

Brussels, Belgium

February 22, 1967

J.R. Buchanan, St Louis
J.E. Filer, Brussels
D.V. Hardy, MCL
Eugene Wilde, St Louis

THIS COPY FOR

DW

Dr. R. Emmet Kelly
St. Louis

Dear Dr. Kelly,

I should like to refer to your letter dated the 10th of February and our telephone discussion of yesterday's date.

I enclose a copy of Sören Jensen's original paper which in fact was presented in English - Dr. Jensen apologises for the typing etc. but this copy is taken from his original paper which has not yet been re-typed and tidied up.

I asked our agent in Sweden to dig out the various pieces of information that you required and I attach a copy of his reply dated February 17th which I think fully covers the point raised in your letter.

If there are any small points outstanding, please let me know and I will try to get you further information.

I am sending the only copy of Mr. Jensen's paper to you and therefore if any of the other recipients of this letter needs to have access, they can take a copy from yourself.

I would however emphasize the post script on page 3 of Ola Palm's letter in which he states Mr. Jensen has consented to send us the full copy of his paper on the understanding that this is used only internally within the Monsanto organization.

Best wishes.

E. Wood

EXHIBIT
WBP M-11

MON-MT-008559

RISING & STRAND

CP/BO

AKTIEBOLAG

TELEFON: 34 01 85

TELEGRAM: HENNIS

TELEX: 1624

POSTGIRO: 5 44 43

BANKGIRO: 73-0448

STOCKHOLM VA February 17, 1967
SVEAVAGEN 47

Mr. David Wood
Monsanto Europe
BRUSSELS 3
Belgium

Dear David,

re: AROCLORS

Reference is made to our telephone conversation and I have tried to dig out the information you asked for. Before going into any detail, however, I would like to refer to your letter to Mr. Sören Jensen, dated February 8.

Mr. Jensen would be interested in samples of as many isomers as you can put at his disposal. The quantities of course are very small indeed and he would need only about 10 mg of each. In theory you could put the lot behind a stamp and mail it to Mr. Sören Jensen but in actual practice it is of course not as easy as that.

Anyway, Mr. Jensen would be interested in both the low-chlorinated and the high-chlorinated types since this would help him to get a better picture of the metabolism.

Now to the various points you raised. Enclosed you will find the original articles as published in "Dagens Nyheter" and "Svenska Dagbladet" on November 23, 1966. The people who organised the meeting where Mr. Jensen's paper was presented was a Committee - 1964 års Naturvårdskommitté - operating under Statens Naturvetenskapliga Forskningsråd (The Swedish Natural Science Research Council). The Committee (for Conservation of Natural Resources of 1964) is working under the chairmanship of a Dr. B. Lundholm.

The meeting was organised under the auspices of Naturvårdskommittén and was called apparently only in order to give Mr. Sören Jensen an opportunity to publish his findings. The timing was made so as to coincide with a visit of Dr. A. Holden from Scotland.

MONS 090516

EXHIBIT
WBPM-11.1

Dr. Alan Holden - address Fresh Water Fisheries Laboratory, Pitlochry Pearcher, Scotland - is the coordinator for the twelve OECD countries, as far as the influence of biocides on Nature goes. Dr. Holden apparently is a specialist on such questions and particularly on fish. There is no intention that Dr. Holden, or the group of twelve OECD countries as such, should carry on any further investigations on the particular problem unearthed by Mr. Jensen. Any continuation will be carried on in Stockholm by Mr. Jensen or cooperating Swedish laboratories.

The meeting of November 23 offered only one paper, i.e. that of Mr. Jensen. No decisions were made on any further action.

The work done by Mr. Jensen was paid for by the above-mentioned Committee for Conservation of Natural Resources. The further studies planned for on toxicology have not been started on yet since there is no money available. It is possible, however, that the same Committee of 1964 will be asked to subsidise even that work.

If toxicological studies are to be made, these probably will be carried out at Karolinska Institutet, Division of Toxicology under Professor Bo Holmstedt. The full address of this institute is

Kgl. Karolinska Institutet
Avd. Toxikologi
Solnavägen 1
Stockholm 60.

Since no decision has been made on toxicological investigations, obviously no information can be given as to the scope of the planned investigations.

The LKB press release of January 10, 1967 mentioned the research of twelve OECD countries. As was mentioned above, there is no question of any central investigations to be made under the auspices of the twelve countries. There is only the question of coordinated efforts by way of interchange of information.

Enclosed you will find photo copies of the original paper of Mr. Sören Jensen. We have taken this copy here and Mr. Jensen apologizes for the state of the paper which is his own typing. He has not yet had an opportunity to have it properly retyped for publication.

I hope the above answers your questions.

Yours sincerely,

MONS 090517

Enc.

P.S.

David:-

For the sake of good order, I would like to mention that Mr. Sören Jensen has sent us these papers with the understanding that they are only for internal use within the Monsanto organisation.

Ola.

MONS 090518

Mr Chairman, Ladies and gentlemen.

In honor to our British guest 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

Th

EXHIBIT
WBP M-112

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 the 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.

MUNS 049048

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 $0,5 \text{ mg/m}^3$. (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.

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 10 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 brack) 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.

MONS 049049

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:

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, potensation in an ecological serie, concentration levels and examples of samples which have been proved to contain PCB.

A residue analysis can be divided in:

1. Extraction of the pesticides from the biological material, followed by a careful cleaning-up to take away interfering substances, most often fat.
2. Identification analysis by mean 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

MONS 049050

solvent reaches the upper part of the glass.

7 ELUTION
tube

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.

5 go

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.

6 go

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 an 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.

MONS 049051

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-radiant. The n-

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 chlirine 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 registrated 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 microcloumetric one, which is specific for chlorine. The compound is burned in a furnace and teh generated chlorine titrated directly.

