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FINAL REPORT

DETERMINATION OF DECABROMODIPHENYL OXIDE (DBDPO) IN RAT LIVERS

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Submitted to

Tracor Jitco
Rockville, MD 20852

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HAZLETON
LABORATORIES AMERICA, INC.
9200 LEESBURG TURNPIKE, VIENNA, VIRGINIA 22180, U.S.A.

May 2, 1980

HAZLETON
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SPONSOR: Tracor Jitco

DATE: May 2, 1980

MATERIAL: Decabromodiphenyl Oxide in Rat Livers

SUBJECT: FINAL REPORT
Determination of Decabromodiphenyl Oxide (DBDPO) in Rat Livers
Project Number 976-605

OBJECTIVE

The objective of this study was to quantitate the amounts of DBDPO in the livers of male rats fed DBDPO.

EXPERIMENTAL

Sample Preparation

Male rat livers stored in 10% neutral buffered formalin were weighed and an aliquot homogenized for analysis. Each liver was homogenized in 25 to 50 ml of tetrahydrofuran (THF). The sample was filtered through Whatman #41 filter paper and diluted to a final volume of 100 ml with THF. Duplicate 5-ml aliquots of the liver extracts were concentrated under a stream of nitrogen to 1 ml and each pulled through a Waters Silica Sep-Pak®. The Sep-Pak® was washed twice with 1-ml aliquots of THF, and the eluate combined in a 15-ml test tube. The total volume was adjusted to obtain an optimal final concentration of 5-15 ng of DBDPO per microliter of eluate.

Sample Analysis

Each sample of liver extract was analyzed by high pressure liquid chromatography using the following parameters:

Instrument:	Waters HPLC, Model 440 with 254 nm UV detector
Column:	Waters μBondapak-C_{18} (3.9 mm x 30 cm)
Mobile phase:	acetonitrile/water (99:1, v/v), isocratic
Chart speed:	0.5 cm/min
Flow rate:	1.0 ml/min
Attenuation:	0.005
Injection volume:	5 μl to 20 μl
Retention time:	7.5 min

Concentration of each sample was quantitated by linear regression estimation calculated for peak height values.

RESULTS AND DISCUSSION

The values of DBDPO concentration in male rat livers are summarized in Table 1 (3 diet levels). Also listed are "spiked" recovery values which ranged from 91.5% to 97.8%. The mean interference, expressed as DBDPO equivalents, from ten control livers is 0.21 μg DBDPO/g of liver. The analytical data for the control level are summarized in Table 2.

The analytical data for the 10 mid-level (2.5%) samples are listed in Table 3. These values range from 0.66 μg to 3.29 μg/g DBDPO of liver with a mean of 2.09 μg/g. Two values are not averaged in with the others, one was spuriously high, and the other had no detectable DBDPO.

The analytical data for the high level (5.0%) samples are listed in Table 4. All samples were processed and analyzed in duplicate. The values range from 3.95 µg to 13.07 µg/g DBDPO of liver with a mean of 8.6 µg/g.

Figure I is a representative standard curve of decabromodiphenyl oxide utilizing high pressure liquid chromatography. Figure II is a typical chromatogram of the standard. Figure III is a control liver sample which shows no detectable DBDPO. A chromatogram of a high level liver sample is shown in Figure IV.

It is concluded from the above data that DBDPO is found in the liver of rats in concentrations proportional to the amounts added to diet feed.

Submitted by: Joyce Rogers
JOYCE ROGERS
Chemist

Approved by: J. T. Hill
JIM T. HILL, Ph.D.
Director of Chemistry

HLA Notebook No. 217, pp.4-10

Summary Table 1

Determination of Decabromodiphenyl Oxide (DBDPO) in Male Rat Livers*

Level of Concentration	Number of Values Averaged[a]	Mean Values ±S.D. µg/g	Percent Recovery	Average %
Control (0%)	9	0.21 ± 0.43		
Mid-Level (2.5%)	8	2.09 ± 0.89		
High-Level (5.0%)	20	8.6 ± 2.83		
Spike Control I (8.19 mg DBDPO)	1	-	91.5	
Spike Control II (8.81 mg DBDPO)	1	-	97.8	94.65

[a] At each level, ten liver tissues were analyzed, but not all the values were averaged (see Tables 2-4).

* All liver tissue was stored in 10% neutral buffered formalin.

