		154		
1	<1	188	35	17.90
2		222	70	31.5
3		256	105	41
4		290	140	48
5		324	175	54
6		358	210	59
7		392	245	62.5

606	AK-38	603	602
	.09		
.11	.26	.08	.07
4.43	10.18	9.24	4.94
39.35	26.70	29.1	33.75
14.22	14.63	13.95	14.36
1.27	2.19	1.87	1.69
54.8	54.00	54.2	54.8

REPORT SHEET

PPM levels of Chlordane in 1254

X (Cl #)	KI-02-602	KI-02-603
2	< 1	< 1
3	< 1	< 1
4	< 1	< 1
5	3.4	3.8
6	5.6	6.0
7	1.1	1.8
	.1	

GC/MS - 77-43

SIGNATURE R H Kelly II

DATE 3/10/77

NOTE BOOK

MONS 213532

WORK REQUEST

TO

APPLIED SCIENCES
RESEARCH CENTER LABORATORIES
MONSANTO INDUSTRIAL CHEMICALS CO.

WORK REQUESTED

DBF in PCB's

REASON/COMMENTS

SAMPLE IDENTIFICATION

2, 3

MEURE
REQUESTER

BLDG. _____ LAB./OFFICE _____

MAIL ZONE _____

PHONE NUMBER _____

PRODUCT AROCLOR

BUSINESS GROUP EP

DATE SUBMITTED 3/9

DATE NEEDED BY 3/18

JOB NUMBER 6.160

SAMPLE DISPOSAL ☒ RETURN
☐ DISCARD

4-100 REV. 7-72

(SUBMIT WHITE, YELLOW AND GREEN COPIES)

MONS 213533

WORK REQUEST

TO

APPLIED SCIENCES
RESEARCH CENTER LABORATORIES
MONSANTO INDUSTRIAL CHEMICALS CO.

WORK REQUESTED

DBF's in PCB's

REASON/COMMENTS

SAMPLE IDENTIFICATION

2, 3

MEURE
REQUESTER

BLDG. _____ LAB./OFFICE _____

MAIL ZONE _____

PHONE NUMBER _____

PRODUCT Aroclor 1254

BUSINESS GROUP FP

DATE SUBMITTED 2/9

DATE NEEDED BY 3/18

JOB NUMBER 16150

SAMPLE DISPOSAL ☒ RETURN ☐ DISCARD

A-108 REV. 7-72

(SUBMIT WHITE, YELLOW AND GREEN COPIES)

MONS 213534

REPORT SHEET

PPM levels of Cl$_x$ DBF — 12-4

x (Cl#)	KI-02-602	KI-02-603
2	< 1	< 1
3	< 1	< 1
4	< 1	< 1
5	3.4	3.8
6	5.6	6.0
7	1.1	1.8

