United States
Environmental Protection
Agency

EPA-600/7-84-095b

September 1984

EPA

Research and Development

ENVIRONMENTAL ASSESSMENT
OF A FIRETUBE BOILER FIRING
COAL/OIL/WATER MIXTURES
Volume II. Data Supplement

Prepared for

Office of Air Quality Planning and Standards

Prepared by

Industrial Environmental Research
Laboratory
Research Triangle Park NC 27711

RESEARCH REPORTING SERIES

Research reports of the Office of Research and Development, U.S. Environmental Protection Agency, have been grouped into nine series. These nine broad categories were established to facilitate further development and application of environmental technology. Elimination of traditional grouping was consciously planned to foster technology transfer and a maximum interface in related fields. The nine series are:

1. Environmental Health Effects Research
2. Environmental Protection Technology
3. Ecological Research
4. Environmental Monitoring
5. Socioeconomic Environmental Studies
6. Scientific and Technical Assessment Reports (STAR)
7. Interagency Energy-Environment Research and Development
8. "Special" Reports
9. Miscellaneous Reports

This report has been assigned to the INTERAGENCY ENERGY-ENVIRONMENT RESEARCH AND DEVELOPMENT series. Reports in this series result from the effort funded under the 17-agency Federal Energy/Environment Research and Development Program. These studies relate to EPA's mission to protect the public health and welfare from adverse effects of pollutants associated with energy systems. The goal of the Program is to assure the rapid development of domestic energy supplies in an environmentally-compatible manner by providing the necessary environmental data and control technology. Investigations include analyses of the transport of energy-related pollutants and their health and ecological effects; assessments of, and development of, control technologies for energy systems; and integrated assessments of a wide range of energy-related environmental issues.

EPA REVIEW NOTICE

This report has been reviewed by the participating Federal Agencies, and approved for publication. Approval does not signify that the contents necessarily reflect the views and policies of the Government, nor does mention of trade names or commercial products constitute endorsement or recommendation for use.

This document is available to the public through the National Technical Information Service, Springfield, Virginia 22161.

EPA-600/7-84-095a

September 1984

ENVIRONMENTAL ASSESSMENT OF A FIRETUBE BOILER FIRING COAL/OIL/WATER MIXTURES

Volume II
Data Supplement

by

R. DeRosier
Acurex Corporation
Energy & Environmental Division
555 Clyde Avenue
P.O. Box 7555
Mountain View, California 94039

Contract No. 68-02-3188

Project Officer

R.E. Hall
Combustion Research Branch
Energy Assessment and Control Division
Industrial Environmental Research Laboratory
Research Triangle Park, North Carolina 27711

OFFICE OF RESEARCH AND DEVELOPMENT
U.S. ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, DC 20460

ABSTRACT

This report is a compendium of detailed emission and test data obtained from field tests of a firetube industrial boiler burning a coal/oil/water (COW) mixture. The boiler was tested while burning COW fuel, and the COW with soda ash (sodium carbonate) added to serve as an SO_2 sorbent. The test data includes preliminary equipment calibration data, boiler operating data for both tests, fuel analysis results, and complete flue gas emission measurement and laboratory analysis results. Flue gas emission measurements included continuous monitoring for criteria gas pollutants; gas chromatography (GC) of gas grab samples for volatile organics (C_1-C_6); EPA Method 5 for particulate; controlled condensation system for sulfur oxide emissions; and source assessment sampling system (SASS) for total organics in two boiling point ranges (100 to 300°C and >300°C), organic compound category information using infrared spectrometry (IR) and low resolution mass spectrometry (LRMS), specific quantitation of the semivolatile organic priority pollutants using gas chromatography/mass spectrometry (GC/MS), liquid chromatography (LC) separation of organic extracts into seven polarity fraction with total organic and IR analyses of eluted fractions, flue gas concentrations of trace elements by spark source mass spectrometry (SSMS) and atomic absorption spectroscopy (AAS), and biological assays of organic sample extracts. All field and laboratory data for these measurements are included in the volume.

CONTENTS

1. INTRODUCTION 1-1
2. PRELIMINARY EQUIPMENT CALIBRATION DATA 2-1
3. BOILER OPERATING DATA 3-1
4. SAMPLING DATA SHEETS 4-1

4.1 CONTINUOUS EMISSION MONITORING DATA (ACUREX) 4-2
4.2 CONTINUOUS EMISSION MONITORING DATA (ADELPHI) 4-8
4.3 FIELD DATA SHEETS FOR SASS AND CONTROLLED CONDENSATION (ACUREX) 4-9
4.4 SUMMARY OF FIELD DATA FOR METHOD 5 (ADELPHI) 4-28

5. ANALYTICAL LABORATORY RESULTS 5-1

5.1 ULTIMATE FUEL ANALYSES 5-2
5.2 PARTICULATE EMISSIONS FROM SASS SAMPLES 5-3
5.3 SULFUR OXIDE EMISSIONS FROM CONTROLLED CONDENSATION SAMPLES 5-6
5.4 TRACE ELEMENT ANALYSES 5-8
5.5 TOTAL CHROMATOGRAPHABLE ORGANICS (TCO), GRAVIMETRIC ORGANICS (GRAV), INFRARED (IR) SPECTRA, AND GAS CHROMATOGRAPHY/MASS SPECTROMETRY (GC/MS) OF TOTAL SAMPLE EXTRACTS 5-24
5.6 LIQUID CHROMATOGRAPHY (LC) SEPARATION, INFRARED (IR) SPECTRA OF LC FRACTIONS, AND LOW RESOLUTION MASS SPECTROMETRY (LRMS) OF SELECTED LC FRACTIONS AND TOTAL SAMPLE EXTRACTS 5-35
5.7 C_1 - C_6 CHROMATOGRAPHY 5-60
5.8 RADIOMETRIC ANALYSES 5-72
5.9 BIOLOGICAL ASSAY REPORT 5-74

SECTION 1

INTRODUCTION

The purpose of this data supplement is to document data in greater detail than was practical in Volume I (Technical Results) of this report. It is intended to provide sufficient detail for researchers to perform their own analysis of the data obtained. Readers are referred to the technical volume for objectives, description of the source emission results, interpretation, and conclusions.

The remaining sections of this data supplement contain the following information:

2 -- Preliminary Equipment Calibration Data

3 -- Boiler Operating Data. Water, stack, air, and fuel temperatures, and water and fuel flowrates.

4 -- Sampling Data Sheets. Continuous flue gas monitor data; operating data for EPA Method 5 (for particulate mass emissions), controlled condensation (for SO_2 and SO_3 sampling), and SASS (for particulate, trace element and organic sampling).

5 -- Analytical Laboratory Results. Ultimate analysis of the coal-oil-water mixtures used in the test program; SASS particulate emissions, sulfur oxides emissions by turbidometric analysis; trace element emissions by Spark Source Mass Spectrometry (SSMS) analysis and

Atomic Absorption Spectroscopy (AAS) analysis; Total Chromatographable Organic (TCO) and gravimetry results; infrared (IR) spectra of total sample extracts; determination of organic compounds by Gas Chromatography/Mass Spectrometry (GC/MS); liquid chromatography (LC) separation with IR spectra of LC fractions; low resolution mass spectrometry (LRMS) of selected fractions and total sample extracts; radiometric analysis results; and biological assay reports on the SASS train sample extracts.

SECTION 2

PRELIMINARY EQUIPMENT CALIBRATION DATA

Date 11-24-80
Time 1000
Barometric Pressure
Ambient Temperature 68°

Orifice Meter . 357
Orifice Magnehelic 90214WW67
Primary Calibration Meter 433583
Control Module C67

Operators ARMAN
Wet Bulb Temperature
Leak check ok

METER CALIBRATION DATA

Orifice Manometer ΔH_{ref} (in wg)	Orifice Magnehelic ΔH_o (in wg)	Primary Meter ΔH_p (in wg.)	Dry Test Meter P_{dg} (in. wg.)	Gas Volume Primary Meter V_p (ft.³)	Gas Volume Dry Test Meter V_d (ft.³)	Temperature: Primary Meter Inlet, T_{pi} (°F)	Primary Meter Outlet, T_{po} (°F)	Primary Meter Avg., T_{pa} (°F)	Dry Test Meter Inlet, T_{di} (°F)	Dry Test Meter Outlet, T_{do} (°F)	Dry Test Meter Avg., T_{da} (°F)	Time t (min)	α	E_c
.2	0.2			296.239	223.759	72	66		64	64		180		
				271.228	248.258	79	69		65	64				
	0.4													
.6	0.6			271.228	248.258	81	67		65	64		11.0		
				296.629	273.172	91	71		66	65				
	0.8													
	1.0			296.629	273.172	96	71		66	65		13.0		
				334.931	310.675	97	75		67	66				
	1.2													
	1.4			334.931	310.675	94	75		68	66		7.2		
				360.119	335.343	92	77		68	66				
	1.6													
	1.6			360.119	335.343	92	77		69	66		6.0		
				385.963	360.762	93	78		70	67				
	2.0													
	2.2			385.963	360.762	93	79		71	67		5.8		
				410.961	385.477	94	80		71	67				
	2.4													
	2.6													
	2.8													
	3.0													
	3.2													
	3.4													
	3.6													
	3.8													
	4.0													
	4.2													
	4.4													
	4.6													
	4.8													
	5.0													
												Average		

A-11-81

Date 7-13-81, 7-14-81
Time 2:20, 8:30
Barometric Pressure 29.79, 29.76
Ambient Temperature 78°F

Orifice Meter
Orifice Manometer 40430 AM 43
Primary Calibration Meter 433583
Control Module 5015
Orifice Plate # 0049

Operators Seegmiller
Wet Bulb Temperature

METER CALIBRATION DATA

	Orifice Manometer ΔH_{ref} (in. wg.)	Orifice Manometer ΔH_D (in wg.)	Primary Meter ΔH_p (in. wg.)	Dry Test Meter P_{eg} (in. wg.)	Gas Volume Primary Meter V_p (ft.³)	Gas Volume Dry Test Meter V_d (ft.³)	Temperature: Primary Meter Inlet. T_{p1} (°F)	Primary Meter Outlet. T_{p2} (°F)	Primary Meter Avg. T_{pa} (°F)	Dry Test Meter Inlet. T_{d1} (°F)	Dry Test Meter Outlet. T_{d2} (°F)	Dry Test Meter Avg. T_{da} (°F)	Time t (min.)	α	K_D	
Large	.60	.6			043.568 023.254	300.857 280.798	112 113	85 84	98.5	83 83	83 82	82.75	8.8	1.018	3.757	α a 1.05
					20.314	20.059										
358	1.59	1.6			063.931 043.568	320.936 300.857	121 113	85 85	101	84 83	83 83	83.25	6.4	1.049	3.102	
					19.863	20.079										Ko av
	2.60	2.6			083.072 063.931	341.097 320.936	126 119	86 86	104.25	85 84	84 83	84	4.5	1.077	3.409	
					19.641	20.161										3.3
	3.60	3.6			102.189 083.072	361.077 341.097	130 125	87 86	107	85 85	84 84	84.5	3.9	1.098	3.252	
					19.117	19.980										
Med.	.60	.6			122.754 102.189	381.100 361.077	104 115	88 87	98.5	85 85	85 84	84.75	17.8	1.000	1.877	α av
					20.565	20.023										
.281	1.64	1.6			143.078 122.754	401.104 381.100	111 105	88 88	98	85 85	85 85	85	11.0	1.012	1.815	1.015
					20.324	20.004										
	2.60	2.6			164.690 143.078	422.500 401.104	103 111	89 88	97.75	85 85	86 85	85.25	9.5	1.019	1.775	Ko av
					21.612	21.396										
	3.60	3.6			184.847 164.690	442.537 422.500	104 114	89 88	98.75	85 85	86 86	85.5	7.7	1.027	1.735	1.80
					20.157	20.037										
Small	.62	.6			276.990 256.762	534.347 514.343	86 93	83 80	85.5	83 80	82 79	81	45.4	1.000	.713	
					20.208	20.004										α avg
.187	1.67	1.6			297.132 276.990	554.353 534.347	91 89	85 84	86.75	82 83	82 82	82.25	26.0	1.005	.687	1.00
					20.162	20.006										
Calib	2.62	2.6			317.311 297.132	574.365 554.353	95 91	86 85	89.25	83 83	83 83	83	22.7	1.010	.692	Ko av
7-14-81					20.179	20.012										
	3.64	3.6			337.505 317.311	594.375 574.365	94 96	86 86	90.5	84 83	84 83	83.5	19.4	1.013	.687	
Pbar					20.194	20.010										.712
29.76														Average		

SECTION 3

BOILER OPERATING DATA

- Temperatures
  - -- Water return
  - -- Water supply
  - -- Stack
  - -- Air
  - -- Fuel
- Flowrates
  - -- Water
  - -- Fuel

BOILER OPERATING CONDITIONS: TEST 1 (COW)[a]

	Temperatures (°F)						Flows	
Minutes into test	Water return	Water supply	Stack	Air	Fuel	Coal	H_2O flow (gal/min)	Fuel flow (kg/hr)[b]
40	166.6	244.9	276	77	77	40	285.2	420.5
60	167.7	244.0	279	77	77	51	290.3	431.8
80	170.1	248.8	286	77	78	57	288.8	438.4
100	172.2	249.2	287	74	79	56	296.3	433.1
120	175.2	249.7	288	73	78	56	297.0	434.1
140	177.7	252.6	291	74	78	54	297.5	431.2
160	179.1	253.3	292	74	78	59	296.9	431
180	180.0	255.8	295	73	78	54	297.1	431.3
200	180.0	255.0	295	73	78	41	298.0	430.9
220	181.0	256.6	297	73	76	42	294.9	432.4
240	182.0	257.7	299	73	76	48	296.5	433.1
260	183.9	260.2	302	74	76	43	290.3	435.3
280	185.8	262.8	306	73	76	38	285.2	433.3
300	--[c]	--	--	--	--	--	--	440
320	--	--	--	--	--	--	--	438.6
340	195.1	275.2	319	72	74	40	283.3	436.8
360	194.4	270.4	315	72	75	40	298.2	435.6

[a]Summary of computer printout -- averaged every 20 minutes.
[b]Averaged using offscale readings = 441 kg/hr.
[c]Unable to average due to poor quality copy.

BOILER OPERATING CONDITIONS: TEST 2 (COW+SA)[a]

	Temperatures (°F)						Flows	
Minutes into test	Water return	Water supply	Stack	Air	Fuel	Coal	H_2O flow (gal/min)	Fuel flow (kg/hr)[b]
20	173	248	325	76	88	45	277.2	430.0
40	173	249	339	75	89	48	289.8	420.1
60	172	246	343	75	91	48	293.8	435.5
80	173	245	344	74	91	49	294.9	438.8
100	173	244	348	74	90	50	293.9	433.0
120	176	245	356	75	92	51	303.1	435.7
140	176	245	365	75	90	52	306.6	440.0
160	178	245	373	75	91	47	305.8	435.3
180	181	246	384	75	91	49	305.8	434.4
200	184	249	399	76	89	52	308.8	389.9

[a]Summary of computer printout -- averaged every 20 minutes.
[b]Averaged using offscale = 441 kg/hr.

SECTION 4

SAMPLING DATA SHEETS

		Page
4.1	CONTINUOUS EMISSION MONITORING DATA (ACUREX)	4-2
	O_2, CO_2, CO, NO_x	
4.2	CONTINUOUS EMISSION MONITORING DATA (ADELPHI)	4-8
	N_2, O_2, Excess Air, CO_2, H_2O, SO_2	
4.3	FIELD DATA SHEETS FOR SASS AND CONTROLLED CONDENSATION (ACUREX)	4-9
4.4	SUMMARY OF FIELD DATA FOR METHOD 5 (ADELPHI)	4-28

4.1 CONTINUOUS EMISSION MONITORING DATA (ACUREX)

FIELD DATA SHEET

JREX Airotherm

Plant: Adelphi university

Location: Boiler COM

Operator: M. Chips

Date: 2-12-81

Load Range: 100%

Ambient Temperature:

Barometric Pressure:

Static Pressure Duct:

Fuel: coal oil water Mix

Time	Sample Point	Load MW	Steam Flow lb/hr x1000	DRY UNCORRECTED O_2 %	CO_2 %	CO ppm	SO_2 ppm	NO ppm	NO_x ppm	NO_2 ppm	CORRECTED TO % O_2 DRY NO ppm	NO_x ppm	NO_2 ppm	HYDROCARBONS (HC) Total HC	Methane	Non Methane HC	H_2O %	Stack Gas Temp °F	COMMENTS
0800				0.5	0.3	0		0											Zero N_2 initial cal
0810				7.73	17.4	740		867											7.73 % O_2 cal, 867 ppm
																			NO cal
1000				3.4	13.6	15		455											Sample C.C.W.
1005				3.6	13.5	12		460											CC spikes to 100 ppm
1010				3.5	13.6	17		462											[illegible] 3.5 [illegible]
1015				3.4	13.6	18		470											(soot Blowing)
1020				3.6	13.6	18		470											
1025				3.5	13.6	20		477											
1030				21%	0.2	<0		60											ambient air [illegible] (Pow
1035				3.6	13.5	20		480											removed from stack)
1040				3.4	13.6	20		475											
1045				3.4	13.6	18		470											
1047				0.4	0.2	0		7											Zero
1050				7.4	17.4	750		855										Span	cal 7.4 [illegible]
1052				7.73	17.9	740		867									Span adj		17.9% [illegible]
1100				3.6	13.6	30		470											
1105				3.7	13.7	30		470											
1110				3.5	13.6	30		465											
1115				3.2	13.5	30		465											
																			AVERAGE

Form EED 061 12 78

FIELD DATA SHEET

JREX Aerotherm

Plant: Adelphi University

Location: Foley

Operator: M. Chips

Date: 2-12-81

Load Range: 100%

Ambient Temperature:

Barometric Pressure:

Static Pressure Duct:

Fuel: coal oil water mix

Time	Sample Point	Load MW	Steam Flow lb/hr x 1000	Dry Uncorrected O_2 %	CO_2 %	CO ppm	SO_2 ppm	NO ppm	NO_x ppm	NO_2 ppmn	Corrected to 3% O_2 Dry NO ppm	NO_x ppm	NO_2 ppm	Hydrocarbons (HC) Total HC	Methane	Non Methane HC	H_2O %	Stack Gas Temp °F	COMMENTS
1120				3.3	13.4	30		465											
1125				3.1	13.4	30		475											
1130				3.1	13.3	30		470											
1135				3.2	13.2	30		465											
1140				3.2	13.2	25		465	470	5									
1145				3.3	13.3	20		440											
1150				3.4	13.3	20		440											
1155				3.4	13.3	20		450											
1200				3.4	13.4	20		450											
1205				3.4	13.4	25		455											
1210				3.3	13.5	25		460											
1215				3.3	13.4	25		460											
1220				3.3	13.5	25		450											
1225				3.2	13.5	20		445											
1230				3.2	13.5	20		445											
1235				3.3	13.5	25		455											
1240				3.3	13.5	25		455											
1245				3.2	13.5	25		460											
1250				3.2	13.5	30		465											
1255				3.0	13.5	35		475	480	5									
1300				3.0	13.4	35		475											
1305				3.0	13.3	35		480											
1310				3.1	13.2	35		480											
1315				3.0	13.2	30		480											
1320				3.0	13.2	30		480											
																			AVERAGE

Form EED-063 12/78

FIELD DATA SHEET

JREX Aerotherm

Plant: Adelphi university

Location: Reiter

Operator: M. Chips

Date: 2-12-81

Load Range: 100%

Ambient Temperature:

Barometric Pressure:

Static Pressure Duct:

Fuel: Coal oil water mix

Time	Sample Point	Load MW	Steam Flow lb/hr x1000	Dry Uncorrected O_2 %	CO_2 %	CO ppm	SO_2 ppm	NO ppm	NO_x ppm	NO_2 ppm	Corrected to 3% O_2 Dry NO ppm	NO_x ppm	NO_2 ppm	Hydrocarbons Dry Total HC	Methane	Non Methane HC	H_2O %	Stack Gas Temp °F	COMMENTS
1325				3.0	13.2	30		480											
1330				2.9	13.3	35		485											
1335				2.5	13.6	60		485											Severe CO spike
1340				2.4	13.6	110		485											> 1000 ppm
1345				2.6	13.4	45		480											
1350				2.5	13.5	65		470											
1355				3.0	13.3	90		440											
1400				2.5	13.6	90		470											
1405				2.5	13.7	85		475											
1410				0.4	0	-10		-5											Zero
1415				7.1	17.2	740		840											Spike
1420				7.1	17.9	740		860											Steam burst
1425				2.5	14.6	440		450											Sample
1430				2.4	14.7	180		470											CO spike
1435				2.2	14.8	320		470											
1440				2.3	14.6	460		470											
1445				2.3	14.5	300		455											
1450				2.1	14.7	470		480											
1455				1.9	14.9	>1000		470											
1500				1.6	15.0	>1000		460											
1505				1.8	14.9	>1000		470											
1510				6.6	11.0	65		420											Boiler off
																			AVERAGE

Form EED (R) 12/78

FIELD DATA SHEET

JREX Acrotherm

Plant: Adelphi University

Location: Boiler

Operator: M. Chips

Date: 2-13-81

Load Range: 100%

Ambient Temperature:

Barometric Pressure:

Static Pressure Duct:

Fuel: Coal Oil Water + Soda Ash

Time	Sample Point	Load MW	Steam Flow lb/hr x1000	DRY UNCORRECTED O_2 %	CO_2 %	CO ppm	SO_2 ppm	NO ppm	NO_x ppm	NO_2 ppm	CORRECTED TO 3% O2 DRY NO ppm	NO_x ppm	NO_2 ppm	HYDROCARBON (DRY) Total HC	Methane	Non Methane HC	H_2O %	Stack Gas Temp °F	COMMENTS
0749				0.3	0.5	0		0											Zero
0805				7.73	17.9	740		867											Span 7.73% O_2 867 ppm
																			17.9% CO_2, 740 ppm CO
0850				2.4	14.1	160		450											
0855				2.4	14.0	140		455											CO spiking
0900				3.3	13.4	140		440											(following soot blow)
0905				3.0	13.3	590		400											
0910				3.4	12.9	620		400											
0915				3.3	12.9	720		340											
0920				3.1	13.0	>1000		340											
0925				3.1	12.9	920		360											
0930				3.5	12.5	460		400											
0935				3.8	12.6	150		430											
0955				3.2	12.9	460		445											
1000				3.1	12.8	470		445											
1005				3.2	—	—		435											CO, CO_2 lines crossed
1010				3.4	12.9	330		440											
1015				3.4	12.9	440		430											
1020				3.3	13.0	600		430											
1025				3.2	13.0	760		425											
1030				3.2	13.0	640		430											
1035				3.1	13.1	720		425											
1040				3.0	13.3	>1000		420											
1045				3.0	13.2	760		425											
1050				3.2	13.0	570		435											
																			AVERAGE

