

cc: R. E. Keller - QUEENY.

February 27, 1967

Dr. M. J. Thomas
Research Division
Building No. 33
National Cash Register
Dayton 2, Ohio

Dear Dr. Thomas:

Attached is a photostat of the original paper of
Dr. Jensen in Sweden, relating to polychlorinated
biphenyls. I will be happy to have your ideas after
you read it.

As far as the section on toxicology is concerned, it
is true that chloracne and liver trouble can result
from large doses. Whether or not this is at all relevant
to small quantities existing in human fat is, of course,
an entirely different question.

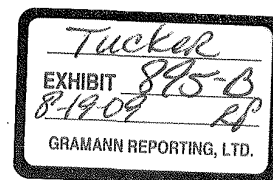
At any rate, I believe before we worry about the
toxicological part of the problem, we should settle
the analytical part.

Sincerely,

R. Emmet Kelly, M. D.
Medical Director

RMK/ln
att.

0631149



PHGNCR 2000087

MONSFOX00000088

WATER_PCB-00044033

Mr Chairman, Ladies and gentlemen.

In honor to our British ^{host} I will try to hold this lecture in English.

As the title of this lecture states, I am today going to tell about the discovery of some hitherto unobserved chlorinated hydrocarbons having up to eight chlorine in the molecule and found in residue analysis. The chemical name of polychlorinated biphenyls (in the following called PCB). To get familiar with PCB I will start with the chemistry and toxicology.

Chemistry

En

The main characteristic of PCB is 1. Their very high stability. As an example they can be boiled with nitric acid without being destroyed. 2. They are hardly metabolised in living organism. 3. If more than 4 chlorine are present they are non inflammable. It is clear that these three characteristics does it easy to understand that when they have entered the living organism they will have a low persistence. But it is difficult to explain how they find their way into the living organism. One thing seems to be clear, they don't come from agricultural use, but from a technical one and most probable it comes to the nature via wastes that are tried to be burnt up, because then we have them at once in the air, because of their non inflammability.

Toxicology

The PCB were introduced in 1929 and as early as 1936 Jones and Alden reported that 23 out of 24 men employed in manufacturing of PCB suffered from an acne form eruption of the skin. Acne did not appear until 6 to 8 months after the material was first used. In 1937 Drinker reported that rats exposed to chlorinated biphenyls in concentration of approximately 1 mg/m^3 for 16 hours a day for 6 weeks showed damage of the liver. After that time the allowed concentration of PCB in air is 0.5 mg/m^3 . (For DDt the same value is $0.5 - 1 \text{ mg/m}^3$). The same authors finished their experiments in 1938, and related that these compounds have an injurious effect, manifested solely in the liver. Chlorinated biphenyls appeared to be the most injurious chlorinated compounds of all tested.

0281420

PHCNCR 2000088

MONSFOX00000089

WATER_PCB-00044034

Greenburg, Mayer and Smith 1939 reported that PCB and polychlorinated naphthalenes are blamed for the death of three young workers, and that pregnant women and persons who have at any time had any liver diseases are particularly susceptible.

Wedel, Haller and Denton gave 1942 animals PCB including administration by inhalation, ingestion and skin absorption. Histological examination of the viscera showed important toxic effect only in the skin and liver, and the degeneration effects in the liver are essentially the same whatever was the method for the administration. Paribok (1955) found as an occupational poison in the electrical industry, mixed tetra and penta chlorobiphenyl causes folliculitis, comedo, pyoderma and other skin affections, and that its principal toxic effect is fatty degeneration of the liver.

Miller (1944) injected 69 mg PCB (4 and 5 chlorine) subcutaneously in 32 guinea pigs. Eight to ten days after injection, fat droplets were noted in the liver cells, and after 16 days they were present in moderate or very large numbers. Rabbits and rats were also tested in this investigation, as well as the PCB was administered both continuously, subcutaneously or ingested in the food. In the feeding experiment 8 guinea pigs received 2 doses of 69 mg of the chlorinated biphenyl 1 week apart. Death occurred in 11 to 29 days.

Finally Mc Laughlin 1964 reported a method to test the chemical toxicity and teratogenic effect by injection into the yolk sac of fertile eggs prior to incubation. PCB was found between the eight compounds among 100 tested having the highest order of toxicity. No hatch was found at a level of 25 mg per egg. At a level of 50 mg per egg, one chick hatched out of 20 injected eggs, but died 2 days later. Some embryos which were examined after they died, showed weak deformities (often a short upper trunk) and growth retardation. Lead acetate resulted as an example in no hatch at a level of 1 mg per egg. Autopsy of the dead embryos have showed extensive brain damage. Mercuric chloride showed no hatch even at a level of 0.5 mg per egg.

As the analytical chemistry is a pronounced service science I have been in contact with many scientists from other fields during the work with residue analysis, and I have always found this contact very stimulating for my own work. This co-operation often demands that we are talking the same scientific language. Because of this need I will today try to give a lecture in low level analytical chemistry for biologists, illustrated by the residue analysis of polychlorinated biphenyls.

The lecture will be divided in the following three sub-divisions:

0281421

PHGNCR 2000089

MONSFOX00000090

WATER_PCB-00044035

1. Chemistry of PCB and their toxicology.
2. Analytical methods for residue analysis and proof of structures.
3. Behaviour of PCB in nature, differences in metabolising rate of the PCB components, potentiation in an ecological series, concentration levels and examples of samples which have been proved to contain PCB.

A residue analysis can be divided into:

1. Extraction of the pesticides from the biological material, followed by a careful cleaning-up to take away interfering substances, most often fats.
2. Identification analysis by means of gas chromatography, Thin-layer chromatography and mass spectrometry.
3. Quantitative analysis.

At an ecological laboratory in Riksmuseet in Stockholm 1-2 g of a sample is cut out of the biological material and transferred into a weighed and carefully cleaned test tube, and stored at -20° until analysis. Smaller samples have been used, min. 5 mg of body fat, and with dry materials such as hair, feathers, pine needles 100 mg are sufficient to reach the desired 10 ng/g level in residue analysis. In cases of water proofs 1 l. is used for reaching the 10 pg/g. level.

B.1(homog) In order to facilitate complete extraction of the fatty materials from the biological sample, the double amount of finely powdered anhydrous magnesium sulphate is added to the sampling tube, and the whole is homogenised with an insertable homogenizer. The resulting powder is transferred into a special Soxhlet extractor. After 4 hours of extraction the solvent is evaporated, leaving the fat in a small weighed test tube at the bottom of the extractor. This fat is dissolved in methylene chloride in such a way that 100 ul (0,1 ml) contain 20 mg of fat.

Sox.-tube)

The 100 ul solution is now transferred to a little object glass, 3 x 7 cm, covered with a silicagel layer 1 mm thick, in order to form a line 0,7 cm from one end of the slide. Inserting this thin-layer plate into a vessel the bottom of which is covered by a few mm of methylene chloride, the solvent will be sucked up in the dry layer of silicagel, and at least reach the upper end of the plate. The fact is that the fat has a greater affinity to the powder on the plate than the chlorinated hydrocarbons have, — and we get a separation. The fat being more polar than the chlorinated hydrocarbons will never go longer than 2 cm before the

0281422

PHGNCR 2000090

MONSFOX00000091

WATER_PCB-00044036

ELUTION
tube

solvent reaches the upper part of the glass.