GC/MS - 77-43

SIGNATURE R. H. Kelly, II | DATE 3/16/77 | NOTE BOOK

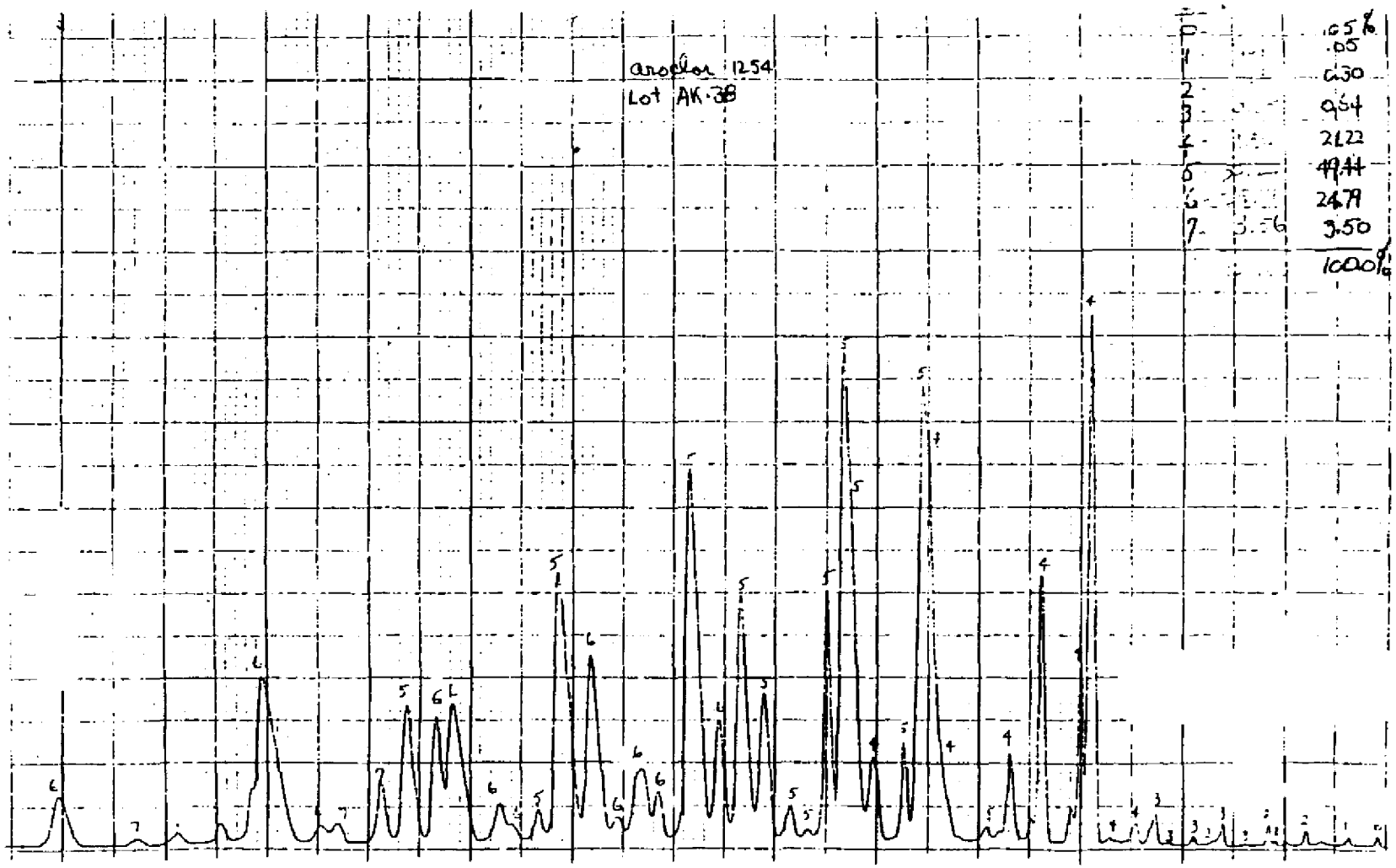
MONS 213535

Arodor 1254 corrected Results -- area correction factors

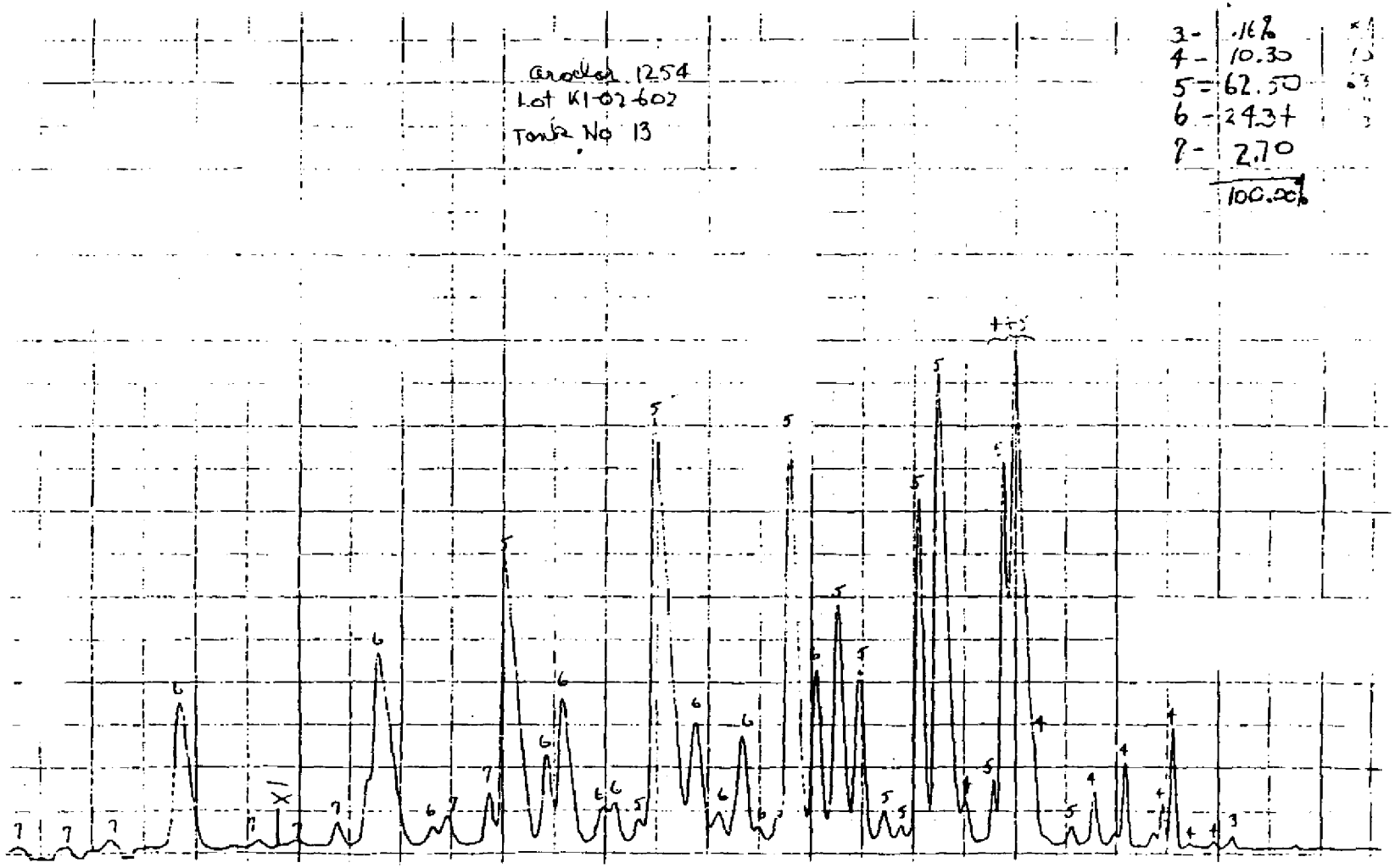
Cl	AK-38	K1-02-602	K102-603	K1-03-606
0	.03%			
1	.03			
2	.21			
3	.50	.13%	.16%	.22%
4	18.80	9.00	17.02	8.17
5	49.02	61.24	53.32	62.30
6	27.21	26.41	25.90	26.14
7	4.21	3.21	3.60	3.20