Table 2

Analytical Data, Control Level
Project Number 976-605

Sample ID	Volume Injected µl	DBDPO Calc. ng	Amount of Liver Extract Injected mg	DBDPO ppm	Final Volume ml	Dilution Factor	Total DBDPO in Sample µg	Weight of Liver Sample g	DBDPO per g of Liver µg/g
1616	5.0	ND	1.25	ND	2.0	4.0	-	5.0	-
1617	5.0	1.40	1.25	1.12	2.0	4.0	5.6	5.1	1.10
1618	5.0	ND	1.25	ND	2.0	4.0	-	5.3	-
1619	5.0	ND	1.25	ND	2.0	4.0	-	5.0	-
1620	5.0	1.07	1.25	0.86	2.0	4.0	4.28	5.3	0.81
1621	5.0	ND	1.25	ND	2.0	4.0	-	4.9	-
1622	5.0	6.32	1.25	5.06	2.0	4.0	25.28	5.0	5.06
1623	5.0	ND	1.25	ND	2.0	4.0	-	4.9	-
1624	5.0	ND	1.25	ND	2.0	4.0	-	5.0	-
1625	5.0	ND	1.25	ND	2.0	4.0	-	5.0	-
									Average 0.21 ± 0.
Spike 1620 8.81 mg	5.0	8.62	0.00025	-	10.0	1×10^6	8.62 (97.8% recovery)		

* Sample value 1622 was not averaged in with the other values.

ND - Not detected

Table 4

Analytical Data, 5.0% Level
Project Number 976-605

Sample ID	Volume Injected µl	DBDPO Calc. ng	Amount of Liver Extract Injected mg	DBDPO ppm	Final Volume ml	Dilution Factor	Total DBDP in Sample µg	Weight of Liver Sample g	DBDPO µg of L µg/g
1716A	20.0	10.56	0.80	13.20	5.0	12.5	132.00	10.1	13.0
1716B	20.0	10.15	0.80	12.69	5.0	12.5	126.86	10.1	12.5
1717A	20.0	3.16	0.80	3.95	5.0	12.5	39.50	10.0	3.9
1717B	20.0	3.16	0.80	3.95	5.0	12.5	39.50	10.0	3.9
1718A	20.0	5.22	0.80	6.53	5.0	12.5	65.25	10.0	6.5
1718B	20.0	4.81	0.80	6.01	5.0	12.5	60.13	10.0	6.0
1719A	20.0	5.63	0.80	7.04	5.0	12.5	70.38	10.2	6.90
1719B	20.0	5.63	0.80	7.04	5.0	12.5	70.38	10.2	6.90
1720A	20.0	7.68	0.80	9.60	5.0	12.5	96.00	10.1	9.51
1720B	20.0	6.86	0.80	8.58	5.0	12.5	85.75	10.1	8.49
1721A	20.0	10.15	0.80	12.69	5.0	12.5	126.88	9.8	12.95
1721B	20.0	9.74	0.80	12.18	5.0	12.5	121.75	9.8	12.42
1722A	20.0	5.22	0.80	6.53	5.0	12.5	65.25	9.8	6.66
1722B	20.0	8.09	0.80	10.11	5.0	12.5	101.13	9.8	10.32
1723A	20.0	5.22	0.80	6.53	5.0	12.5	65.25	10.0	6.53
1723B	20.0	5.22	0.80	6.53	5.0	12.5	65.25	10.0	6.53
1724A	20.0	6.86	0.80	8.58	5.0	12.5	85.75	9.8	8.75
1724B	20.0	8.50	0.80	10.63	5.0	12.5	106.25	9.8	10.84
1725A	20.0	6.86	0.80	8.58	5.0	12.5	85.75	9.2	9.32
1725B	20.0	7.27	0.80	9.09	5.0	12.5	90.88	9.2	9.88

Average 8.60 ± 2.