The front of the fat appears quite visible against a lamp, and with the aid of a razor blade the zone above the fat is transferred to the elution tube and the chlorinated biocides absorbed on the powder can now be eluted by one ml of ether. The concentration is sufficient for detection of the chlorinated hydrocarbons down to the 10^{-12} g level.

The next step in the analytical procedure concerns the separation of the different chlorinated hydrocarbons that the sample may contain. As a matter of fact, this is a troublesome task. It is easy to estimate what is not present, but more difficult to say exactly one is present. We suffer from the negative demonstration, as will be shown later.

At first a few words about the separation of the components present in the sample and their visualization.

The separation is accomplished by means of a gas chromatograph fitted to a detector that transfers its impulse to a recorder.

The system is shortly described:

A spirally formed glass tube with an inner diameter of 2 mm and about 2 m in length is filled up by a support, covered with a thin layer of an oil. The tube is heated in the chromatograph to about 200° . Through the tube a stream of nitrogen continuously follows. When about 10 μ l (1/100 of 1 ml) of the purified sample is injected into the tube, the components of the sample will be evaporized and go forward through the column with the gas stream. As the constituents have different affinity to the column filling they will pass the column with different speed and it will take different time for them to reach the detector at the other end of the glass tube. If the temperature and the nitrogen flow are held constant this time, the retention time, has a specific value for a certain compound. This is true, but unfortunately it is also a fact that two components can have the same retention time. This is one of the bigger problems in gas chromatographic analysis of unknown samples, as will soon be obvious. To make it possible to estimate the retention time it is necessary to visualize the chlorinated hydrocarbons. For that purpose more or less specific detectors are used. The detector most often used in pesticide analysis is the so called electron capture detector, which can detect down to one picogram ($= 10^{-12}$ g of lindan). Unfortunately this detector is not specific for chlorine, but gives answer also for oxygencontaining compounds. The response here is much lower but can be counterbalanced if the concentration of the oxygen containing is much higher.

0281423

The principle for the electron capture detector is shortly:

At the end of the gas chromatographic tube is placed a little tube containing a foil made of titanium tritide. This is an α -radiant. The α -

PHGNCR 2000091

MONSFOX00000092

WATER_PCB-00044037

particles are reacting with the nitrogen molecules coming from the column. Then we get $+ N_2 \rightarrow e^- + N_2^+$. Over the detector we have a tension of 90 volt and by mean of the electrons we will get a constant electrical current over the detector. This standing current is transferred to a one-mV recorder as a constant baseline. When now a chlorinated hydrocarbon leaves the column this compound has a high affinity to the electrons and this means that the amount of electrons will diminish, and they will diminish proportionally to the amount of chlorine. The electrical current will also diminish and this is noted as a peak on the recorder. The area of the peak will be proportional to the amount of substance in the sample. By mean of a standard injection it is now possible to compare the retention time and the area of an unknown component with the retention time and area of the known standard. As said before this detector is not specific for chlorine but anyhow very useful, because of its high sensitivity. The system described has, as we have seen, two disadvantages:

1. Two different compounds can have the same retention time and be detected as one peak.
2. A registered peak does not need to be chlorinated, because the detector is not specific.

If the sample is injected in two different columns with different chemical properties we have increased the chance for a good separation. If two compounds have the same retention time on one column they may not have it on another. When a result seems doubtful, - if the compound being responsible for a certain peak contains chlorine or not - it is possible to concentrate the sample and analyse it on a less sensitive detector such as the microcoulometric one, which is specific for chlorine. The compound is burned in a furnace and the generated chlorine titrated directly.

As is seen from the two last mentioned possibilities it is anyhow possible to get a rather high degree of certainty in residue analysis, but it is a rather time-consuming work. When using this method just described, we very often found that many chromatograms from residue analysis of most carefully purified samples still contain a large number of peaks. Many of these have retention times that do not agree with any known chlorinated pesticides, or their metabolites. This chromatogram can serve as an example. It was obtained by residue analysis of a sea-eagle found dead in the archipelago of Stockholm. In the range of the known peaks, there are so many unidentified that there also must be an obvious risk of the known peaks to be covered by unknown ones.

If this remark is found true, the reported results of many previous anal-

0281424

PHGNCR 2000092

MONSFOX00000093

WATER_PCB-00044038

titative analysis must be brought into question. In the present investigation it is shown that most of the unknown peak of chromatograms at residue analysis of chlorinated pesticides are due to polychlorinated biphenyls.

I will show a chromatogram of human fat analysed on a so called SP 96 column, the most often used type in pesticide analyses. Early retention times were in agreement with DDE, DDT_{op} and DDT_{pp}. Next slide shows the same sample analysed on a QP-1 column. Now the former 2 DDT peaks have divided into 4 peaks, and two of them are still in agreement with DDE_{pp} and op., the two new were unknown.

Logically, these unknown components were at first thought to be metabolites of the insecticides. Against that spoke that neither treatment nor concentrated sulfuric acid in ether. This treatment made it rather sure that the compounds did not contain oxygen. In Sweden residues of organic mercury have been investigated rather intensively in the Swedish fauna. As these compounds give very high responses to the electron capture detector it was also investigated if the unknown peaks could have a mercuric origin.

It was found that the water-ecological series had high residues of both mercury (Westermark, Johnels) and the unknown ones, when the same individuals were analysed. Anyhow, the pheasant suffering most from mercury poisoning only contained low levels of electron capturing compounds and these belonged

to the normal insecticides. Therefore the unknown could hardly be mercurials or metabolites of them.

As the eagle sample giving the chromatogram shown in fig. 10. could be estimated to contain DDT and DDE up to 13 g/kg in extractable fat, the amount of unknown compounds also were suggested to be in the same range, and then sufficiently high to do a run on the combined gas chromatograph - mass spectrometer. If this could be done successfully it would be possible to get very important informations about the chemical nature of the unknown, for ex. the molecular weight numbers of chlorine etc. This method is up to now the method giving the highest degree of certainty in the low level analytical chemistry, amounts of 100 ng substance being enough.

As this method for identification of totally unknown residues surely will be very important in the future (when i.e. a biologist has found that fishes in a river die) it may be possible by means of this method to find out exactly what compounds are responsible for the death.

For this reason, I will go into some details with this method.

In the actual case we took the extract from 20 mg eagle and concentrated

0281425

PHGNCR 2000093

MONSFOX00000094

WATER_PCB-00044039

8

it as much as possible and made an injection on the gas chromatograph combined with the mass spectrometer. The result was the chromatogram shown on the next slide. Every time the recorder showed that a compound is leaving the column, the effluent is led to the mass spectrometer. Now just a few words about the mass spec.

The molecules leaving the column are bonded with electrons at E. We have now got the molecule positive charged, but with the same mass as before. This M^+ is accelerated in a vacuum and will then get a kinetic energy where v is the speed. Next comes the magnetic field that tries to bend the direction of the molecule. This will be big for a small molecule and less for

If we have a sieve in the other end we can directly read the molecular weight. Added to this parent molecule M^+ we will also get addition informations, because of the fact that M^+ may not be stable, a part of them will be broken down before they reach the sieve in the other end.

