None of the strains tested produced these compounds with the exception of a very few strains which produced cyclopiazonic acid. Manable (1977.3) observed that some Koji mold belonging to Aspergillus oryzae and A. sojae produced cyclopiazonic acid. This cyclopiazonic acid is very toxic, lethal to ducklings and rats. $LD_{50}$ for rats males 36 mg/Kg and females 63 mg/ Kg

## D. MUTAGENIC SUBSTANCES IN SHOYU

Although the noncarcinogenicity of fermented shoyu has long been known from long-term animal studies, the mutagenicity of heated products of amino acids or proteins such as Trip-P-1, Trip-P-2, Glu-P-1, and Glu-P-2 has been established more recently. Secliff and Mower (1977) reported that soy sauce produces mutagens upon the heating of glucose, galactose, and arabinose in shoyu.

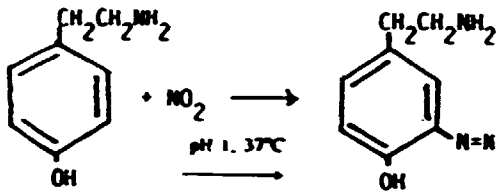
Using the salmonella/mammalian microsome mutagenicity test, Lin *et al.* (1978) found that when treated with nitrite at 2000 ppm, soybean sauce produced a mutagenic substance. As fermented shoyu sometimes contains a small amount of amines (e g. histamine and tyramine), the formation of mutagenic substances as a result of reaction between amines and an abundance of nitrite is possible. Shibamoto (1983) mixed soy sauce with 100, 500, 1000, and 2000 ppm of sodium nitrite, adjusting pH at 3.0, and heated the mixture for 2 hr at 25°C and then for an additional 30 min at 80°C. Only at the highest concentration, 2000 ppm, was mutagenicity exhibited in the Ames test. Shibamoto concluded that the formation of nitrosamines may not be significant because the quantity of nitrite used in the study was excessive compared with actual food systesm. It is generally reported that the nitrite concentration remaining in the human stomach after a meal is estimated to be about 5 ppm, or about 15 ppm at most. It was also reported that just after ingestion of cured ham, the concentration in the stomach is about 70 ppm. According to Nagahara *et al.* (1984), shoyu itself did not represent mutagenicity. 1-methyl-1,2,3,4-tetrahydro-β-carboline-3-carboxylic acid (MTCA) decreased in a buffer solution when treated with more than 10 ppm of nitrite for 1 hr at 37°C and pH 3.0, but in shoyu, it decreased with more than 250 ppm of nitrite. Tyramine decreased in a buffer solution when treated with more than 50 ppm of nitrite for 1 hr at 37°C and pH 1.0, but in shoyu, it did not decrease even when treated with 2300 ppm of nitrite. Nagahori *et al.* (1980) reported that the addition of 5–7% fermented shoyu to a mixture of dimethylamine and nitrite at pH 3.6 suppressed the formation of *N*-nitrosodimethylamine by 60–80%. Moreover, the quantity of nitrosamine formation hindering substances in fermented shoyu increased with the advance of fermentation and aging of the mash. These substances were identified as the amino acids present in shoyu, which react more easily with nitrite than with dimethylamine. Ochiai *et al.* (1982) and Wakabayashi *et al.* (1983) isolated a nitrosable precursor of mutagens from shoyu. Its chemical structure was confirmed to be 1.2.3.4-tetrahydroharman-3-carboxylic acid (1-methyl-1,2.3,4-tetrahydro-β-carboline-3-carboxylic acid, MTCA). When this compound was treated with 3450 ppm of nitrite for 1 hr at 37°C and pH 3.0, the nitration product was strongly mutagenic to *Salmonella typhimurium* TA 100. Wakabayashi *et al.* (1983) determined the tyramine content of shoyu to be 17–

NITROSABLE PREMUTAGENS ISOLATED FROM SHO



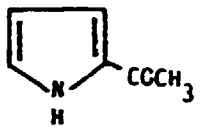

Mutagenic (*Salmonella typhimurium* TA 100)
Eighty percent of nitrosation product is not mutagenic. From Ochiai *et al.* (1982)

(−) − (1*R*,3*S*)-MTCA and its isomer
Content in shoyu is 0.03–0.7 ppm.




Reaction is one-third at pH 3. No reaction at pH 5–6. From Nagao *et al.* (1983)

Tyramine 3-Diazotyramine
Content in shoyu is 0–1000 ppm.




Yan and Lee (1984)

2-Acetylpyrrole
Content in shoyu is 3 ppm.

2250 ppm, and when tyramine was treated with 2300 ppm of nitrite for 1 hr at 37°C and pH 1.0, strong mutagenicity to TA 100 was observed. Yen and Lee (1984) isolated 2-acetylpyrrole as a nitrosable premutagen from shoyu. These are given in Table XLVI.

## E. BACTERICIDAL ACTION OF SHOYU

Ujiie *et al.* (1956) identified the bactericidal nature of a commercial fermented shoyu with respect to nine kinds of intestinal pathogenic bacteria, such as *Escherichia coummunis*, *Shigella flexneria*, *Vibrio cholerae* Inaba, *Salmonella typhimurium* Shikata, *Bacillus subtilis* (B-31). The kinds of bacteria present were attributed to the acidity, high osmotic pressure, and some of the chemicals contained in the shoyu. To the sample, 0.005% of butyl-*p*-hydroxybenzoate was added as a preservative. Sakaguchi *et al.* (1975) tested the fate of staphylococci during incubation in a normal shoyu and in a milder shoyu containing 17% (w/v) and 9% (w/v) of sodium chloride, respectively. No chemical preservatives

were added to either sample. The normal shoyu which initially contained $10^6$ staphylococci per milliliter was nearly free of viable staphylococci within 3 hr. In the milder shoyu, over 90% of the cells were destroyed within 22–30 min, while in the normal shoyu, only 13–14 min were required. That sodium chloride contributes to the destruction of staphylococci in soy sauce is evident because the rate of killing in normal shoyu is greater than in milder shoyu. The fate of staphylococci in phosphate buffer saline solutions with a pH level of 4.7 containing 10 and 17% sodium chloride, respectively, was tested under the same conditions. The time taken to destroy over 90% of the cells in the 10% solution and in the 17% solution was 980–1440 min and 460–530 min, respectively. These results suggest the participation of some factor other than sodium chloride in the destruction of staphylococci in shoyu. The activity of *Clostridium botulinum* in shoyu was also tested during months of storage at 30°C. Neither *C. botulinum* 62A (Type A) nor *C. botulinum* Okre (Type B) grew during this time. The number of Type A spores remained the same, but those of Type B decreased slightly in number after the 3 months.