MONS 049052

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 penko to be covered by unknown ones.

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

titativo 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 SF 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 QF-1 column. Now the former 2 DDT peaks have divided into 4 peaks, and two of them are still in agreement with DDT_{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 other. 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 (Westermarck, 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.

MONS 049053

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 information 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 f.ex. 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

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 r 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.

For 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

MUNS 049054

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



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

$M_{\text{PHC}} = M - x M_{\text{Cl}} + x M_{\text{H}}$, where M is the molecular weight of the component having x chlorine atoms. For $m = 426$ and 8 Cl we will get $M_{\text{PHC}} = 426 - 280 + 8 = 154$ and equal with the other molecules.

The most probable formula with carbon and hydrogen giving this molecular weight is $\text{C}_{12}\text{H}_{10}$ 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 them I want to say come back in a year.

MONS 049055

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 Riksmuseet 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 find 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.

MONS 049056

1. Mixture of insecticides and PCB. from eagle sample

2. Same sample after nitration. Only PCB remain.

3. PCB-standard.

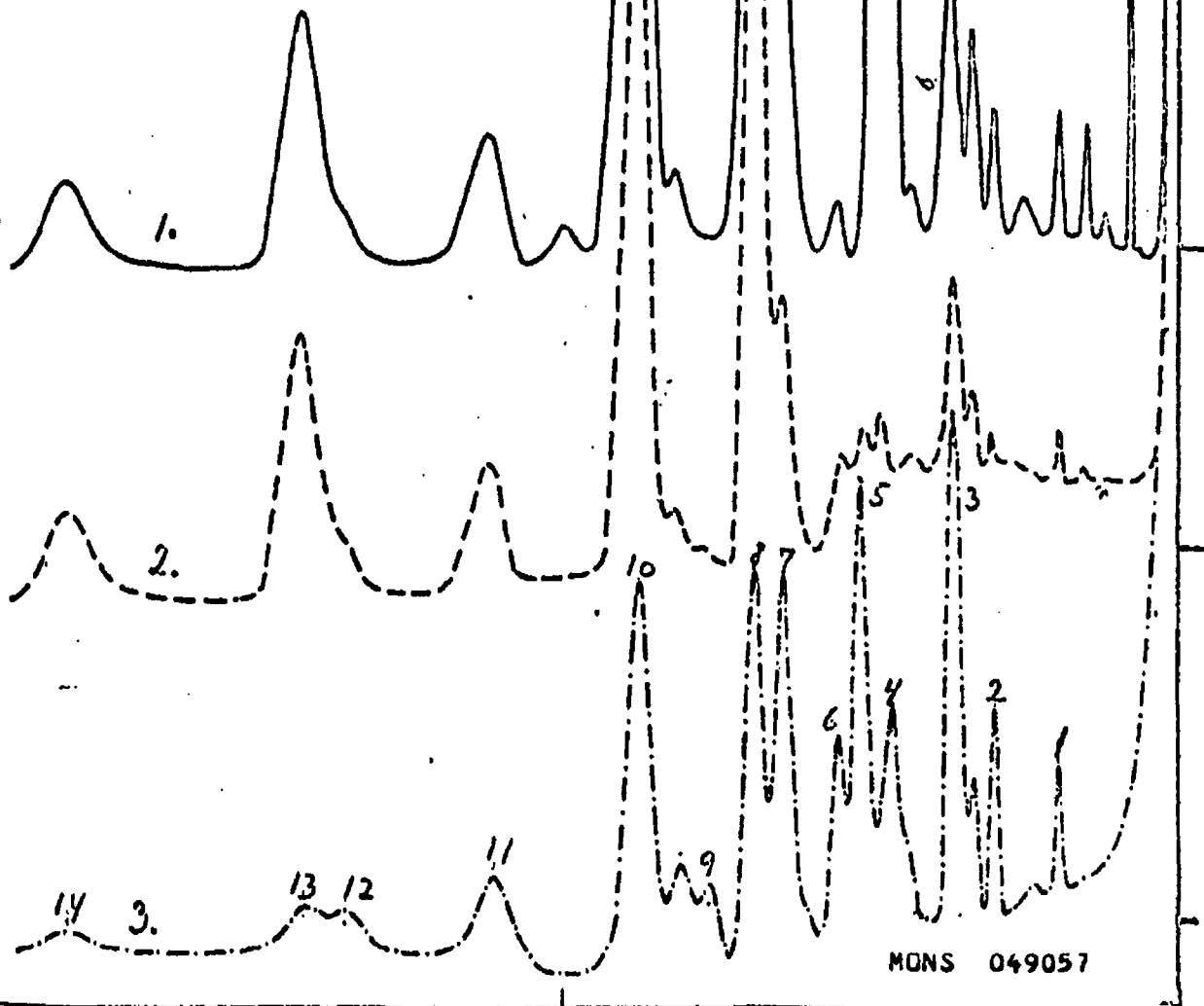
Column: QFI 6% on gaschrom P 100-120 mesh silanized, sedimentated.

130cm in ϕ . 1.8cm i ϕ . allglos.

N_2 -carrier gas 15cm/sec.

eq. to 30 ml/min.

Det. ECD. Col. temp. 188°.



850640 SNOW

P.P.OOE ALLS

P.P'DOT

5 min.