Form EED 063 12/78

FIELD DATA SHEET

UREX Aerotherm

Plant: Adelphi university

Location: Boiler

Operator: M. Chips

Date: 2-13-81

Load Range: 100 %

Ambient Temperature:

Barometric Pressure:

Static Pressure Duct:

Fuel: Coal oil blend + [illegible]

Time	Sample Point	Load MW	Steam Flow lb/hr x 1000	Dry Uncorrected O_2 %	CO_2 %	CO ppm	SO_2 ppm	NO ppm	NO_x ppm	NO_2 ppm	Corrected to 3% O_2 Dry NO ppm	NO_x ppm	NO_2 ppm	Hydrocarbons ppm Total HC	Methane	Non Methane HC	H_2O %	Stack Gas Temp °F	COMMENTS
1055				3.4	12.9	340		430											
1100				3.7	12.6	100		430											
1105				3.3	12.7	160		430											
1110				3.6	12.7	50		425											
1115				3.6	12.8	20		405											
1120				3.5	12.9	60		390											
1125				3.5	13.0	40		400											
1130				3.3	13.2	100		420											
1135				3.1	13.3	140		430											
1138				0.4	0.3	<0		0											zero
1142				7.4	17.2	770		800											Spike 7.7% O_2 [illegible]
																			NO 17% O_2 [illegible]
																			AVERAGE

Form EED 001 12/78

4.2 CONTINUOUS EMISSION MONITORING DATA (ADELPHI)

CONTINUOUS MONITORS -- ADELPHI[a]

Time minutes	N_2 percent	O_2 percent	CO_2 percent	H_2O percent	SO_2 ppm	E.A.[b] percent
Test 1 (COW)						
40	76.8	3.3	12.7	6.4	1,324	19.7
60	76.5	2.4	13.2	6.7	1,484	13.9
80	76.5	2.7	13.1	6.6	1,389	15.6
100	76.6	3.1	12.9	6.5	1,299	18.0
120	76.6	3.0	12.9	6.5	1,286	17.8
140	76.5	3.1	12.9	6.5	1,285	18.1
160	76.5	3.1	12.9	6.5	1,296	18.0
180	76.5	3.1	12.9	6.5	1,296	17.9
200	--[c]	--[c]	--[c]	--[c]	--[c]	--[c]
220	76.5	3.1	12.9	6.5	1,288	18.2
240	76.5	3.1	12.9	6.5	1,309	18.0
260	76.4	2.8	13.1	--[c]	--[c]	16.5
280	76.5	2.8	13.1	6.6	1,338	16.3
300	76.4	2.3	13.6	6.7	1,425	12.9
320	--[c]	--[c]	--[c]	6.8	1,620	--[c]
340	76.2	2.0	13.8	6.9	1,640	10.9
360	76.3	1.8	13.9	7.0	1,548	10.2
Test 2 (COW+SA)						
20	76.5	2.9	12.9	6.8	44	16.9
40	76.6	3.0	13.0	6.5	138	17.7
60	76.5	3.0	13.1	6.5	68	17.3
80	76.5	2.8	13.2	6.5	40	16.3
100	76.5	3.0	13.1	6.5	39	17.9
120	76.5	2.9	13.2	6.5	34	16.6
140	76.4	2.7	13.3	6.6	51	15.5
160	76.4	3.1	13.1	6.5	30	18.5
180	76.5	3.8	13.1	6.4	2	43.1
200	76.9	2.7	13.0	6.4	35	15.7

[a]Average of previous 20 minutes of data.

[b]Percent excess air $100 \left(\frac{79}{21} \times O_2\right) / \left(\%N - \frac{79}{21} \times O_2\right)$.

[c]Unable to read from printout.

4.3 FIELD DATA SHEETS FOR SASS AND CONTROLLED CONDENSATION (ACURE/)

FIELD DATA

Plant ADELPHI Twin.
Date 8-12-81
Sample Location STACK
Sample Type SASS
Run Number 1-SASS
Operator KAUFMANN
Ambient Temperature
Barometric Pressure 30.62 at 10:30
Static Pressure, (H_2O) +.5" H_2O
Filter Number(s)

Impinger Volumes

Initial	Final
500	
500	
500	

Silica Gel

803.6	1658 g H_2O Final
655.8	

Probe Length and Type 3 ft - PYREX
Nozzel I.D. (No.) .655
Assumed Moisture 8%
Molecular Weight, Dry, (M_d)
Meter Box Number 067
Meter Coefficient K_o 3744
α Factor 0.974
K =
$K(Nd)^4$ = ___ x (___)^4 = ___
$\Delta H = K(N_d)^4 \left(\frac{Tm}{Ts}\right) (\Delta P)$ Cp - .795

Leak Check; Initial at ____" Hg, ____ CFM From Organic mod .015 at 23"/Entire train .035 at 10"
Final at ____" Hg, ____ CFM

Traverse Point Number	Clock Time (24-hr) Clock / Sampling Time, min	Gas Meter Reading (V_m), ft^3	Velocity Head (ΔP_s), in. H_2O	Orifice Pressure Differential (ΔH), in. H_2O Desired	Actual	Temperature °F Stack	Probe	Impinger	Organic Module	Oven	Gas Meter In	Out	Pump Vacuum in. Hg	Avg. $\sqrt{\Delta P}$
	1021 / 0	Init. 43.554												
	15	107	.37		2.1	286	429	58	64	390	66	70	16/16	.608
	30	158.1	.37		1.9	286	416	59	66	395	84	70	20/20	.608
	45	209.933	.37		1.6	289	417	68	73	405	87	72	21/21	.608
	60	269.7	.37		2.1	290	416	58	72	406	82	71	17/17	.608
	75	324.0	.37		1.8	293	417	57	65	405	88	74	20/20	.608
	90	374.200	.37		1.5	294	417	58	66	403	85	74	22/22	.608
	105		.37		2.1	294	417	62	59	407	71	71	16/16	.608
	120	487.0	.37		1.8	296	417	64	69	405	90	74	20/20	.608
	135	536.20	.37		1.5	297	417	66	62	404	87	73	22/22	.608

Comments: Changing Filter 45 min. restart 1112/change filter 90 min restart 1205
change filter 135 min. [illegible] restart 1251.

Traverse Point Number	Clock Time (24-hr) Clock / Sampling Time, min	Gas Meter Reading (V_m), ft 3	Velocity Head (ΔP_s), in. H_2O	Orifice Pressure Differential (ΔH), in. H_2O Desired	Orifice Pressure Differential (ΔH), in. H_2O Actual	Temperature °F Stack	Temperature °F Probe	Temperature °F Impinger	Temperature °F Organic Module	Temperature °F Oven	Gas Meter In	Gas Meter Out	Pump Vacuum in. Hg	Avg. $\sqrt{\Delta P}$
	1331 / 135	Init. 536.20												
	150	593.0	.37		2.1	300	410	65	50	408	66	70	15/15	.608
	165	642.643	.37		1.6	303	410	65	63	405	85	71	20/20	.608
	180	699.2	.37		2.1	308	410	63	53	407	66	68	17/17	.608
	195	748.493	.37		1.6	311	410	68	59	404	83	69	21/21	.608
	210	804.7	.35		2.1	315	410	55	53	408	73	69	21/17	.592
Stop	225	851.0258	.35		1.4	314	410	57	62	404	85	71		.592
											92.2			
											78.8	71.1		
Leak check		807.574			1.82	298.4					75.7			.608

Run No. 1 - SASS Date 2-12-81 Sampling Location STACK

Comments: Change filter + silica gel 1.5 min restart 1345; change filter 195 min restart 1417
final test

ISOKINECTIC NOZZLE CALCULATION
AND
SAMPLING RATE CALCULATION

Plant ADELPHI UNIVERSITY Performed by P. KAUFMANN

Date 2-12-81

Sample Location STACK

Test No./Type 1 - SASS

$$N_d = \left(\frac{\Delta H\ T_s}{K\ T_m\ \Delta P}\right)^{.25}$$

where: N_d = Nozzel diameter (inches)

Average pressure differential across the orifice meter (in. H_2O)	ΔH	2.1
Temperature stack gas, average (^oF)	T_s	260
Temperature of gas meter, average (^oF)	T_m	85
Stack gas velocity pressure (in H_2O)	ΔP	.3
$\left(\frac{(2.1) \cdot (260 + 460)}{\cancel{.8844}\ (85 + 460)(.3)}\right)^{.25}$	N_d	.740

use .685

$$\Delta H = K\ (N_d)^4\ \frac{T_m}{T_s}\ (\Delta P)$$

where: ΔH = Pressure differential across the orifice meter (in H_2O)

Nozzel diameter, actual (inches)	N_d	
Temperature of gas meter (^oF)	T_m	
Temperature of stack gas (^oF)	T_s	
Stack gas velocity pressure (in H_2O)	ΔP	
$\left((___)\ (___)^4\ \frac{(___ + 460)}{(___ + 460)}\ (___)\right)$	ΔH	
Magic number ( )^4	$K(N_d)^4$	

ISOKINECTIC SAMPLING WORKSHEET

Plant ADELPHI UNIVERSITY Peformed by P. KAUFMANN

Date 2-12-81

Sample Location STACK

Test No./Type 1 / SASS

$$K = \frac{782.687\ (Cp)^2\ (1-B_{wo})^2\ P_s\ M_d}{K_o^2\ M_s\ P_m}$$

where: K = Contant of fixed and assumed parameters (dimensionless)

Pitot coefficient (dimensionless)	C_p	.795	
Water vapor in the gas stream (proportion by volume)	B_{wo}	.08	
Absolute stack gas pressure (in. Hg)	P_s	30.66	+.5" H_2O
Molecular weight, stack gas dry (lb/lb-mole)	M_d	29.0	
Orifice coefficient (dimensionless)	K_o	3.744	
Molecular weight, stack gas wet (lb/lb-mole) $M_d(1-B_{wo}) + 18(B_{wo})$	M_s	28.12	
Abolute meter pressure (in. Hg)	P_m	30.66	
$\frac{782.687\ (.795)^2\ (1-.08)^2\ (30.66)\ (29.00)}{(3.744)^2\ (28.12)\ (30.62)}$	K	30.844	

ISOKINETIC PERFORMANCE WORKSHEET & PARTICULATE CALCULATIONS

Plant Adelphi Univ Long Island NY Performed by John Linsum

Date 2-12-81

Sample Location Stack

Test No./Type 1-Bass

Barometric Pressure (in. Hg)	P_b	
Meter volume (std), $17.64 \left(\frac{V_m}{\alpha}\right)\left(\frac{P_b + \frac{\Delta H}{13.6}}{T_m + 460}\right)$ $17.64 \left(\frac{(327.57)}{(0.974)}\right)\left(\frac{(30.62) + \frac{(1.83)}{13.6}}{(75.7) + 460}\right)$	V_m std	339.652
Volume of liquid collected (grams)	Vl_c	1658
Volume of liquid at standard condition (scf) $Vl_c \times 0.04707$	V_w std	78.04206
Stack gas proportion of water vapor $\frac{V_w \text{ std}}{V_w \text{ std} + V_m \text{ std}}$, $\frac{(78.04)}{(78.04) + (339.6)}$	B_{wo}	0.08
Molecular weight, stack gas dry (lb/lb-mole) (% CO_2 x 0.44) + (% O_2 x 0.32) + (% N_2 + % CO x 0.28) (1.0 x 0.44) + (3.3 x 0.32) + (82.6 + — x 0.28)	M_d	30.34
Molecular weight, stack gas wet (lb/lb-mole) $Md(1-B_{wo}) + 18(B_{wo})$, (30.34)(1-.08) + 18(.08)	M_s	29.35
Absolute stack pressure (in. Hg) $P_b + \frac{P_{stack} \text{ (in. } H_2O)}{13.6}$, $(30.62) + \frac{(.5)}{13.6}$	P_s	30.66

7602/5/81/Rev 1

Temperature stack gas, average (°F)	T_s	293.4
Stack velocity (fps) $85.49\ (C_p)\ (\sqrt{\Delta P_{s\ avg}})\ \sqrt{\dfrac{T_{s\,avg} + 460}{P_s\ \ M_s}}$ $85.49\ (.795)(.606)\ \sqrt{\dfrac{(293.4) + 460}{(30.66)(29.35)}}$	$V_{s(avg)}$	37.81
Total sample time (minutes)	θ	225
Nozzle diameter, actual (inches)	N_d	.685
Percent isokinetic (%) $\dfrac{17.33\ (T_s + 460)(V_w\ std + V_m\ std)}{\theta\ \ V_s\ \ P_s\ \ N_d^2}$ $\dfrac{17.33\ (293.4 + 460)((78.04) + (837.60))}{(225)(37.81)(30.66)(.685)}$	%I	98.05
Area of stack (ft^2) $\pi = 3.1416$ $\pi r^2 \div 144$, $\pi(1.1/2)^2 \div 144$	A_s	1.9689
Stack gas volume at standard conditions (dscfm) $60\ (1 - B_{wo})Vs_{avg}\ A_s \left(\dfrac{528}{T_s\ avg + 460}\right) \left(\dfrac{Ps}{29.92}\right)$ $60\ (1 - .08)(37.81)(1.9689)\left(\dfrac{528}{293.4 + 460}\right) \left(\dfrac{(30.66)}{(29.92)}\right)$	Q_s	2932
Particulate matter concentration, dry (gr/dscf) $15.432\ \dfrac{M_p(grams)}{Vm_{std}}$, $15.432\ \dfrac{(____)}{(____)}$	$C_{s(std)}$	
Emission rate of particulate matter (lb/hr) $0.00857\ (Q_s)\ C_{s(std)}$, $0.00857\ (____)(____)$	E_p	

7602/5/81/Rev 1

176 1212

FIELD DATA

Plant ADELPHI UNIV.
Date 2-13-81
Sample Location STACK
Sample Type SASS
Run Number 2
Operator KAUFMANN
Ambient Temperature 75°
Barometric Pressure 31.06
Static Pressure, (H_2O)
Filter Number(s)

Impinger Volumes

Initial	Final
500	
500	
500	945

Silica Gel

	Moisture Catch

Probe Length and Type 3 ft Pyrex
Nozzel I.D. (No.) .685
Assumed Moisture 8%
Molecular Weight, Dry, (M_d) See Computer Data
Meter Box Number 067
Meter Coefficient K = 3.744
α Factor 0.974
K =
$K(Nd)^4$ = ___ x (___)^4 = ___
$\Delta H = K(N_d)^4 \left(\frac{T_m}{T_s}\right) (\Delta P)$

Leak Check: Initial at ____" Hg, ____ CFM .011 at 22" from Orifice Mech. .047 at 10" entire train
Final at ____" Hg, ____ CFM

Traverse Point Number	Clock Time (24-hr) Clock / Sampling Time, min	Gas Meter Reading (V_m), ft³	Velocity Head (ΔP_s), in. H_2O	Orifice Pressure Differential (ΔH), in. H_2O Desired	Actual	Temperature °F Stack	Probe	Impinger	Organic Module	Oven	Gas Meter In	Gas Meter Out	Pump Vacuum in. Hg	Avg. $\sqrt{\Delta P}$
	910	Init. 852 0.25												
Change filter	5 0920	866.5	.33		1.5	318	402	69	60	363	73	71	22/22	.57
	10	885	.33		1.4	331	413	73	59	404	71	70	22/22	
Change filter	15 0936	895.72	.33		0.6	335	412	73	57	403	75	70	23/23	
	20	910.3	.33		1.0	335	414	75	60	406	71	68	23/23	
change filter	22.5 0952	915.375	.33		0.5	336	413	73	57	405	73	66	23/23	
	25	924.3	.33		1.0	338	406	70	56	408	68	67	22/22	
change filter	30 1007	939.27	.33		0.5	339	406	69	56	407	71	67	23/23	
	35	950.3	.33		1.1	342	415	70	50	408	67	66	20/20	
change filter	37.5	955.215	.33			343	405	70	51	406	71	66	23/23	

Filter plug almost instantly can hold a ΔH of 2.1 for about ½ min

Comments:

Traverse Point Number	Clock Time (24-hr) Clock / Sampling Time, min	Gas Meter Reading (V_m), ft 3	Velocity Head (ΔP_s), in. H_2O	Orifice Pressure Differential (ΔH), in. H_2O Desired	Orifice Pressure Differential (ΔH), in. H_2O Actual	Temperature °F Stack	Temperature °F Probe	Temperature °F Impinger	Temperature °F Organic Module	Temperature °F Oven	Gas Meter In	Gas Meter Out	Pump Vacuum in. Hg	Avg. $\sqrt{\Delta P}$
	1021 / 37.5	Init. 955.215												
change Filter	45 1035	975.207	.33		1.0	347	415	71	50	408	67	65	20/20	.57
change Filter	52.5 1054	994.975	.33		1.0	354	412	71	52	409	68	65	23/23	
change Filter	60 1108	1014.92	.33		0.9	367	416	69	56	408	70	65	23/23	
change Filter	67.5 1123	1034.86	.33		0.9	380	410	73	56	406	68	66	23/23	
stop	75 1131	1055.242	.33		0.9	388	417	68	53	407	72	66	23/23	
TOTAL											70.4	67.3		
AVERAGE		202.614			0.95	376.6					68.8			.57

Run No. 2-SA55 Date 2-13-81 Sampling Location SDASL

Comments:

ISOKINETIC PERFORMANCE WORKSHEET & PARTICULATE CALCULATIONS

Plant ADELPHI UNIV. Performed by JOHN UNGER

Date 2-13-81

Sample Location STACK

Test No./Type 2 - GLASS

Barometric Pressure (in. Hg)	P_b	
Meter volume (std), $17.64 \left(\frac{V_m}{\alpha}\right)\left(\frac{P_b + \frac{\Delta H}{13.6}}{T_m + 460}\right)$ $17.64 \left(\frac{(226.614)}{(.974)}\right)\left(\frac{(31.06) + \frac{(0.95)}{13.6}}{(68.8) + 460}\right)$	V_m std	216.04
Volume of liquid collected (grams)	Vl_c	400.72 CALCULATED FROM BLOC
Volume of liquid at standard condition (scf) Vl_c x 0.04707	V_w std	18.8602 CALCULATED FROM BLOC
Stack gas proportion of water vapor $\frac{V_{w\ std}}{V_{w\ std} + V_{m\ std}}$, $\frac{(___)}{(___) + (___)}$	B_{wo}	ASSUME .03
Molecular weight, stack gas dry (lb/lb-mole) (% CO_2 x 0.44) + (% O_2 x 0.32) + (% N_2 + % CO x 0.28) (14.0 x 0.44) + (3.2 x 0.32) + (82.3 + - x 0.28)	M_d	30.23
Molecular weight, stack gas wet (lb/lb-mole) $Md(1-B_{wo}) + 18(B_{wo})$, (30.23)(1-.03) + 18(.03)	M_s	29.25
Absolute stack pressure (in. Hg) $P_b + \frac{P_{stack}\ (\text{in. } H_2O)}{13.6}$, $(31.06) + \frac{(-.50)}{13.6}$	P_s	30.1

7602/5/81/Rev 1

Temperature stack gas, average (°F)	T_s	316.6
Stack velocity (fps) $85.49\ (C_p)\ (\sqrt{\Delta P_{s\ avg}})\ \sqrt{\dfrac{T_s avg + 460}{P_s\ \ M_s}}$ $85.49\ (.775)(.574)\ \sqrt{\dfrac{(316.6) + 460}{(30.1)(29.25)}}$	$V_{s(avg)}$	37.34
Total sample time (minutes)	θ	75
Nozzle diameter, actual (inches)	N_d	.685
Percent isokinetic (%) $\dfrac{17.33\ (T_s + 460)(V_w\ std + V_m\ std)}{\theta\ \ V_s\ \ P_s\ \ N_d^2}$ $\dfrac{17.33\ (316.6 + 460)(13.86) + (26.04)}{(75)(37.34)(30.1)(.685^2)}$	%I	83.02
Area of stack (ft^2) $\pi = 3.1416$ $\pi r^2 \div 144$, $\pi(\frac{14}{2})^2 \div 144$	A_s	1.9689
Stack gas volume at standard conditions (dscfm) $60\ (1 - B_{wo})Vs_{avg}\ A_s \left(\dfrac{528}{T_s\ avg + 460}\right) \left(\dfrac{Ps}{29.92}\right)$ $60\ (1 - .08)(37.34)(1.9689)\left(\dfrac{528}{316.6 + 460}\right) \left(\dfrac{(30.1)}{(29.92)}\right)$	Q_s	2672
Particulate matter concentration, dry (gr/dscf) $15.432\ \dfrac{M_p(grams)}{Vm_{std}}$, $15.432\ \dfrac{(____)}{(____)}$	$C_{s(std)}$	
Emission rate of particulate matter (lb/hr) $0.00857\ (Q_s)\ C_{s(std)}$, $0.00857\ (____)(____)$	E_p	

ASSUMED SAME AS PREVIOUS TEST

7602/5/81/Rev 1

CONTROLLED CONDENSATION SYSTEM (CCS)
FIELD DATA SHEET

Plant [illegible]
Date [illegible]
Sample Location [illegible]
Run No. [illegible]
Operator [illegible]

Ambient Temperature [illegible]
Barometric Pressure [illegible]
Meter Box Number 0015
Meter Orifice Coefficient
Meter α Factor 1.012 [illegible]

Sampling Time, min	Clock Time (24-hr) clock	Gas Meter Reading (V_m), ft³ Init. 272.670	Temperature (°F) Stack	Probe	Filter Skin	Filter Out	°C Recirc Water	Exit Coil	Dry Gas Meter In	Dry Gas Meter Out
5	1115	274.500	290	405	800	590	60	114	72	73
1/ 5	1123	INITIAL 274.500	—	—	—	—	—	—	74	74
2/ 10	1128	No Reading						116	70	71
15	1143	278.000	290	N/A	760	520	60	115	70	71
15	1151	INITIAL 278.00	—	—	—	—	—	—	70	71
20	1156	279.800	290	N/A	1100	649	60	115	69	70
35	1201	281.250	290	N/A	1150	658	60	113	69	70
30	1206	282.500	290	N/A	1150	674	60	112	69	70
35	1211	283.900	290	N/A	—	681	60	112	69	71
40	1216	285.610	290	N/A	—	689	60	111	71	71
45	1221	287.010	290	N/A	—	694	60	110	71	71
50	1226	286.100	290	N/A	—	701	60	112	71	71
55	1231	289.000	290	N/A		692	60	110	71	71
Average	55 MIN	16.33	290	N/A		654.8	60	112.7	71.0	