According to Yamanoto *et al.* (1978), the time needed for the total destruction of *Escherichia coli* 215 or *Staphylococcus aureus* 209P (ATCC 11522) inoculated in fermented shoyu was dependent upon the initial number of cells in these bacteria; 4–6 hr for $10^3$/ml, 24–48 hr for $10^5$/ml, and 5–7 hr for $10^7$/ml. A high salt content was the dominant factor in accelerating the speed of sterilization; the pH value and amount of alcohol, total nitrogen, and ether-soluble compounds were judged to be supplementary factors.

## F. BIOLOGICAL TESTS OF SHOYU

The long-term effects of Japanese shoyu (Kikkoman) on the gastric mucosa of intact rats and those with a fundasectomy were studied by MacDonald and Dueck (1976) in Canada. At the end of the test period, the animals that had been fed shoyu were smaller than the controls; the 15 intact rats that received the shoyu were healthier, more active, and lived 33 months longer than did the 7 controls. Breast tumors developed in 10 control rats, but in none of the experimental animals given shoyu. These findings suggest that shoyu does not appear to be carcinogenic in rats; its prolonged use impaired neither health nor longevity. Oshita *et al.* (1977) studied the acute and long-term effects of large amounts of Kikkoman shoyu on mice and rats. The acute toxicity of shoyu was attributed to the toxicity of its sodium chloride component. The oral $LD_{50}$ values for shoyu were 20.6 ml/kg for rats and 27.3 mg/kg for mice. In long-term feeding tests (1.5 years for mice and 6 months for rats), the food intake of animals given a diet containing shoyu was otherwise comparable to that of the control group. This was true even for animals given a diet containing 10% powdered shoyu (corre-

sponding to ~25% liquid shoyu). Although the animals that were fed shoyu were smaller than the controls, no significant differences in mortality were observed between the two groups. In addition, male rats given a diet containing 5% or 2% powdered shoyu grew faster than rats fed an equivalent amount of sodium chloride alone (i.e., diets containing 2.25% or 0.9% sodium chloride). At the highest dose level, 10% powdered shoyu, there were significant differences in the urinary systems of experimental and control animals. While both rats and mice developed enlarged kidneys and bladders, rats developed higher concentrations of in serum, and mice gave evidence of hydronephrosis after 1.5 years. The same effects were observed in animals who received sodium chloride in the same concentrations as those fed the highest level of shoyu. There was no indication of carcinogenic effects at any level of shoyu feeding.

## RESEARCH NEEDS

### RAW MATERIALS

The precise mixture of soybeans and wheat used as the raw materials in shoyu production is the result of technological know-how developed over hundreds of years. But the shoyu-like seasonings can be prepared from a mixture of plant proteins and starches other than soybeans and wheat, and these are available worldwide. The by-products of oil pressing and extraction, such as peanut cake, copra meal, cottonseed meal, rapeseed protein, and sesame protein, have been experimentally substituted, with good results. Many kinds of mung beans also seem to have good potential. On the other hand, wheat kernel is considered to be the best starch raw material for shoyu, but barley, rye, oats, rice, and corn are sometimes used. The superiority of the wheat kernel lies in its high source of protein (glutamine) and its high concentration of glucosides which give koikuchi its destructive bran flavor. The use of a mixture of wheat bran and starches other than wheat, such as rice, corn, and potato, would be worth exploring for those nations that do not have wheat. Steaming and puffing corn kernels, mung beans, and other raw materials which have hard plant tissues or extruding these moistened powders may also give good results in shoyu preparation.

FLAVOR COMPONENTS FOUND IN SHOYU

| Compound | Molecular weight | Molecular formula | Reference number[a] |
|---|---|---|---|
| I. Hydrocarbons (37) | | | |
| 1. Benzene | 78 | $C_6H_6$ | 1 |
| 2. Toluene | 92 | $C_7H_8$ | 1 |
| 3. Styrene | 104 | $C_8H_8$ | 1 |
| 4. *o*-Xylene | 106 | $C_8H_{10}$ | 1 |
| 5. *m*-Xylene | 106 | $C_8H_{10}$ | 1 |
| 6. *p*-Xylene | 106 | $C_8H_{10}$ | 1 |
| 7. Ethylbenzene | 106 | $C_8H_{10}$ | 1 |
| 8. Mesitylene | 120 | $C_9H_{12}$ | 1 |
| 9. 1,2,3-Trimethylbenzene | 120 | $C_9H_{12}$ | 1 |
| 10. 1,2,4-Trimethylbenzene | 120 | $C_9H_{12}$ | 1 |
| 11. 1-Ethyl-2-methylbenzene | 120 | $C_9H_{12}$ | 1 |
| 12. Cumene | 120 | $C_9H_{12}$ | 1 |
| 13. Naphthalene | 128 | $C_{10}H_8$ | 1 |
| 14. 4-Methylindan | 132 | $C_{10}H_{12}$ | 1 |
| 15. 5-Methylindan | 132 | $C_{10}H_{12}$ | 1 |
| 16. 1,2,3,4-Tetrahydronaphthalene | 132 | $C_{10}H_{12}$ | 2 |
| 17. 1-Ethyl-2,3-dimethylbenzene | 134 | $C_{10}H_{14}$ | 1 |
| 18. 1-Ethyl-2,4-dimethylbenzene | 134 | $C_{10}H_{14}$ | 1 |
| 19. 1-Ethyl-3,5-dimethylbenzene | 134 | $C_{10}H_{14}$ | 1 |
| 20. 2-Ethyl-1,3-dimethylbenzene | 134 | $C_{10}H_{14}$ | 1 |
| 21. 2-Ethyl-1,4-dimethylbenzene | 134 | $C_{10}H_{14}$ | 1 |
| 22. 2-Ethyl-1,2-dimethylbenzene | 134 | $C_{10}H_{14}$ | 1 |
| 23. 1-Methyl-2(or 4)-propylbenzene | 134 | $C_{10}H_{14}$ | 1 |
| 24. 1,2,3,5-Tetramethylbenzene | 134 | $C_{10}H_{14}$ | 1 |
| 25. 1,2,4,5-Tetramethylbenzene | 134 | $C_{10}H_{14}$ | 1 |
| 26. 1,2-Diethylbenzene | 134 | $C_{10}H_{14}$ | 1 |
| 27. 1,3-Diethylbenzene | 134 | $C_{10}H_{14}$ | 1 |
| 28. 1,4-Diethylbenzene | 134 | $C_{10}H_{14}$ | 1 |
| 29. Butylbenzene | 134 | $C_{10}H_{14}$ | 1 |
| 30. Cyclohexylcyclohexane | 134 | $C_{10}H_{14}$ | 1 |
| 31. 1-Methylnaphthalene | 142 | $C_{11}H_{10}$ | 1 |
| 32. 2-Methylnaphthalene | 142 | $C_{11}H_{10}$ | 1 |
| 33. 2,3,5(or 6)-Trimethylnaphthalene | 170 | $C_{13}H_{14}$ | 1 |
| 34. Tetradecane | 198 | $C_{14}H_{30}$ | 1 |
| 35. Pentadecane | 212 | $C_{15}H_{32}$ | 1 |
| 36. Hexadecane | 226 | $C_{16}H_{34}$ | 1 |
| 37. 5-Phenyldodecane | 246 | $C_{18}H_{30}$ | 1 |
| II. Alcohols (30) | | | |
| 1. Methanol | 32 | $CH_4O$ | 2, 3 |
| 2. Ethanol | 46 | $C_2H_6O$ | |