20

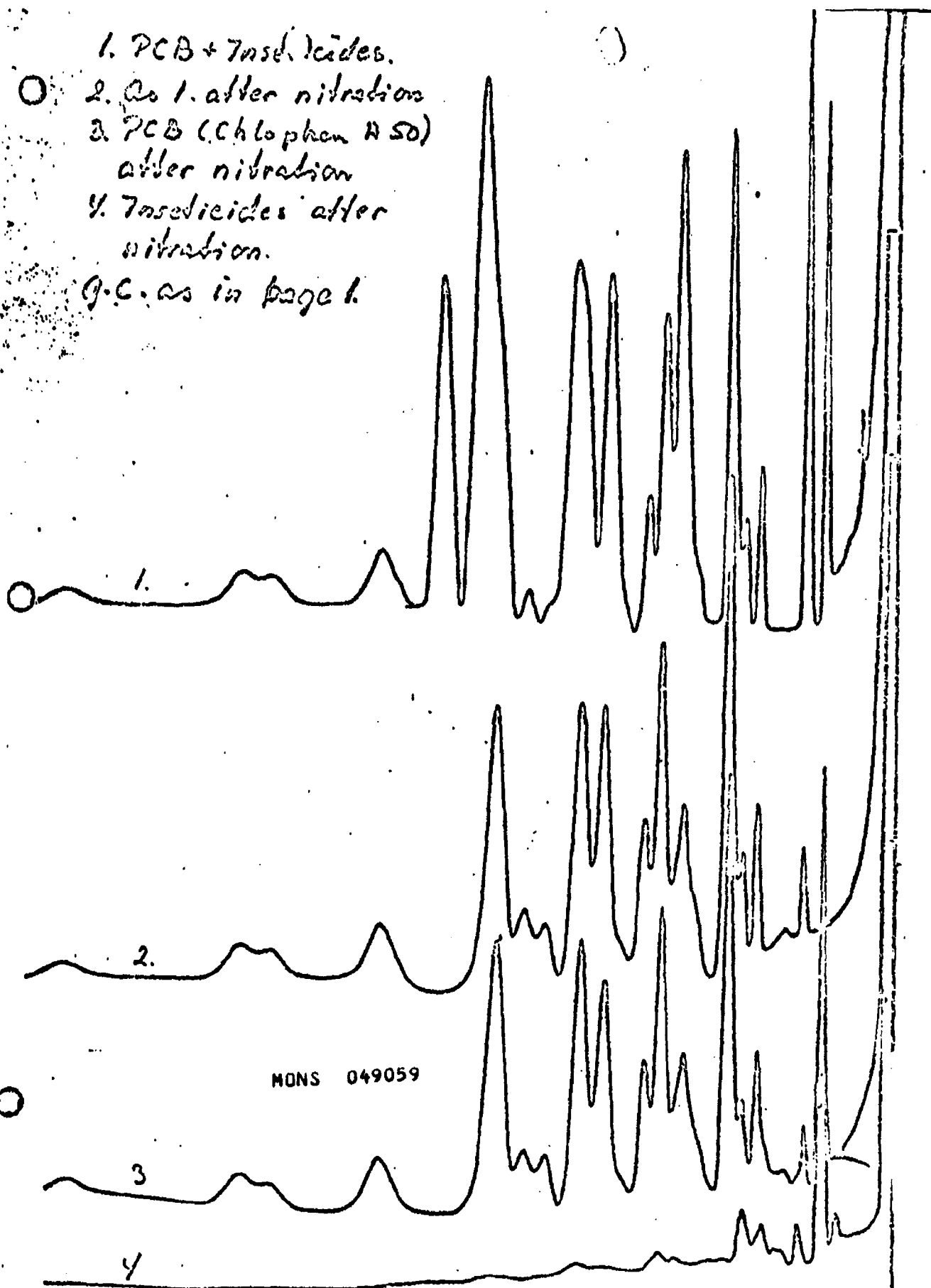
1. PCB + Insecticides.

2. As 1. after nitration

3. PCB (Chlophen ASD)
after nitration

4. Insecticides after
nitration.

G.C. as in page 1.