1/ [illegible]
2/ [illegible]

CONTROLLED CONDENSATION SYSTEM (CCS)
FIELD CHECKPOINT SHEET

Checkpoint	Initials		Remarks
	Supervisor	QA Inspector	
LABORATORY PREPARATION			
• Inspect and clean CCC. Both filter holder and CCC are cleaned with hot chromic acid solution and D.I. H_2O.		✓	
• Rinse with acetone and air dry CCC.		✓	
• Place Tissuequartz filter in filter housing.		✓	
• Check seal between end of joint and filter.		✓	
• Do not use grease on joints.		✓	
• Inspect and clean all glass joints.		✓	
SITE SETUP.			
• Rinse the inside of probe prior to run.		✓	
• Rinse probe with acetone until rinse solution is clear.		✓	
• Perform leak test.		✓	.001 @ 22"
• Leak rate must be less than 80 ml/min (0.003 cfm).		✓	
• Thermocouple leads attched to probe and filter.		✓	
• CCC water bath held at 60°C (140°F) ±1°C.		✓	
• Leak test train.		✓	
• Probe temperature maintained at 316°C (600°F) ±17°C.		No	Probe [illegible] in [illegible]
• Gas temperature out of filter holder held at 228°C (550°F).		✓	
• Fresh solutions placed in impingers.		✓	
• Fresh absorbent replaced in final impinger.		✓	
• Adjust flowrate in system to 8 lpm.		✓	

CONTROLLED CONDENSATION SYSTEM (CCS)
FIELD CHECKPOINT SHEET -- Continued

Checkpoint	Initials		Remarks
	Supervisor	QA Inspector	
SAMPLING RUN			
• Turn vacuum pump on just before inserting probe in stack.		✓	
• Check seal between probe and port to prevent any outside air from entering stack.		✓	
• Run test for 1 hour or until coils are frosted to 1/2 or 2/3 their length.		✓	1 hr
• After run, cap both ends of probe and lay in horizontal position.		✓	
• Rinse the CCC coils into the modified Erlenmeyer flask with a maximum of 40 ml D.I. H_2O.		✓	
• Was any of the solution lost (Ø ml estimated)?		✓	
• After probe has cooled, it is rinsed with a maximum of 40 ml D.I. H_2O into a 25-ml Erlenmeyer flask.		✓	
- Was any solution lost (Ø ml estimated)?			
Clean support equipment priot to next run.		✓	
- Save filter for titration.		✓	

Comments:

CONTROLLED CONDENSATION SYSTEM (CCS)
LABORATORY CHECKPOINTS

Checkpoint	Initials		Remarks
	Supervisor	QA Inspector	
LABORATORY ANALYSIS			
• Clean glassware prior to titration.		✓	
• Use Bromphenol Blue indicator.		✓	
• Is the NaOH buret protected with a CO_2 absorbent tube?		✓	
• When was NaOH standardized last (Date)?		N/A	
• Filter any solution that has suspended particulate.		✓	
• Use same number of indicator drops for each titration.		✓	
• Perform indicator blank on a volume of D.I. H_2O equal to sample aliquot used.		✓	
• Indicator blank added to H_2SO_4 milli-equivalents found.		✓	
• Perform all analyses in triplicate.		✓	
DATA ANALYSIS VERIFICATION			
• Obtain and titrate test samples from main laboratory.		✓	

Comments:

3-12-81 [illegible]

See Field Log Notebook for analysis

CONTROLLED CONDENSATION SYSTEM (CCS)
FIELD DATA SHEET

Plant Adelphi University
Date 2-12-81
Sample Location Stack
Run No. 2- CDW/SIDE ACC
Operator Dukos

Ambient Temperature ~70°F
Barometric Pressure 31.96
Meter Box Number 0015
Meter Orifice Coefficient
Meter α Factor 1.012

Clock Time (24-hr) clock / Sampling Time, min	Gas Meter Reading (V_m), ft^3	Temperature (°F)							
		Stack	Probe	Filter Skin	Filter Out	°C Recirc Water	Exit Coil	Dry Gas Meter In	Dry Gas Meter Out
1109 / 0	Init. 296.100								
1124 / 25	296.900	350 388	—	—	680	60	115	71	71
Average 24 min	≈ 8	369		680		60	115	71	71

71

CONTROLLED CONDENSATION SYSTEM (CCS)
FIELD CHECKPOINT SHEET

Checkpoint	Initials		Remarks
	Supervisor	QA Inspector	
LABORATORY PREPARATION			
• Inspect and clean CCC. Both filter holder and CCC are cleaned with hot chromic acid solution and D.I. H_2O.		✓	
• Rinse with acetone and air dry CCC.		✓	
• Place Tissuequartz filter in filter housing.		✓	
• Check seal between end of joint and filter.		✓	
• Do not use grease on joints.		✓	
• Inspect and clean all glass joints.		✓	
SITE SETUP			
• Rinse the inside of probe prior to run.		✓	
• Rinse probe with acetone until rinse solution is clear.		✓	
• Perform leak test.		✓	[illegible]
• Leak rate must be less than 80 ml/min (0.003 cfm).		✓	
• Thermocouple leads attched to probe and filter.		✓	
• CCC water bath held at 60°C (140°F) ±1°C.		✓	
• Leak test train.		✓	[illegible]
• Probe temperature maintained at 316°C (600°F) ±17°C.		[illegible]	[illegible]
• Gas temperature out of filter holder held at 228°C (550°F).		✓	
• Fresh solutions placed in impingers.		✓	
• Fresh absorbent replaced in final impinger.		✓	
• Adjust flowrate in system to 8 lpm.		✓	

[illegible]

CONTROLLED CONDENSATION SYSTEM (CCS)
FIELD CHECKPOINT SHEET -- Continued

Checkpoint	Initials		Remarks
	Supervisor	QA Inspector	
SAMPLING RUN • Turn vacuum pump on just before inserting probe in stack.		✓	
• Check seal between probe and port to prevent any outside air from entering stack.		✓	
• Run test for 1 hour or until coils are frosted to 1/2 or 2/3 their length.		Run for 24 min @ very low ? low water	
• After run, cap both ends of probe and lay in horizontal position.		✓	
• Rinse the CCC coils into the modified Erlenmeyer flask with a maximum of 40 ml D.I. H_2O.		✓	
• Was any of the solution lost (Ø ml estimated)?		✓	
• After probe has cooled, it is rinsed with a maximum of 40 ml D.I. H_2O into a 25-ml Erlenmeyer flask. Was any solution lost (Ø ml estimated)?		✓	
Clean support equipment priot to next run.		✓	
- Save filter for titration.		✓	

Comments:

CONTROLLED CONDENSATION SYSTEM (CCS)
LABORATORY CHECKPOINTS

Checkpoint	Initials		Remarks
	Supervisor	QA Inspector	
LABORATORY ANALYSIS			
• Clean glassware prior to titration.		✓	
• Use Bromphenol Blue indicator.		✓	
• Is the NaOH buret protected with a CO_2 absorbent tube?		✓	
• When was NaOH standardized last (Date)?		N/A	
• Filter any solution that has suspended particulate.		✓	
• Use same number of indicator drops for each titration.		✓	
• Perform indicator blank on a volume of D.I. H_2O equal to sample aliquot used.		✓	
• Indicator blank added to H_2SO_4 milli-equivalents found.		✓	
• Perform all analyses in triplicate.		✓	
DATA ANALYSIS VERIFICATION			
• Obtain and titrate test samples from main laboratory.		✓	

Comments:

3-12-81 [illegible].

See Field Log Notebook - for Analysis -

ADELPHI CENTER FOR ENERGY STUDIES
A division of
ADELPHI RESEARCH CENTER, INC.
ADELPHI UNIVERSITY
GARDEN CITY, L.I., NEW YORK 11530
516-294-8700 ext. 7618-7619

DR JOHN P DOOHER
DIRECTOR

October 22, 1981

Mr. Bob DeRosier
Acurex Corporation
485 Clyde Avenue
Mountain View, CA 94042

Dear Mr. DeRosier:

Please find enclosed the particulate information you requested:

wt of sample collected	0.7240g
wt of water collected	12g
Dry Gas Volume	6.872 ft^3
Stack Temperature	375 ^0F
Temperature at meter	70 ^0F

If you have any questions, please call me.

Sincerely,

Terry Kanabrocki

/mh

Enclosure

SECTION 5

ANALYTICAL LABORATORY RESULTS

		Page
5.1	ULTIMATE FUEL ANALYSES	5-2
5.2	PARTICULATE EMISSIONS FROM SASS SAMPLES	5-3
5.3	SULFUR OXIDE EMISSIONS FROM CONTROLLED CONDENSATION SAMPLES	5-6
5.4	TRACE ELEMENT ANALYSES	5-8
5.5	TOTAL CHROMATOGRAPHABLE ORGANICS (TCO), GRAVIMETRIC ORGANICS (GRAV), INFRARED (IR) SPECTRA, AND GAS CHROMATOGRAPHY/MASS SPECTROMETRY (GC/MS) OF TOTAL SAMPLE EXTRACTS	5-24
5.6	LIQUID CHROMATOGRAPHY (LC) SEPARATION, INFRARED (IR) SPECTRA OF LC FRACTIONS, AND LOW RESOLUTION MASS SPECTROMETRY (LRMS) OF SELECTED LC FRACTIONS AND TOTAL SAMPLE EXTRACTS	5-35
5.7	C_1 - C_6 CHROMATOGRAPHY	5-60
5.8	RADIOMETRIC ANALYSES	5-72
5.9	BIOLOGICAL ASSAY REPORT	5-74

5.1 ULTIMATE FUEL ANALYSES

LABORATORY CERTIFICATE

COPY

CURTIS & TOMPKINS, LTD.

ESTABLISHED 1870

ANALYTICAL - CHEMISTS - CONSULTING

SAMPLERS — INSPECTORS

MEMBERS OFFICIAL CHEMISTS AND/OR SAMPLERS FOR MANY COMMODITY AND TRADE ORGANIZATIONS

CORPORATE OR STAFF MEMBERSHIP IN PRINCIPAL SCIENTIFIC SOCIETIES

290 DIVISION STREET
SAN FRANCISCO, CALIF 94103
U.S.A.
Telephone (415) 861-1863

CABLE ADDRESS ANALYST

REFEREE ANALYSES
RESEARCH — INVESTIGATIONS
VITAMIN ASSAYS — BIOCHEMISTRY

SPECIALISTS IN BULK COMMODITIES

Laboratory No. 81h100
Preliminary No. 6487

Reported 8/13/81
Sampled ------
Received 7/07/81

For ACUREX CORPORATION

Report on 5 samples of Fuel Product

Mark Project No. 7734.12, 7/06/81, Blanket Subcontract RB59186A, Release No. 2.

DRY BASIS EXCEPT AS NOTED

	COW 813895			COW+SA 813893		
	1st Test	2nd Test	3rd Test	1st Test	2nd Test	3rd Test
Carbon (C), % --------	78.02	----	----	71.74	----	----
Hydrogen (H), % ------	8.60	----	----	7.83	----	----
Oxygen (O), (by difference),%--------	6.70	----	----	4.65	----	----
Nitrogen (N), % ------	0.75	0.77	0.75	0.72	0.72	0.74
Sulfur (S), % --------	1.93	1.90	1.95	1.89	1.90	1.90
Heating Value: BTU/Pound ----------	15,985	----	----	13,705	----	----
Specific Gravity, 75°F (as rec'd) ----	1.061	----	----	1.131	----	----
Ash, % ----------	4.00	----	----	13.17	----	----
Moisture,% (as rec'd) ----------	15.60	----	----	15.54	----	----

SAMPLES DISCARDED 30 DAYS AFTER RECEIPT UNLESS OTHERWISE REQUESTED

This report applies only to the sample, or samples, investigated and is not necessarily indicative of the quality or condition of apparently identical or similar products. As a mutual protection to clients, the public and this Laboratory, this report is submitted and accepted for the exclusive use of the client to whom it is addressed and upon the condition that it is not to be used, in whole or in part, in any advertising or publicity matter without prior

5.2 PARTICULATE EMISSIONS FROM SASS SAMPLES

ACUREX Corporation
ANALYSIS LABORATORIES

DATA REPORTING FORM

CUSTOMER CMEA DATE

CUSTOMER CONTRACT NO. 307605.02 ACUREX CONTRACT NO. A81-04-004

RESULTS REPORT TO C. Castaldini TELEPHONE

ADDRESS

Adelphi I 23.776 dscm

SAMPLE ID (CUSTOMER)	398	399	420	-	407					
SAMPLE ID (LAB)	10μ	3μ	1μ	Filters	Probe					
PARAMETER										UNITS
Ash Weight	18.9052	13.8240	11. 7542	2.4102	1.3175					g

ANALYST

REVIEWER

Form EED-057 4 80

ACUREX Corporation
ANALYSIS LABORATORIES

DATA REPORTING FORM

CUSTOMER CMEA DATE

CUSTOMER CONTRACT NO. 307605.62 ACUREX CONTRACT NO. A81-04-004

RESULTS REPORT TO C. Castaldini TELEPHONE

ADDRESS

Adelphi 2 6.142 dscm

SAMPLE ID (CUSTOMER)	439	438	437	-	441					
SAMPLE ID (LAB)	10μ	3μ	1μ	Filters	Probe					
PARAMETER										UNITS
Ash weight	3.2482	0.2809	0.7805	15.6518	2.7962					g

ANALYST

REVIEWER

Form EED 057 4 80

5.3 SULFUR OXIDE EMISSIONS FROM CONTROLLED CONDENSATION SAMPLES

CONTROLLED CONDENSATION SYSTEM (CCS) LABORATORY DATA SHEET

Plant _____ Analyst _____
Date _____ Date Lab Analysis Completed _____
Sample Location _____
Run No. _____

Titration Data

Method _____ Titrant _____ Normality _____ Indicator _____

Sample Description	Probe, Nozzle and Filter Rinse	G/R Coil Rinse	Impinger Contents and Rinse	H_2O Blank	3% H_2O_2 Blank
Sample No.					
Vol. of Sample					
Vol. of Aliquot					
Vol. of Titrant Used					
Average Vol. of Titrant Used					

Calculations

Vol. of Gas Sampled (V_M) _____ ft^3, Avg. Meter Temp (T_M) _____ °F,
Meter Pressure (P_M) _____ "Hg, Meter α Factor _____ dimensionless

Below

$$PPM\ SO_4 = \frac{48.15\ (___,\ MgSO_4)(___,\ T_M+460)}{96\ (___,\ V_M)(___,\ P_M)}\ (___,\ \alpha)$$

ppm SO_4 = _____

PROBE AND FILTER RINSE

$\left(\frac{.05-.03}{10}\right).02N=.00004N$

$.00004N \times 132.0=.00528\ MeqSO_4/Sample$

$\left(\frac{.00528}{2}\right) \times 96=.253\ MgSO_4/Sample$

$$PPM\ SO_2 = \frac{48.15\ (___,\ MgSO_2)(___,\ T_M+460)}{64\ (___,\ V_M)(___,\ P_M)}\ (___,\ \alpha)$$

ppm SO_2 = _____

CONTROLLED CONDENSATION SYSTEM (CCS)
LABORATORY DATA SHEET

Plant _____ Analyst _____
Date _____ Date Lab Analysis Completed _____
Sample Location _____
Run No. _____

Titration Data

Method _____ Titrant _____ Normality _____ Indicator _____

Sample Description	Probe, Nozzle and Filter Rinse			G/R Coil Rinse			Impinger Contents and Rinse			H_2O Blank			3% H_2O_2 Blank		
Sample No.															
Vol. of Sample															
Vol. of Aliquot															
Vol. of Titrant Used															
Average Vol. of Titrant Used															

Calculations

Vol. of Gas Sampled (V_M) _____ ft^3, Avg. Meter Temp (T_M) _____ °F,
Meter Pressure (P_M) _____ "Hg, Meter α Factor _____ dimensionless

$$\text{PPM } SO_4 = \frac{48.15\ (___,\ MgSO_4)(___,\ T_M+460)}{96\ (___,\ V_M)(___,\ P_M)}\ (___,\alpha)$$

ppm SO_4 =

$$\text{PPM } SO_2 = \frac{48.15\ (___,\ MgSO_2)(___,\ T_M+460)}{64\ (___,\ V_M)(___,\ P_M)}\ (___,\alpha)$$

ppm SO_2 =

COMMERCIAL TESTING & ENGINEERING CO.
GENERAL OFFICES. 228 NORTH LA SALLE STREET, CHICAGO, ILLINOIS 60601 AREA CODE 312 726-8434

Reply to
Instrument Analysis Division
490 Orchard Street
Golden, CO 80401

Phone: 303-278-9521

October 29, 1981

Mr. Larry Waterman
Acurex Corporation
485 Clyde Avenue
Mountain View, CA 94042

Re: IAD #97-H479-116-05
Re-logged in from 97-G452-116-30

Analytical Report

In an October 2, 1981, phone conversation (L. Waterman/J. Oldham) we were asked to analyze five (5) samples for Sulfur that had been reported originally as part of IAD Batch #97-G452-116-30. These samples were either impinger solutions or ash in which Sulfur had been determined as a major component by spark source mass spectrometry (SSMS).

Five millilitres of the impinger solutions and ≅0.5 g of the ash samples were fused with sodium carbonate in platinum. The fusion was dissolved in hot water, acidified, and the Sulfur precipitated as barium sulfate which was filtered out and weighed. Data from these gravimetric determinations were calculated to give results in weight per cent (wt %) Sulfur and are presented in Table No. I.

Table No. I
(wt %)

Sample #	Sulfur
Adelphi 2 1μ & filter	16
Adelphi - 1 3 & 10μ	1.1
Adelphi - 1 Imp. 1	1.3
Adelphi - 2 3 & 10μ	11
Adelphi - 2 Imp. 1	0.40

If there are any questions concerning these results, please call.

Bruce A. Hale
Section Supervisor

M. L. Jacobs, Ph.D., Mngr.
Instrumental Analysis Div.

Charter Member

OVER 40 BRANCH LABORATORIES STRATEGICALLY LOCATED IN PRINCIPAL COAL MINING AREAS, TIDEWATER AND GREAT LAKES PORTS, AND RIVER LOADING FACILITIES

COMMERCIAL TESTING & ENGINEERING CO.

GENERAL OFFICES: 228 NORTH LA SALLE STREET, CHICAGO ILLINOIS 60601 AREA CODE 312 726-8434
Reply to INSTRUMENTAL ANALYSIS DIVISION 14335 WEST 44TH AVENUE GOLDEN COLORADO 80401 PHONE 303 278-9521

To: Mr. R. Belletto
Acurex Corporation
485 Clyde Avenue
Mountain View, CA 94042

Date June 23, 1981

Analyst J. Oldham

P. O. No.: Subcontract SW 59159A

Sample No.: 813896 Adelphi (OW(42/42/16)

SPARK SOURCE MASS SPECTROGRAPHIC ANALYSIS

CONCENTRATION IN PPM WEIGHT

IAD No 97-G452-116-30

ELEMENT	CONC.	ELEMENT	CONC.	ELEMENT	CONC	ELEMENT	CONC.
Uranium	0.7	Terbium		Ruthenium		Vanadium	15
Thorium	0.4	Gadolinium		Molybdenum	1	Titanium	MC
Bismuth		Europium	0.05	Niobium	0.5	Scandium	0.3
Lead	4	Samarium	0.4	Zirconium	0.6	Calcium	MC
Thallium	0.4	Neodymium	0.6	Yttrium	3	Potassium	MC
Mercury	NR	Praseodymium	0.3	Strontium	8	Chlorine	1
Gold		Cerium	1	Rubidium	2	Sulfur	MC
Platinum		Lanthanum	2	Bromine	0.7	Phosphorus	35
Iridium		Barium	22	Selenium	3	Silicon	MC
Osmium		Cesium	0.3	Arsenic	4	Aluminum	>20
Rhenium		Iodine	0.4	Germanium	0.6	Magnesium	92
Tungsten		Tellurium		Gallium	1	Sodium	>51
Tantalum		Antimony		Zinc	6	Fluorine	≈7
Hafnium		Tin	0.05	Copper	3	Oxygen	NR
Lutetium		Indium	STD	Nickel	8	Nitrogen	NR
Ytterbium		Cadmium	0.04	Cobalt	2	Carbon	NR
Thulium		Silver	0.08	Iron	MC	Boron	2
Erbium		Palladium		Manganese	10	Beryllium	0.3
Holmium		Rhodium		Chromium	6	Lithium	1
Dysprosium						Hydrogen	NR

Note: Sample thermally ashed at 450°C for 1 hour prior to analysis

STD — Internal Standard
NR — Not Reported
All elements not detected < 0.02ppm
MC — Major Component >100ppm
INT — Interference

Approved: M Specht

COMMERCIAL TESTING & ENGINEERING CO.
GENERAL OFFICES 228 NORTH LA SALLE STREET, CHICAGO, ILLINOIS 60601 AREA CODE 312 726-8434
Reply to INSTRUMENTAL ANALYSIS DIVISION, 14335 WEST 44TH AVENUE GOLDEN, COLORADO 80401 PHONE 303-278-9521

To: Mr. R. Belletto
Acurex Corporation
485 Clyde Avenue
Mountain View, CA 94042

Date: June 18, 1981

Analyst: J. Oldham

P. O. No.: Subcnontract SW 59159A

Sample No.: Adelphi-1 3+ 10μ 2.0456/ 32.7292g

SPARK SOURCE MASS SPECTROGRAPHIC ANALYSIS

CONCENTRATION IN PPM WEIGHT

IAD No. 97-G452-116-30

ELEMENT	CONC.	ELEMENT	CONC.	ELEMENT	CONC.	ELEMENT	CONC.
Uranium	80	Terbium	2	Ruthenium		Vanadium	460
Thorium	17	Gadolinium	5	Molybdenum	10	Titanium	MC
Bismuth	0.7	Europium	2	Niobium	12	Scandium	53
Lead	420	Samarium	19	Zirconium	150	Calcium	MC
Thallium	10	Neodymium	25	Yttrium	30	Potassium	MC
Mercury	NR	Praseodymium	12	Strontium	200	Chlorine	51
Gold		Cerium	53	Rubidium	35	Sulfur	>570
Platinum		Lanthanum	42	Bromine	20	Phosphorus	MC
Iridium		Barium	430	Selenium	110	Silicon	MC
Osmium		Cesium	3	Arsenic	48	Aluminum	>91
Rhenium		Iodine	2	Germanium	9	Magnesium	MC
Tungsten	2	Tellurium	0.3	Gallium	30	Sodium	>230
Tantalum	0.3	Antimony	0.7	Zinc	150	Fluorine	≈14
Hafnium	1	Tin	0.6	Copper	31	Oxygen	NR
Lutetium	0.2	Indium	STD	Nickel	38	Nitrogen	NR
Ytterbium	2	Cadmium	0.7	Cobalt	7	Carbon	NR
Thulium	0.3	Silver	≤0.2	Iron	MC	Boron	21
Erbium	2	Palladium		Manganese	310	Beryllium	2
Holmium	3	Rhodium		Chromium	100	Lithium	13
Dysprosium	4					Hydrogen	NR

STD — Internal Standard
NR — Not Reported
All elements not detected < 0.1ppm
MC — Major Component
INT — Interference

Approved:

COMMERCIAL TESTING & ENGINEERING CO.