Ex. M^+ DDT M^+ DDT - CCl_3

Mass spectrograms from the different unknown peaks in the eagle sample as shown. The mass numbers equal to the molecular weights of the unknowns could be read to 426, 392, 358, 324. Astonishingly, the molecular differences were constantly 34 mass units. This difference shows a familiarity in origin of the unknown. Now the fact is that chlorine exists as a mixture of two isotopes with atom weights 35 and 37 in proportion 75:25. If the molecule has one chlorine, this will give two molecule peaks, one for Cl_{35} and one for Cl_{37} . If there are two chlorine we have the possibility of one with only Cl_{35} , one with both Cl_{35} and 37 and one with 2 Cl_{37} and therefore

0281426

PHGNCR 2000094

MONSFOX00000095

WATER_PCB-00044040

The relation of the peaks found on the different mass spec were:

Molecular weight	324	358	392	426
Chlorine content	5	6	7	8

An explanation of the familiarity of the compounds can be given if one substance is built from the former by substituting a hydrogen with chlorine

RH RO1 HC1
M⁺ + 012 M⁺ + 94

Then it is possible to calculate the molecular weight of the parent hydrocarbon PBC.

$M_{PBC} = M - x M_{Cl} + x M_H$, where M is the molecular weight of the component having x chlorine atoms. For example for m = 426 and 8 Cl we will get $M_{PBC} = 426 - 280 + 8 = 154$ and equal with the other molecule.

The most probable formula with carbon and hydrogen giving this molecular weight is $C_{12}H_{10}$ and this can only be satisfied when the parent-hydrocarbon is biphenyl, and the unknown being polychlorinated biphenyls.

This explanation was later fully verified by injection of a synthetic PBC on the mass spec.

Furthermore extensive gas chromatographic investigations proved that the PBC standard gave peaks with the same retention time as the unknown peaks from the sea eagle.

With the method just described I suppose that we have a new possibility to study the residues in the air because the pine needles can always be

. We have had great difficulty in quantifying the PCB, but when getting a little more time it will be possible. We have done a few calculations on a few species, and I suppose they are right within a factor 2. We have found the residue to be from

It has been my statement here to-day to present this method for studies of defiling of the nature, and with this method a new type of defiling agents has been found to be present in nature, and a few experiments have shown where they may be found.

Now this method is going to be used in the first hand to estimate how the situation is in nature as a whole, and in the other hand to find the leaks through which they find its way to nature. Soem maybe are present here today to get news about the leaks, and to then I want to say come back in a year.

0281427

PHGNCR 2000095

MONSFOX00000096

WATER_PCB-00044041

So much I think I can say again that the PCB hardly can come from agriculture. As support for this suggestion I can say that we have found PCB in eagle feathers from Hikanuseet from 1944, where hardly any chlorinated pesticides were used in agriculture. One more thing that I find important to say is that in contrast to the mercury problem this does not seem to be a pure Swedish problem. I have just studied chromatograms taken from London air, and they clearly contain PCB, and Dr. Holden has told me that he also finds them in his fishsamples. But finally in waiting at more results I should like to point ^{out} one more thing. It is proved that PCB comes to nature, we don't know now where they are used, but they are very persistent to chemicals and to fire. I think the poison jury should try to state that a content of PCB shall always be found in an open declaration.

0281428

PHGNCR 2000096

MONSFOX00000097

WATER_PCB-00044042

1. Mixture of insecticides and PCB. from eagle sample.

2. Same sample after nitration. Only PCB remain.

3. PCB-standard.

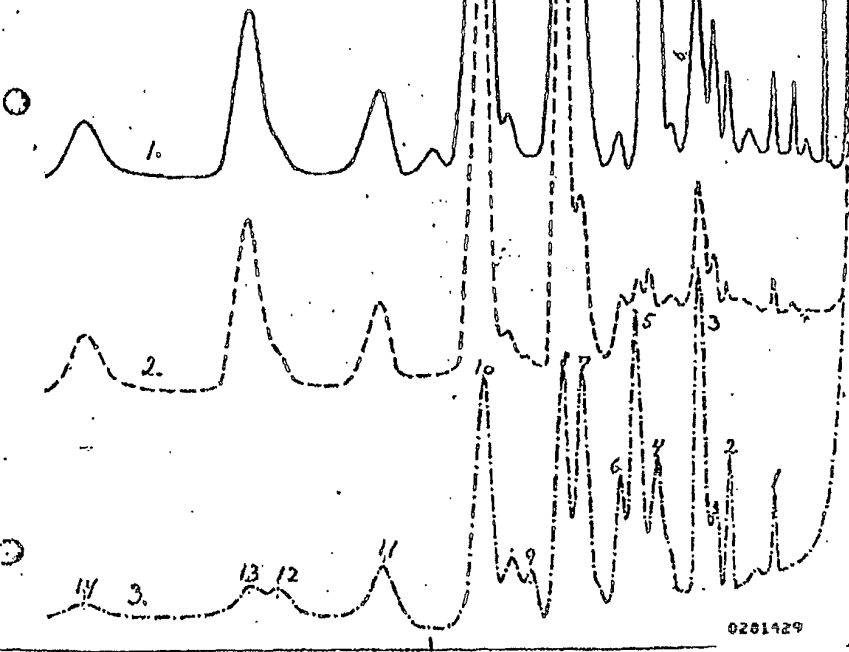
Column: QFI 6% on gaschrom P 100-120 mesh silanized, sedimented.

150cm in 0.12cm i.d. allgls.

N₂-carrier gas 15cm/sec.

eg. to 30 ml/min.