G. Gasser

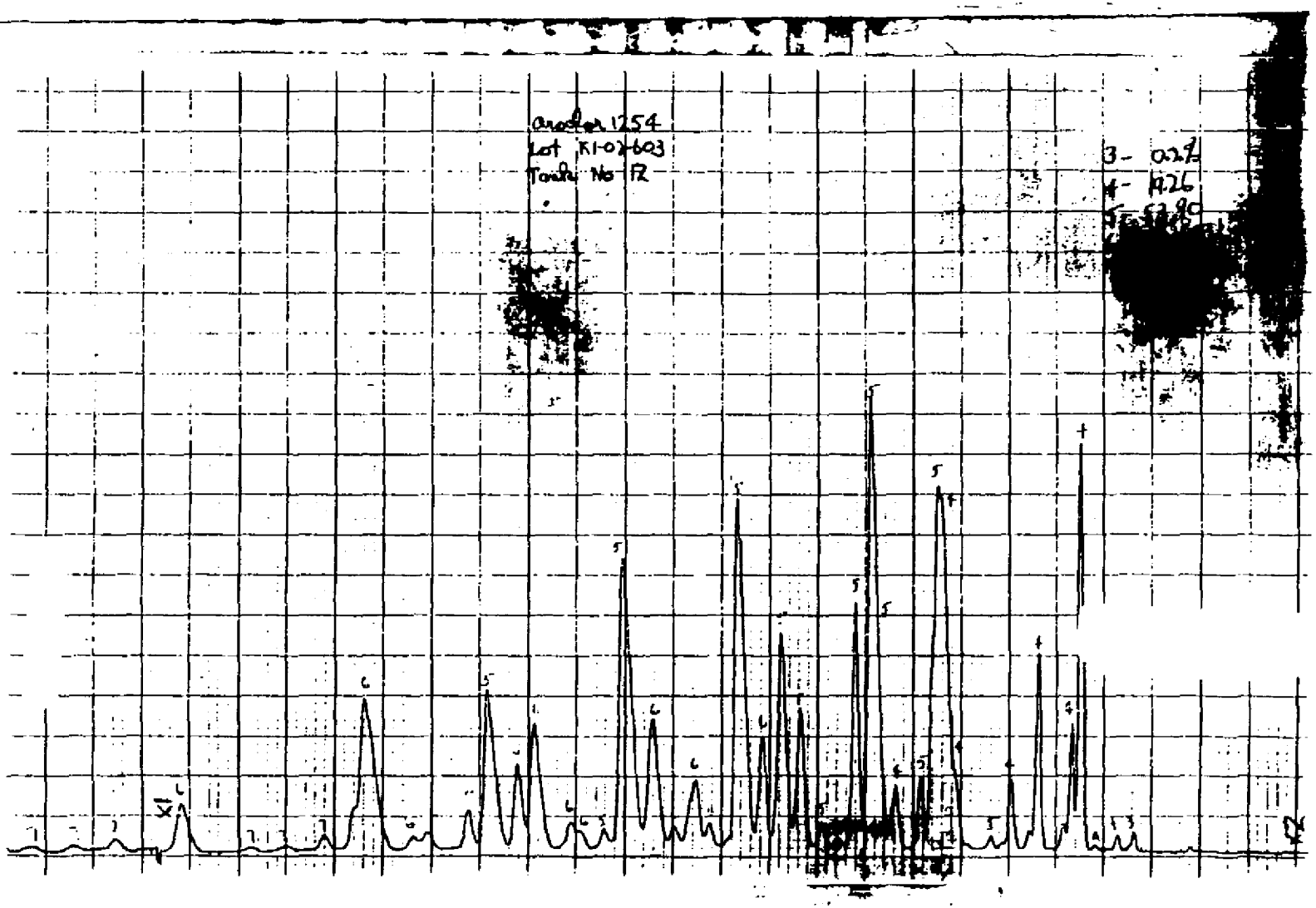
MONS 213536


aroclor 1254
Lot AK-38
0 .05%
1 .05
2 0.30
3 0.64
4 21.22
5 49.44
6 24.79
7 3.56 3.50
100.0%