090640 SNOW

14

Curve 1

13

12

11

10

9

8

7

5

6

3

2

1

14

Curve 2

13

12

11

10

9

8

7

6

5

4

3

2

1

14

Curve 3

13

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4

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1

Curve 4

P.P'-DDT

P.P'-D₂

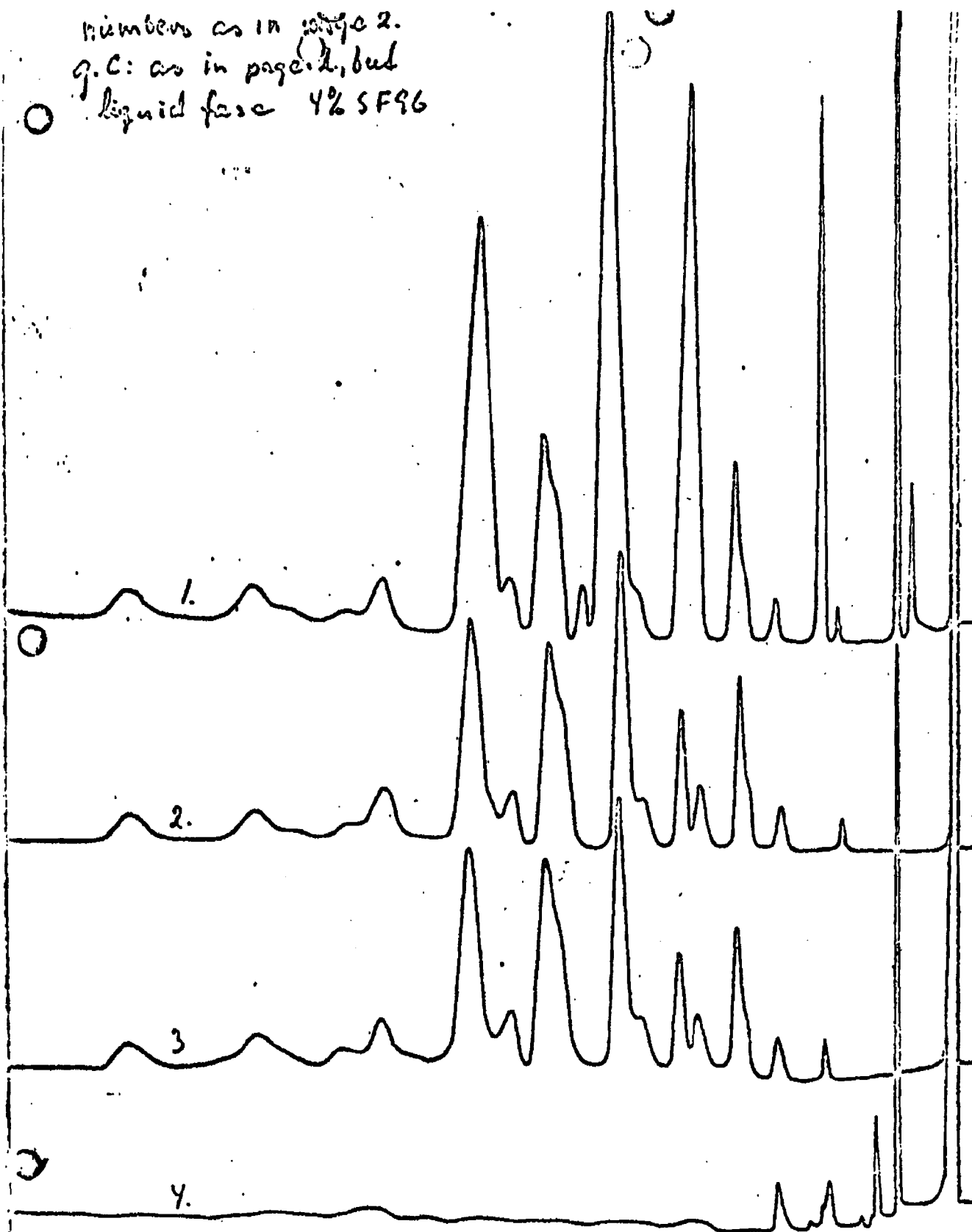
DIELDRIN

o.p'-DDT+7

P.P'-DDE

ALDRIN
LINDRIN

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



MONS 049061

14

14

14

Curve A

Curve B

Curve C

Curve D

13

13

13

13

12

12

12

12

11

11

11

11

10

10

10

10

o,p'-DDT

10-o,p'-DDT

pp'-DDT

DIELDRIN
p,p'-DDE

ALORIN

LINDANE

LINDANE

MONS 049062

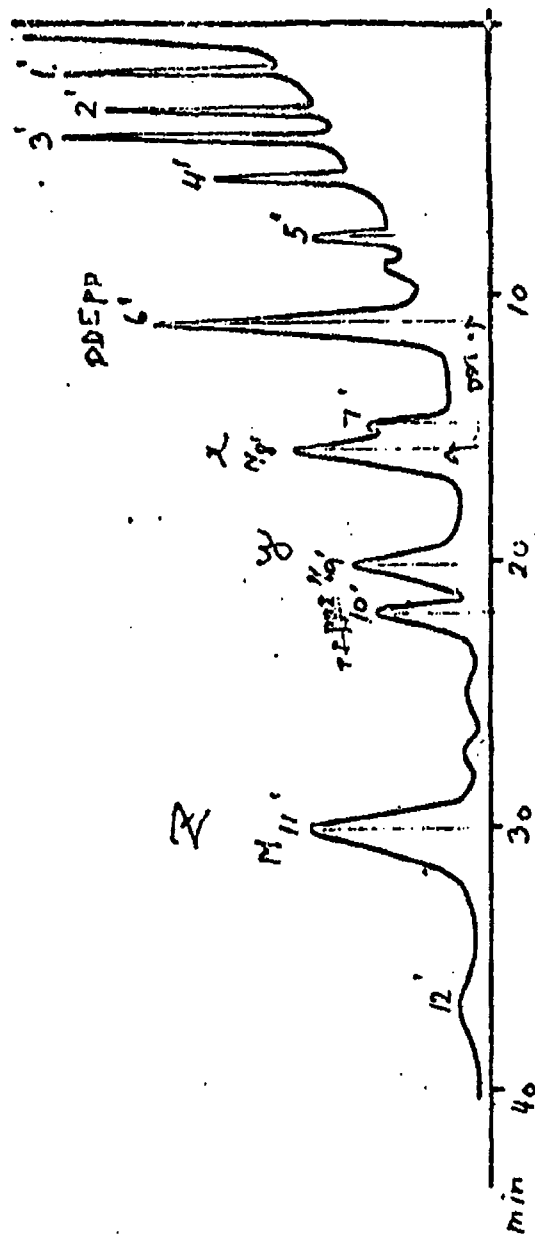


Fig 3. GC-MS chromatogram from eagle sample. Peak numbers represent in points of scanning.