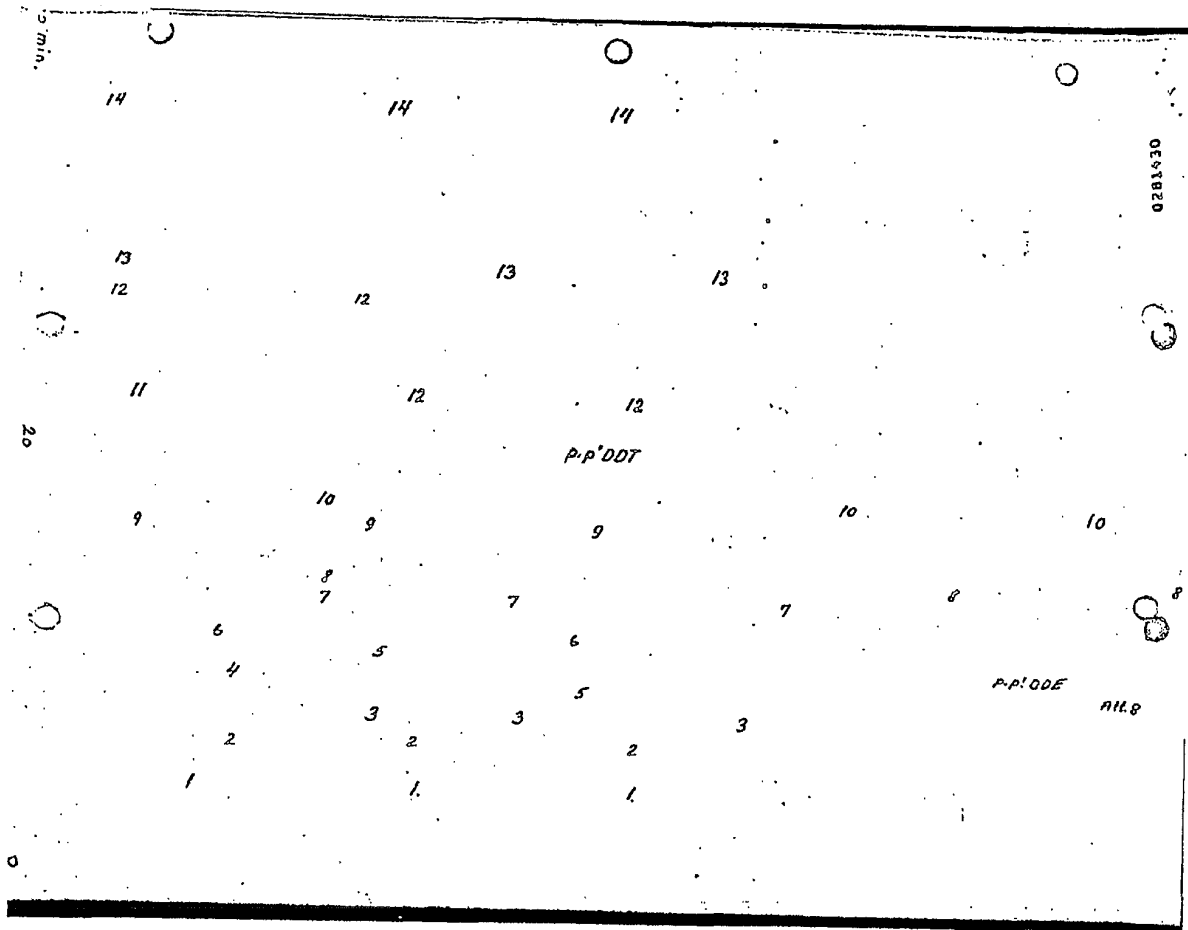
Det. ECD. Col. temp. 188°.



PHGNCR 2000097

MONSFOX00000098

WATER_PCB-00044043



PHGNCR 2000098

MONSFOX00000099

WATER_PCB-00044044

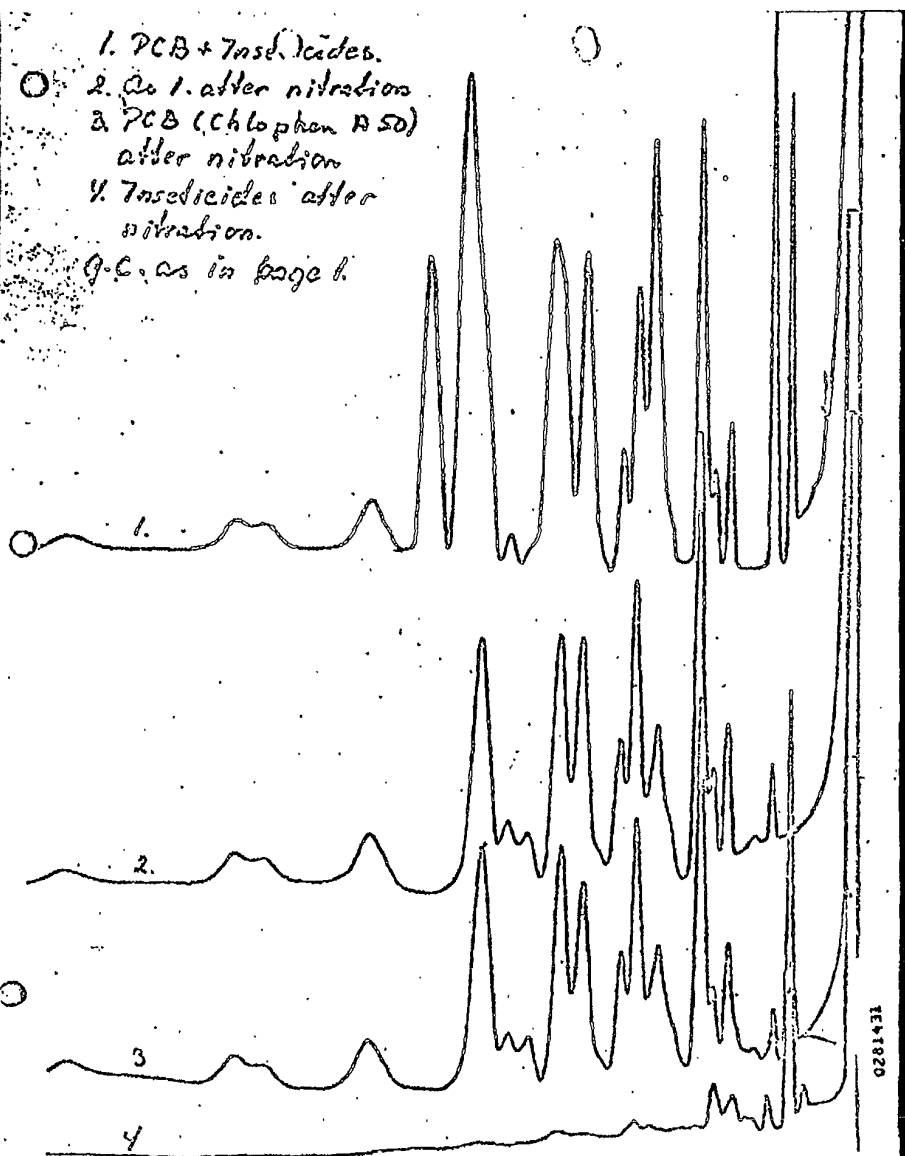
1. PCB + Insecticides.

2. As 1. after nitration

3. PCB (Chlophen ASD)
after nitration

4. Insecticides after
nitration.

G.C. as in page 1.