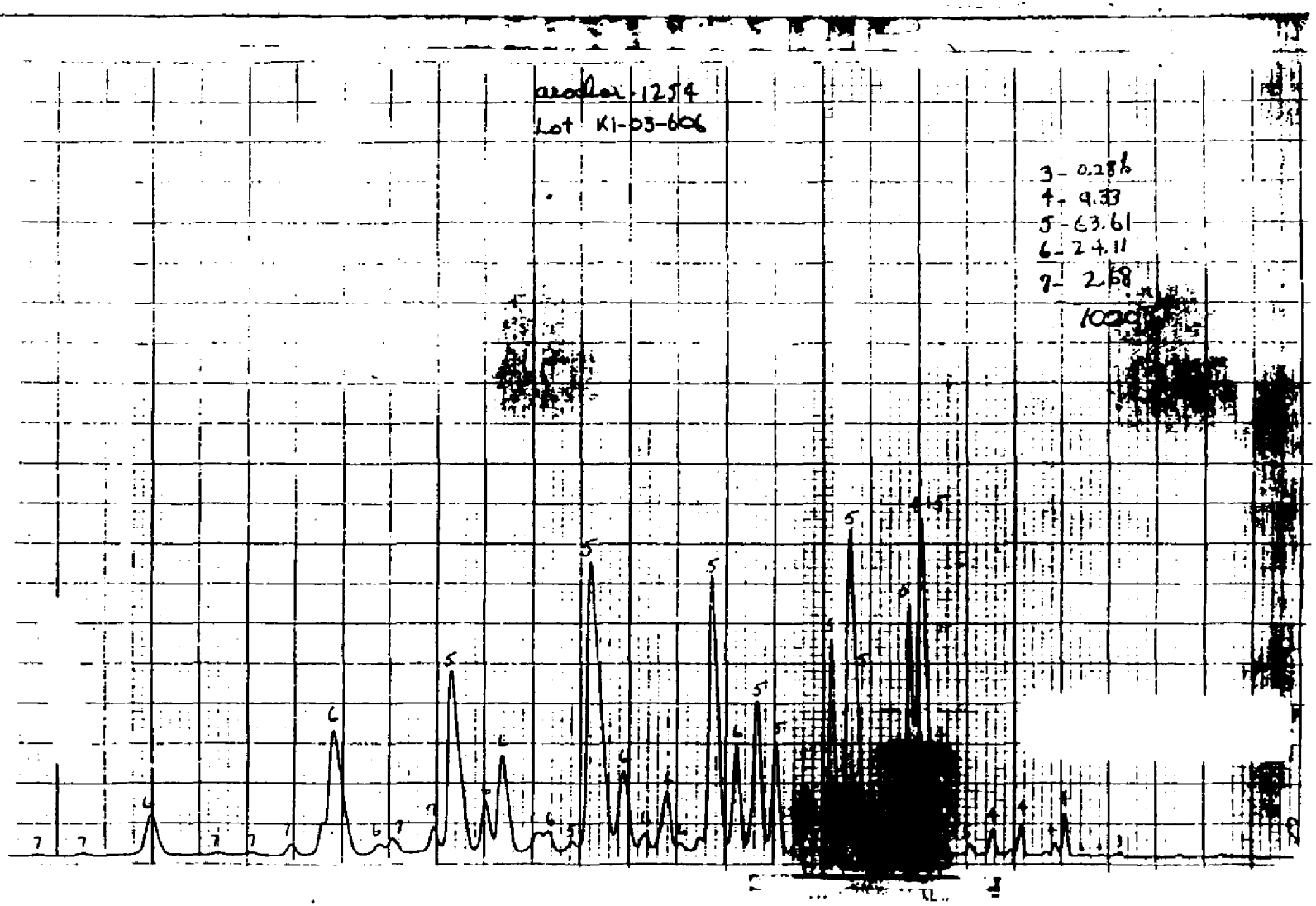
MONS 213537


Crocker 1254
Lot K1-02-602
Tank No 13
3- .16%
4- 10.30
5- 62.50
6- 24.3+
7- 2.70
100.00%

MONS 213538



MONS 213539



MONS 213540

[illegible]

[illegible]nk F. M. Greenwell and L. M. Carpenter for collecting [illegible] also thank Linda Guy for providing access to the [illegible]her house. Finally, we are grateful to L. F. Stickel [illegible] reading of the manuscript.

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[illegible] E., W. L. REICHEL, L. N. LOCKE, A. A. BELISLE, T. E. [illegible], T. G. LAMONT, B. M. MULHERN, R. M. PROUTY, and D. [illegible]NEFORD: Past. Monit. J. (in press).
[illegible] J. Mamm. 55, 1 (1974).

Determination of Chlorinated Dibenzofurans in Kanechlors and "Yusho Oil"

by JUNYA NAGAYAMA, MASANORI KURATSUNE
Department of Public Health
Faculty of Medicine
Kyushu University
Fukuoka, Japan
and
YOSHITO MASUDA
Dentistry College of Pharmaceutical Sciences
Fukuoka, Japan

Polychlorinated biphenyls (PCB) are widely distributed in the environment, especially in the tissues of fish, wild lives and man. They are also known as the causal agent of a mass food poisoning which occured among more than 1,000 persons in western Japan in 1968. The disease induced by this poisoning is called "Yusho", namely oil disease, because the patients were proved to have consumed a commercial rice oil contaminated with a large amount of Kanechlor-400 (KC-400), a brand of PCB of Japanese make (TSUKAMOTO et al., 1969). Feeding experiment using broilers demonstrated, however, that the rice oil taken by patients with Yusho were twice or more toxic than expected from the amount of KC-400 presented in the rice oil (IKEDA, 1972). This suggested that the "Yusho oil" must contain some more toxic substance or substances besides PCB. VOS et al. (1970), on the other hand, found by the chick embryo assay that toxicity of commercial PCB preparations is greatly affected by their contaminants, polychlorinated dibenzofurans (PCDF). Bauer et al. (1961) also demonstrated that structurally related polychlorinated dibenzodioxins (PCDD) were extremely toxic and acnegenic to man. Approximately 1 ppm of PCDF was estimated to contain in KC-400 (ROACH and POMERANTZ, 1974).

All these facts necessitated an analysis of Kanechlors and the rice oil taken by patients with Yusho for their possible content of PCDF and PCDD.

EXPERIMENTAL

In order to separate PCB, PCDF and PCDD from the "Yusho oil", 5 - 10 g of the oil was saponificated with ethanol containing an excess amount of sodium hydroxide for 1 hour at 80°C and the n-hexane extract of the reaction mixture was chromatographed on a column of silica gel (Wakogel S-1, activated by heating at 130°C for 3 hours) with 100 ml of n-hexane. The eluate was concentrated to a small volume.