DIETZGEN CORPORATION

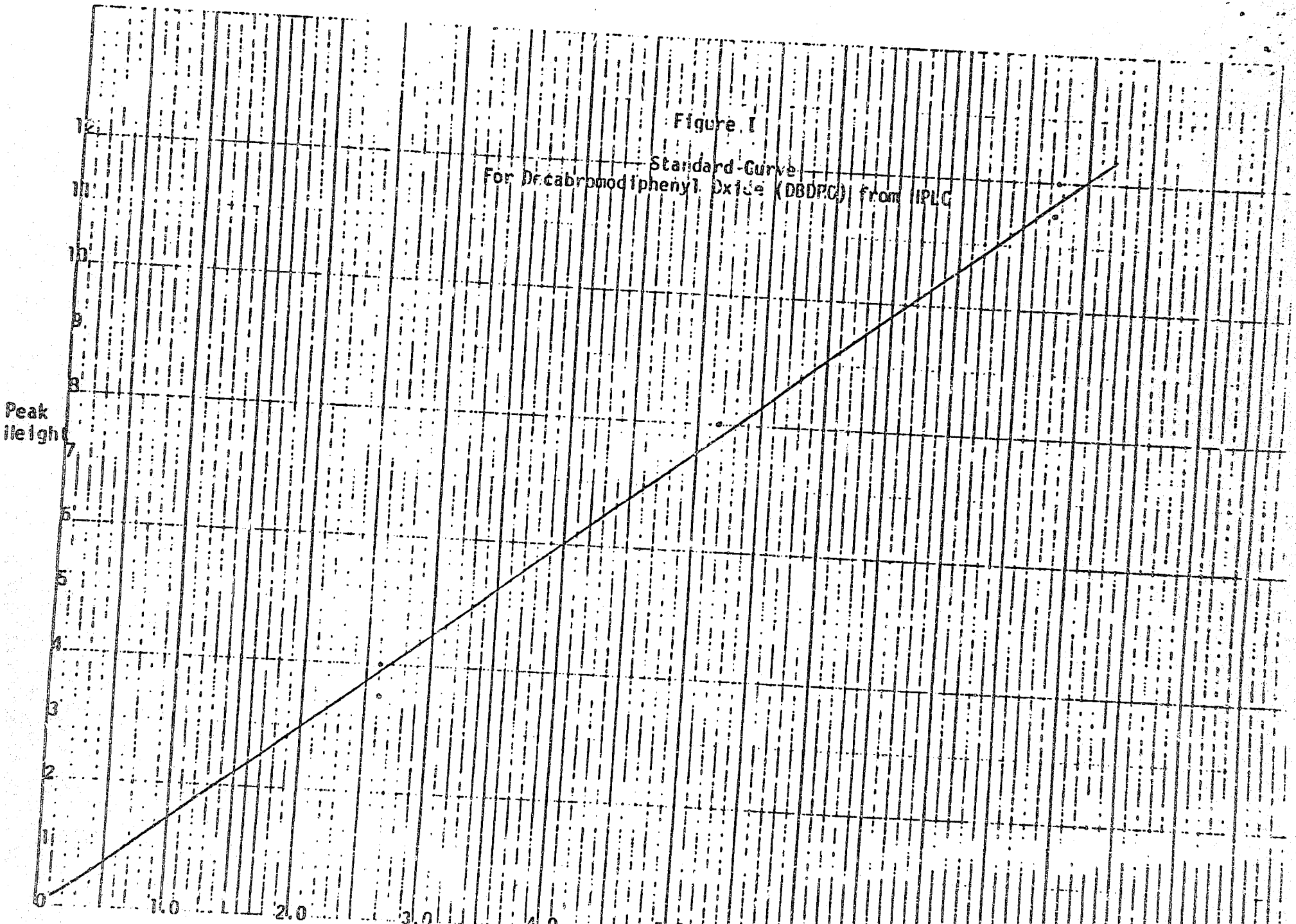

Figure I

Standard Curve
For Decabromodiphenyl Oxide (DBDPO) from HPLC

Figure II