PHGNCR 2000099

MONSFOX00000100

WATER_PCB-00044045

Curve 4

Curve 3

Curve 2

Curve 1

0201432

14

14

14

13

13

13

12

12

12

11

11

11

9

10

9

10

9

8

7

8

7

6

4

5

3

2

1

1

5

5

6

3

2

P-p'-DDT

P-p'-D₁

DIELDRIN

p-p'-DDT+7

P-p'-DDE

3

ALDRIN

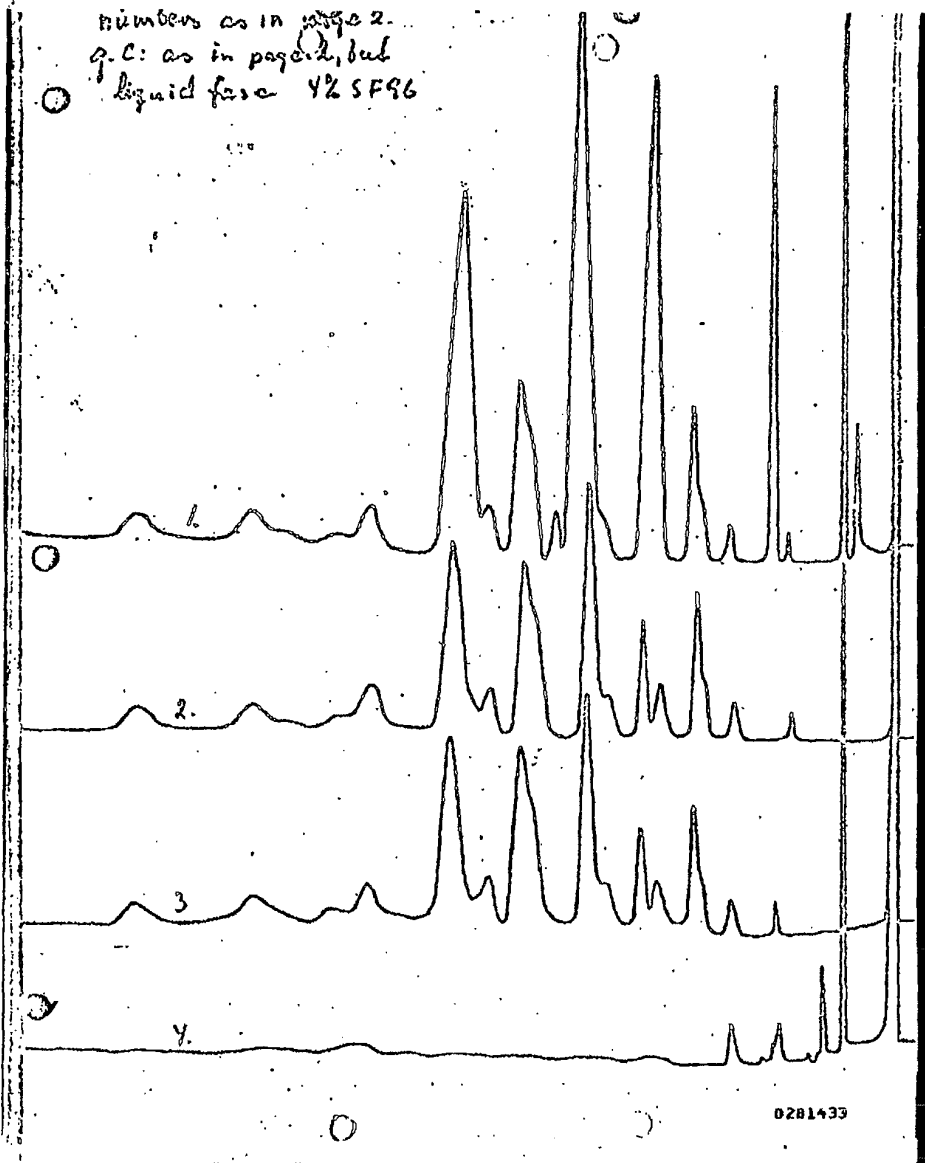
LINDRIN

PHGNCR 2000100

MONSFOX00000101

WATER_PCB-00044046

numbers as in page 2.
g.c. as in page 2, but
liquid phase 4% SF96

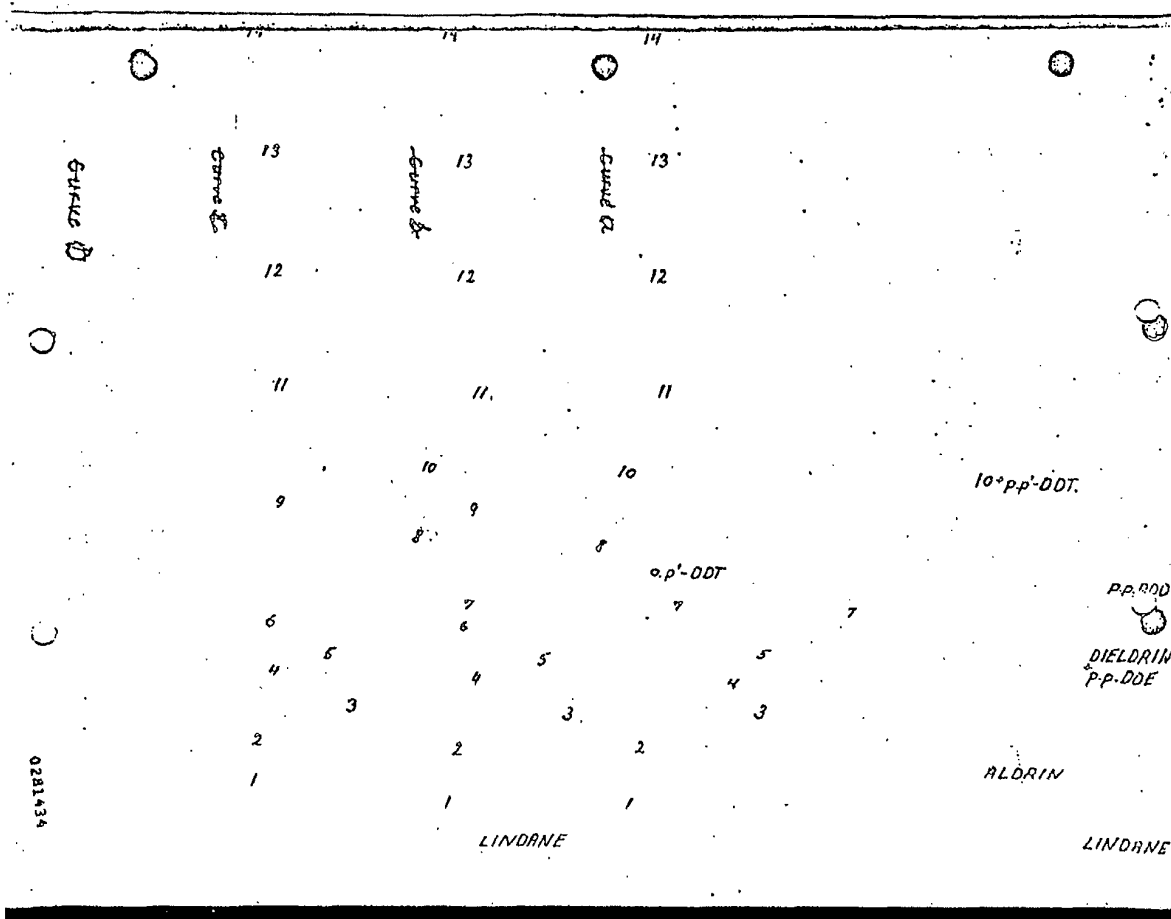


0201433