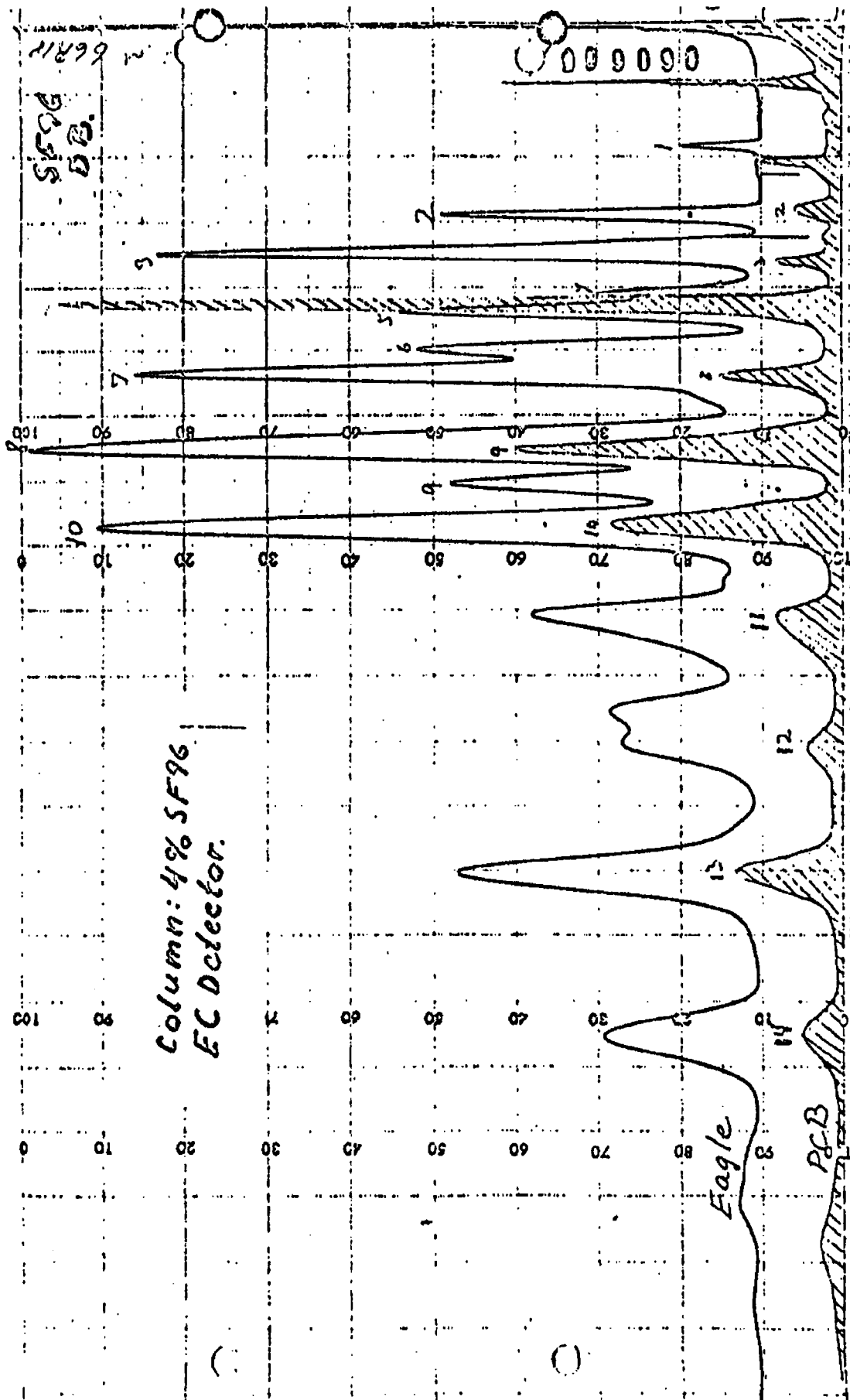
MONS 049063

Column: X70406:
 4% (SF₆, OF₂, SO₂, W₂)
 EC Detector

The chromatogram displays several peaks labeled with numbers 9 through 15. Peak 15 is the largest and is labeled "Eagle PCB". Other peaks are labeled 9, 10, 11, 12, 13, and 14. The x-axis represents retention time in minutes, with markings at 0, 10, 20, 30, 40, 50, 60, 70, 80, 90, and 100. The y-axis represents detector response, with markings at 0, 10, 20, 30, 40, 50, 60, 70, 80, 90, and 100.