(*continued*)

| | Compound | Molecular weight | Molecular formula | Reference numbers |
|---|---|---|---|---|
| II | Alcohols (30) (*continued*) | | | |
| | 3 1-Propen-3-ol | 58 | $C_3H_6O$ | [illegible] |
| | 4 2-Propen-1-ol (allyl alcohol) | 58 | $C_3H_6O$ | 9 |
| | 5 1-Propanol | 60 | $C_3H_8O$ | 2 |
| | 6 2-Propanol | 60 | $C_3H_8O$ | 5, 6 |
| | 7 2-Methyl-1-propanol | 74 | $C_4H_{10}O$ | 5, 3 |
| | 8 1-Butanol | 74 | $C_4H_{10}O$ | 2 |
| | 9 2-Methyl-2-buten-1-ol | 86 | $C_5H_{10}O$ | 1 |
| | 10 1-Penten-3-ol | 86 | $C_5H_{10}O$ | 2 |
| | 11 3-Penten-2-ol | 86 | $C_5H_{10}O$ | 1 |
| | 12 2-Methyl-1-butanol | 88 | $C_5H_{12}O$ | 2, 7 |
| | 13 3-Methyl-1-butanol | 88 | $C_5H_{12}O$ | 2, 3 |
| | 14 1-Pentanol | 88 | $C_5H_{12}O$ | 2 |
| | 15 3-Pentanol | 88 | $C_5H_{12}O$ | 2 |
| | 16 3-Buten-1,2-diol | 88 | $C_4H_8O_2$ | 1 |
| | 17 2-Ethoxyethanol | 90 | $C_4H_{10}O_2$ | 6 |
| | 18 (-)-2,3-Butanediol | 90 | $C_4H_{10}O_2$ | 2, 10, 11 |
| | 19 *meso*-2,3-Butanediol | 90 | $C_4H_{10}O_2$ | 2, 10, 11 |
| | 20 (*E*)-2-Hexen-1-ol | 100 | $C_6H_{12}O$ | 8 |
| | 21 1-Hexanol | 102 | $C_6H_{14}O$ | 3 |
| | 22 Benzyl alcohol | 108 | $C_7H_8O$ | 5 |
| | 23 2,3-Dimethyl-2-pentanol | 116 | $C_7H_{16}O$ | 1 |
| | 24 2,4-Dimethyl-3-pentanol | 116 | $C_7H_{16}O$ | 1 |
| | 25 3-Methyl-3-hexanol | 116 | $C_7H_{16}O$ | 1 |
| | 26 2-Phenylethanol | 122 | $C_8H_{10}O$ | 5, 12 |
| | 27 1-Octen-3-ol | 128 | $C_8H_{16}O$ | 8 |
| | 28 5-Nonanol | 144 | $C_9H_{20}O$ | 1 |
| | 29 2-Phenyl-1-butanol | 150 | $C_{10}H_{14}O$ | 1 |
| | 30 2-Undecanol | 172 | $C_{11}H_{24}O$ | 3 |
| III | Esters (41) | | | |
| | 1 Methyl acetate | 74 | $C_3H_6O_2$ | 4 |
| | 2 Ethyl formate | 74 | $C_3H_6O_2$ | 13, 4 |
| | 3 Ethyl acetate | 88 | $C_4H_8O_2$ | 5, 14, 15 |
| | 4 2-Oxopropyl acetate (acetol acetate) | 100 | $C_5H_8O_2$ | 2 |
| | 5 Ethyl propionate | 102 | $C_5H_{10}O_2$ | 2 |
| | 6 Butyl formate | 102 | $C_5H_{10}O_2$ | 8 |
| | 7 1-Methylpropyl acetate | 116 | $C_6H_{12}O_2$ | 2 |
| | 8 2-Methylpropyl acetate | 116 | $C_6H_{12}O_2$ | 1 |
| | 9 Butyl acetate | 116 | $C_6H_{12}O_2$ | 6 |
| | 10 Ethyl 2-hydroxypropanoate (ethyl lactate) | 118 | $C_5H_{10}O_3$ | 5, 16 |
| | 11 3-Methylbutyl acetate | 130 | $C_7H_{14}O_2$ | 17, 5 |
| | 12 Pentyl acetate | 130 | $C_7H_{14}O_2$ | 18 |