PHGNCR 2000101

MONSFOX00000102

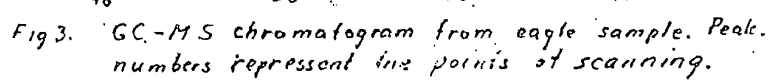
WATER_PCB-00044047



PHGNCR 2000102

MONSFOX00000103

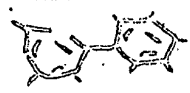
WATER_PCB-00044048



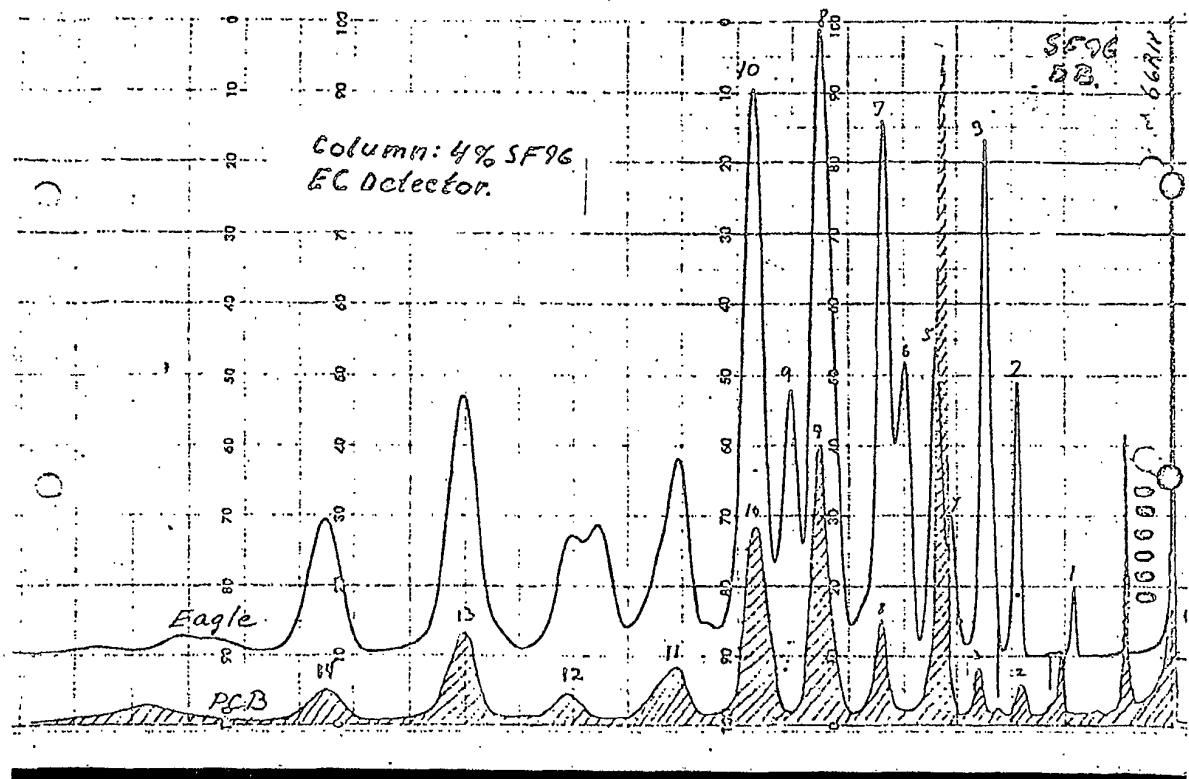
0281435

WATER_PCB-00044049

WATER_PCB-00044050



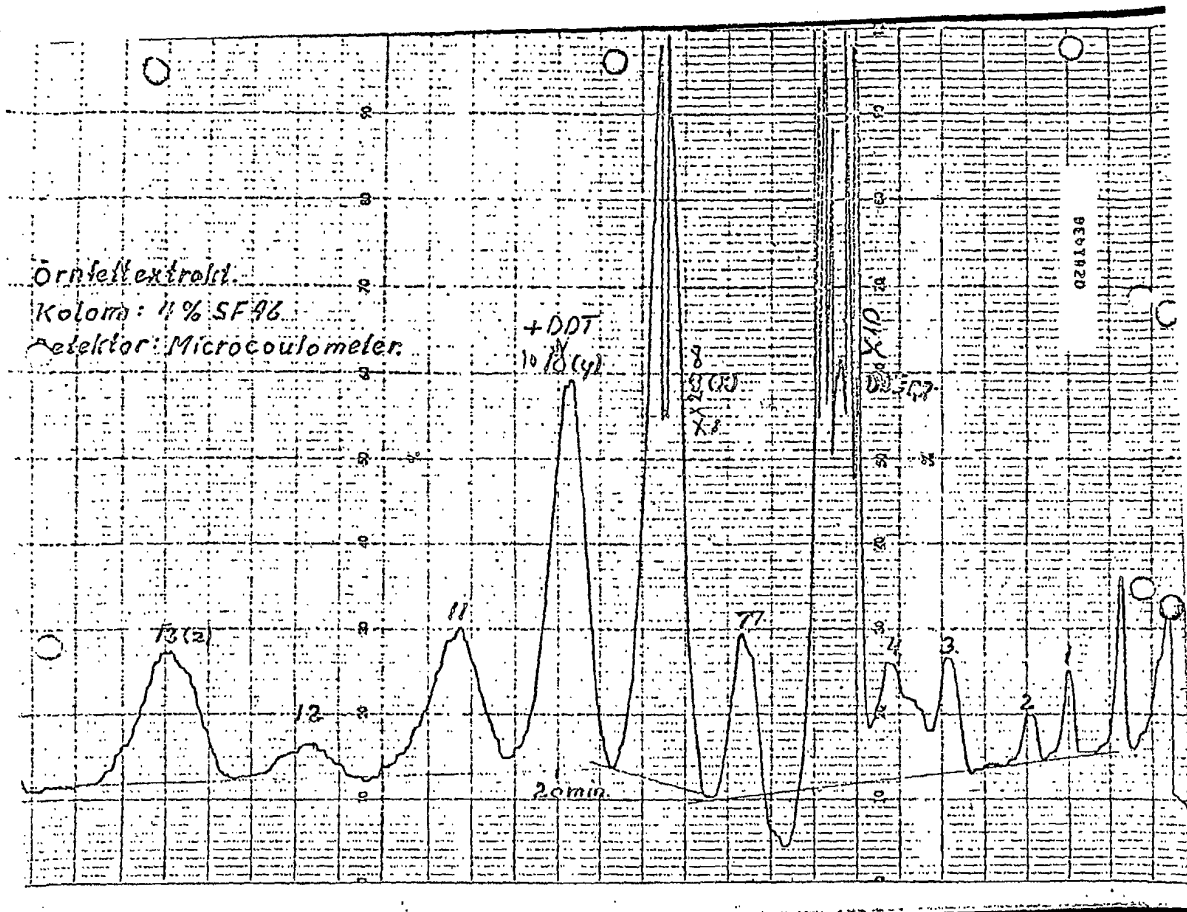
0281437



PHGNCR 2000105

MONSFOX00000106

WATER_PCB-00044051



PHGNCR 2000106

MONSFOX00000107

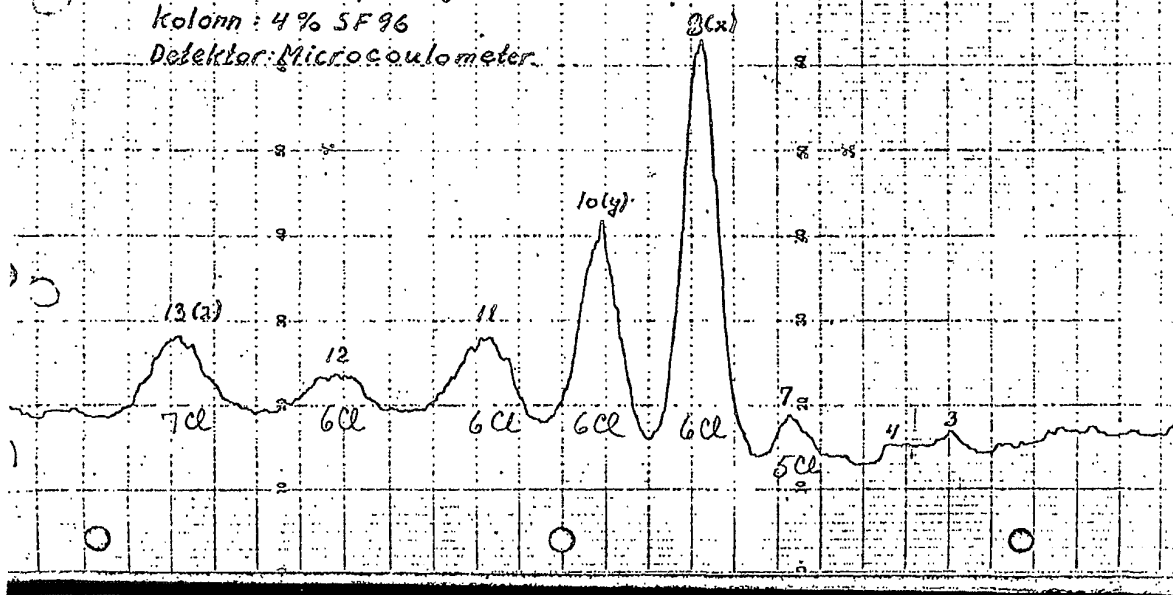
WATER_PCB-00044052

örnfettextrakt.