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This concentrated eluate and samples of KC-300, 400, 500 and 600 (0.1 - 0.5 g) were chromatographed respectively, on a column of alumina (300 mesh, Wako Pure Chemical Ind. Ltd., 5 g) activated by heating at 150°C for 12 hours, using as eluents, first 20 ml of n-hexane, then 120 ml of n-hexane containing 20 % carbon tetrachloride, 10 ml of n-hexane and finally 30 ml of n-hexane containing 20 % methylene chloride. The last eluate was evaporated to dryness, dissolved in n-hexane and subjected to gas chromatography with an electron capture detector (Beckman GC 72-5) and gas chromatography/mass spectrometry (JEOL O-100) for qualitative and quantitative determination of PCDF and PCDD. When the separation of PCDF and PCDD from PCB was not adequate, a similar chromatographic fractionation of the last eluate was repeated using a smaller amount of alumina (2 g) and a smaller volume of the same solvent systems, prior to the determination of PCDF and PCDD. Amount of individual di-, tri-, tetra-, penta-, hexa- and hepta-chlorodibenzofurans was calculated from respective peak heights comparing with those of tetra-, penta- and hexachlorodibenzofurans which were synthesized by chlorination of dibenzofuran. In this determination, the individual peaks were confirmed by GC-MS and their heights were assumed to have the same sensitivity. The total amount of PCDF was also determined by the perchlorination method. The PCDF from each sample were chlorinated to octachlorodibenzofuran (OCDF) with BMC reagents (HUTZINGER et al., 1972). The amount of OCDF was determined by ECD-GC comparing with standard OCDF (Analabs, Inc.) and converted to the amount of tetra-, hexa- or hepta-chlorodibenzofuran which was the main component of PCDF in the sample.

Possible formation of PCDF from PCB during the saponification process was denied by comparing the determined amount of PCDF in KC-400 with that of PCDF in a sample of saponified vegetable oil containing KC-400. The recovery of PCDF in the whole analytical procadure was examined by analyzing 10 g of vegetable oil containing 1 µg of PCDF, finding more than 90 %.

RESULTS AND DISCUSSION

Presence of PCDF was demonstrated in all samples of Kanechlors and "Yusho oils" analyzed, as shown in Table 1 and Figure 1, but PCDD was not detected. As might be expected, Kanechlors with higher chlorine contents such as KC-500 and 600 contained more chlorinated dibenzofurans than those with lower chlorine contents. The concentration of total PCDF in KC-400

TABLE 1

Concentration of chlorinated dibenzofurans in Kanechlors and "Yusho oil".

Samples		Chlorodibenzofurans							Concentration (ppm)	
		Di-	Tri-	Tetra-	Penta-	Hexa-	Hepta-		a	b
Kanechlors	300			+	+				1	1.5
	400	+++	++	++	+++				10	17
	500				+	+++	+		4	2.5
	600			+	+	++	++		5	3
"Yusho oil"	A		+	++	+++	+			5	4.5
	B		+	++	++	+			4	5
	C		+	++	+++	+			5	5

a: Calculated from peak heights, b: Calculated by perchlorination method.

MONS 213542

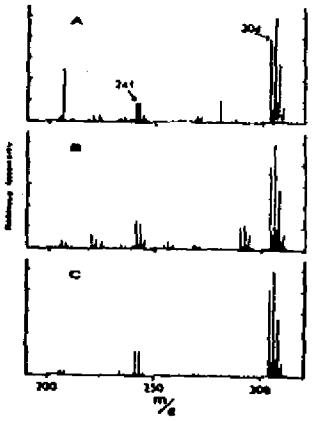

Figure 1. GC-MS of Tetrachlorodibenzofuran
A: Kanechlor-400, B: "Yusho oil",
C: Authentic specimen.

was estimated to be 18 ppm, the highest of the Kanechlors tested. GC peak patterns of PCDF present in three samples of the "Yusho oil" were very similar each other consisting of tri- to hexa-chlorodibenzofurans and their concentrations were 5 ppm. The PCB concentration in the "Yusho oil" analyzed by us was about 1,000 ppm. Therefore, PCDF concentration in the PCB in the oil was calculated to be 5,000 ppm. In other words, PCDF in the oil seemed to have been 250 times concentrated as compared with its concentration in KC-400. VOS et al. (1970) showed in their chick embryo assays that PCDF was far more toxic than PCB. According to ARAKI's (1974) animal experiment, hepatic enzyme-inducing activity of PCDF was 170 times that of KC-400. In view of all these facts, the authors consider that the toxic role of this specific type of contaminants in the "Yusho oil" should not be dismissed in the causation of Yusho, even if their quantity was small in the "Yusho oil".

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