GENERAL OFFICES 228 NORTH LA SALLE STREET, CHICAGO ILLINOIS 60601 AREA CODE 312 728-8434
Reply to INSTRUMENTAL ANALYSIS DIVISION, 14335 WEST 44TH AVENUE GOLDEN, COLORADO 80401 PHONE 303 278-9521

To: Mr. R. Belletto
Acurex Corporation
485 Clyde Avenue
Mountain View, CA 94042

Date June 18, 1981

Analyst J. Oldham

P. O. No.: Subcontract SW 59159A

Sample No.: Adelphi-1 Filter +1u 1.7494/ 14/1644 g

SPARK SOURCE MASS SPECTROGRAPHIC ANALYSIS

CONCENTRATION IN PPM WEIGHT

IAD No 97-G452-116-30

ELEMENT	CONC.	ELEMENT	CONC.	ELEMENT	CONC.	ELEMENT	CONC
Uranium	68	Terbium	1	Ruthenium		Vanadium	480
Thorium	18	Gadolinium	5	Molybdenum	27	Titanium	MC
Bismuth	0.7	Europium	2	Niobium	22	Scandium	56
Lead	230	Samarium	20	Zirconium	62	Calcium	MC
Thallium	10	Neodymium	27	Yttrium	140	Potassium	MC
Mercury	NR	Praseodymium	22	Strontium	390	Chlorine	24
Gold		Cerium	120	Rubidium	83	Sulfur	>600
Platinum		Lanthanum	100	Bromine	17	Phosphorus	MC
Iridium		Barium	MC	Selenium	12	Silicon	MC
Osmium		Cesium	6	Arsenic	160	Aluminum	>96
Rhenium		Iodine	2	Germanium	48	Magnesium	MC
Tungsten	5	Tellurium		Gallium	64	Sodium	>250
Tantalum	0.8	Antimony	6	Zinc	160	Fluorine	≈150
Hafnium	2	Tin	4	Copper	58	Oxygen	NR
Lutetium	0.5	Indium	STD	Nickel	400	Nitrogen	NR
Ytterbium	6	Cadmium	0.3	Cobalt	33	Carbon	NR
Thulium	0.8	Silver	1	Iron	MC	Boron	91
Erbium	4	Palladium		Manganese	MC	Beryllium	16
Holmium	5	Rhodium		Chromium	280	Lithium	140
Dysprosium	8					Hydrogen	NR

STD — Internal Standard
NR — Not Reported
All elements not detected < 0.1ppm
MC — Major Component
INT — Interference

5-11

Approved: M. Hacoots

COMMERCIAL TESTING & ENGINEERING CO.
GENERAL OFFICES: 228 NORTH LA SALLE STREET, CHICAGO ILLINOIS 60601 AREA CODE 312 726-8434
Reply to INSTRUMENTAL ANALYSIS DIVISION, 14335 WEST 44TH AVENUE, GOLDEN, COLORADO 80401 PHONE 303-278-9521

To: Mr. R. Belletto
Acurex Corporation
485 Clyde Avenue
Mountain View, CA 94042

Date: June 18, 1981

Analyst: J. Oldham

P. O. No.: Subcontract SW 59159A

Sample No.: Adelphi-1 XAD 3/130 g

IAD No. 97-G452-116-30

SPARK SOURCE MASS SPECTROGRAPHIC ANALYSIS

CONCENTRATION IN PPM WEIGHT

ELEMENT	CONC.	ELEMENT	CONC.	ELEMENT	CONC.	ELEMENT	CONC.
Uranium	<0.5	Terbium		Ruthenium		Vanadium	0.1
Thorium	<0.7	Gadolinium		Molybdenum	*6	Titanium	0.9
Bismuth		Europium		Niobium		Scandium	≤0.1
Lead	1	Samarium		Zirconium	0.5	Calcium	110
Thallium		Neodymium		Yttrium		Potassium	48
Mercury	NR	Praseodymium		Strontium	1	Chlorine	13
Gold		Cerium	0.4	Rubidium	<0.1	Sulfur	24
Platinum	0.8	Lanthanum	0.4	Bromine	3	Phosphorus	6
Iridium		Barium	7	Selenium	0.1	Silicon	12
Osmium		Cesium		Arsenic	≤0.1	Aluminum	4
Rhenium		Iodine		Germanium		Magnesium	4
Tungsten		Tellurium		Gallium	0.7	Sodium	66
Tantalum		Antimony		Zinc	3	Fluorine	≈3
Hafnium		Tin		Copper	3	Oxygen	NR
Lutetium		Indium	STD	Nickel	3	Nitrogen	NR
Ytterbium		Cadmium		Cobalt	≤0.1	Carbon	NR
Thulium		Silver		Iron	8	Boron	0.2
Erbium		Palladium		Manganese	1	Beryllium	
Holmium		Rhodium		Chromium	2	Lithium	0.2
Dysprosium						Hydrogen	NR

STD — Internal Standard
NR — Not Reported
All elements not detected < 0.1ppm
MC — Major Component
INT — Interference

* Heterogeneous

Approved: M Specht

COMMERCIAL TESTING & ENGINEERING CO.
GENERAL OFFICES: 228 NORTH LA SALLE STREET, CHICAGO ILLINOIS 60601 AREA CODE 312 726-8434
Reply to INSTRUMENTAL ANALYSIS DIVISION 14335 WEST 44TH AVENUE GOLDEN COLORADO 80401 PHONE 303-278-9521

To: Mr. R. Belletto
Acurex Corporation
485 Clyde Avenue
Mountain View, CA 94042

Date June 18, 1981

Analyst J. Oldham

P. O. No.: Subcontract SW 59159A

Sample No.: Adelphi-1 Imp 100/1780 ml

SPARK SOURCE MASS SPECTROGRAPHIC ANALYSIS

IAD No 97-G452-116-30

CONCENTRATION IN µg/ml

ELEMENT	CONC.	ELEMENT	CONC.	ELEMENT	CONC.	ELEMENT	CONC
Uranium	<0.003	Terbium		Ruthenium		Vanadium	0.009
Thorium	<0.004	Gadolinium		Molybdenum	0.2	Titanium	0.05
Bismuth		Europium		Niobium	0.002	Scandium	<0.001
Lead	0.8	Samarium		Zirconium	0.007	Calcium	0.7
Thallium		Neodymium		Yttrium	<0.001	Potassium	0.7
Mercury	NR	Praseodymium		Strontium	0.003	Chlorine	0.03
Gold		Cerium	<0.001	Rubidium	<0.001	Sulfur	>3
Platinum		Lanthanum	≤0.001	Bromine	≤0.008	Phosphorus	0.2
Iridium		Barium	0.02	Selenium	≤0.01	Silicon	0.4
Osmium		Cesium	<0.001	Arsenic	0.003	Aluminum	0.2
Rhenium		Iodine	<0.001	Germanium	0.001	Magnesium	0.08
Tungsten		Tellurium	<0.001	Gallium	0.004	Sodium	>1
Tantalum		Antimony	0.004	Zinc	MC	Fluorine	≈0.2
Hafnium		Tin	0.004	Copper	0.6	Oxygen	NR
Lutetium		Indium	STD	Nickel	MC	Nitrogen	NR
Ytterbium		Cadmium	<0.001	Cobalt	0.002	Carbon	NR
Thulium		Silver	0.02	Iron	MC	Boron	0.001
Erbium		Palladium		Manganese	0.5	Beryllium	
Holmium		Rhodium		Chromium	MC	Lithium	0.06
Dysprosium						Hydrogen	NR

STD — Internal Standard
NR — Not Reported
All elements not detected < 0.001µg/ml
MC — Major Component >10µg/ml
INT — Interference

Approved: M. Yocco

COMMERCIAL TESTING & ENGINEERING CO.
GENERAL OFFICES: 228 NORTH LA SALLE STREET, CHICAGO ILLINOIS 60601 AREA CODE 312 726-8434
Reply to INSTRUMENTAL ANALYSIS DIVISION, 14335 WEST 44TH AVENUE, GOLDEN, COLORADO 80401, PHONE 303-278-9521

To: Mr. R. Belletto
Acurex Corporation
485 Clyde Avenue
Mountain View, CA 94042

Date: June 19, 1981

Analyst: J. Oldham

P. O. No.: Subcontract SW 59159A

Sample No.: Adelphi-1 Imp 1 Blank-2 90ml

IAD No. 97-G452-116-30

SPARK SOURCE MASS SPECTROGRAPHIC ANALYSIS

CONCENTRATION IN μg/ml

ELEMENT	CONC.	ELEMENT	CONC.	ELEMENT	CONC.	ELEMENT	CONC.
Uranium	<0.007	Terbium		Ruthenium		Vanadium	<0.001
Thorium	<0.009	Gadolinium		Molybdenum		Titanium	0.003
Bismuth		Europium		Niobium		Scandium	<0.001
Lead	≤0.004	Samarium		Zirconium	0.002	Calcium	0.3
Thallium		Neodymium		Yttrium		Potassium	0.2
Mercury	NR	Praseodymium		Strontium	<0.001	Chlorine	0.02
Gold		Cerium		Rubidium		Sulfur	2
Platinum		Lanthanum		Bromine	0.003	Phosphorus	0.2
Iridium		Barium	0.005	Selenium	≤0.003	Silicon	0.3
Osmium		Cesium		Arsenic	*0.003	Aluminum	0.05
Rhenium		Iodine		Germanium		Magnesium	0.1
Tungsten		Tellurium		Gallium	<0.001	Sodium	>3
Tantalum		Antimony		Zinc	0.07	Fluorine	≤0.04
Hafnium		Tin		Copper	0.006	Oxygen	NR
Lutetium		Indium	STD	Nickel	0.004	Nitrogen	NR
Ytterbium		Cadmium		Cobalt	<0.001	Carbon	NR
Thulium		Silver	0.2	Iron	0.03	Boron	<0.001
Erbium		Palladium		Manganese	0.001	Beryllium	
Holmium		Rhodium		Chromium	0.01	Lithium	
Dysprosium						Hydrogen	NR

STD — Internal Standard
NR — Not Reported
All elements not detected < 0.001μg/ml
MC — Major Component >10μg/ml
INT — Interference

* Heterogeneous

Approved: M Specht

COMMERCIAL TESTING & ENGINEERING CO.

GENERAL OFFICES: 228 NORTH LA SALLE STREET, CHICAGO ILLINOIS 60601 AREA CODE 312 726-8434
Reply to INSTRUMENTAL ANALYSIS DIVISION, 14335 WEST 44TH AVENUE GOLDEN COLORADO 80401 PHONE 303 278-9521

To: Mr. R. Belletto
Acurex Corporation
485 Clyde Avenue
Mountain View, CA 94042

Date June 18, 1981

Analyst J. Oldham

P. O. No.: Subcontract SW 59159A

Sample No.: 813892 Adelphi (OW+SA(38.6/39 .2/14.5/7.7)

SPARK SOURCE MASS SPECTROGRAPHIC ANALYSIS

CONCENTRATION IN PPM WEIGHT

IAD No.: 97-G452-116-30

ELEMENT	CONC.	ELEMENT	CONC.	ELEMENT	CONC.	ELEMENT	CONC
Uranium	14	Terbium	0.2	Ruthenium		Vanadium	68
Thorium	4	Gadolinium	0.5	Molybdenum	5	Titanium	MC
Bismuth		Europium	0.2	Niobium	2	Scandium	0.6
Lead	2	Samarium	0.8	Zirconium	12	Calcium	MC
Thallium	≤0.2	Neodymium	2	Yttrium	13	Potassium	MC
Mercury	NR	Praseodymium	2	Strontium	29	Chlorine	2
Gold		Cerium	11	Rubidium	≤0.7	Sulfur	MC
Platinum		Lanthanum	11	Bromine	2	Phosphorus	*MC
Iridium		Barium	MC	Selenium	5	Silicon	MC
Osmium		Cesium	1	Arsenic	NR	Aluminum	41
Rhenium		Iodine		Germanium	0.9	Magnesium	MC
Tungsten	0.9	Tellurium		Gallium	1	Sodium	MC
Tantalum		Antimony	NR	Zinc	3	Fluorine	≈0.6
Hafnium	0.2	Tin		Copper	5	Oxygen	NR
Lutetium		Indium	STD	Nickel	17	Nitrogen	NR
Ytterbium		Cadmium		Cobalt	0.1	Carbon	NR
Thulium		Silver		Iron	MC	Boron	1
Erbium	<0.1	Palladium		Manganese	52	Beryllium	0.2
Holmium	0.2	Rhodium		Chromium	MC	Lithium	0.1
Dysprosium	0.4					Hydrogen	NR

STD — Internal Standard
NR — Not Reported
All elements not detected < 0.1ppm
MC — Major Component >100ppm
INT — Interference

* Heterogeneous

5-15

Approved: M Yocoots

COMMERCIAL TESTING & ENGINEERING CO.
GENERAL OFFICES 228 NORTH LA SALLE STREET, CHICAGO, ILLINOIS 60601 AREA CODE 312 726-8434
Reply to INSTRUMENTAL ANALYSIS DIVISION, 14335 WEST 44TH AVENUE, GOLDEN, COLORADO 80401 PHONE 303-278-9521

To: Mr. Jim Steiner
Acurex Corporation
485 Clyde Avenue
Mountain View, CA 94042

Date: July 24, 1981

Analyst: J. Oldham

P. O. No.: Subcontract SW 59159A
Sample No.: Adelphi-2
3+10μ + F.H.

SPARK SOURCE MASS SPECTROGRAPHIC ANALYSIS

IAD No. 97-G452-116-30

CONCENTRATION IN PPM WEIGHT

ELEMENT	CONC.	ELEMENT	CONC.	ELEMENT	CONC.	ELEMENT	CONC.
Uranium	34	Terbium	0.4	Ruthenium		Vanadium	480
Thorium	4	Gadolinium	3	Molybdenum	24	Titanium	900
Bismuth	≤0.2	Europium	0.6	Niobium	5	Scandium	6
Lead	440	Samarium	5	Zirconium	62	Calcium	MC
Thallium	3	Neodymium	5	Yttrium	31	Potassium	MC
Mercury	NR	Praseodymium	2	Strontium	68	Chlorine	540
Gold		Cerium	45	Rubidium	21	Sulfur	>590
Platinum		Lanthanum	44	Bromine	21	Phosphorus	410
Iridium		Barium	450	Selenium	12	Silicon	MC
Osmium		Cesium	1	Arsenic	20	Aluminum	>95
Rhenium		Iodine	2	Germanium	10	Magnesium	MC
Tungsten	0.9	Tellurium	0.3	Gallium	14	Sodium	>240
Tantalum		Antimony	0.6	Zinc	63	Fluorine	≈8
Hafnium		Tin	0.6	Copper	29	Oxygen	NR
Lutetium	0.2	Indium	STD	Nickel	99	Nitrogen	NR
Ytterbium	2	Cadmium	0.3	Cobalt	2	Carbon	NR
Thulium	0.3	Silver	2	Iron	MC	Boron	72
Erbium	0.9	Palladium		Manganese	320	Beryllium	2
Holmium	1	Rhodium		Chromium	49	Lithium	13
Dysprosium	2					Hydrogen	NR

STD — Internal Standard
NR — Not Reported
All elements not detected < 0.1 ppm
MC — Major Component
INT — Interference

Approved: M. Gerald

COMMERCIAL TESTING & ENGINEERING CO.

GENERAL OFFICES: 228 NORTH LA SALLE STREET, CHICAGO, ILLINOIS 60601 AREA CODE 312 726-8434
Reply to INSTRUMENTAL ANALYSIS DIVISION, 14335 WEST 44TH AVENUE GOLDEN COLORADO 80401 PHONE 303-278-9521

To: Mr. R. Belletto
Acurex Corporation
485 Clyde Avenue
Mountain View, CA 94042

Date June 19, 1981

Analyst J. Oldham

P. O. No.: Subcontract SW 59159A

Sample No.: Adelphi-2 1μ + Filter 1.8721/ 16.4323g

SPARK SOURCE MASS SPECTROGRAPHIC ANALYSIS

CONCENTRATION IN PPM WEIGHT

IAD No. 97-G452-116-30

ELEMENT	CONC.	ELEMENT	CONC.	ELEMENT	CONC.	ELEMENT	CONC
Uranium	8	Terbium		Ruthenium		Vanadium	68
Thorium		Gadolinium		Molybdenum	25	Titanium	270
Bismuth		Europium		Niobium	1	Scandium	≤0.1
Lead	170	Samarium		Zirconium	3	Calcium	MC
Thallium	2	Neodymium		Yttrium	3	Potassium	MC
Mercury	NR	Praseodymium	0.5	Strontium	15	Chlorine	MC
Gold		Cerium	5	Rubidium	20	Sulfur	>560
Platinum		Lanthanum	4	Bromine	20	Phosphorus	160
Iridium		Barium	220	Selenium	5	Silicon	MC
Osmium		Cesium	0.6	Arsenic	25	Aluminum	>90
Rhenium		Iodine	0.4	Germanium	5	Magnesium	420
Tungsten	2	Tellurium	≤0.1	Gallium	7	Sodium	>230
Tantalum		Antimony	0.4	Zinc	26	Fluorine	≈140
Hafnium		Tin	0.3	Copper	12	Oxygen	NR
Lutetium		Indium	STD	Nickel	17	Nitrogen	NR
Ytterbium		Cadmium		Cobalt	2	Carbon	NR
Thulium		Silver	0.4	Iron	MC	Boron	21
Erbium		Palladium		Manganese	20	Beryllium	0.3
Holmium		Rhodium		Chromium	83	Lithium	6
Dysprosium						Hydrogen	NR

STD — Internal Standard
NR — Not Reported
All elements not detected < 0.1ppm
MC — Major Component
INT — Interference

Approved: [signature]

COMMERCIAL TESTING & ENGINEERING CO.
GENERAL OFFICES 228 NORTH LA SALLE STREET, CHICAGO, ILLINOIS 60601 AREA CODE 312 726-8434
Reply to INSTRUMENTAL ANALYSIS DIVISION, 14335 WEST 44TH AVENUE, GOLDEN, COLORADO 80401, PHONE: 303-278-9521

To: Mr. R. Belletto
485 Clyde Avenue
Mountain View, CA 94042

Date: June 19, 1981

Analyst: J. Oldham

P. O. No.: Subcontract SW 59159A

Sample No.: Adelphi-2
XAD 3/130g

SPARK SOURCE MASS SPECTROGRAPHIC ANALYSIS
CONCENTRATION IN PPM WEIGHT

IAD No. 97-G452-116-30

ELEMENT	CONC.	ELEMENT	CONC.	ELEMENT	CONC.	ELEMENT	CONC.
Uranium	<0.8	Terbium		Ruthenium		Vanadium	<0.1
Thorium	<1	Gadolinium		Molybdenum	1	Titanium	3
Bismuth		Europium		Niobium		Scandium	<0.1
Lead	0.5	Samarium		Zirconium	0.7	Calcium	17
Thallium		Neodymium		Yttrium	0.1	Potassium	7
Mercury	NR	Praseodymium		Strontium	<0.1	Chlorine	2
Gold		Cerium	0.3	Rubidium		Sulfur	9
Platinum	*1	Lanthanum	0.3	Bromine	1	Phosphorus	1
Iridium		Barium	1	Selenium	0.6	Silicon	17
Osmium		Cesium		Arsenic	0.1	Aluminum	0.6
Rhenium		Iodine		Germanium		Magnesium	5
Tungsten		Tellurium		Gallium	0.1	Sodium	6
Tantalum		Antimony		Zinc	2	Fluorine	≈1
Hafnium		Tin		Copper	0.7	Oxygen	NR
Lutetium		Indium	STD	Nickel	18	Nitrogen	NR
Ytterbium		Cadmium		Cobalt	0.2	Carbon	NR
Thulium		Silver	0.5	Iron	7	Boron	0.3
Erbium		Palladium		Manganese	0.6	Beryllium	
Holmium		Rhodium		Chromium	5	Lithium	<0.1
Dysprosium						Hydrogen	NR

STD — Internal Standard
NR — Not Reported
All elements not detected < 0.1ppm
MC — Major Component
INT — Interference

* Heterogeneous

Approved: M Yeeoes

COMMERCIAL TESTING & ENGINEERING CO.
GENERAL OFFICES: 228 NORTH LA SALLE STREET, CHICAGO ILLINOIS 60601 AREA CODE 312 726-8434
Reply to INSTRUMENTAL ANALYSIS DIVISION, 14335 WEST 44TH AVENUE GOLDEN, COLORADO 80401 PHONE 303 278-9521

To: Mr. R. Belletto
Acurex Corporation
485 Clyde Avenue
Mountain View, CA 94042

Date June 19, 1981

Analyst J. Oldham

P. O. No.: Subcontract SW 59159A

Sample No.: Adelphi-2 1st Imp 100/940ml

SPARK SOURCE MASS SPECTROGRAPHIC ANALYSIS

IAD No 97-G452-116-30

CONCENTRATION IN µg/ml

ELEMENT	CONC.	ELEMENT	CONC.	ELEMENT	CONC.	ELEMENT	CONC
Uranium	<0.01	Terbium		Ruthenium		Vanadium	0.001
Thorium	<0.01	Gadolinium		Molybdenum	0.05	Titanium	0.002
Bismuth		Europium		Niobium	0.004	Scandium	≤0.002
Lead	0.07	Samarium		Zirconium	0.003	Calcium	0.6
Thallium		Neodymium		Yttrium		Potassium	1
Mercury	NR	Praseodymium		Strontium	0.003	Chlorine	0.09
Gold		Cerium		Rubidium	0.004	Sulfur	MC
Platinum		Lanthanum		Bromine	0.01	Phosphorus	0.03
Iridium		Barium	0.02	Selenium	0.2	Silicon	0.1
Osmium		Cesium	0.002	Arsenic		Aluminum	0.2
Rhenium		Iodine		Germanium		Magnesium	*0.7
Tungsten		Tellurium		Gallium	0.002	Sodium	>4
Tantalum		Antimony		Zinc	1	Fluorine	≈0.1
Hafnium		Tin	0.005	Copper	0.5	Oxygen	NR
Lutetium		Indium	STD	Nickel	2	Nitrogen	NR
Ytterbium		Cadmium	0.005	Cobalt	≤0.002	Carbon	NR
Thulium		Silver	0.02	Iron	MC	Boron	<0.001
Erbium		Palladium		Manganese	0.08	Beryllium	
Holmium		Rhodium		Chromium	0.2	Lithium	0.02
Dysprosium						Hydrogen	NR

STD — Internal Standard
NR — Not Reported
All elements not detected < 0.001 µg/ml
MC — Major Component >10 µg/ml
INT — Interference

* Heterogeneous

5-19 Approved: M Specht

COMMERCIAL TESTING & ENGINEERING CO.