Beh. med $H_2SO_4 - HNO_3$ 1:1

kolonn: 4% SF96

Detektor: Microcoulometer.



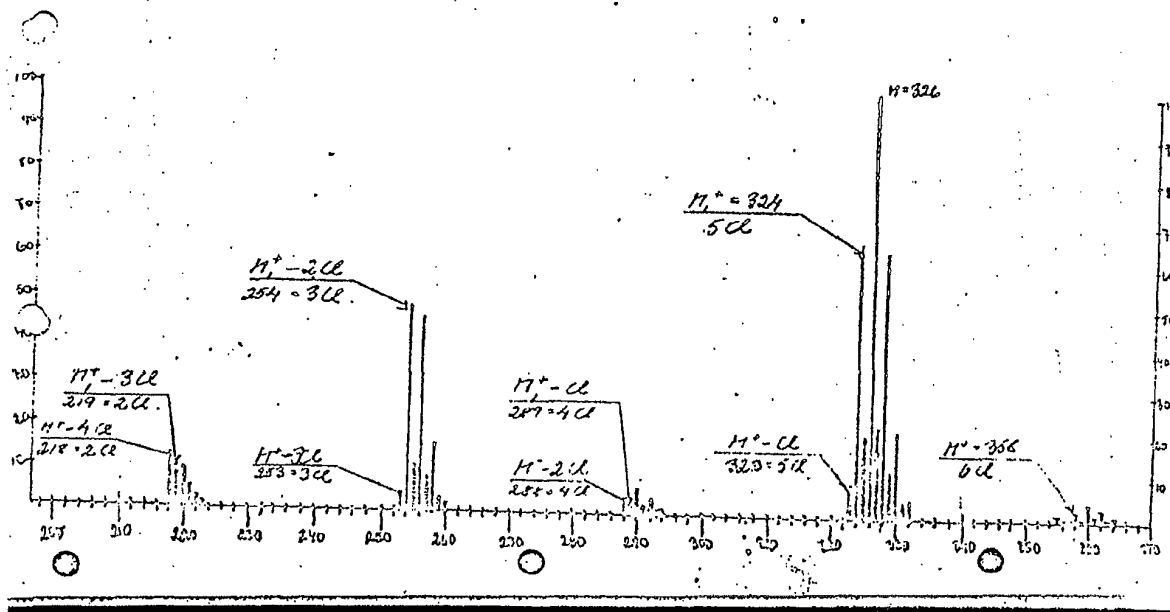
PHGNCR 2000107

MONSFOX00000108

WATER_PCB-00044053

Glophen ASD
topp nr. 7.