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009090



Eagle

Pg. 13

Örnekleştirilmiştir.

Kolom: 1107031 76.5 %

Defektor: Microcoulometer.

700

10187

8225

10

127

21

11

2.2 min.

22

— 34 —

and

2

7

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48	49	50	51	52	53	54	55	56	57	58	59	60	61	62	63	64	65	66	67	68	69	70	71	72	73	74	75	76	77	78	79	80	81	82	83	84	85	86	87	88	89	90	91	92	93	94	95	96	97	98	99	100
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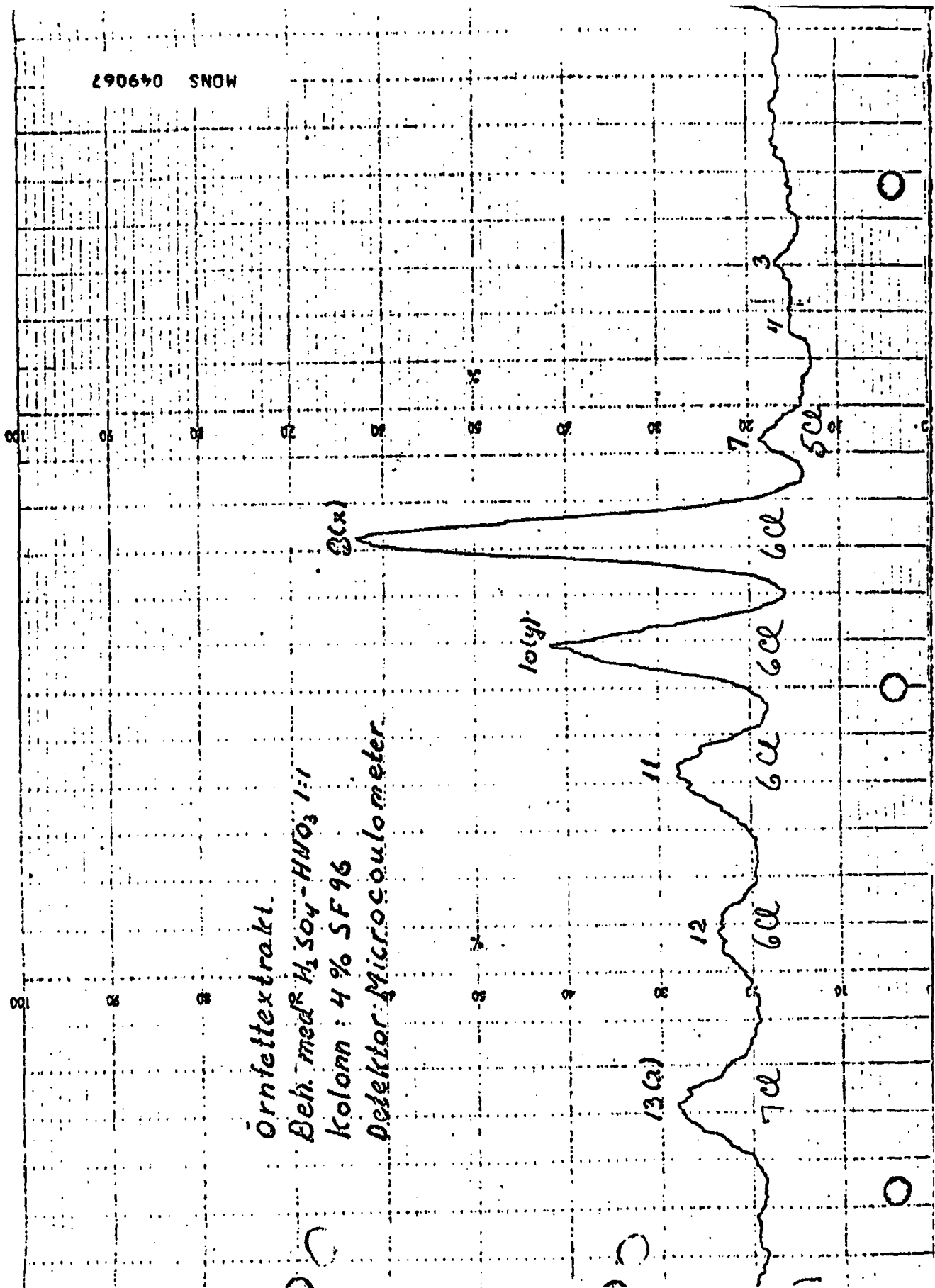
290640 SNOW