HPLC Chromatogram of DBDPO Standard

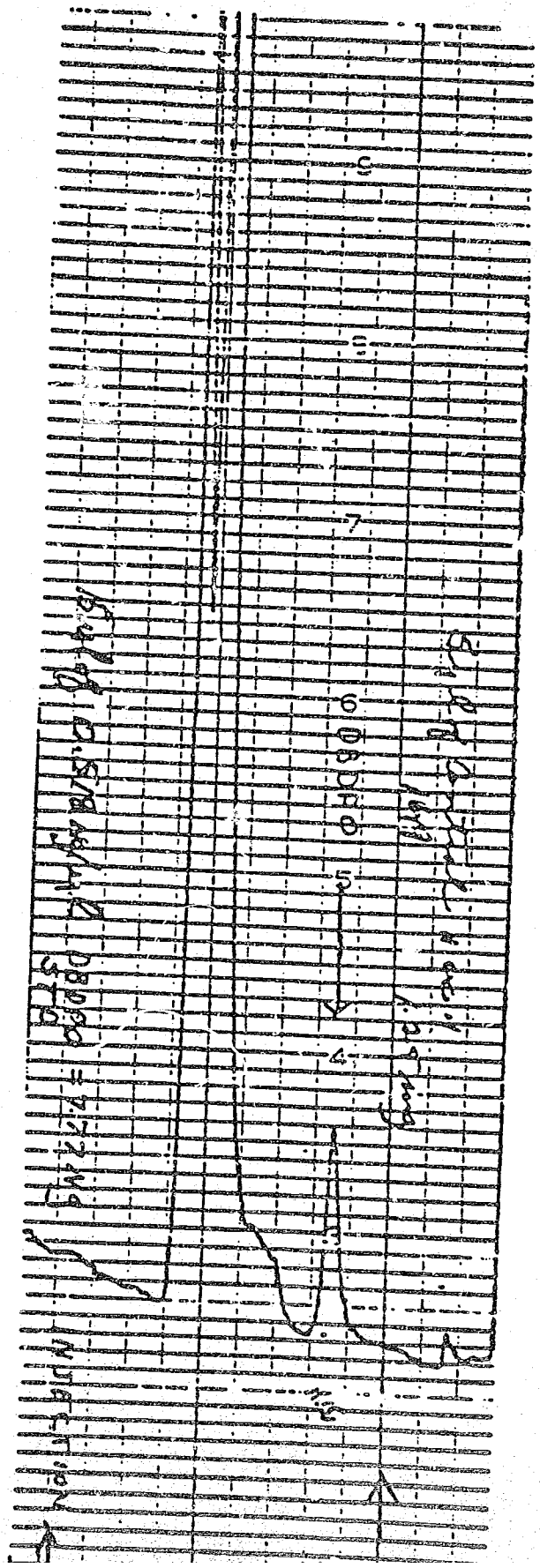

Figure III

HPLC Chromatogram of Control Liver Sample

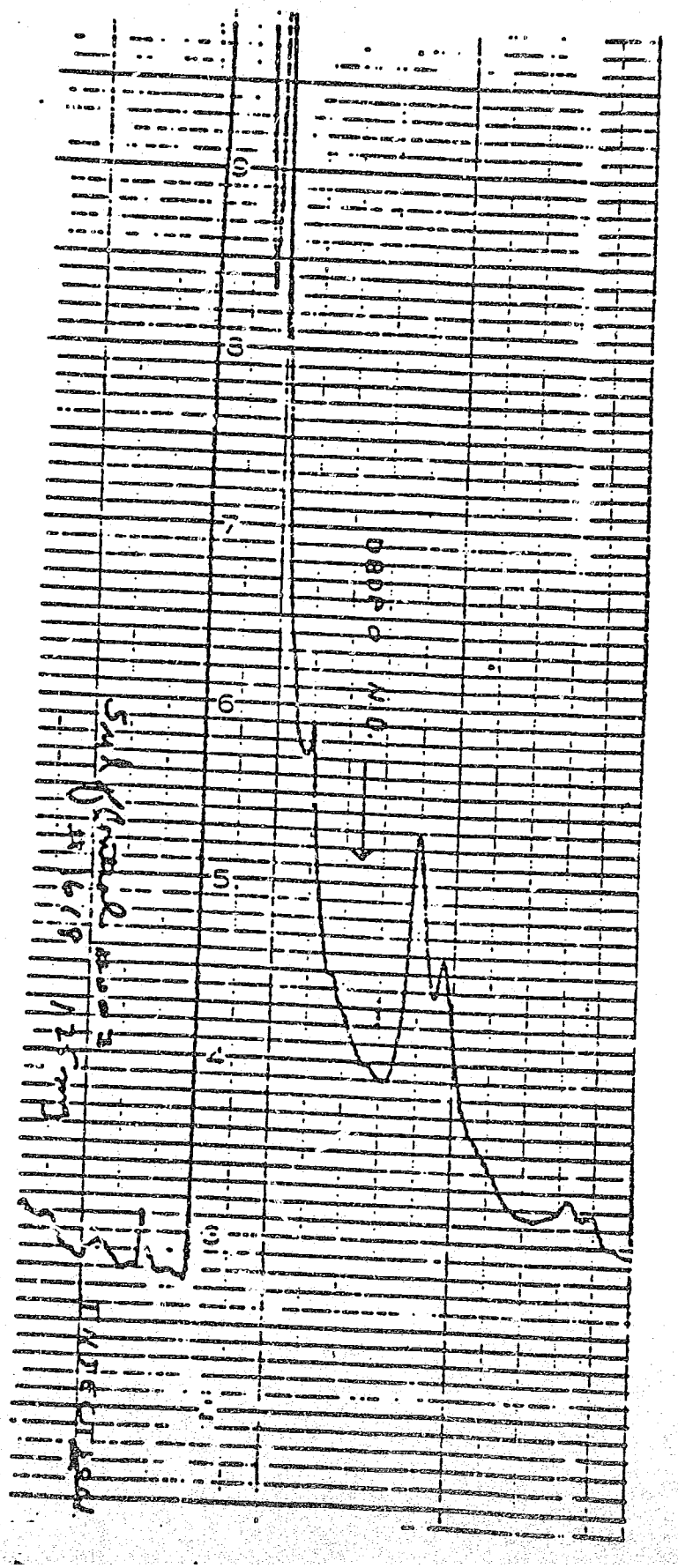