0281440



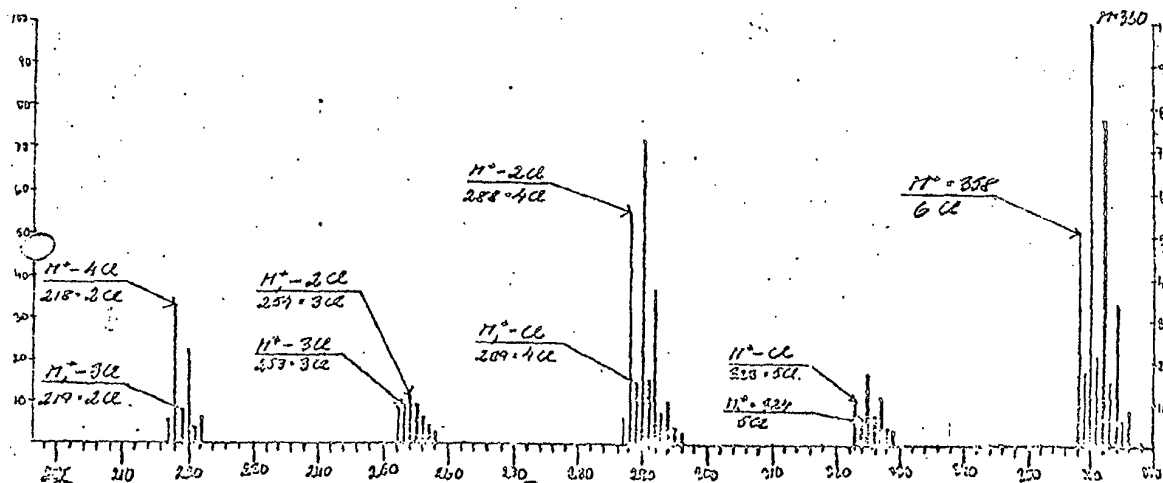
PHGNCR 2000108

MONSFOX00000109

WATER_PCB-00044054

Clophen A50
topp nr 8

0281661



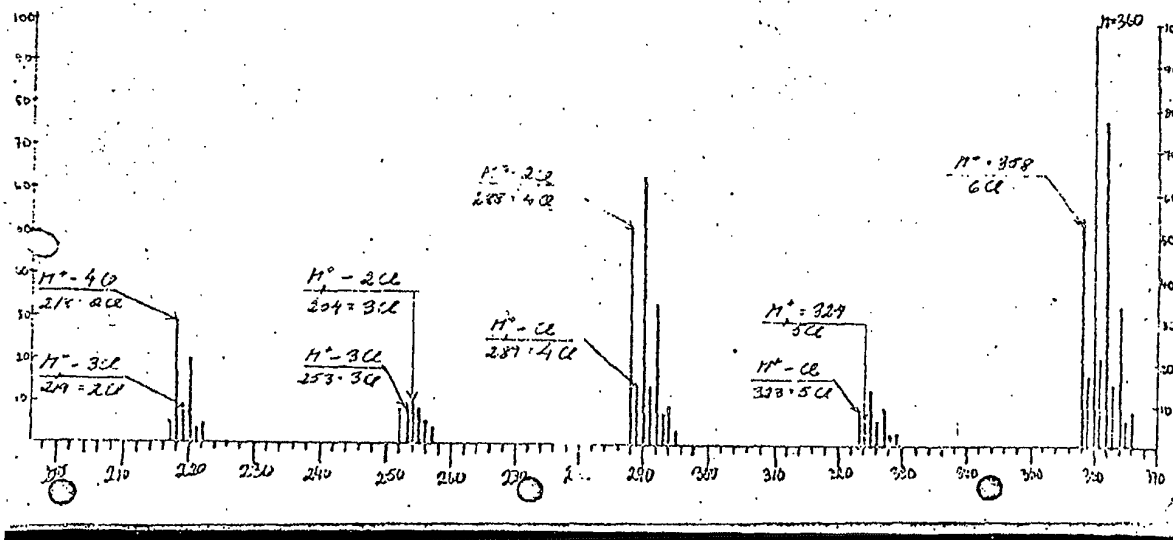
PHGNCR 2000109

MONSFOX00000110

WATER_PCB-00044055

Clophen A50
topp. nr 10

0281452



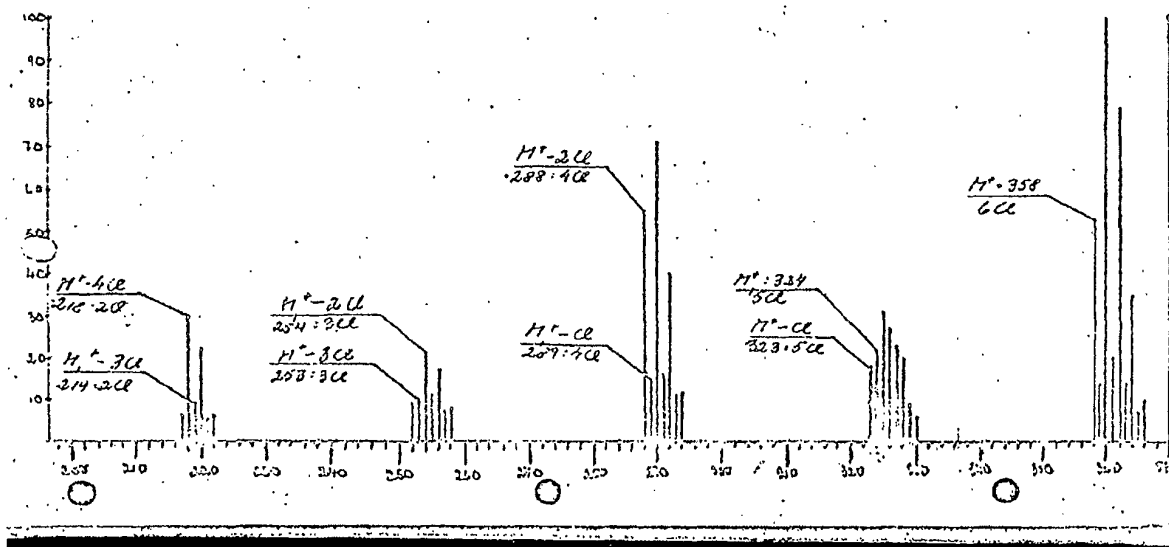
PHGNCR 2000110

MONSFOX00000111

WATER_PCB-00044056

Glophen A50.
 topp nr. 11.

0201820



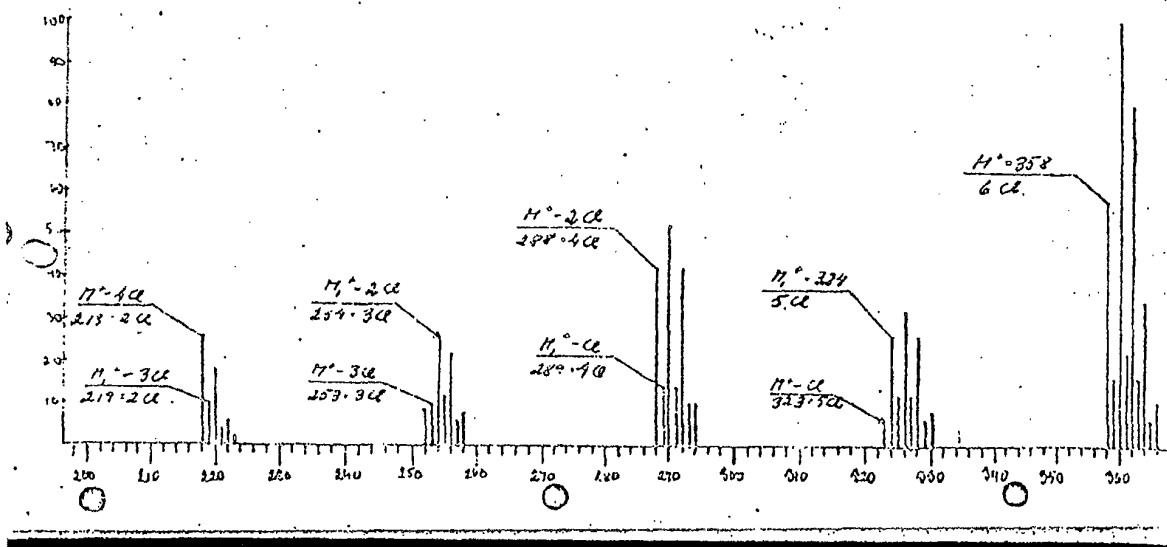
PHGNCR 2000111

MONSFOX00000112

WATER_PCB-00044057

Clophen A50
 1opp nr. 12

0281444



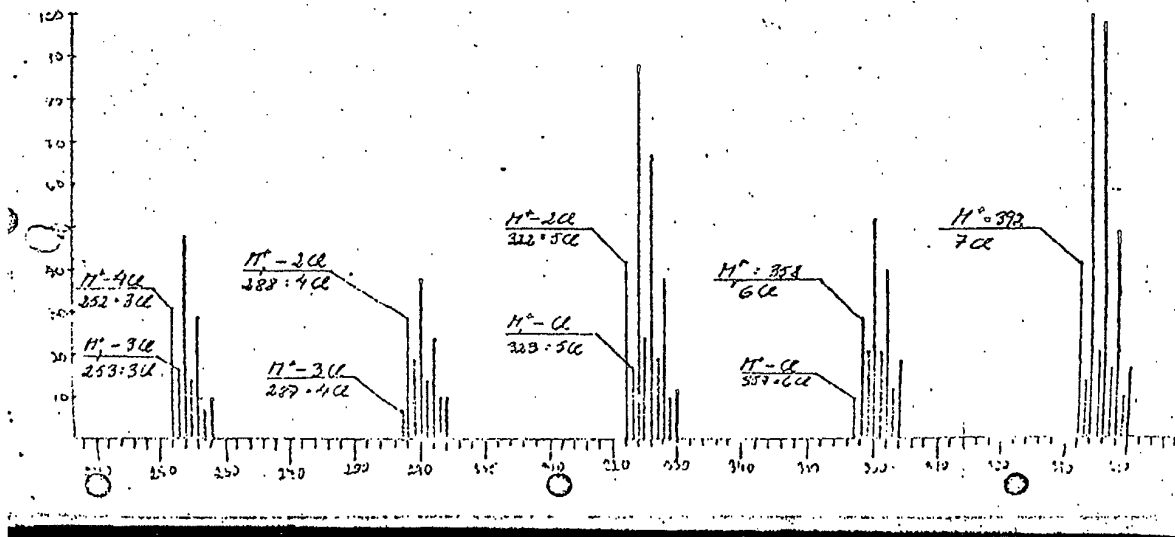
PHGNCR 2000112

MONSFOX00000113

WATER_PCB-00044058

Glophen A50
topp nr. B

0281049



PHGNCR 2000113

MONSFOX00000114

WATER_PCB-00044059