GENERAL OFFICES 228 NORTH LA SALLE STREET, CHICAGO ILLINOIS 60601 AREA CODE 312 726-8434
Reply to INSTRUMENTAL ANALYSIS DIVISION, 14335 WEST 44TH AVENUE, GOLDEN, COLORADO 80401 PHONE 303-278-9521

To: Mr. R. Belletto
Acurex Corporation
485 Clyde Avenue
Mountain View, CA 94042

Date: June 23, 1981

Analyst: J. Oldham

P. O. No.: Subcontract SW 59159A

Sample No.: CMEA Filter Blank

SPARK SOURCE MASS SPECTROGRAPHIC ANALYSIS

IAD No. 97-452-116-30

CONCENTRATION IN $\mu g/cm^2$

ELEMENT	CONC.	ELEMENT	CONC.	ELEMENT	CONC.	ELEMENT	CONC
Uranium	<0.001	Terbium		Ruthenium		Vanadium	<0.001
Thorium	<0.002	Gadolinium		Molybdenum		Titanium	0.02
Bismuth		Europium		Niobium		Scandium	<0.001
Lead	0.003	Samarium		Zirconium	*0.005	Calcium	MC
Thallium		Neodymium		Yttrium	<0.001	Potassium	>2
Mercury	NR	Praseodymium		Strontium	0.002	Chlorine	0.1
Gold		Cerium	<0.001	Rubidium	<0.001	Sulfur	0.02
Platinum		Lanthanum	<0.001	Bromine	0.04	Phosphorus	0.3
Iridium		Barium	0.02	Selenium	<0.001	Silicon	MC
Osmium		Cesium		Arsenic	<0.001	Aluminum	>0.2
Rhenium		Iodine	<0.001	Germanium		Magnesium	0.8
Tungsten		Tellurium		Gallium	<0.001	Sodium	0.5
Tantalum		Antimony		Zinc	0.01	Fluorine	≈0.06
Hafnium		Tin		Copper	0.002	Oxygen	NR
Lutetium		Indium	STD	Nickel	0.002	Nitrogen	NR
Ytterbium		Cadmium		Cobalt	0.001	Carbon	NR
Thulium		Silver		Iron	0.06	Boron	0.2
Erbium		Palladium		Manganese	<0.001	Beryllium	<0.001
Holmium		Rhodium		Chromium	<0.001	Lithium	0.002
Dysprosium		* Heterogeneous				Hydrogen	NR

STD — Internal Standard
NR — Not Reported
All elements not detected < 0.001 $\mu g/cm^2$
MC — Major Component
INT — Interference

5-20

Approved:

COMMERCIAL TESTING & ENGINEERING CO.

GENERAL OFFICES 228 NORTH LA SALLE STREET, CHICAGO ILLINOIS 60601 AREA CODE 312 726-8434

Reply to INSTRUMENTAL ANALYSIS DIVISION, 14335 WEST 44TH AVENUE GOLDEN COLORADO 80401 PHONE 303 278-9521

To: Mr. R. Belletto
Acurex Corporation
485 Clyde Avenue
Mountain View, CA 94042

Date June 23, 1981

Analyst J. Oldham

P. O. No.: Subcontract SW 59159A

Sample No.: XAD Blank

SPARK SOURCE MASS SPECTROGRAPHIC ANALYSIS

IAD No 97-G452-116-30

CONCENTRATION IN PPM WEIGHT

ELEMENT	CONC	ELEMENT	CONC.	ELEMENT	CONC	ELEMENT	CONC
Uranium	<0.3	Terbium		Ruthenium		Vanadium	<0.1
Thorium	<0.4	Gadolinium		Molybdenum	1	Titanium	0.8
Bismuth		Europium		Niobium	<0.1	Scandium	<0.1
Lead	0.2	Samarium		Zirconium	0.1	Calcium	37
Thallium		Neodymium		Yttrium	<0.1	Potassium	*30
Mercury	NR	Praseodymium		Strontium	<0.1	Chlorine	1
Gold		Cerium		Rubidium		Sulfur	4
Platinum	0.5	Lanthanum	0.1	Bromine	1	Phosphorus	7
Iridium		Barium	11	Selenium	0.1	Silicon	74
Osmium		Cesium	<0.1	Arsenic	≤0.1	Aluminum	*1
Rhenium		Iodine		Germanium		Magnesium	1
Tungsten		Tellurium		Gallium	≤0.2	Sodium	6
Tantalum		Antimony		Zinc	3	Fluorine	≈0.2
Hafnium		Tin	0.1	Copper	2	Oxygen	NR
Lutetium		Indium	STD	Nickel	9	Nitrogen	NR
Ytterbium		Cadmium		Cobalt	<0.1	Carbon	NR
Thulium		Silver	0.2	Iron	5	Boron	0.1
Erbium		Palladium		Manganese	0.2	Beryllium	
Holmium		Rhodium		Chromium	0.6	Lithium	<0.1
Dysprosium						Hydrogen	NR

STD — Internal Standard
NR — Not Reported
All elements not detected < 0.1ppm
MC — Major Component
INT — Interference

* Heterogeneous

5-21

Approved: M Speeds

ACUREX Corporation
ANALYSIS LABORATORIES

DATA REPORTING FORM

CUSTOMER CMEA DATE

CUSTOMER CONTRACT NO. 307605.62 ACUREX CONTRACT NO. A81-04-004

RESULTS REPORT TO C. Castaldini TELEPHONE

ADDRESS

Adelphi 1 23.776

SAMPLE ID (CUSTOMER)	398	420	424	406	429					
SAMPLE ID (LAB)	10μ+3μ	Filter +1μ	XAD	Imp 1	Imp 2+3					
PARAMETER										UNITS
Hg Aliquot	16	830	7	260	86					ng
Hg Blank	< 10	< 10	8	170	50					ng
Hg	0.011	0.28	< 0.004	0.13	0.051					ug/dscm
As Aliquot	-	-	-	-	< 10					ug/L
As	-	-	-	-	< 0.7					ug/dscm
Sb Aliquot	-	-	-	-	< 10					ug/L
Sb	-	-	-	-	< 0.7					ug/dscm

ANALYST

REVIEWER

Form EED-057 4 80

ACUREX Corporation
ANALYSIS LABORATORIES

DATA REPORTING FORM

CUSTOMER CMEA DATE

CUSTOMER CONTRACT NO. 307605.62 ACUREX CONTRACT NO. A81-04-004

RESULTS REPORT TO C. Castaldini TELEPHONE

ADDRESS

Adelphi 2 6.142 dscm

SAMPLE ID (CUSTOMER)	439	437	436	440	448					
SAMPLE ID (LAB)	10μ+3μFHR	Filter+1μ	XAD	Imp 1	Imp 2+3					
PARAMETER										UNITS
Hg Aliquot	< 10	< 10	38	440	14					ng
Hg blank	< 10	< 10	8	170	50					ng
Hg	< 10	< 10	63	0.83	< 0.2					ug/dscm
As Aliquot	-	-	-	-	< 10					ug/L
As	-	-	-	-	< 2					ug/dscm
Sb Aliquot	-	-	-	-	< 10					ug/L
Sb	-	-	-	-	< 2					ug/dscm

ANALYST

REVIEWER

Form EED 057 4-80

5.5 TOTAL CHROMATOGRAPHABLE ORGANICS (TCO), GRAVIMETRIC ORGANICS (GRAV), INFRARED (IR) SPECTRA, AND GAS CHROMATOGRAPHY/MASS SPECTROMETRY (GC/MS) OF TOTAL SAMPLE EXTRACTS

ACUREX Corporation

Energy & Environmental Division

May 22, 1981
Lab ID Number: A81-04-004
Customter P.O. Number: 307605

CMEA
MS #2-2260

Attention: C. Castaldini

Sample: 5 SASS Trains, received April 7, 1981

The above referenced samples were received and analyzed per Level 1 protocol. Arsenic and antimony were determined by furnace AAS as per directive of B. Higginbotham.

Polynuclears were determined by a modified method 625. 1 ul of a sample was injected on a SE-54 J+W 30 meter capillary column using Grob injection. The column was held at 35° C for 5 minutes then ramped at 10° C/minute to 300° C.

Prepared by: Greg Nicoll
Greg Nicoll
Manager, Organics

Athorized by: William L. Fitch
William L. Fitch, Ph. D.
Technical Director

485 CLYDE AVENUE. MOUNTAIN VIEW. CA 94042 PHONE (415) 964-3200 TELEX: 34-6391 TWX 910-3796593

ACUREX Corporation
ANALYSIS LABORATORIES

DATA REPORTING FORM

CUSTOMER CMEA DATE

CUSTOMER CONTRACT NO. 307605.62 ACUREX CONTRACT NO. A81-04-004

RESULTS REPORT TO C. Castaldini TELEPHONE

ADDRESS

Adelphi 1 23.776 dscm

SAMPLE ID (CUSTOMER)	398	420	424	427						
SAMPLE ID (LAB)	10μ+3μ	Filter +7μ	XAD	OMC						
PARAMETER										UNITS
GRAV Aliquot	< 4	< 4	87	< 4						mg
GRAV Blank	< 4	< 4	19	< 4						mg
GRAV	< 200	< 300	2900	< 200						ug/dscm
TCO Aliquot	-	-	1.4	< 0.1						mg
TCO Blank	-	-	1	< 0.1						mg
TCO	-	-	< 40	< 4						ug/dscm
Fluoranthene Aliquot	< 1	< 1	1	< 1						ng
Phenanthene Aliquot	< 1	< 1	3	< 1						ng
Fluoranthene	< 0.05	< 0.07	0.05	< 0.04						ug/dscm
Phenanthene	< 0.05	< 0.07	0.1	< 0.04						ug/dscm
Other Poylnuclears	< 0.05	< 0.07	< 0.05	< 0.04						ug/dscm

ANALYST

REVIEWER

Form EED-057 4-80

IR REPORT

SAMPLE: A81-04-004-424 Adelphi I XAD

Wave Number (cm^{-1})	Intensity	Assignment	Comments
3460-3040	w	O-H (COOH)	
2910	s	C-H Alkane	
2850	s	C-H Alkane	
1770	s	C=O (COOH)	
1468	m	C-H Alkane	
1450	m	C-H Alkane	
1280	s	C-O (COOH)	
1130	s	C-O	
1080	s	C-O	
970	m	C-C Alkane	

IR REPORT

SAMPLE: A81-04-004-398 Adelphi 1 10 μ + 3 μ

Wave Number (cm^{-1})	Intensity	Assignment	Comments
3440-2960	w	O-H (COOH)	
2910	s	C-H Alkane	
2850	s	C-H Alkane	

IR REPORT

SAMPLE: A81-04-004-420 Adelphi 1 Filter + 1μ

Wave Number (cm^{-1})	Intensity	Assignment	Comments
3480-2960	m	O-H (COOH)	Weak Spectrum
2920-2360	s	C-H Alkane	

IR REPORT

SAMPLE: A81-04-004-427 Adelphi - 1 OMC

Wave Number (cm^{-1})	Intensity	Assignment	Comments
3540-3000	m	O-H (COOH)	Weak Spectrum
2920-2320	s	C-H Alkane	

ACUREX Corporation
ANALYSIS LABORATORIES

DATA REPORTING FORM

CUSTOMER CMEA DATE

CUSTOMER CONTRACT NO. 307605.62 ACUREX CONTRACT NO. A81-04-004

RESULTS REPORT TO C. Castaldini TELEPHONE

ADDRESS

Adelphi 2 6.142 dscm

SAMPLE ID (CUSTOMER)	439	437	436	435						
SAMPLE ID (LAB)	10μ+3μ+FHR	1μ+Filter	XAD	OMC						
PARAMETER										UNITS
GRAV Aliquot	8	7	48	< 4						mg
GRAV Blank	< 4	< 4	19	< 4						mg
GRAV	2000	2000	5000	< 700						ug/dscm
TCO Aliquot	-	-	0.3	< 0.1						mg
TCO Blank	-	-	1	< 0.1						mg
TCO	-	-	< 200	< 20						ug/dscm
Phenanthene Aliquot	< 1	< 1	4	< 1						ng
Phenanthene	< 0.3	< 0.3	0.7	< 0.2						ug/dscm
Other Polynuclears	< 0.3	< 0.3	< 0.2	< 0.2						ug/dscm

ANALYST

REVIEWER

Form EED-057 4 80

IR REPORT

SAMPLE: A81-04-004-439 Adelphi 2 10μ+3μ

Wave Number (cm^{-1})	Intensity	Assignment	Comments
3620-2980	w	O-H (COOH)	
2910	s	C-H Alkane	
2840	s	C-H Alkane	
1730	w	C=O (COOH)	

IR REPORT

SAMPLE: A81-04-004-437 Adelphi 2 Filter + 1μ

Wave Number (cm^{-1})	Intensity	Assignment	Comments
3460-2960	w	O-H (COOH)	
2920	s	C-H Alkane	
2840	s	C-H Alkane	
1100	m	C-O	
970	w	C-C Alkane	

IR REPORT

SAMPLE: A81-04-004-436 Adelphi 2 XAD

Wave Number (cm^{-1})	Intensity	Assignment	Comments
3440-3000	w	O-H (COOH)	
2920	s	C-H Alkane	
2840	s	C-H Alkane	
1730	m	C=O (COOH)	
1270	w	C-O	
1070	w	C-O	
970	w	C-C Alkane	

IR REPORT

SAMPLE: A81-04-004-435 Adelphi 2 OMC

Wave Number (cm^{-1})	Intensity	Assignment	Comments
3440-2980	s	O-H (COOH)	Weak Spectrum
2920-2390	m	C-H Alkane	

5.6 LIQUID CHROMATOGRAPHY (LC) SEPARATION, INFRARED (IR) SPECTRA OF LC FRACTIONS, AND LOW RESOLUTION MASS SPECTROMETRY (LRMS) OF SELECTED LC FRACTIONS AND TOTAL SAMPLE EXTRACTS

SAMPLE: Adelphi 1 XAD #424

	TCO mg	GRAV mg	TCO + GRAV Total mg	Concentration mg/dscm
Total Sample[1]	< 1	68	68	2.9
Taken for LC[2]	-	34	34	1.4
Recovered[3]	-	23	23	0.97

Fraction	TCO in mg				GRAV in mg				TCO + GRAV Total mg	Concentration mg/dscm
	Found in Fraction	Blank	Corrected	Total[4]	Found in Fraction	Blank	Corrected	Total[4]		
1					7	<1	7	14	14	0.59
2					6.0	<0.8	6.0	12	12	0.50
3					5.2	<0.8	5.2	10	10	0.42
4					1.8	<1.2	<0.8	< 2	<2	< 0.08
5					<0.8	<0.8	<0.8	< 2	<2	< 0.08
6					3.2	<0.8	3.2	6	6	0.25
7					1.4	<0.8	1.4	3	3	0.13
Sum					25	<6	23	45	45	1.9

1. Quantity in entire sample, determined before LC
2. Portion of whole sample used for LC, actual mg
3. Quantity recovered from LC column, actual mg
4. Total mg computed back to total sample

IR REPORT

SAMPLE: Adelphi 1 XAD 424 F1

Wave Number (cm^{-1})	Intensity	Assignment	Comments
2920	s	CH Aliphatic hydrocarbons	
2840	s	CH Aliphatic Hydrocarbons	
1740	m	C=O Aliphatic hydrocarbons	
1270	m	CO Aliphatic hydrocarbons	

IR REPORT

SAMPLE: Adelphi 1 XAD 424 F2

Wave Number (cm^{-1})	Intensity	Assignment	Comments
2920	s	CH Aliphatic oxygen compounds	
2840	s	CH Aliphatic oxygen compounds	
1730	s	C=O Aliphatic oxygen compounds	
1270	s	CO Aliphatic oxygen compounds	

IR REPORT

SAMPLE: Adelphi 1 XAD 424 F3

Wave Number (cm^{-1})	Intensity	Assignment	Comments
2920	s	CH Aliphatic oxygen compounds	
2860	s	CH Aliphatic oxygen compounds	
1730	s	C=O Aliphatic oxygen compounds	
1280	s	CO Aliphatic oxygen compounds	

IR REPORT

SAMPLE: Adelphi 1 XAD 424 F4

Wave Number (cm^{-1})	Intensity	Assignment	Comments
		No Peaks	

IR REPORT

SAMPLE: Adelphi 1 XAD 424 F5

Wave Number (cm^{-1})	Intensity	Assignment	Comments
		No Peaks	

IR REPORT

SAMPLE: Adelphi 1 XAD 424 F6

Wave Number (cm^{-1})	Intensity	Assignment	Comments
2860	s	CH Alcohols	
1110-1070	m	CO Alcohols	

IR REPORT

SAMPLE: Adelphi 1 XAD 424 F7

Wave Number (cm^{-1})	Intensity	Assignment	Comments
		No Peaks	

LRMS REPORT

SAMPLE: 424 F1 Adelphi 1 XAD

Major Categories

Intensity	Category	MW Range
1	Aliphatic hydrocarbons	

Sub-Categories, Specific Compounds

Intensity	Category	m/e	Composition

Other

LRMS REPORT

SAMPLE: 424 F2 Adelphi 1 XAD

Major Categories

Intensity	Category	MW Range
	None found	

Sub-Categories, Specific Compounds

Intensity	Category	m/e	Composition

Other

LRMS REPORT

SAMPLE: 424 F3 Adelphi 1 XAD

Major Categories

Intensity	Category	MW Range
	None found	

Sub-Categories, Specific Compounds

Intensity	Category	m/e	Composition

Other

SAMPLE: Adelphi 2 XAD #436

	TCO mg	GRAV mg	TCO + GRAV Total mg	Concentration mg/dscm
Total Sample[1]	< 1	29	29	4.7
Taken for LC[2]	-	20	20	3.3
Recovered[3]	-	12	12	2.0

Fraction	TCO in mg				GRAV in mg				TCO + GRAV Total mg	Concentration mg/dscm
	Found in Fraction	Blank	Corrected	Total[4]	Found in Fraction	Blank	Corrected	Total[4]		
1					< 1	< 1	< 1	< 1	< 1	< 0.2
2					4.0	< 0.8	4.0	6	6	1
3					6.2	< 0.8	6.2	9	9	1
4					1.6	< 1.2	< 0.8	< 1	< 1	< 0.2
5					< 0.8	< 0.8	< 0.8	< 1	< 1	< 0.2
6					1.4	< 0.8	1.4	2	2	0.3
7					< 0.8	< 0.8	< 0.8	< 1	< 1	< 0.2
Sum					13	< 6	12	18	18	2.9

1. Quantity in entire sample, determined before LC
2. Portion of whole sample used for LC, actual mg
3. Quantity recovered from LC column, actual mg
4. Total mg computed back to total sample

IR REPORT

SAMPLE: Adelphi 2 XAD 436 F1

Wave Number (cm^{-1})	Intensity	Assignment	Comments
2940-2380	s	CH Aliphatic hydrocarbons	

IR REPORT

SAMPLE: Adelphi 2 436 F2

Wave Number (cm^{-1})	Intensity	Assignment	Comments
2920	s	CH Aliphatic oxygen compounds	
2840	s	CH Aliphatic oxygen compounds	
1730	s	C=O Aliphatic oxygen compounds	
1270	s	CO Aliphatic oxygen compounds	

IR REPORT

SAMPLE: Adelphi 2 XAD 436 F3

Wave Number (cm^{-1})	Intensity	Assignment	Comments
2920-2380	s	CH Aliphatic oxygen compounds	
1730	m	C=O Aliphatic oxygen compounds	
1270	m	CO Aliphatic oxygen compounds	

IR REPORT

SAMPLE: Adelphi 2 XAD 436 F4

Wave Number (cm^{-1})	Intensity	Assignment	Comments
3460-2380	s	OH, CH Aliphatic oxygen compounds	