Örnfettextrakt.

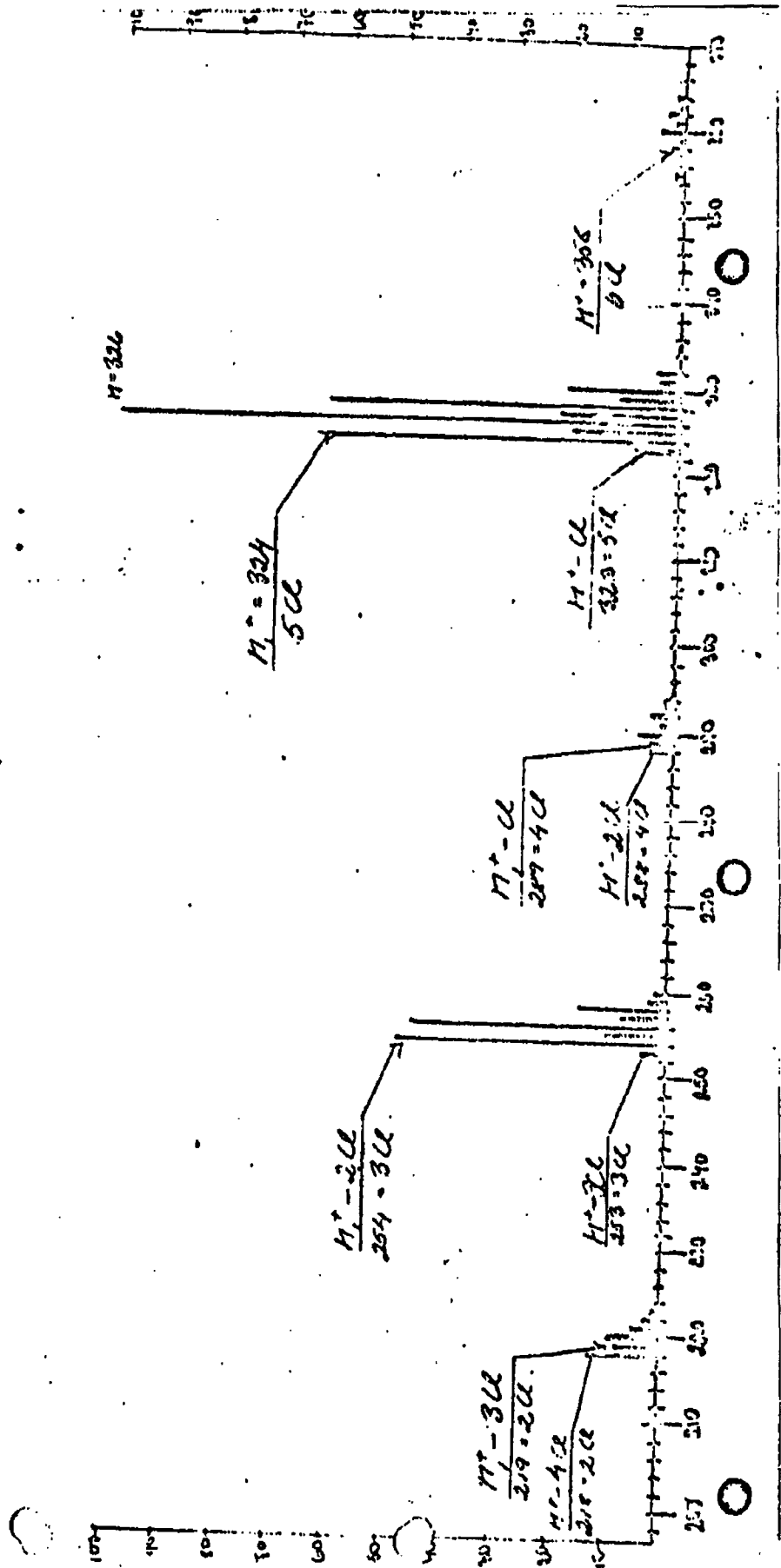
Beh. med $H_2SO_4-HNO_3$ 1:1

Kolonn : 4 % SF96

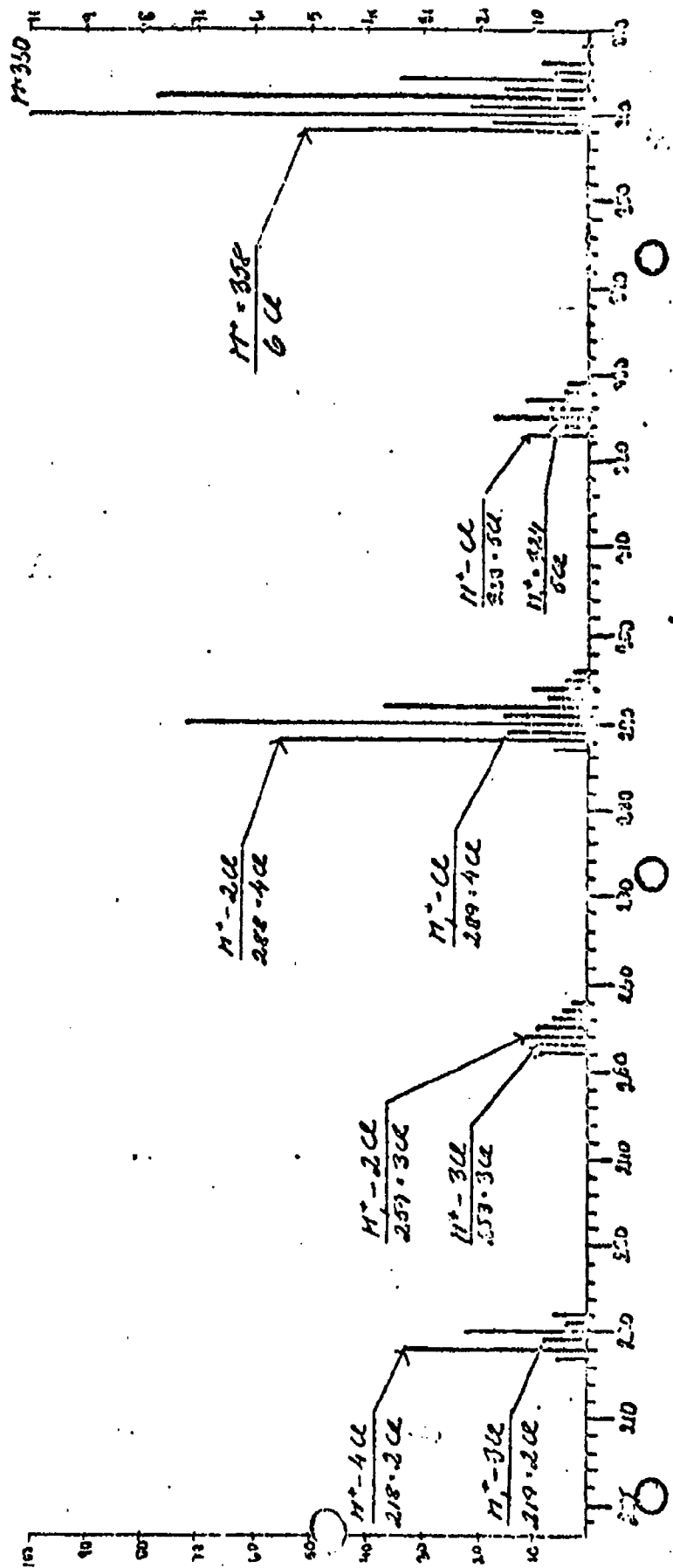
Detektor: Microcoulometer



Clophen A53
topp nr. 7.