| Compound | Molecular weight | Molecular formula | Reference number[a] |
|---|---|---|---|
| III. Esters (41) (*continued*) | | | |
| 13. 2-Methylpropyl propionate | 130 | $C_7H_{14}O_2$ | 1 |
| 14. Ethyl 3-methylbutanoate | 130 | $C_7H_{14}O_2$ | 19, 20 |
| 15. Ethyl 2-methylbutanoate | 130 | $C_7H_{14}O_2$ | 9 |
| 16. Ethyl pentanoate | 130 | $C_7H_{14}O_2$ | 17 |
| 17. 2-Ethyoxyethyl acetate | 132 | $C_6H_{12}O_3$ | 2 |
| 18. Ethyl 4-oxopentanoate (ethyl levulinate) | 144 | $C_7H_{12}O_3$ | 6 |
| 19. Ethyl hexanoate (ethyl caproate) | 144 | $C_8H_{16}O_2$ | 19 |
| 20. Diethyl oxalate | 146 | $C_6H_{10}O_4$ | 48 |
| 21. 2-Phenylethyl formate | 150 | $C_9H_{10}O_2$ | 1 |
| 22. Ethyl benzoate | 150 | $C_9H_{10}O_2$ | 5, 19, 20 |
| 23. Diethyl malonate | 160 | $C_7H_{12}O_4$ | 6 |
| 24. 2-Phenylethyl acetate | 164 | $C_{10}H_{12}O_2$ | 5 |
| 25. Ethyl phenylacetate | 164 | $C_{10}H_{12}O_2$ | 5 |
| 26. 3-Methylbutyl 3-methylbutanoate | 172 | $C_{10}H_{20}O_2$ | 17 |
| 27. Ethyl octnoate (ethyl caprylate) | 172 | $C_{10}H_{20}O_2$ | 49 |
| 28. Diethyl maleate | 172 | $C_8H_{12}O_4$ | 6 |
| 29. Diethyl succinate | 174 | $C_8H_{14}O_4$ | 5, 6 |
| 30. Ethyl 3-phenylpropenoate (ethyl cinnamate) | 176 | $C_{11}H_{12}O_2$ | 1 |
| 31. Pentyl hexanoate (amyl caproate) | 186 | $C_{11}H_{22}O_2$ | 17 |
| 32. Ethyl nonanoate (ethyl pelargonate) | 186 | $C_{11}H_{22}O_2$ | 17 |
| 33. 2-Phenylethyl butanoate | 192 | $C_{12}H_{16}O_2$ | 1 |
| 34. 4-Formyl-2-methoxyphenyl acetate (vanillin acetate) | 194 | $C_{10}H_{10}O_4$ | 2 |
| 35. Ethyl 4-hydroxy-3-methoxybenzoate (ethyl vanillate) | 196 | $C_{10}H_{12}O_4$ | 2, 7 |
| 36. Ethyl dodecanoate (ethyl laurate) | 228 | $C_{14}H_{28}O_2$ | 17 |
| 37. Ethyl tetradecanoate (ethyl myristate) | 256 | $C_{16}H_{32}O_2$ | 17, 5 |
| 38. Ethyl hexadecanoate (ethyl palmitate) | 284 | $C_{18}H_{36}O_2$ | 21, 22 |
| 39. Ethyl 9,12-octadecadienoate (ethyl linoleate) | 308 | $C_{20}H_{36}O_2$ | 22 |
| 40. Ethyl 9-octadecenoate (ethyl oleate) | 310 | $C_{20}H_{38}O_2$ | 22 |
| 41. Ethyl octadecanoate (ethyl stearate) | 312 | $C_{20}H_{40}O_2$ | 21 |
| IV. Aldehydes (15) | | | |
| 1. Acetaldehyde | 44 | $C_2H_4O$ | 5, 14, 23, 24 |
| 2. Propanal | 58 | $C_3H_6O$ | 5, 24 |
| 3. 2-Methylpropanal | 72 | $C_4H_8O$ | 5, 25, 24 |
| 4. Butanal | 72 | $C_4H_8O$ | 24, 26 |
| 5. 2-Methylbutanal | 86 | $C_5H_{10}O$ | 1 |
| 6. 3-Methylbutanal | 86 | $C_5H_{10}O$ | 5, 14, 25, 24 |
| 7. Pentanal | 86 | $C_5H_{10}O$ | 27 |
| 8. Hexanal | 100 | $C_6H_{12}O$ | 17, 2 |

(*continued*)

| Compound | Molecular weight | Molecular formula | Reference number |
|---|---|---|---|
| IV. Aldehydes (15) *(continued)* | | | |
| 9. Benzaldehyde | 106 | $C_7H_6O$ | 5, 20 |
| 10. 2,3-Dihydro-4*H*-pyran-2-carbaldehyde | 112 | $C_6H_8O_2$ | 1 |
| 11. Phenylacetaldehyde | 120 | $C_8H_8O$ | 5 |
| 12. 3-Phenyl-2-propenal (cinnamaldehyde) | 132 | $C_9H_8O$ | 17 |
| 13. 2,5-Dimethyl-2,3-dihydro-5*H*-pyran-2-carbaldehyde | 140 | $C_8H_{12}O_2$ | 2 |
| 14. 2-Methyl-3-phenyl-2-propenal (α-methylcinnamaldehyde) | 146 | $C_{10}H_{10}O$ | 1 |
| 15. 4-Hydroxy-3-methoxybenzaldehyde (vanillin) | 152 | $C_8H_8O$ | 3, 26 |
| V. Acetals (4) | | | |
| 1. 1,1-Diethoxyethane | 118 | $C_6H_{14}O_2$ | 2, 28 |
| 2. 1,1-Diethoxy-3-methylbutane | 160 | $C_9H_{20}O_2$ | 29 |
| 3. 1,1-Diethoxy-2-methylbutane | 160 | $C_9H_{20}O_2$ | 9 |
| 4. 1,1-Diethoxy-4-methyl-2-pentanol | 190 | $C_{10}H_{22}O_3$ | 29 |
| VI. Ketones (19) | | | |
| 1. Acetone | 58 | $C_3H_6O$ | 5 |
| 2. 2-Butanone | 72 | $C_4H_8O$ | 1 |
| 3. Hydroxyacetone (acetol) | 74 | $C_3H_6O_2$ | 2 |
| 4. 2,3-Butanedione (diacetyl) | 86 | $C_4H_6O_2$ | 2, 30 |
| 5. 3-Hydroxy-2-butanone (acetoin) | 88 | $C_4H_8O_2$ | 5, 14, 11 |
| 6. 2-Cyclohexin-1-one | 96 | $C_6H_8O$ | 33 |
| 7. 4-Methyl-3-penten-2-one | 98 | $C_6H_{10}O$ | 1 |
| 8. 4-Methyl-2-pentanone | 100 | $C_6H_{12}O$ | 1 |
| 9. 2-Hexanone | 100 | $C_6H_{12}O$ | 5 |
| 10. 2,3-Pentanedione | 100 | $C_5H_8O_2$ | 2, 30 |
| 11. 3-Hydroxy-2-pentanone | 102 | $C_5H_{10}O$ | 31 |
| 12. 2-Hydroxy-3-methyl-2-cyclopenten-1-one (cyclotene) | 112 | $C_6H_8O_2$ | 2 |
| 13. 5-Methyl-2-hexanone | 114 | $C_7H_{14}O$ | 1 |
| 14. 2,3-Hexanedione | 114 | $C_6H_{10}O_2$ | 5, 30 |
| 15. Acetophenone | 120 | $C_8H_8O$ | 2 |
| 16. 3-Octanone | 128 | $C_8H_{16}O$ | 2 |
| 17. 2,6-Dimethyl-4-heptanone | 142 | $C_9H_{18}O$ | 1 |
| 18. 2-Methyl-3-octanone | 142 | $C_9H_{18}O$ | 1 |
| 19. 3-Methyl-3-decen-2-one | 168 | $C_{11}H_{20}O$ | 33 |
| VII. Acids (7) | | | |
| 1. Formic acid | 46 | $CH_2O_2$ | 19 |
| 2. Acetic acid | 60 | $C_2H_4O_2$ | 5, 32, 35 |
| 3. Propionic acid | 74 | $C_3H_6O_2$ | 2, 19 |
| 4. (*E*)-2-Butenoic acid (crotonic acid) | 86 | $C_4H_6O_2$ | 2 |
| 5. 2-Methylpropanoic acid (*iso*-butyric acid) | 88 | $C_4H_8O_2$ | 17, 5 |