IR REPORT

SAMPLE: Adelphi 2 XAD 436 F5

Wave Number (cm^{-1})	Intensity	Assignment	Comments
		No Peaks	

IR REPORT

SAMPLE: Adelphi 2 XAD 436 F6

Wave Number (cm^{-1})	Intensity	Assignment	Comments
		No Peaks	

IR REPORT

SAMPLE: Adelphi 2 XAD 436 F7

Wave Number (cm^{-1})	Intensity	Assignment	Comments
		No Peaks	

LRMS REPORT

SAMPLE: 436 F1 Adelphi 2 XAD

Major Categories

Intensity	Category	MW Range
1	Aliphatic hydrocarbons	

Sub-Categories, Specific Compounds

Intensity	Category	m/e	Composition

Other

LRMS REPORT

SAMPLE: 436 F2 Adelphi 2 XAD

Major Categories

Intensity	Category	MW Range
	None found	

Sub-Categories, Specific Compounds

Intensity	Category	m/e	Composition

Other

LRMS REPORT

SAMPLE: 436 F3 Adelphi 2 XAD

Major Categories

Intensity	Category	MW Range
	None found	

Sub-Categories, Specific Compounds

Intensity	Category	m/e	Composition

Other

LRMS REPORT

SAMPLE: 439 Adelphi 2 10μ and 3μ

Major Categories

Intensity	Category	MW Range
1	Aliphatic hydrocarbons	

Sub-Categories, Specific Compounds

Intensity	Category	m/e	Composition

Other

LRMS REPORT

SAMPLE: 437 Adelphi 2 lu and filter

Major Categories

Intensity	Category	MW Range
1	Phenols	
1	Aliphatic hydrocarbons	

Sub-Categories, Specific Compounds

Intensity	Category	m/e	Composition

Other

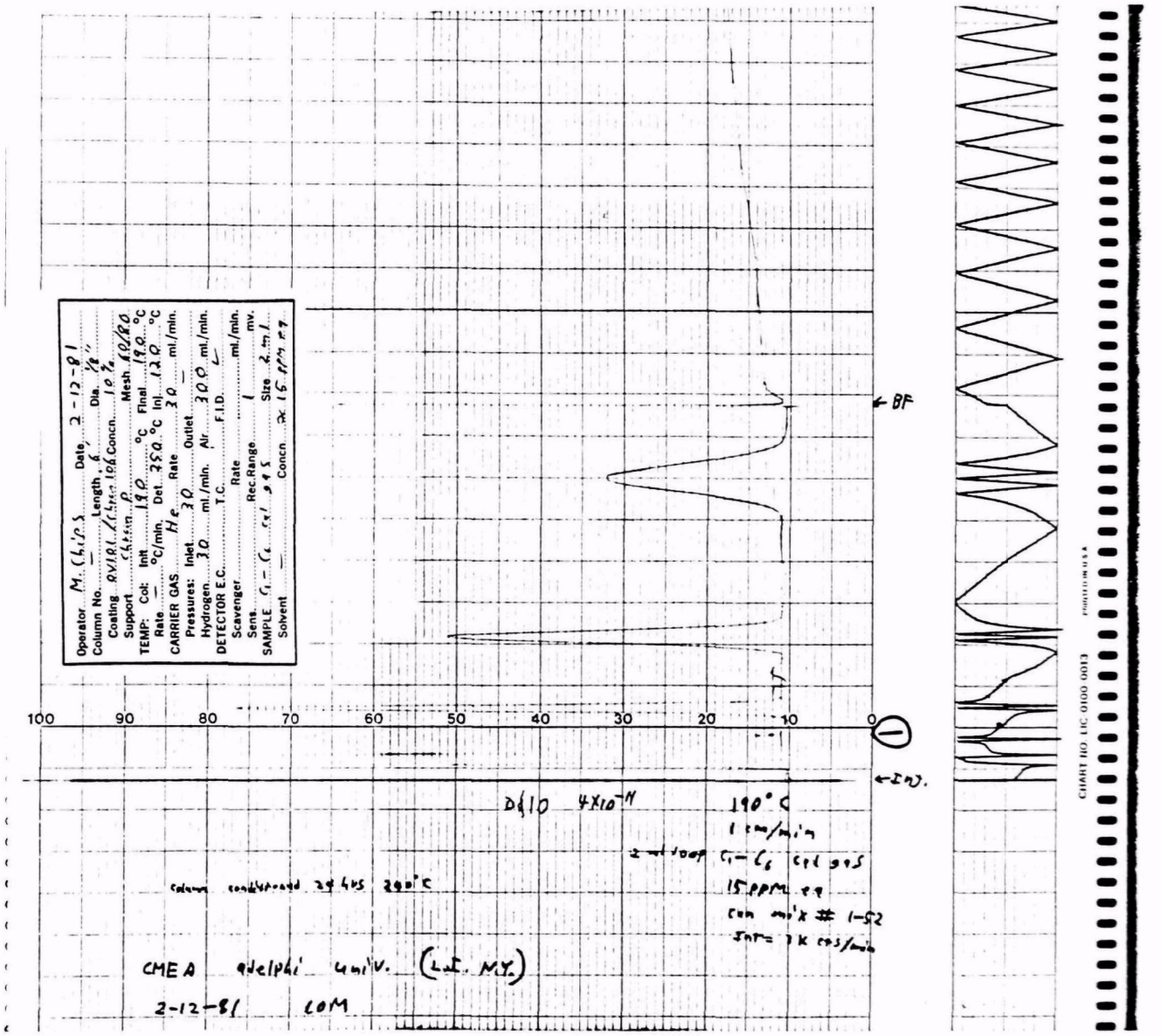
5.7 C_1 - C_6 CHROMATOGRAPHY

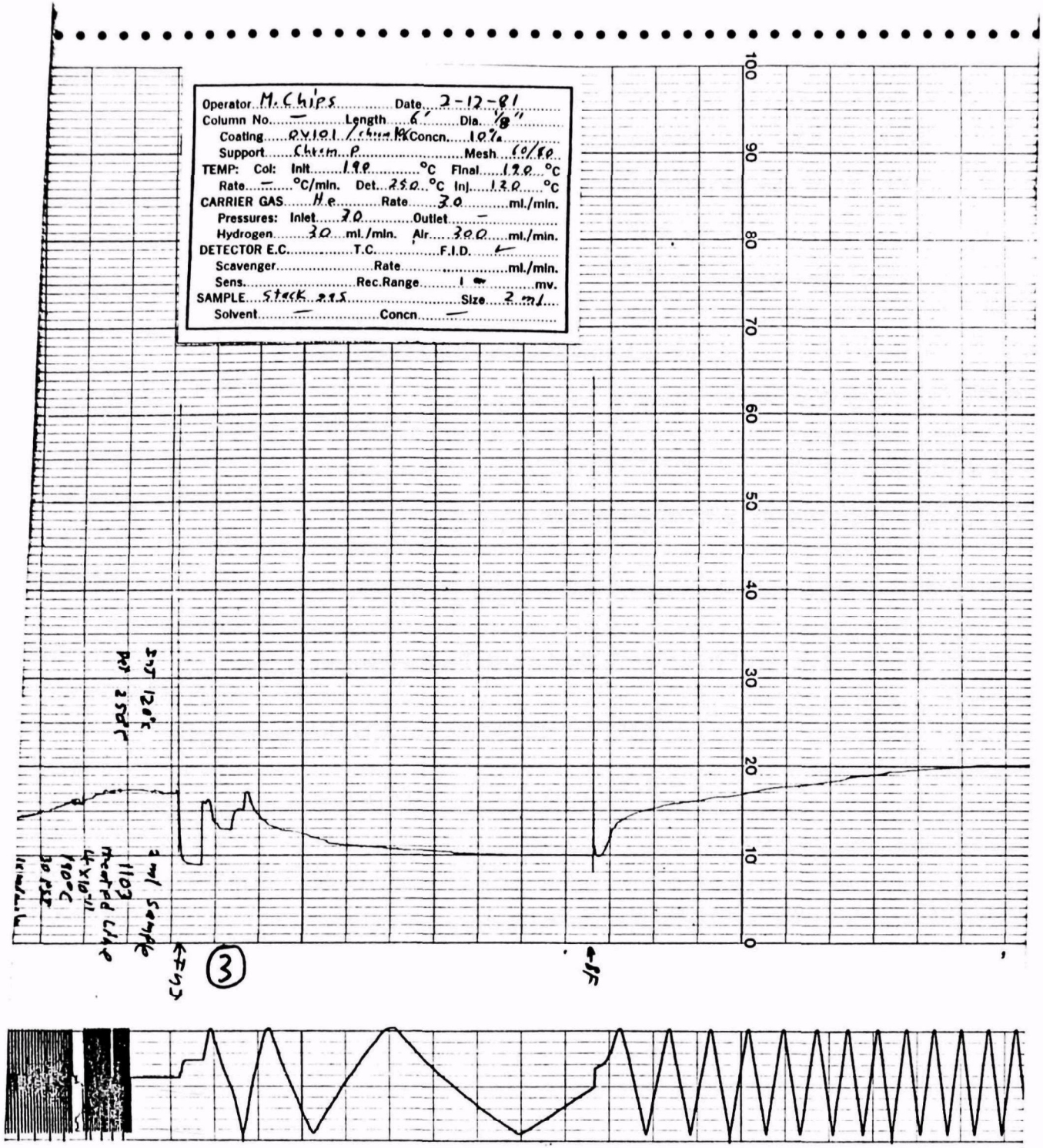
ADELPHI UNIV. RESULTS:

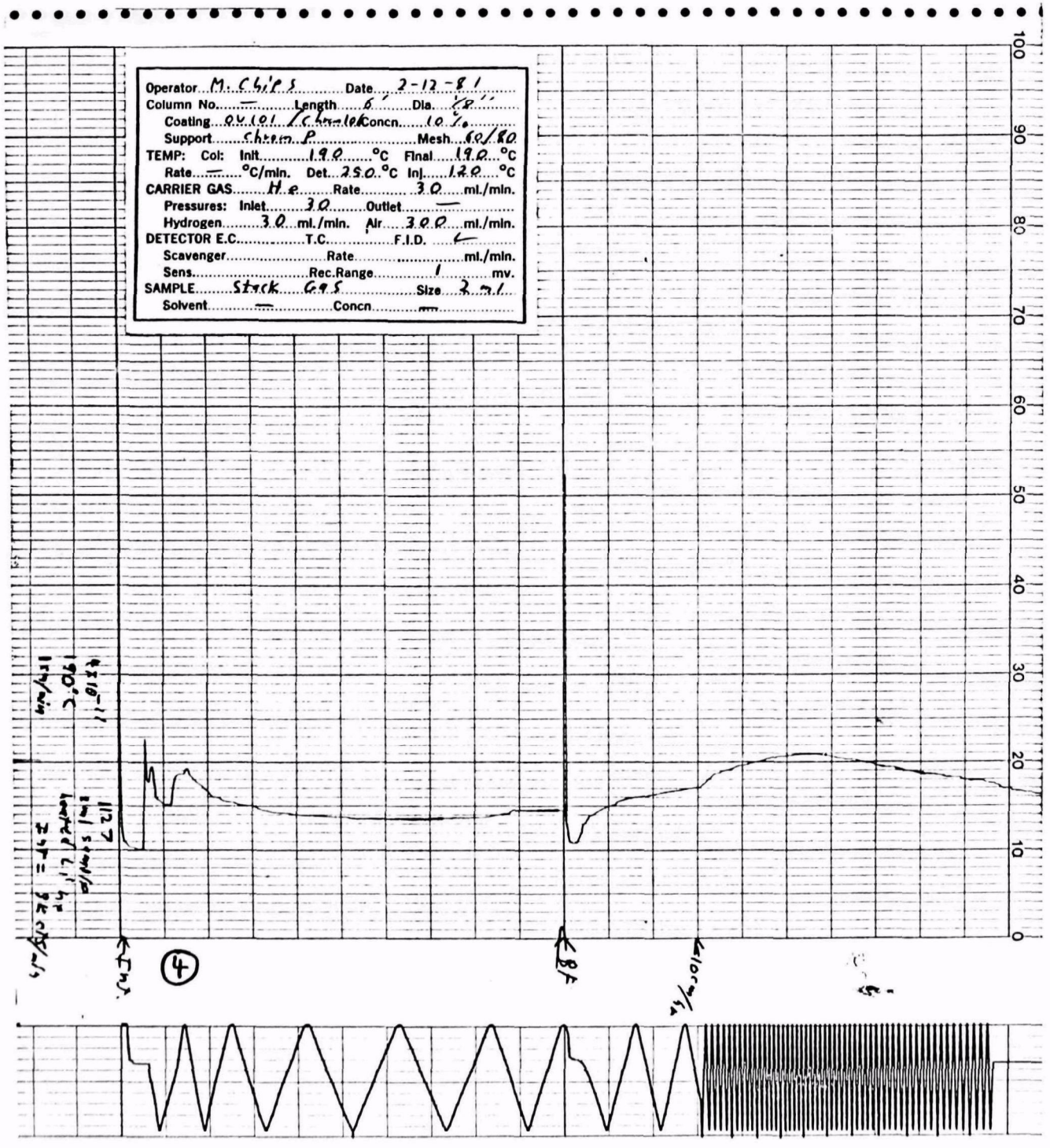
	2/12/81 Time	Chroma-togram #		COW Test 1, ppm C_1	C_2	C_3	C_4	C_5	C_6
Cal	0810	1	avg	15.1	14.6	15.6	15.2	15.6	15.9
Cal	1429	7	avg	80.5	131	174	133.5	81.5	43
Sample	1103	3		--	1.4	--	1.5	--	--
Sample	1127	4		3.1	1.1	--	1.1	--	--
Sample	1239	5		1.8	--	--	1.3	--	--
Sample	1405	6		2.4	--	--	1.4	--	--

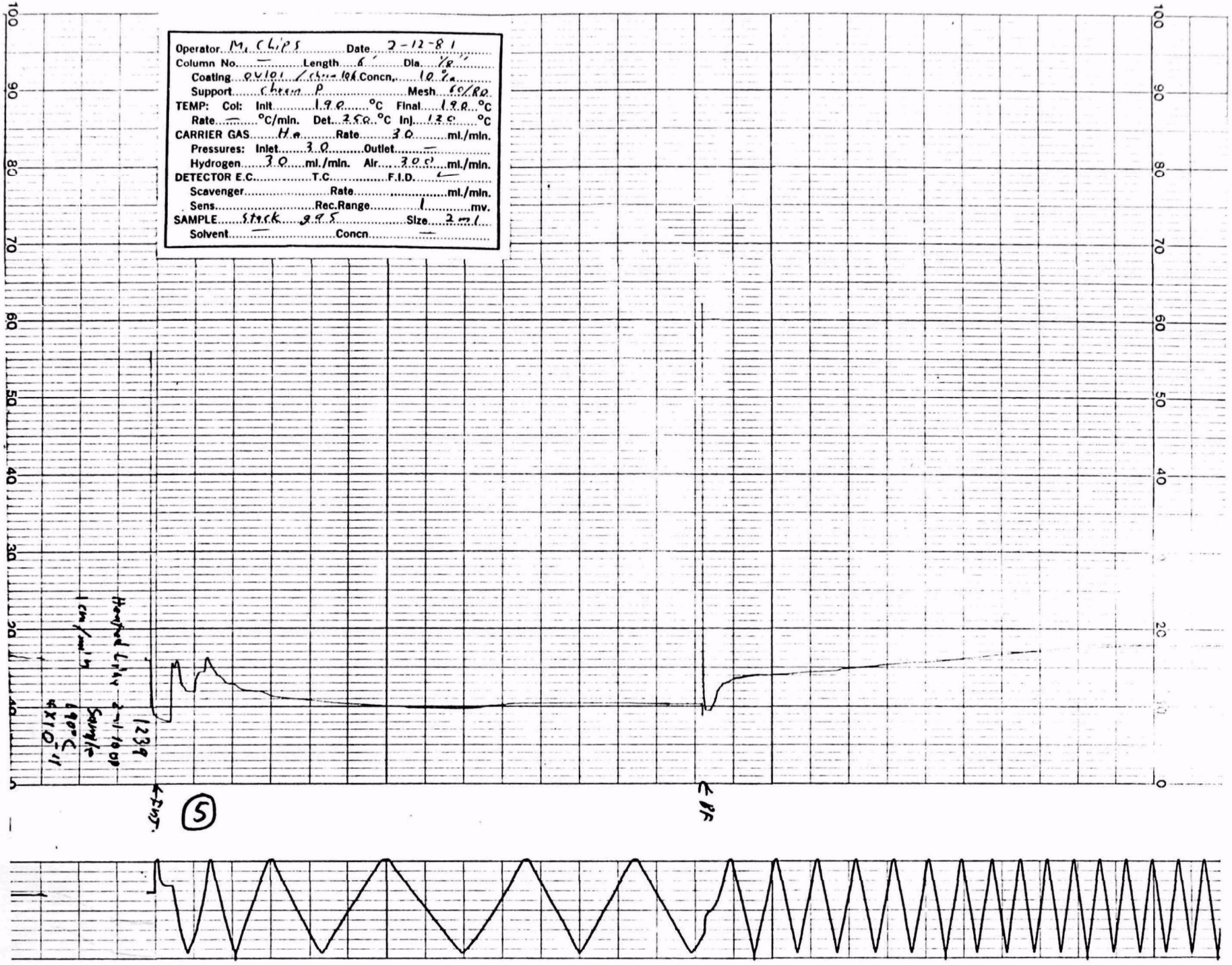
Soda Ash Injection

	2/13/81 Time	Chroma-togram #		Test 2, ppm C_1	C_2	C_3	C_4	C_5	C_6
Cal	0951	10	avg	15.1	14.6	15.6	15.2	15.6	15.9
Cal	1328	13	avg	80.5	139.5	173	132	82.5	43
Blank	0827	9		3.3	0.5	0.2	--	--	--
Sample	1002	11		1.8	--	--	2.0	--	--
Sample	1023	12		1.3	--	--	1.3	--	--


Operator M. Chips Date 2-12-81
Column No. — Length 6' Dia. 1/8"
Coating Rvibl Carbon 10% Concn. 10%
Support Chrom P Mesh 80/100
TEMP: Col: Int. 190 °C Final 190 °C
Rate — °C/min. Det. 250 °C Inj. 120 °C
CARRIER GAS He Rate 30 ml/min.
Pressures: Inlet 30 Outlet —
Hydrogen 30 ml./min. Air 300 ml./min.
DETECTOR E.C. T.C. F.I.D.
Scavenger Rate ml./min.
Sens. Rec.Range 1 mv.
SAMPLE C1 – C6 Cyl gas Size 2 ml.
Solvent — Concn. 15 ppm
BF
Inj.
100 90 80 70 60 50 40 30 20 10 0
D&10 4X10-11
190° C
1 cm/min
2 ml loop C1 – C6 cyl gas
15 ppm
column conditioned 24 hrs 290°C
CME A adelphi univ. (L.I. N.Y.)
2-12-81 COM
CHART NO. LIC 0100 0013

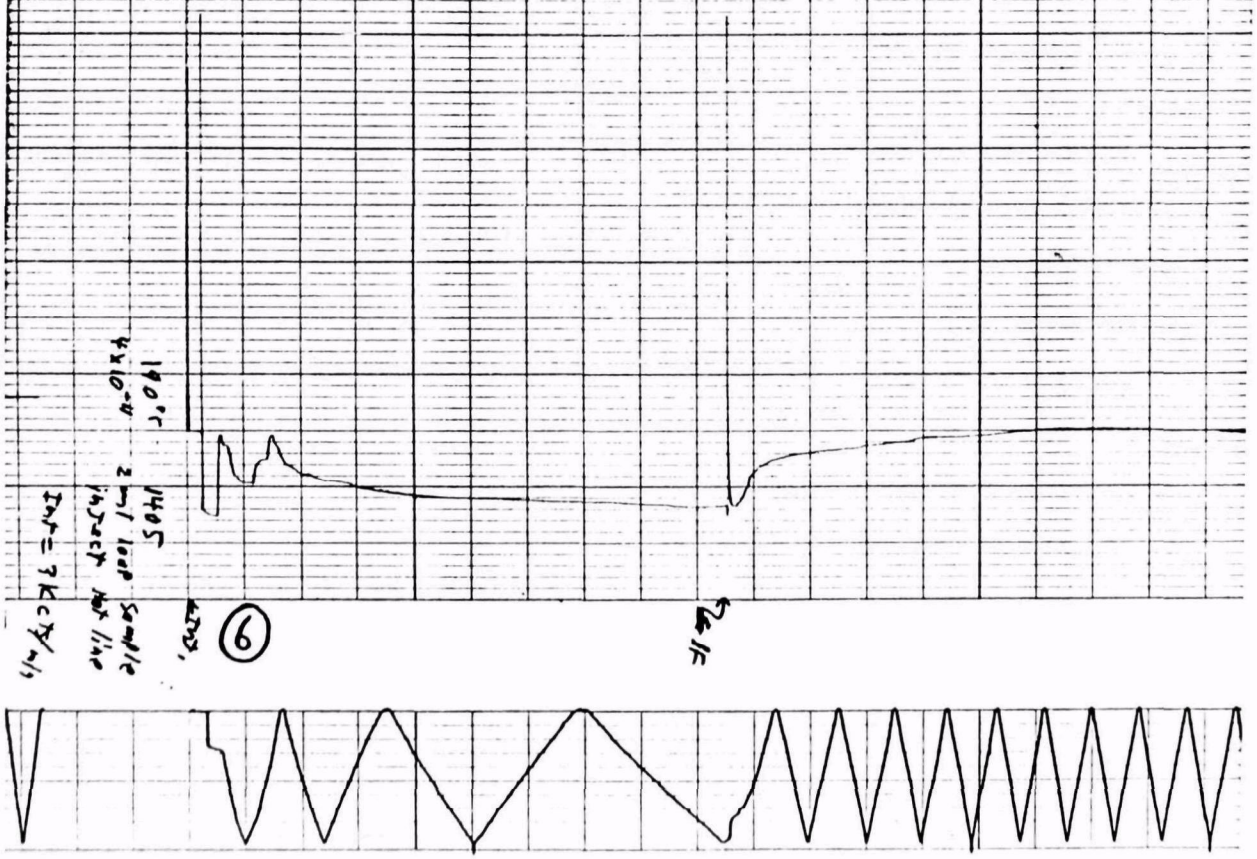

Operator M. Chips Date 2-12-81
Column No. — Length 6' Dia. 1/8"
Coating OV101 / Chem P Concn. 10%
Support Chem P Mesh 60/80
TEMP: Col: Init. 190 °C Final 190 °C
Rate — °C/min. Det. 250 °C Inj. 120 °C
CARRIER GAS He Rate 30 ml./min.
Pressures: Inlet 30 Outlet —
Hydrogen 30 ml./min. Air 300 ml./min.
DETECTOR E.C. T.C. F.I.D. ←
Scavenger Rate ml./min.
Sens. Rec.Range 1 mv.
SAMPLE Stack gas Size 2 ml
Solvent — Concn —
100
90
80
70
60
50
40
30
20
10
0
Inj 120°C
Det 250°C
3 ml sample
1103
Heated line
4x10⁻¹¹
190°C
30 psi
Initial
③
←Inj
←Air
CHART NO. LIC-0100-0013
PRINTED IN U.S.A.