0
3
7
6
5
4
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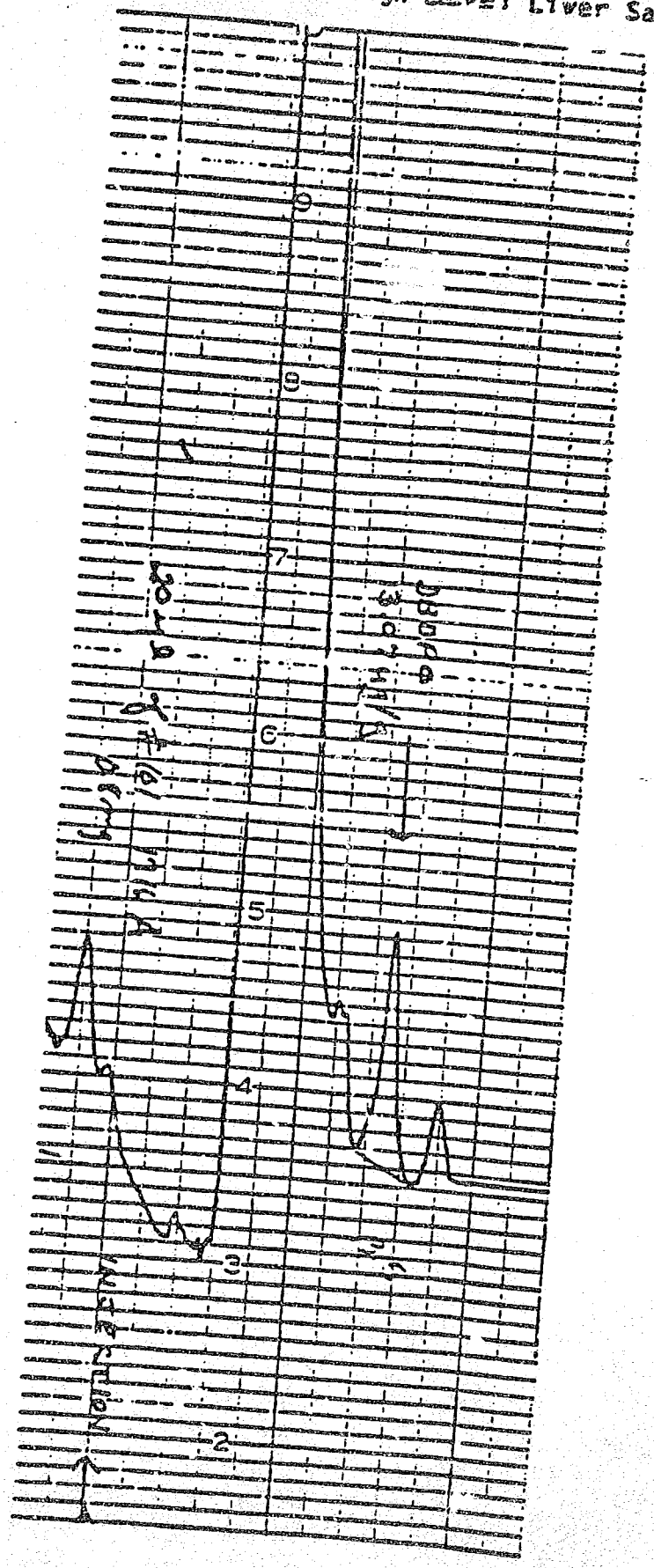
Figure 14

HPLC Chromatogram of High Level Liver Sample