| Compound | Molecular weight | Molecular formula | Reference number[a] |
|---|---|---|---|
| VII. Acids (24) (*continued*) | | | |
| 6. Butanoic acid (*sec*-butyric acid) | 88 | $C_4H_8O_2$ | 5, 19 |
| 7. 2-Oxopropanoic acid (pyruvic acid) | 88 | $C_3H_4O_3$ | 24 |
| 8. 2-Hydroxypropanoic acid (lactic acid) | 90 | $C_3H_6O_3$ | 17 |
| 9. 2-Methyl-2-butenoic acid | 100 | $C_5H_8O_2$ | 2 |
| 10. 2-Methylbutanoic acid | 102 | $C_5H_{10}O_2$ | 2 |
| 11. 3-Methylbutanoic acid | 102 | $C_5H_{10}O_2$ | 5, 19 |
| 12. Pentanoic acid (*n*-valeric acid) | 102 | $C_5H_{10}O_2$ | 17, 2 |
| 13. 2-Oxobutanoic acid (2-ketobutyric acid) | 102 | $C_4H_6O_3$ | 34 |
| 14. 4-Methylpentanoic acid | 116 | $C_6H_{12}O_2$ | 17, 2 |
| 15. Hexanoic acid (caproic acid) | 116 | $C_6H_{12}O_2$ | 2, 19 |
| 16. 4-Oxopentanoic acid (levulinic acid) | 116 | $C_5H_8O_3$ | 17 |
| 17. Butanedioic acid (succinic acid) | 118 | $C_4H_6O_4$ | 17 |
| 18. Benzoic acid | 122 | $C_7H_6O_2$ | 5, 19 |
| 19. Phenylacetic acid | 136 | $C_8H_8O_2$ | 17, 2, 19 |
| 20. Octanoic acid (caprylic acid) | 144 | $C_8H_{16}O_2$ | 17 |
| 21. Dodecanoic acid (lauric acid) | 200 | $C_{12}H_{24}O_2$ | 17 |
| 22. Hexadecanoic acid (palmitic acid) | 254 | $C_{16}H_{32}O_2$ | 35, 36, 22 |
| 23. 9,12-Octadecadienoic acid (linoleic acid) | 280 | $C_{18}H_{32}O_2$ | 22 |
| 24. 9-Octadecenoic acid (oleic acid) | 282 | $C_{18}H_{34}O_2$ | 22 |
| VIII. Phenols (17) | | | |
| 1. Phenol | 94 | $C_6H_6O$ | 2 |
| 2. 1,2-Benzenediol (pyrocatechol) | 110 | $C_6H_6O_2$ | 2 |
| 3. 4-Vinylphenol | 120 | $C_8H_8O$ | 33 |
| 4. 4-Ethylphenol | 122 | $C_8H_{10}O$ | 5, 12 |
| 5. 2-Methoxyphenol (guaiacol) | 124 | $C_7H_8O_2$ | 5 |
| 6. 4-(2-Hydroxyethyl)phenol (tyrosol) | 138 | $C_8H_{10}2$ | 37 |
| 7. 4-Ethyl-1,3-benzenediol (4-ethylresorcinol) | 138 | $C_8H_{10}2$ | 1 |
| 8. 4-Hydroxybenzoic acid | 138 | $C_7H_6O_3$ | 12 |
| 9. 2-Methoxy-5-vinylphenol | 150 | $C_9H_{10}2$ | 2 |
| 10. 4-Ethyl-2-methoxyphenol (4-ethylguaiacol) | 152 | $C_9H_{12}2$ | 5, 35 |
| 11. 2,6-Dimethoxyphenol | 154 | $C_8H_{10}3$ | 5 |
| 12. 3,4-Dihydroxybenzoic acid (protocatechuic acid) | 154 | $C_7H_6O_4$ | 19 |
| 13. 3-(4-Hydroxyphenyl)propenoic acid (*p*-coumaric acid), (*p*-hydroxycinnamic acid) | 164 | $C_9H_8O_3$ | 12 |
| 14. 4-Hydroxy-3-methoxyacetophenone (acetovanillon) | 166 | $C_9H_{10}3$ | 2, 19 |
| 15. 4-Hydroxy-3-methoxybenzoic acid (vanillic acid) | 168 | $C_8H_8O_4$ | 35, 38 |

(*continued*)