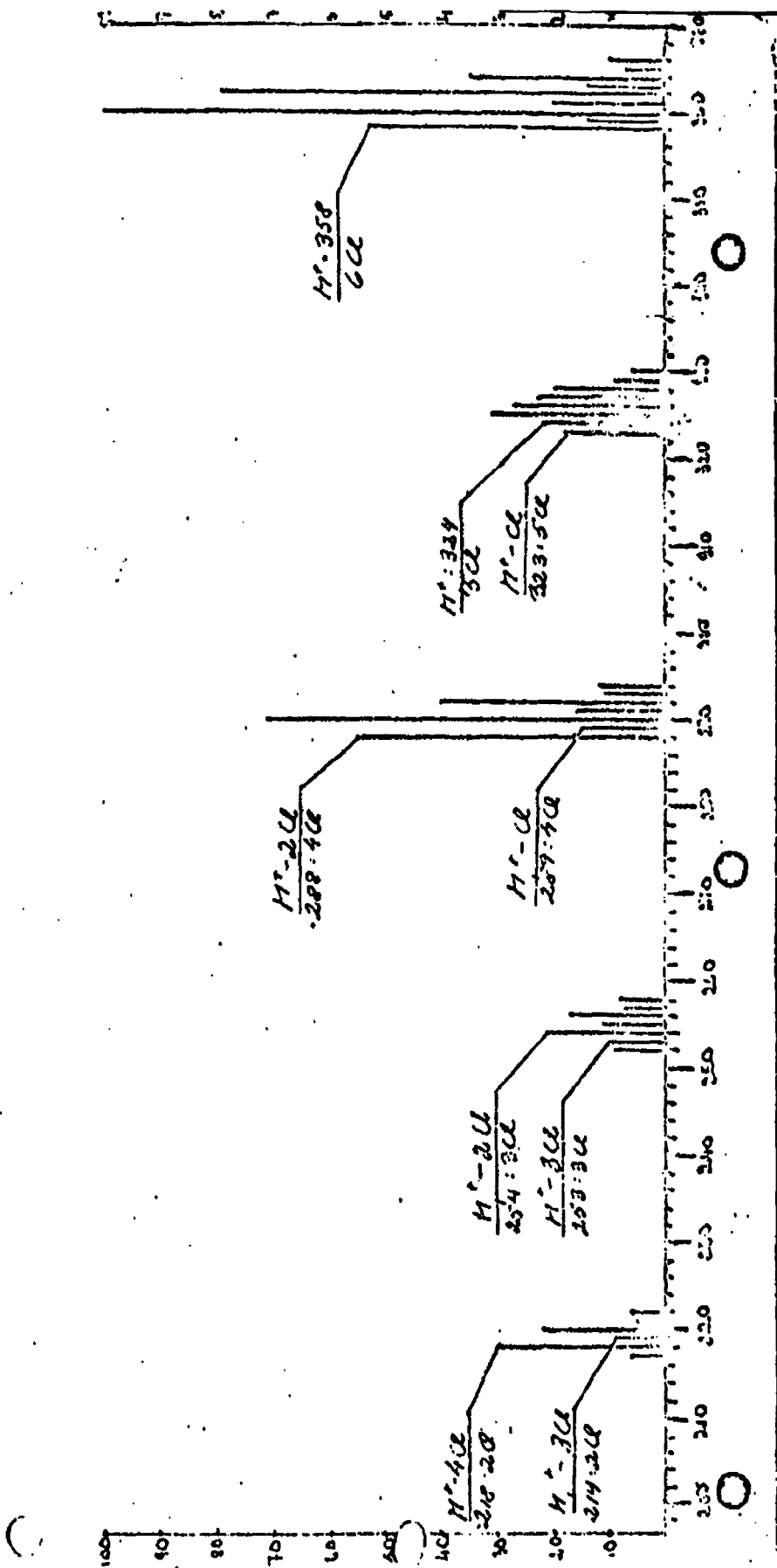


690640 SNOW



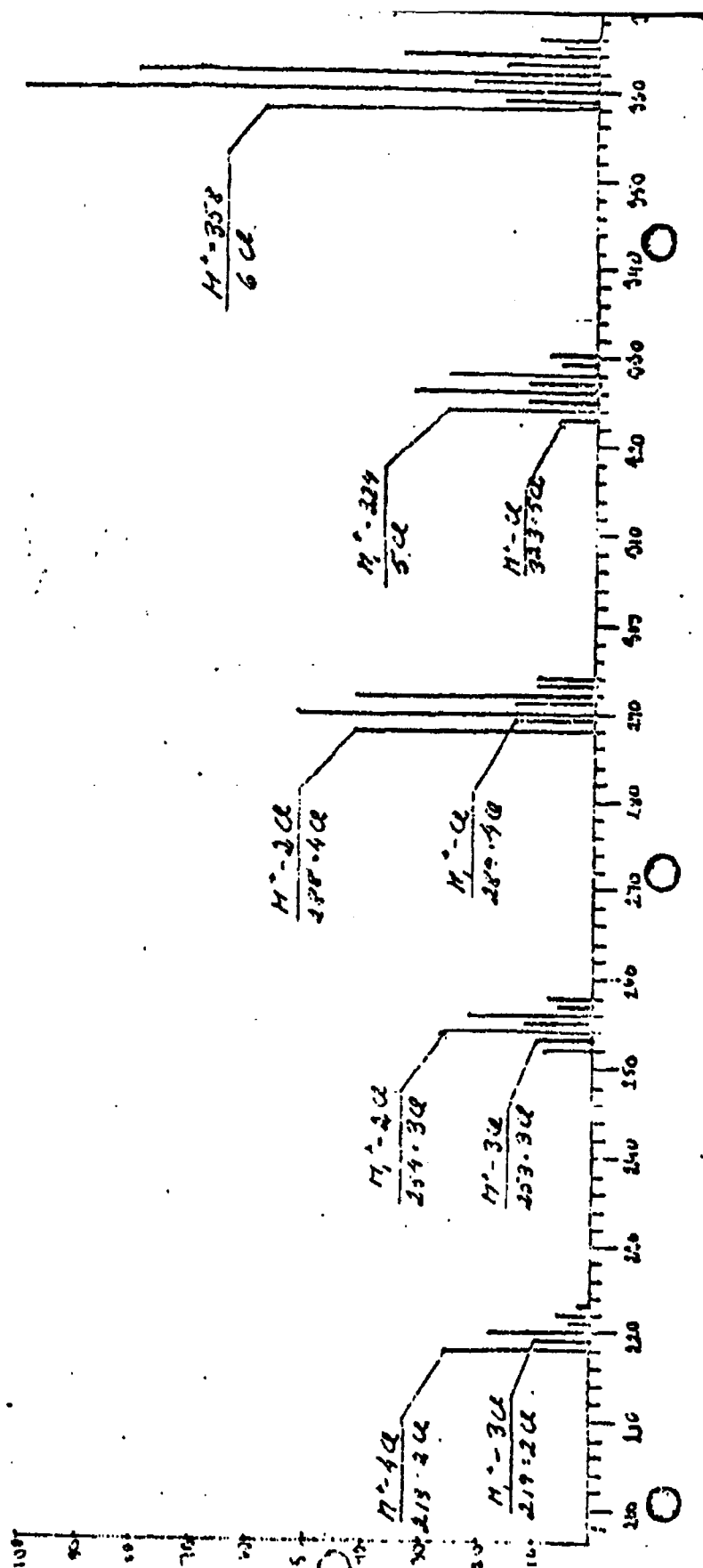
Clophen A50.
 topp nr. 11

MONS 049071



Clophen A50
topp nr 13

220640 SNOW



Clapham A50
topp nr. B

MONS 049073