Operator M. Chiles Date 2-12-81
Column No. — Length 6' Dia. 1/8"
Coating OV-101 / Chromosorb Concn. 10%
Support Chrom P Mesh 60/80
TEMP: Col: Init. 190 °C Final 190 °C
Rate — °C/min. Det. 250 °C Inj. 120 °C
CARRIER GAS He Rate 30 ml./min.
Pressures: Inlet 30 Outlet —
Hydrogen 30 ml./min. Air 300 ml./min.
DETECTOR E.C. T.C. F.I.D. ✓
Scavenger Rate ml./min.
Sens. Rec.Range 1 mv.
SAMPLE Stack Gas Size 2 ml
Solvent — Concn —
Inj.
BF
10 cm/hr
4
0
10
20
30
40
50
60
70
80
90
100
CHART NO. LIC 010



-0100-0013 PRINTED IN U.S.A. CHART NO. LIC-0100-0013

Operator M. Clips Date 2-12-81
Column No. — Length 6' Dia. 1/8"
Coating OV 101 / Chem-6 Concn. 10%
Support Chem-P Mesh 60/80
TEMP: Col: Init. 190 °C Final 190 °C
Rate — °C/min. Det. 250 °C Inj. 120 °C
CARRIER GAS He Rate 30 ml./min.
Pressures: Inlet 30 Outlet —
Hydrogen 30 ml./min. Air 300 ml./min.
DETECTOR E.C. T.C. F.I.D. ✓
Scavenger Rate ml./min.
Sens. Rec.Range 1 mv.
SAMPLE Stock 205 Size 2 ml
Solvent — Concn. —



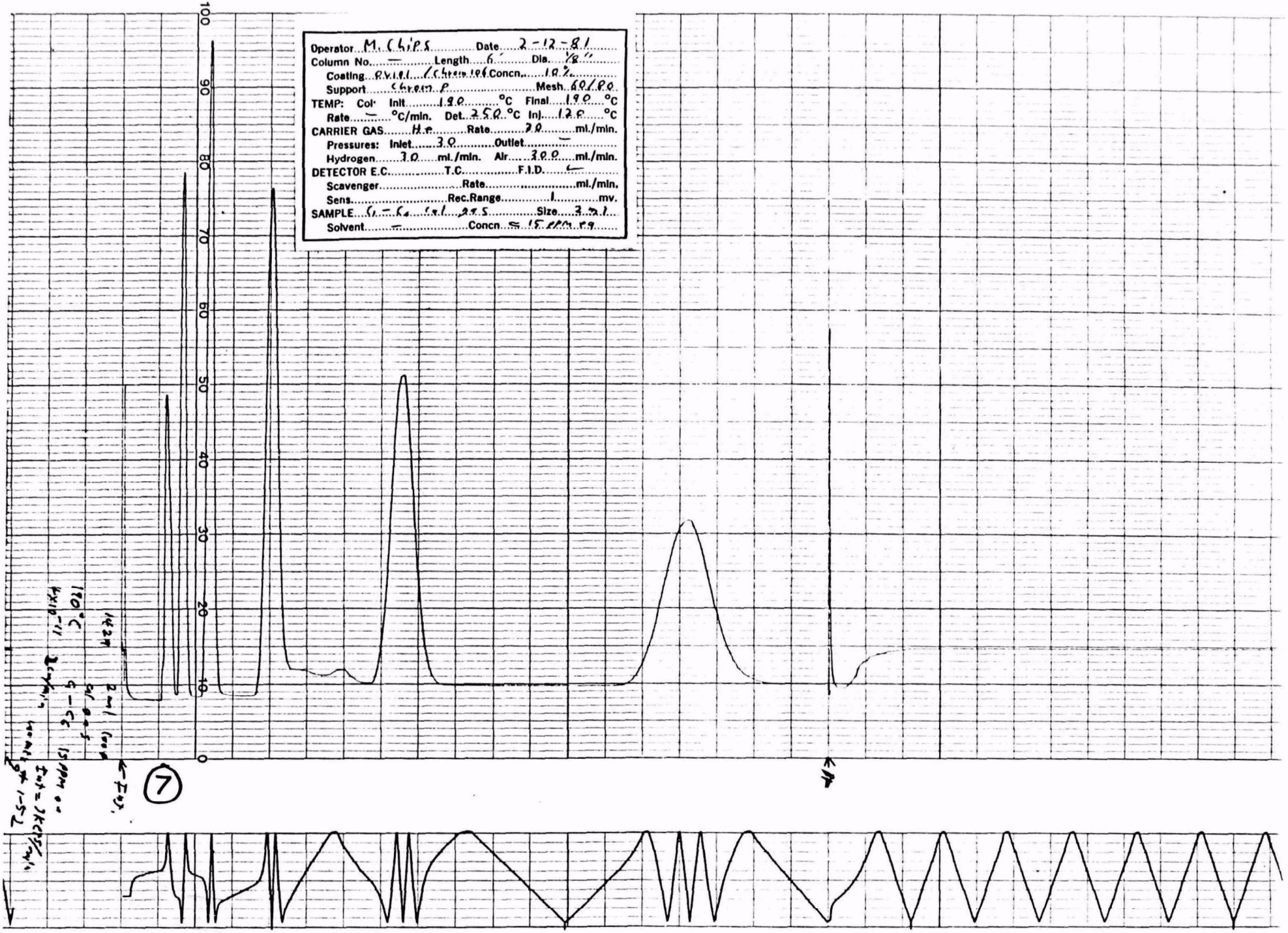
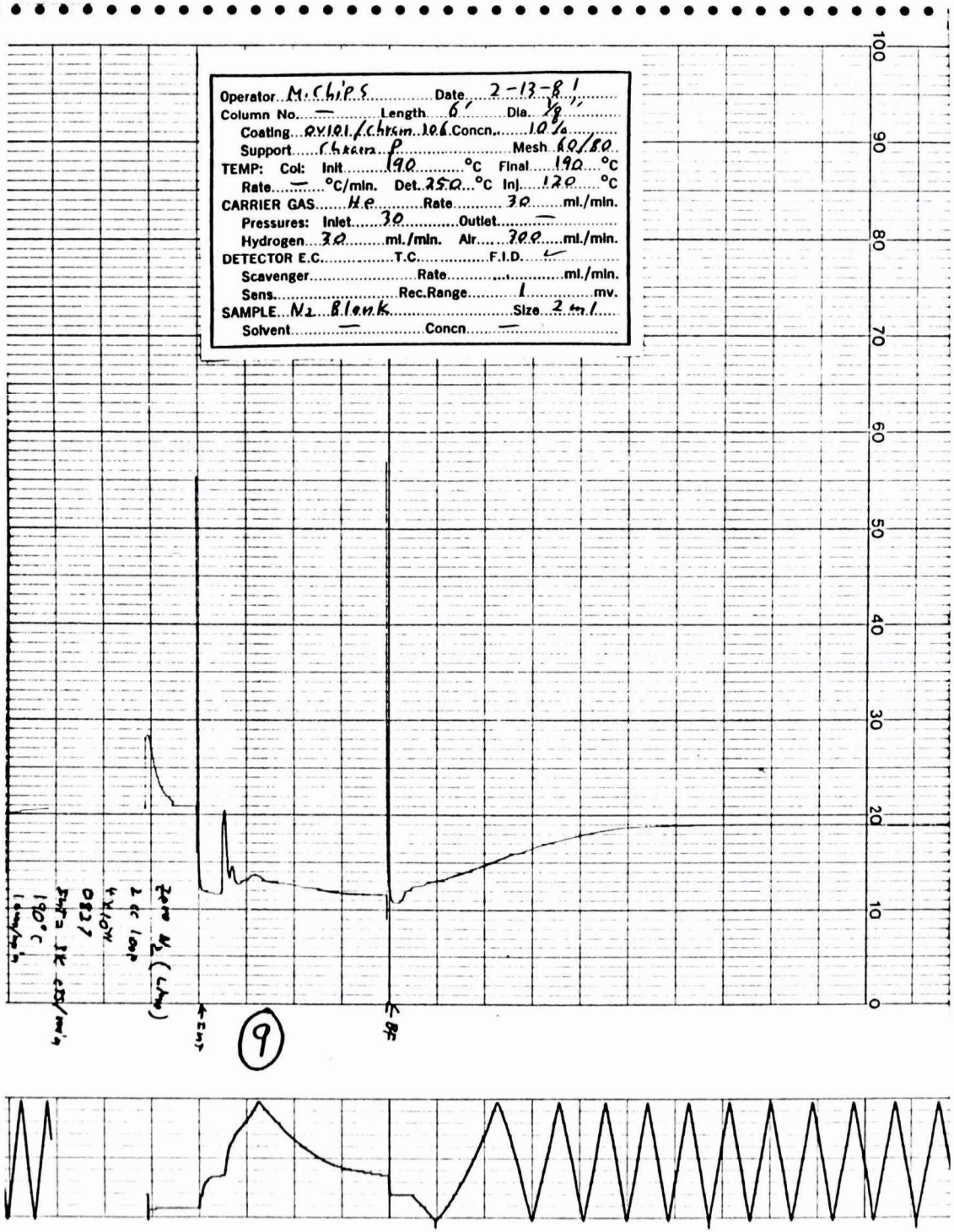
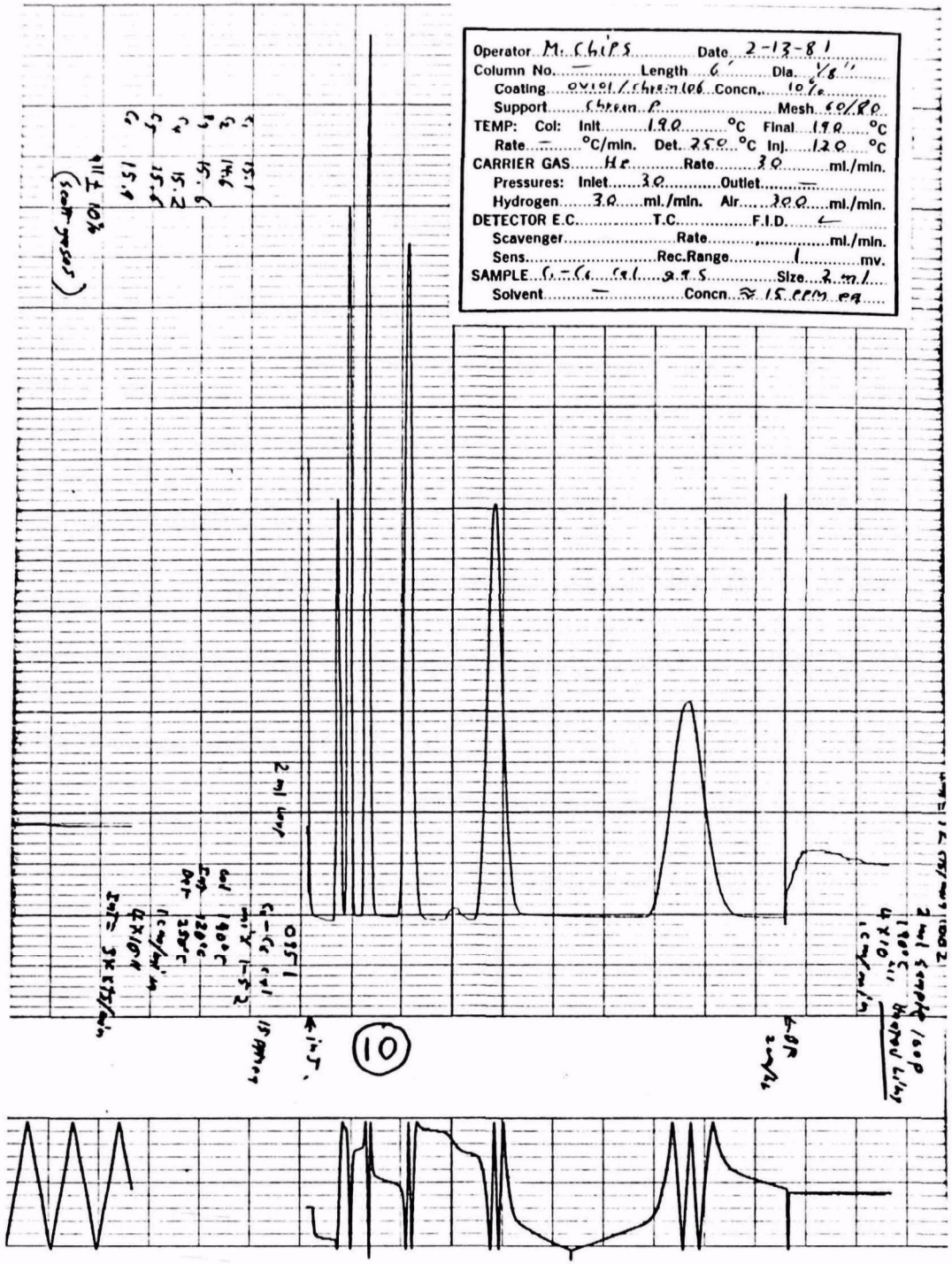


CHART NO. LIC-0100-0013 PRINTED IN U.S.A. CHART


Operator M. Chips Date 2-13-81
Column No. — Length 6' Dia. 1/8"
Coating OV101/Chrom 106 Concn. 10%
Support Chrom P Mesh 60/80
TEMP: Col: Init 190 °C Final 190 °C
Rate — °C/min. Det. 250 °C Inj. 120 °C
CARRIER GAS He Rate 30 ml./min.
Pressures: Inlet 30 Outlet —
Hydrogen 30 ml./min. Air 300 ml./min.
DETECTOR E.C. T.C. F.I.D. ✓
Scavenger Rate ml./min.
Sens. Rec.Range 1 mv.
SAMPLE N2 Blank Size 2 ml
Solvent — Concn —
100
90
80
70
60
50
40
30
20
10
0
Inj
9
BF
CHART NO. LIC 0100 0013



0013 PRINTED IN U.S.A.

Operator M. Clips Date 2-13-81
Column No. — Length 6' Dia. 1/8"
Coating ovisol / chrom 106 Concn. 10 %
Support chrom P Mesh 60/80
TEMP: Col: Init 190 °C Final 190 °C
Rate — °C/min. Det. 250 °C Inj. 120 °C
CARRIER GAS He Rate 30 ml./min.
Pressures: Inlet 30 Outlet —
Hydrogen 30 ml./min. Air 300 ml./min.
DETECTOR E.C. T.C. F.I.D. ✓
Scavenger Rate ml./min.
Sens. Rec.Range 1 mv.
SAMPLE Stock 225 Size 2 ml
Solvent — Concn —

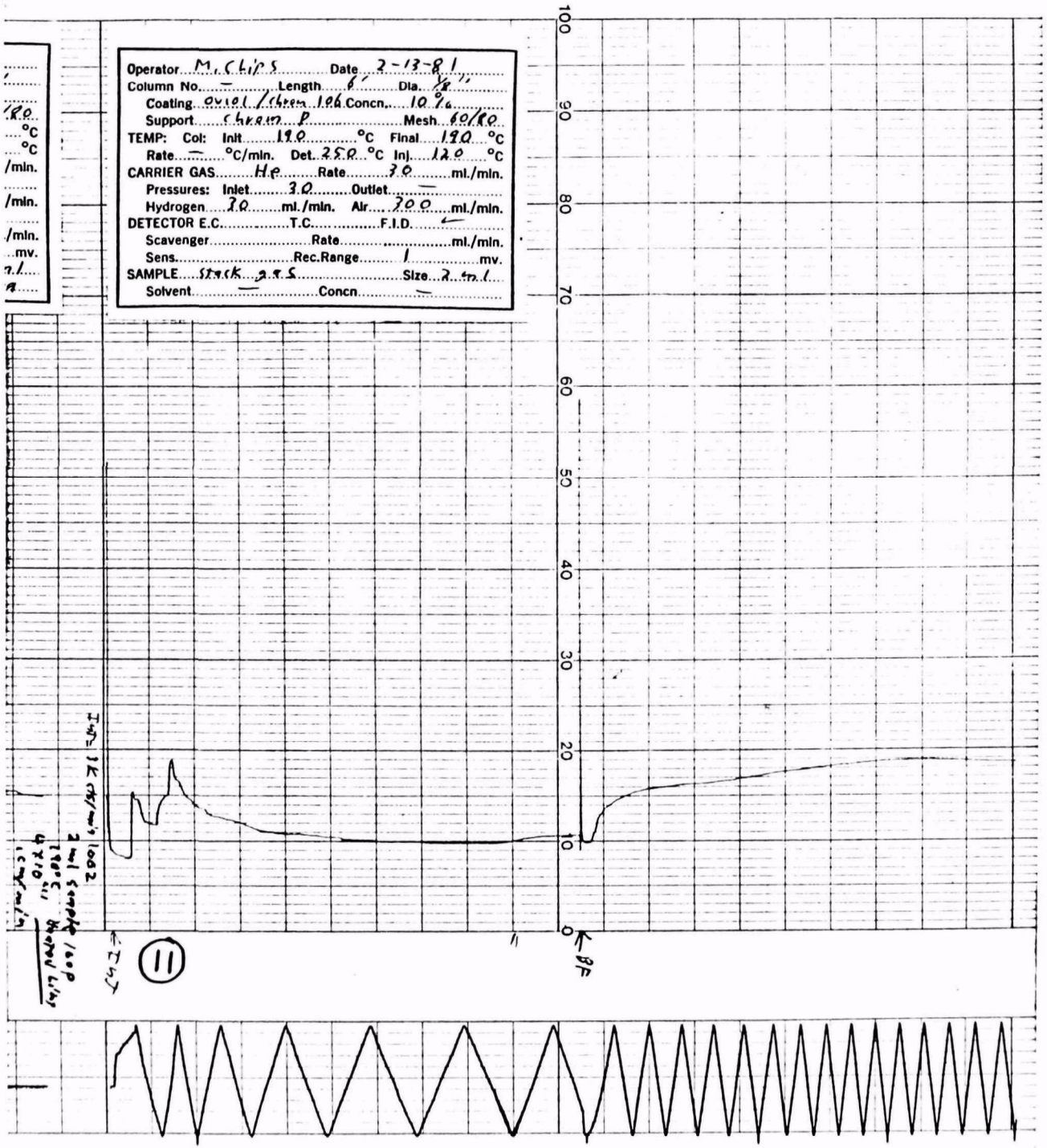
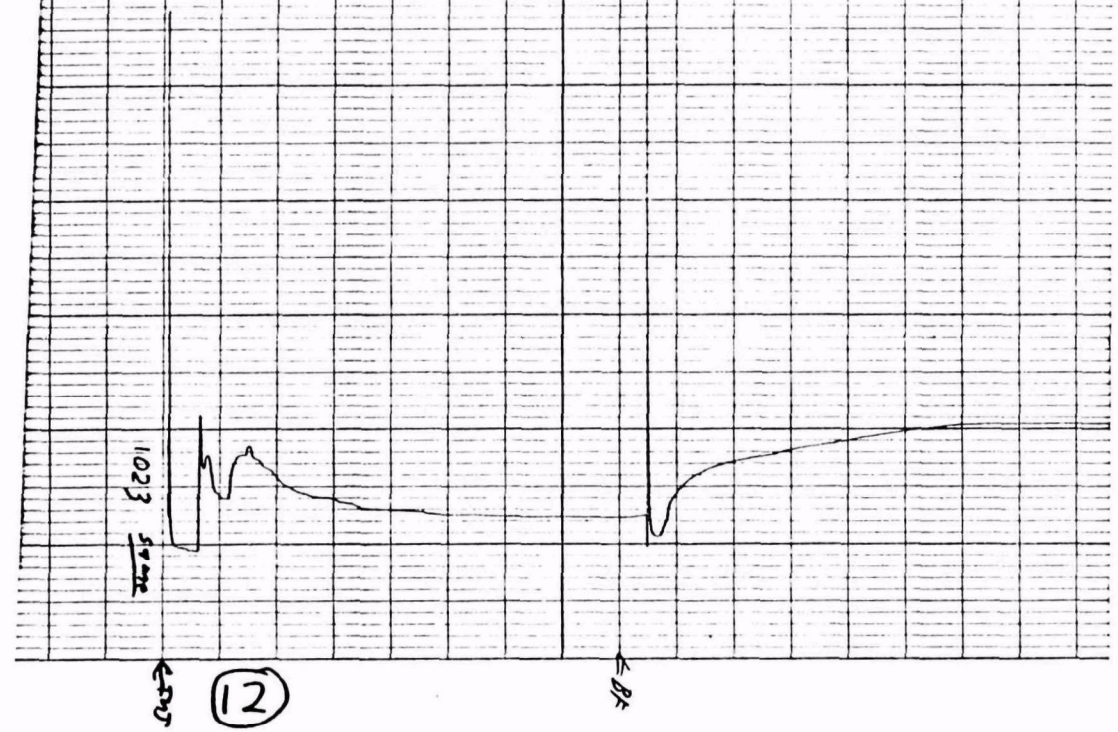


CHART NO. LIC-0100-0013 PRINTED IN U.S.A.

Operator M. Chiles Date 2-12-81
Column No. — Length 6' Dia. 1/8"
Coating OV101 / Chrom 106 Concn. 10%
Support Chrom P Mesh 60/80
TEMP: Col: Init. 190 °C Final 190 °C
Rate — °C/min. Det. 250 °C Inj. 120 °C
CARRIER GAS He Rate 30 ml./min.
Pressures: Inlet 30 Outlet —
Hydrogen 30 ml./min. Air 300 ml./min.
DETECTOR E.C. T.C. F.I.D. ✓
Scavenger Rate ml./min.
Sens. Rec.Range 1 mv.
SAMPLE Stack gas Size 2 ml
Solvent — Concn —



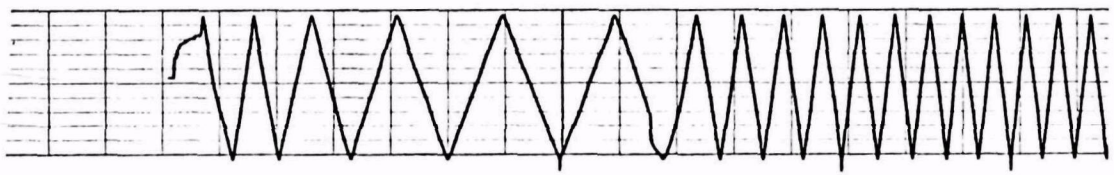
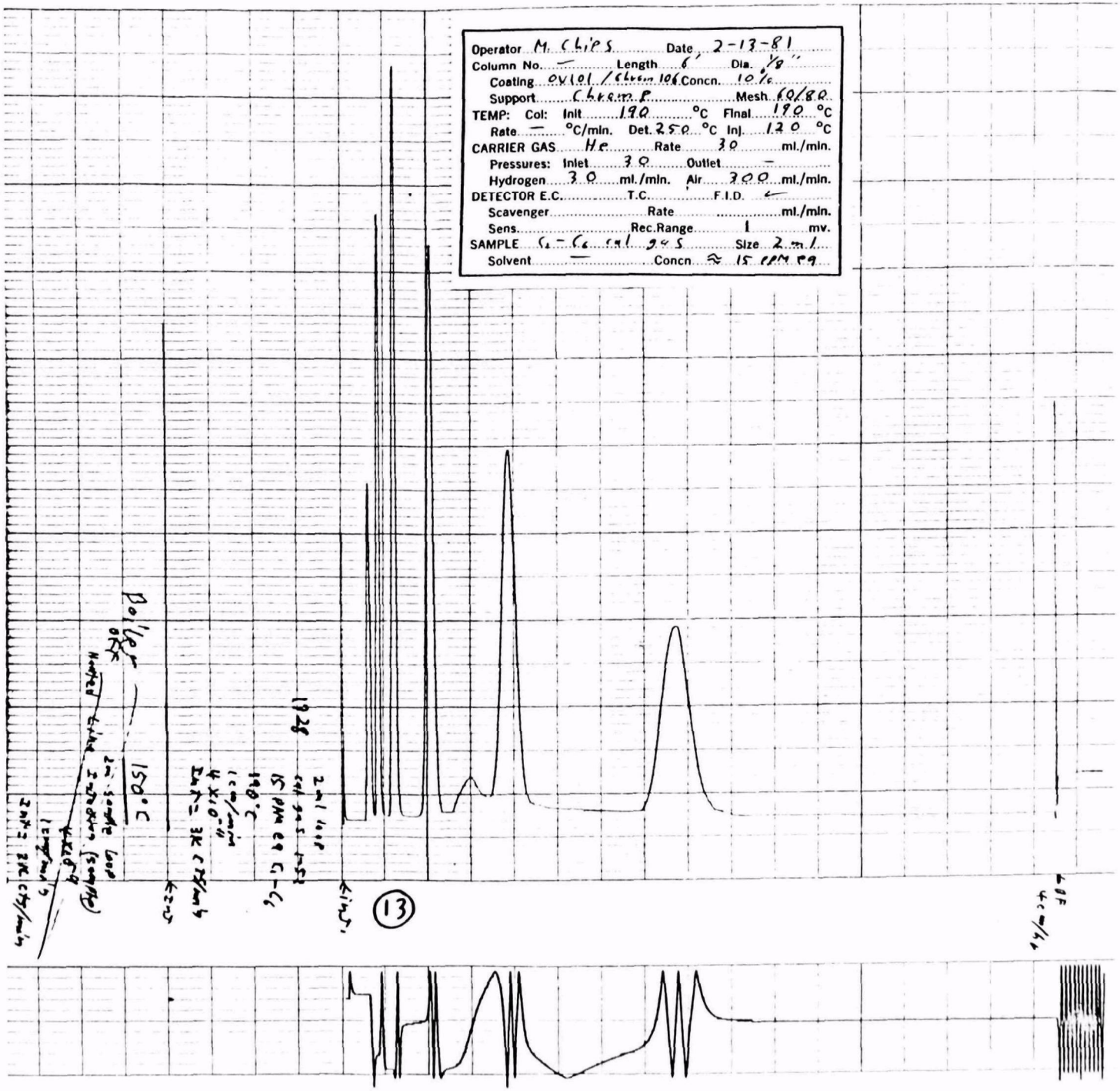


CHART NO


Operator M. Clips Date 2-13-81
Column No. — Length 6' Dia. 1/8"
Coating OV101 / Chrom 106 Concn. 10%
Support Chrom P Mesh 60/80
TEMP: Col: Init 190 °C Final 190 °C
Rate — °C/min. Det. 250 °C Inj. 120 °C
CARRIER GAS He Rate 30 ml./min.
Pressures: Inlet 30 Outlet —
Hydrogen 30 ml./min. Air 300 ml./min.
DETECTOR E.C. T.C. F.I.D. ←
Scavenger Rate ml./min.
Sens. Rec.Range 1 mv.
SAMPLE C1-C6 cal gas Size 2 ml
Solvent — Concn. ≈ 15 ppm ea
13
-0100-0013
PRINTED IN U.S.A.