| Compound | Molecular weight | Molecular formula | Reference number[a] |
|---|---|---|---|
| VIII. Phenols (17) (*continued*) | | | |
| 16. 3-(4-Hydroxy-3-methoxyphenyl) propenoic acid (ferulic acid) | 194 | $C_{10}H_{10}O_4$ | 39 |
| 17. 4-Hydroxy-3,5-dimethoxybenzoic acid (syringic acid) | 198 | $C_9H_{10}O_5$ | 39 |
| IX. Furans (16) | | | |
| 1. Furan | 68 | $C_4H_4O$ | 4 |
| 2. 2-Methylfuran | 82 | $C_5H_6O$ | 4 |
| 3. 2-Furfural | 96 | $C_5H_4O_2$ | 5, 14, 40 |
| 4. Furfuryl alcohol | 98 | $C_5H_6O_2$ | 5, 41 |
| 5. Tetrahydrofurfuryl alcohol | 102 | $C_5H_{10}O_2$ | 2 |
| 6. 1-(2-Furyl)-1-ethanone (2-furyl methyl ketone) | 110 | $C_6H_6O_2$ | 8, 5 |
| 7. 5-Methyl-2-furfural | 110 | $C_6H_6O_2$ | 2 |
| 8. 1-(2-Tetrahydrofuryl)-1-ethanone (2-tetrahydrofuryl methyl ketone) | 114 | $C_6H_{10}O_2$ | 2 |
| 9. 1-(2-Furyl)-1-propanone (ethyl 2-furyl ketone) | 124 | $C_7H_8O_2$ | 1 |
| 10. 2-furfuryl acetate | 126 | $C_6H_6O_3$ | 5 |
| 11. 1-(3-Hydroxy-2-furyl)-1-ethanone (isomaltol) | 126 | $C_6H_6O_3$ | 2 |
| 12. 5-Hydroxymethyl-2-furfural | 126 | $C_6H_6O_3$ | 42 |
| 13. (2,5-Dimethyl-3-furyl)-1-ethanone | 138 | $C_8H_{10}O_2$ | 1 |
| 14. Ethyl 2-furoate | 140 | $C_7H_8O_3$ | 1 |
| 15. 3-Phenylfuran | 144 | $C_{10}H_8O$ | 1 |
| 16. 2-Propenyl 2-furoate | 152 | $C_8H_8O_3$ | 2 |
| X. Lactones (4) | | | |
| 1. 4-Butanolide | 86 | $C_4H_6O_2$ | 2 |
| 2. 2-Penten-4-olide [5-methyl-2(5*H*)-furanone] (β-angelicalactone) | 98 | $C_5H_6O_2$ | 2 |
| 3. 2-Methyl-4-butanolide | 100 | $C_5H_8O_2$ | 2 |
| 4. 4-Pentanolide | 100 | $C_5H_8O_2$ | 5 |
| XI. Furanones (6) | | | |
| 1. 3-Methyl-2(5*H*)-furanone | 98 | $C_5H_6O_2$ | 33 |
| 2. 2-Methyl-3-tetrahydrofuranone | 100 | $C_5H_8O_2$ | 5 |
| 3. 4-Hydroxy-5-methyl-3(2*H*)-furanone | 114 | $C_5H_6O_3$ | 43 |
| 4. 4-Hydroxy-2,5-dimethyl-3(2*H*)-furanone | 128 | $C_6H_8O_3$ | 2 |
| 5. 4,5-Dihydro-5-(1-hydroxyethyl)-2(3*H*) furanone | 130 | $C_6H_{10}O_3$ | 33 |
| 6. 4-Hydroxy-2(or 5)-ethyl-5-(or 2)-methyl-3(2*H*)-furanone | 142 | $C_7H_{10}O_3$ | 4 |

| Compound | Molecular weight | Molecular formula | Reference number[a] |
|---|---|---|---|
| XII. Pyrones (5) | | | |
| 1. 3-Hydroxy-2-methyl-4*H*-pyran-4-one (maltol) | 126 | $C_6H_6O_3$ | 5, 13 |
| 2. 5-Hydroxy-2-methyl-4*H*-pyran-4-one | 126 | $C_6H_6O_3$ | 2 |
| 3. 3-Methoxy-2-methyl-4*H*-pyran-4-one | 140 | $C_7H_8O_3$ | 2 |
| 4. 3,5-Dihydroxy-2-methyl-4*H*-pyran-4-one | 142 | $C_6H_6O_4$ | 2 |
| 5. 3,5-Dihydroxy-6-methyl-2,3-dihydro-4*H*-pyran-4-one | 144 | $C_6H_8O_4$ | 2 |
| XIII. Pyrazines (27) | | | |
| 1. Pyrazine | 80 | $C_4H_4N_2$ | 28 |
| 2. 2-Methylpyrazine | 94 | $C_5H_6N_2$ | 5, 28 |
| 3. 2,3-Dimethylpyrazine | 108 | $C_6H_8N_2$ | 5, 28 |
| 4. 2,5-Dimethylpyrazine | 108 | $C_6H_8N_2$ | 28 |
| 5. 2,6-Dimethylpyrazine | 108 | $C_6H_8N_2$ | 5, 28 |
| 6. 2-Ethylpyrazine | 108 | $C_6H_8N_2$ | 28 |
| 7. 5*H*-Cyclopenta[*b*]pyrazine | 118 | $C_7H_6N_2$ | 28 |
| 8. 2-Methyl-6-vinylpyrazine | 120 | $C_7H_8N_2$ | 2 |
| 9. 6,7-Dihydro-5*H*-cyclopenta[*b*]pyrazine | 120 | $C_7H_8N_2$ | 28 |
| 10. 2,3,5-Trimethylpyrazine | 122 | $C_7H_{10}N_2$ | 28 |
| 11. 2-Ethyl-5-methylpyrazine | 122 | $C_7H_{10}N_2$ | 28 |
| 12. 2-Ethyl-6-methylpyrazine | 122 | $C_7H_{10}N_2$ | 5, 28 |
| 13. 2(or 3)-Methyl-5*H*-cyclopenta[*b*]-pyrazine | 132 | $C_8H_8N_2$ | 28 |
| 14. 6-Methyl-5*H*-cyclopenta[*b*]pyrazine | 132 | $C_8H_8N_2$ | 28 |
| 15. 7-Methyl-5*H*-cyclopenta[*b*]pyrazine | 132 | $C_8H_8N_2$ | 28 |
| 16. Pyrrolo[1,2-*a*]-3-methylpyrazine | 132 | $C_8H_8N_2$ | 33 |
| 17. 2-Methyl-6,7-dihydro-5*H*-cyclopenta[*b*]-pyrazine | 134 | $C_8H_{10}N_2$ | 28 |
| 18. Tetramethylpyrazine | 136 | $C_8H_{12}N_2$ | 5, 28 |
| 19. 3-Ethyl-2,5-dimethylpyrazine | 136 | $C_8H_{12}N_2$ | 5, 28 |
| 20. 2,3-Diethylpyrazine | 136 | $C_8H_{12}N_2$ | 28 |
| 21. 2,6-Diethylpyrazine | 136 | $C_8H_{12}N_2$ | 28 |
| 22. 2(or 3),6(or 7)-Dimethyl-5*H*-cyclopenta[*b*]pyrazine | 146 | $C_9H_{10}N_2$ | 28 |
| 23. Pyrrolo[1,2-*a*]-3,4-dimethylpyrazine | 146 | $C_9H_{10}N_2$ | 33 |
| 24. 2-Ethyl-6,7-dihydro-5*H*-cyclopenta[*b*]-pyrazine | 148 | $C_9H_{12}N_2$ | 28 |
| 25. 2-Ethyl-3,5,6-trimethylpyrazine | 150 | $C_9H_{14}N_2$ | 28 |
| 26. 2,6-Diethyl-3-methylpyrazine | 150 | $C_9H_{14}N_2$ | 28 |
| 27. 2,3,5-Trimethyl-6,7-dihydro-5*H*-cyclopenta[*b*]pyrazine | 162 | $C_{10}H_{14}N_2$ | 28 |

(continued)

| Compound | Molecular weight | Molecular formula | Reference number[a] |
|---|---|---|---|
| XIV. Pyridines (7) | | | |
| 1. Pyridine | 79 | $C_5H_5N$ | 28 |
| 2. 3-Methylpyridine | 93 | $C_6H_7N$ | 28 |
| 3. 2,6-Dimethylpyridine | 107 | $C_7H_9N$ | 28 |
| 4. 2-Ethylpyridine | 107 | $C_7H_9N$ | 28 |
| 5. 2-Pyridylmethanol | 109 | $C_6H_7NO$ | 1 |
| 6. 3-Methoxypyridine | 109 | $C_6H_7NO$ | 28 |
| 7. Ethyl 3-pyridinecarboxylate (ethyl nicotinate) | 151 | $C_8H_9NO_2$ | 28 |
| XV. Miscellaneous nitrogen-containing compounds (8) | | | |
| 1. 1-Methyl-2-pyrrolidinone | 99 | $C_5H_9NO$ | 1 |
| 2. 1-(2-Pyrrolyl)-1-ethanone | 109 | $C_6H_7NO$ | 8, 5, 28 |
| 3. Benzoxazole | 119 | $C_7H_5NO$ | 28 |
| 4. 1-(5-Methyl-2-pyrrolyl)-1-ethanone | 123 | $C_7H_9NO$ | 1 |
| 5. 1,5-Dimethyl-2-pyridone | 123 | $C_7H_9NO$ | 1 |
| 6. 2-Methylbenzoxazole | 133 | $C_8H_7NO$ | 2, 28 |
| 7. Ethyl 2-pyrrolidone-5-carboxylate | 157 | $C_7H_{11}NO_3$ | 33 |
| 8. Ethyl 2-(acetylamino)-4-methyl-pentanoate (*N*-acetylleucine ethyl ester) | 201 | $C_{10}H_{19}NO_3$ | 33 |
| XVI. Sulfur-containing compounds (16) | | | |
| 1. Hydrogen sulfide | 34 | $H_2S$ | 46 |
| 2. Methanethiol | 48 | $CH_4S$ | 29 |
| 3. Dimethyl sulfide | 62 | $C_2H_6S$ | 4 |
| 4. Ethanethiol | 62 | $C_2H_6S$ | 24 |
| 5. 2-Propene-1-thiol | 74 | $C_3H_6S$ | 17 |
| 6. Thiophene | 84 | $C_4H_4S$ | 33 |
| 7. Dimethyl disulfide | 94 | $C_2H_6S_2$ | 2 |
| 8. 4-Methyl-1,3-oxathiolane | 104 | $C_4H_8OS$ | 33 |
| 9. 3-Methylthiopropanal (methional) | 104 | $C_4H_8OS$ | 46 |
| 10. 3-(Methylthio)-1-propanal (methionol) | 106 | $C_4H_{10}OS$ | 5, 16, 47 |
| 11. Phenylmethanethiol | 124 | $C_7H_8S$ | 1 |
| 12. Dimethyl trisulfide | 126 | $C_2H_6S_3$ | 1 |
| 13. 3,4-Dimethyl-2,5-dihydrothiophen-2-one | 128 | $C_6H_8OS$ | 33 |
| 14. 2-Ethyl-6-methyl-1,3-oxathiane | 146 | $C_7H_{14}OS$ | 1 |
| 15. 3-(Methylthio)propyl acetate | 148 | $C_6H_{12}O_2S$ | 1 |
| 16. 1,1-Bis(methylthio)-2-methylpropane | 150 | $C_6H_{14}S_2$ | 24 |
| XVII. Thiazoles (4) | | | |
| 1. 2-Ethoxythiazole | 129 | $C_5H_7NOS$ | 1 |
| 2. 2-Butoxythiazole | 157 | $C_7H_{11}NOS$ | 1 |
| 3. *N*-Acetyl-1*H*-benzothiazol | 179 | $C_9H_9NOS$ | 33 |
| 4. 2-(Methylthio)benzothiazole | 181 | $C_8H_7NS_2$ | 1 |
| XVIII. Terpenes (3) | | | |
| 1. Borneol | 154 | $C_{10}H_{18}O$ | 5 |

| Compound | Molecular weight | Molecular formula | Reference number[a] |
|---|---|---|---|
| XVIII. Terpenes (3) (*continued*) | | | |
| 2. 4-Methyl-2-(2-methyl-1-propenyl)-tetrahydropyran (*cis*-rose oxide) | 154 | $C_{10}H_{18}O$ | 1 |
| 3. Bornyl acetate | 196 | $C_{12}H_{20}O_2$ | 5 |
| XIX. Miscellaneous compounds (3) | | | |
| 1. 1,4-Dioxane | 88 | $C_4H_8O_2$ | 33 |
| 2. β-Methoxystyrene | 134 | $C_9H_{10}O$ | 1 |
| 3. 1,5-Dimethoxynaphthalene | 188 | $C_{12}H_{14}O_2$ | 1 |

[a] References: 1: Nunomura *et al.* (1979); 2: Nunomura *et al.* (1980); 3: Shoji (1936); 4: Sasaki and Nunomura (1979); 5: Nunomura *et al.* (1976a); 6: Morimoto and Murakami (1966); 7: Yamada and Goan (1969); 8: Goto (1973); 9: Sasaki and Nunomura (1981); 10: Taira (1926a); 11: Tomiyasu (1927); 12: Asao *et al.* (1967); 13: Kihara (1940); 14: Akabori (1936); 15: Taira (1926b); 16: Akabori and Kaneko (1936); 17: Ishizu (1969); 18: Yokotsuka (1951a); 19: Yokotsuka (1953b); 20: Yokotsuka (1951c); 21: Fukai (1929); 22: Yokotsuka (1951b); 23: Yamada (1928); 24: Yokotsuka (1953c); 25: Nakajima and Takei (1949); 26: Yokotsuka (1954); 27: Ikeda and Kawaguchi (1922); 28: Nunomura *et al.* (1978); 29: Yokotsuka (1950); 30: Asao and Yokotsuka (1961b); 31: Asao and Yokotsuka (1963); 32: Matsumoto (1921); 33: Nunomura and Sasaki (1981); 34: Yokotsuka and Asao (1961); 35: Yokotsuka (1953a); 36: Yokotsuka (1953d); 37: Yukawa (1917); 38: Yokotsuka (1956); 39: Asao and Yokotsuka (1958a); 40: Shoji and Onuki (1932); 41: Morimoto and Murakami (1967); 42: Yokotsuka (1949); 43: Nunomura *et al.* (1979); 44: Nunomura *et al.* (1976b); 45: Kosuge *et al.* (1971); 46: Nunomura and Sasaki (1982); 47: Morimoto and Murakami (1966); 48: Ishizu (1963).

## Mycotoxins formed by different fungi and their association with foodstuffs

| Toxin | Organism | Foodstuffs affected | Some toxic effects |
|---|---|---|---|
| 1. Aflatoxins* | *Aspergillus flavus* *A. parasiticus* | Groundnuts and products, rice, maize, other nuts and seeds, cottonseeds, coconut, wheat, tree nuts, milk, cheese | Liver and kidney carcinoma, bileduct proliferation, fatty infiltration of liver of animals |
| 2. Sterigmato-cystin* | *A. versicolor* | Cereals | Hepatoma in rats |
| 3. Ochratoxins* | *A. ochraceus* | Cereals | Liver and kidney pathology of rats |
| 4. Aspergillic acid | *A. flavus* | Cereals | Antimicrobial and toxic to mice |
| 5. Kojic acid* | *A. flavus* and other spp. of *Aspergillus* | Cereals | Antimicrobial and mammalian toxicity |
| 6. beta-nitro-propanoic acid | *A. flavus* | Cereals | Toxic to man and animals |
| 7. Tremorgenic toxin | *A. flavus* | Maize and other foodstuffs | Sustained trembling in mice |
| 8. Luteoskyrin | *Penicillium islandicum* | Rice | Liver toxicity Hepatoma |
| 9. Rugulosin | *P. rugulosum* | Rice | Nephrosis and liver damage |
| 10. Chlorine-containing peptide | *P. islandicum* | Rice | Hepatotoxin Hepatoma of animals |
| 11. Islanditoxin | *P. islandicum* | Rice | |

* Mycotoxins detected as natural contaminants

| Toxin | Organism | Foodstuffs affected | Some toxic effects |
|---|---|---|---|
| 12. Citrinin* | P. citrinum | Rice | Automicrobial nephropathy of animals |
| 13. Citreoviridin | P. citreo-viride and other Penicillium spp. | Rice | Mammalian paralysis |
| 14. Rubratoxins | P. rubrum | Maize | Fatty infiltration of liver of rats |
| 15. Patulin* | P. expansum<br>A. clavatus | Apple Rice Feeds | Antimicrobial phytotoxic, carcinogenic in rats |
| 16. Penicillic acid* | P. puberulum<br>P. cyclopium | Maize | Antimicrobial carcinogenic in rats |
| 17. Cyclopiazonic acid | P. cyclopium | Food and foodstuff | Convulsions in rats. Severe lesions in spleen and kidney |
| 18. Psoralens | Solerotinia Solerotiorum | Celery plant | Toxic to man and animals dermatitis |
| 19. Stachybotrys toxin | Stachybotrys atra<br>S. alternans | Straw | Farm and other animals and man. Stachybotryotoxicosis (Dermal Toxicity) |
| 20. ATA toxin | Fusarium sporotrichioides | Oats, Wheats, Barley | Alimentary toxic Aleukia of animals and man (blood dyserasia) |
| 21. Diacetoxy-scirpenol* | F. scirpi<br>F. tricinotum | Wheat, Oats, Rye, Maize | Skin necrosis and eye damage in rats |
| 22. T-2 toxin* | F. tricinotum<br>F. nivale | Cereals, Maize, Fescue grass | Necrosis of the epidermal tissue of rats "Fescue Food of Cattle" |
| 23. Nivalenol*<br>Deoxynivalenol* | F. nivale | Rice | Inhibition of DNA synthesis |

* Mycotoxins detected as natural contaminants

| Toxin | Organism | Foodstuffs affected | Some toxic effects |
|---|---|---|---|
| 24. Fusarenone | F. nivale | Rice and cereals | Inhibition of protein synthesis in mice |
| 25. Butenolide | F. nivale | Maize, Fescue, Cereals, Hay | Fescue foot in cattle and tail necrosis |
| 26. Zearalenone* | F. graminearum | Maize, Hay, Barley, Feed | Hyper-estrogenic in animals |
| 27. Sporidesmins* | Pithomyces chartarum | Pasture | Facial eczema of animals |
| 28. Rhizoctonia toxin | Rhizoctonia leguminicola | Hay, Red Clover | Slobbering in cattle and horses |
| 29. Ergot | Claviceps spp. | | Gangrene |

* Mycotoxins detected as natural contaminants

## REFERENCES


1. Altschul A. M., New protein foods
Academic Press, N. York + London 1974

2. Campbell G., Fermented foods of the world
Butter Worths, London.

3. Yokotsukat, Soysauce biochemistry
Advances in food reseach vol. 30
Academic Press Inc. 1986.

4. FUKUSHIMA, D. (1979) Fermented vegetable (soybean) protein and related foods of Japan and China. *J. Am. Oil Chem. Soc.*, 56, 357–362.

5. SHIBASAKI, K. and HESSELTINE, C. W. (1962) Miso fermentation. *Econ. Bot.*, 16, 180–185.

6. WANG, H.-L. and HESSELTINE, C. W. (1979) Mold-modified foods. In: Peppler, H. J. and Perlman, D. (eds) *Microbial Technology*, 2nd edn, Vol 2, 95–129. New York: Academic Press.

7. WU-LEUNG, W.-T., BUTRUM, R. R. and CHANG, F. H. (1972) *Food Composition Table for Use in East Asia*, 334 pp. Bethesda, MD: USDHEW and FAO, Rome.

8. FUKUSHIMA, D. (1979) Fermented vegetable (soybean) protein and related foods of Japan and China. *J. Am. Oil Chem. Soc.* 56: 357–362.

9. LEE, W. J. and CHO, D. H. (1971) Microbiological studies of Korean native soy-sauce fermentation. I A study of the microflora changes during Korean native soy sauce fermentation. *J. Korea Agric. Chem. Soc.* 14: 137–148. Cited by Kwon, T. W. (ed.) (1972) *Fermented Foods in Korea*, 89–91. Seoul: Korea. Inst. Sci. Tech.

10. STEINKRAUS, K. H. (ed.) (1983) *Handbook of Indigenous Fermented Foods*, 437–467. New York: Marcel Dekker.

**APPENDIX 14**

July 1993

## COMMENTS BY THE BACKSTOPPING OFFICER

The expert contributed effectively to the installation and putting into operation of the equipment, mainly the GLC and the AAS. In his in-country training courses he concentrated on pesticide residues, heavy metals, mycotoxines and food additives as well as on rapid methods for food quality control. In each of these fields he trained 12 people. Since Mr. Kaminski's mission the DTQC laboratory has been operational.

The training manual and the other documents he elaborated will contribute to the sustainability of the laboratory activities. Mr. Kaminski's mission has, therefore, contributed positively to the fulfilment of the project activities. His report is acceptable to the Agro-based Industries Branch and is considered an important tool to strengthen food quality control in Vietnam.