5.? RADIOMETRIC ANALYSES

SAFETY SPECIALISTS, Inc.

3284 F Edward Avenue, Santa Clara, California 95050 • Telephone (408) 988-1111

ASSAY REPORT

Acurex Corporation
485 Clyde Avenue
Attn: Ms. Linda Bohannon, M/S 0-1212
Mountain View, California 94042

Date: June 3, 1981

Date Samples Received: 4/30/81

Customer Order No.: RB59185A, Rel. 10

SSI No.	Client Description	Activity* Gross Alpha pCi/gram	Activity* Gross Beta pCi/gram
C	Adelphi - 1, 1μ + filter	224.1 ± 47.7	128.8 ± 38.6
D	Adelphi - 1, 3μ + 10μ	196.7 ± 20.0	79.3 ± 11.3
E	Adelphi - 2, filter rinse + 10μ + 3μ	93.7 ± 8.5	40.5 ± 14.7
F	Adelphi - 2, 1μ + filter	24.8 ± 4.8	16.3 ± 2.9

Analyst: Pamela S. Shreve

Approved: T. C. Noble, Director
Safety and Health Services Division

*The ± values are the two sigma Poisson standard deviation of the counting error.
The ≤ values are equal to or less than three sigma of the counting error.

SAFETY SPECIALISTS, Inc.

3284 F Edward Avenue, Santa Clara, California 95050 · Telephone (408) 988-111

ASSAY REPORT

Acurex Corporation
Attn: Mr. Larry Waterland
485 Clyde Avenue
Mountain View, California 94042

Date: August 7, 1981

Date Samples Received: 4/30/81

Customer Order No.: RB59185A. Rel. 1C

SSI No.	Client Description	Activity Gross Gamma p Ci/g
C	Adelphi - 1, 1μ and 1, filter	553 ± 300
D	Adelphi - 1, 3μ and 1, 10μ	183 ± 292
E	Adelphi 2, filter rinse; 2, 10μ and 2, 3μ	219 ± 293
F	Adelphi 2, 1μ and 2, filter	100 ± 290

Analyst: Pamela S. Shreve

Approved: T. C. Noble, Director
Safety and Health Services Division

*The ± values are the two sigma Poisson standard deviation of the counting error.
The ≤ values are equal to or less than three sigma of the counting error.

MUTAGENICITY EVALUATION OF 424 (ADELPHI 1 XAD EXTRACT) IN THE EPA LEVEL 1 AMES SALMONELLA/MICROSOME PLATE TEST

FINAL REPORT

SUBMITTED TO:

ACUREX CORPORATION
485 CLYDE AVENUE
MOUNTAIN VIEW, CALIFORNIA 94042

SUBMITTED BY:

LITTON BIONETICS, INC.
5516 NICHOLSON LANE
KENSINGTON, MARYLAND 20895

LBI PROJECT NO. 22064

REPORT DATE: SEPTEMBER 1981

PREFACE

This assay conforms to the standard EPA Level 1 procedure for the Ames Salmonella/microsome mutagenesis assay as described in "IERL-RTP Procedures Manual: Level 1 Environmental Assessment Biological Tests"[1]. The data was evaluated and formatted as recommended in "Level 1 Biological Testing Assessment and Data Formatting"[2].

The Ames Salmonella/microsome mutagenesis assay has been shown to be a sensitive method for detecting mutagenic activity for a variety of chemicals representing various chemical classes[3]. This assay is one of several recommended by EPA to identify, categorize and rank the pollutant potential of influent and effluent streams from industrial and energy-producing processes. This assay has been well validated with a wide range of positive and negative control chemicals and complex environmental samples.

All procedures and documents pertaining to the receipt, storage, preparation, testing and evaluation of the test material shall conform to Litton Bionetics, Inc. standard operating procedures and the Good Laboratory Practices Act of 1979. Deviations from standard procedure shall be fully documented and noted in the report.

All test and control results in this report are supported by fully documented raw data which are permanently maintained in the files of the Department of Molecular Toxicology or in the archives of Litton Bionetics, Inc., 5516 Nicholson Lane, Kensington, Maryland 20895. Copies of raw data will be supplied to the sponsor upon request.

TABLE OF CONTENTS

		Page No.
	PREFACE	i
I.	ASSAY SUMMARY	1
II.	OBJECTIVE	2
III.	TEST MATERIAL	3
	A. Description	3
	B. Handling and Preparation	3
IV.	MATERIALS	4
	A. Indicator Microorganisms	4
	B. Media	4
	C. Activation System	5
	1. S9 Homogenate	5
	2. S9 Mix	5
V.	EXPERIMENTAL DESIGN	6
	A. Dose Selection	6
	B. Mutagenicity Test	6
	1. Nonactivation Assay	6
	2. Activation Assay	6
	C. Control Compounds	8
	D. Recording and Presenting Data	8
VI.	RESULTS	9
	A. Interpretation	9
	B. Tables	9
VII.	ASSAY ACCEPTANCE AND EVALUATION CRITERIA	12
	A. Surviving Populations	12
	B. Dose-Response Phenomena	12
	C. Control Tests	12
	D. Evaluation Criteria for Ames Assay	13
	1. Strains TA-1535 and TA-1537	13
	2. Strains TA-98 and TA-100	13
	3. Pattern	13
	4. Reproducibility	13
	E. Relation Between Mutagenicity and Carcinogenicity	14
	F. Criteria for Ranking Samples in the Ames Assay	14
VIII.	REFERENCES	15

I. ASSAY SUMMARY

A. Sponsor: Acurex Corporation

B. Material (Test Compound): Genetics Assay Number: 5806

1. Identification: 424 (Adelphi 1 XAD extract)

2. Date Received: June 28, 1981

3. Physical Description: Clear, yellow liquid.

C. Type of Assay: EPA Level 1 Ames Salmonella/Microsome Plate Test

D. Assay Design Number: 401 (EPA Level 1)

E. Study Dates:

1. Initiation: August 22, 1981

2. Completion: September 7, 1981

F. Supervisory Personnel:

A. Study Director: D.R. Jagannath, Ph.D.

G. Evaluation:

The test material, 424 (Adelphi 1 XAD extract), was evaluated for mutagenic activity over a concentration range of 10 µl/plate to 200 µl/plate in the Ames Salmonella/microsome assay. Without metabolic activation, the test results were negative for all test strains. With metabolic activation, strains TA-1537, TA-98 and TA-100 were all positive. TA-1535 was negative but was evaluated at only one test concentration because of limited sample. The minimum effective concentration (MEC) was found to be 10 µl/plate in strain TA-98 with activation. This was equivalent to 49 µg organics per plate. This was evaluated as essentially on the borderline between high and moderate (H/M) mutagenicity categories defined for the Level 1 Ames assay[1].

Submitted by:

Study Director

for: D.R. Jagannath, Ph.D. 9/11/81 Date
Section Chief,
Submammalian Genetics,
Department of Molecular Toxicology

Reviewed by:

David J. Brusick, Ph.D. 9/11/81 Date
Director,
Department of Molecular Toxicology

II. OBJECTIVE

The objective of this study was to determine the genetic activity of 424 (Adelphi 1 XAD extract) in the Salmonella/ microsome assay with and without the addition of mammalian metabolic activation preparations. The genetic activity of a sample is measured in these assays by its ability to revert the Salmonella indicator strains from histidine dependence to histidine independence. The degree of genetic activity of a sample is reflected in the number of revertants that are observed on the histidine-free medium.

III. TEST MATERIAL

A. Description

The test material was received as a clear, yellow solution in methylene chloride. The sample contained 17.0 milligrams of organic material in an undetermined volume of methylene chloride. No information on the sampling parameters (such as the equivalent volume of stack gas represented by the sample) was provided.

B. Handling and Preparation

The test material was received at LBI on June 28, 1981. The sample was assigned LBI safety number 7032 and LBI assay number 5806. The sample was stored at +4°C in the dark.

Pretest sample preparation consisted of solvent exchanging the sample into dimethylsulfoxide (DMSO). The sample was transferred with methylene chloride rinses into a graduated conical tube. The methylene chloride was gradually evaporated (50°C under a stream of nitrogen) and DMSO was sequentially added. The sample was brought to volume in 3.5 ml of DMSO, giving a sample concentration of 4.9 mg organics per ml DMSO. The sample was transferred to a glass vial and sealed with a teflon-coated rubber septum.

A total volume of 2.56 ml of test material was used between three trials. In all trials, varying sized aliquots of the test material were added directly to the mixtures to give the desired concentration. The amount of sample used was 0.36 ml for Trial 1, 1.08 ml for Trial 2 and 1.12 ml for Trial 3.

IV. MATERIALS

A. Indicator Microorganisms

The *Salmonella typhimurium* strains used in this assay were obtained from Dr. Bruce Ames, University of California at Berkeley.[4-8] The following four strains were used.

Strain Designation	Gene Affected	Additional Mutations			Mutation Type Detected
		Repair	LPS	R Factor	
TA-1535	*his* G	Δ *uvr* B	*rfa*	-	Base-pair substitution
TA-1537	*his* C	Δ *uvr* B	*rfa*	-	Frameshift
TA-98	*his* D	Δ *uvr* B	*rfa*	pKM101	Frameshift
TA-100	*his* G	Δ *uvr* B	*rfa*	pKM101	Base-pair substitution

All the above strains have, in addition to the mutation in the histidine operon, mutation (*rfa*-) that leads to defective lipopolysaccharide coat, a deletion that covers genes involved in the synthesis of vitamin biotin (*bio*-) and in the repair of ultraviolet (uv) - induced DNA damage (*uvr*B-). The *rfa*- mutation makes the strains more permeable to many large molecules. The *uvr*B- mutation decreases repair of some types of chemically or physically damaged DNA and thereby enhances the strain's sensitivity to some mutagenic agents. The resistant transfer factor plasmid (R factor) pKM101 in TA-98 and TA-100 is believed to cause an increase in error-prone DNA repair that leads to many more mutations for a given dose of most mutagens.[8] In addition, plasmid pKM101 confers resistance to the antibiotic ampicillin, which is a convenient marker to detect the presence of plasmid in the cells.

All indicator strains are kept at 4°C on minimal medium plates supplemented with a trace of biotin and an excess of histidine. In addition, the plates with plasmid-carrying strains contain ampicillin (25 μg/ml) to ensure stable maintenance of plasmid pKM101. New stock culture plates are made as often as necessary from the frozen master cultures or from single colony reisolates that were checked for their genotypic characteristics (*his*, *rfa* *uvr*B, *bio*) and for the presence of plasmid. For each experiment, an inoculum from the stock culture plates is grown overnight at 37°C in nutrient broth (Oxoid CM67) and used.

B. Media

The bacterial strains were cultured in Oxoid Media #2 (Nutrient Broth). The selective medium was Vogen Bonner Medium E with 2% glucose.[10] The

overlay agar consisted of 0.6% purified agar with 0.05 mM histidine, 0.05 mM biotin and 0.1M NaCl according to the methods of Ames *et al.*[9]

C. Activation System

1. S9 Homogenate

A 9,000 x *g* supernatant prepared from Sprague-Dawley adult male rat liver induced by Aroclor 1254 (Ames *et al.*[9]) was purchased from Bionetics Laboratory Products, Litton Bionetics, Inc. and used in these assays.

2. S9 Mix

S9 mix used in these assays consisted of the following components:

Components	Concentration per Milliliter S9 Mix
NADP (sodium salt)	4 µmoles
D-glucose-6-phosphate	5 µmoles
$MgCl_2$	8 µmoles
KCl	33 µmoles
Sodium phosphate buffer pH 7.4	100 µmoles
Organ homogenate from rat liver (S9 fraction)	100 µliters

V. EXPERIMENTAL DESIGN

A. Dosage Selection

Test strategy and dose selection depend upon sample type and sample availability. The Level 1 manual[1] recommends solids to be initially tested at the maximum applicable dose (MAD) of 5 mg per plate and at lower concentrations of 2.5, 1, 0.5, 0.1 and 0.05 mg per plate. Liquids are tested initially at the MAD of 200 µl per plate, and at lower concentrations of 100, 50 and 10 µl per plate. Samples are retested over a narrower range of concentrations with strains showing positive results initially. Alternate dose are employed if sample size is limiting or at the direction of the sponsor.

Because the amount of test material was potentially limiting, a preliminary range-finding trial was run using a single plate per dose with strain TA-98 without metabolic activation. This trial, as did the two subsequent trials, used the recommended dose levels of 200, 100, 50, and 10 µl per plate.

B. Mutagenicity Testing

The procedure used was based on the paper published by Ames *et*. *al*.[9] and was performed as follows:

(1) Nonactivation Assay

To a sterile 13 x 100 mm test tube placed in a 43°C water bath the following was added in order:

- 2.00 ml of 0.6% agar containing 0.05 mM histidine and 0.05 mM biotin.
- 0.01 ml to 0.2 ml of a solution of the test chemical to give the appropriate dose.
- 0.1 ml to 0.2 ml of indicator organism(s).
- 0.50 ml of 0.2M phosphate buffer, pH 7.4.

This mixture was swirled gently and then poured onto minimal agar plates (see IV B, Media). After the top agar had set, the plates were incubated at 37°C for approximately 2 days. The number of *his*+ revertant colonies growing on the plates were counted with an automatic colony counter and recorded.

(2) Activation Assay

The activation assay was run concurrently with the nonactivation assay. The only difference was the addition of 0.5 ml of S9 mix (see IV C, Activation System) to the tubes in place of 0.5 ml of phosphate buffer which was added in nonactivation assays. All other details were similar to the procedure for nonactivation assays.

A detailed flow diagram for the plate incorporation assay is provided in Figure 1.

AMES ASSAY [PLATE INCORPORATION METHOD]

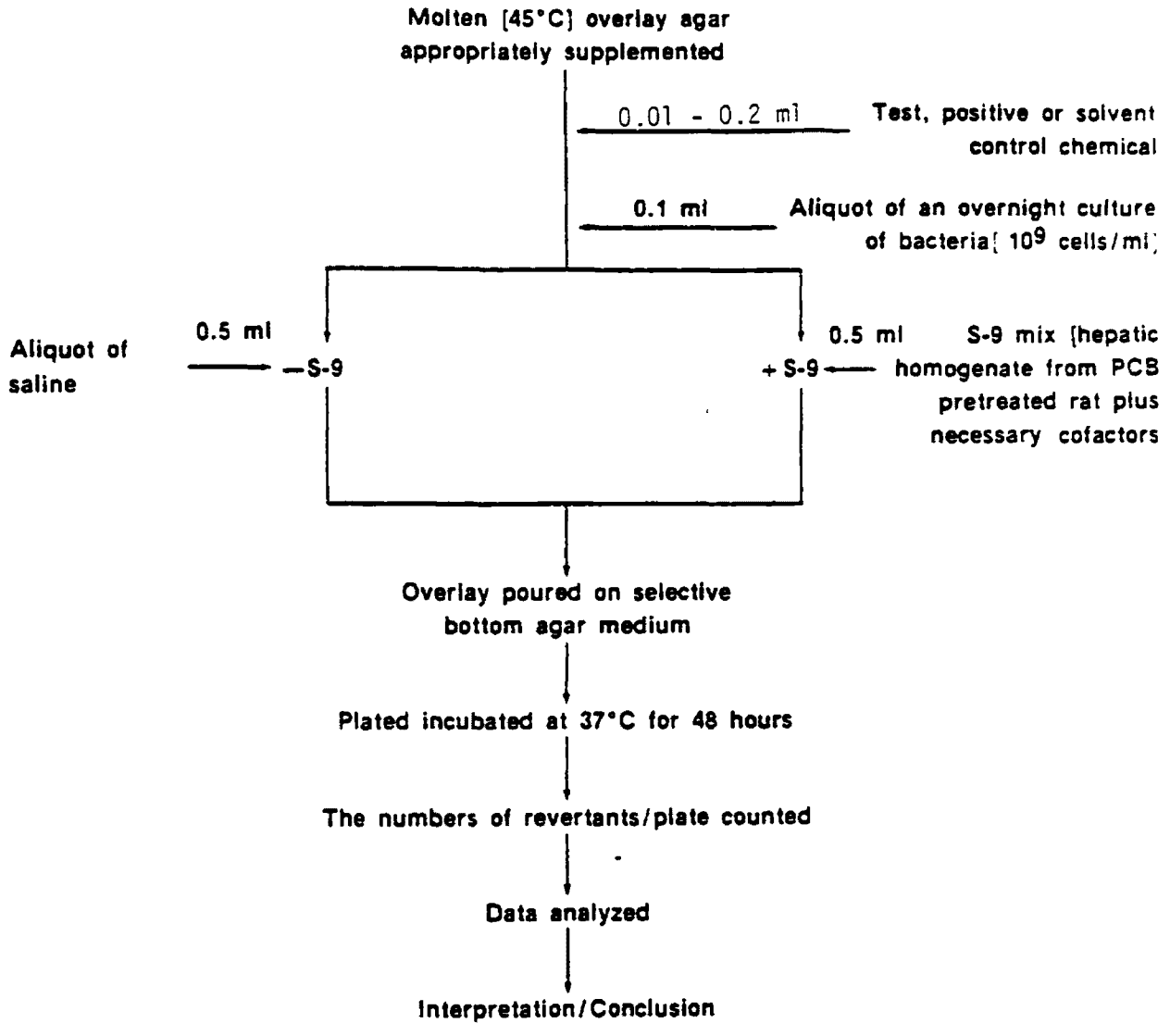


Figure 1 AMES SALMONELLA/MICROSOME MUTAGENESIS ASSAY

C. Control Compounds

A negative control consisting of the solvent used for the test material was also assayed concurrently with the test material. For negative controls, step 'b' of Nonactivation Assays was replaced by 0.05 ml of the solvent. The negative controls were employed for each indicator strain and were performed in the absence and presence of S9 mix. The solvent used to prepare the stock solution of the test material is given in the Results section of this report. All dilutions of the test material were made using this solvent. The amount of solvent used was equal to the maximum volume used to give the appropriate test dose.

Specific positive control compounds known to revert each strain were also used and assayed concurrently with the test material. The concentrations and specificities of these compounds to specific strains are given in the following table:

Assay	Chemical	Solvent	Concentration per plate (µg)	Salmonella Strains
Nonactivation	Sodium azide	Water	10.0	TA-1535, TA-100
	2-Nitrofluorene (NF)	Dimethyl-sulfoxide	10.0	TA-98
	9-aminoacridine (9AA)	Ethanol	50.0	TA-1537
Activation	2-anthramine (ANTH)	Dimethyl-sulfoxide	2.5	For all strains

D. Recording and Presenting Data

The number of colonies on each plate were counted and recorded on printed forms. These raw data were analyzed in a computer program and reported on a printout. The results are presented as revertants per plate for each indicator strain employed in the assay. The positive and solvent controls are provided as reference points.

VI. RESULTS

A. Interpretation

The test material, sample 424, (Adelphi 1 XAD extract), was initially tested in strain TA-98 without activation. This trial served as a range-finding study to help select doses for subsequent testing with all four strains. The dose range for all trials was 200, 100, 50 and 10 µl per plate using a single plate per dose. Trial 2 was with strain TA-98 with activation and strain TA-100 both with and without activation. Trial 3 employed strains TA-1535 and TA-1537 both with and without activation.

The nonactivation results for all strains tested were negative. Slight increases were observed at 100 µl/plate with TA-1537, TA-98 and TA-100 but they were not close to the criteria set for designating a positive response.

The activation test results for strains TA-1537, TA-98, and TA-100 were all positive by established criteria. The mutagenic response was generally dose-related over the range of concentration tested. TA-1535 was not active at the single dose of 200 µl/plate that was tested.

The minimum effective concentration (MEC) was found to be 10 µl per plate with strain TA-98 with activation. This concentration was equivalent to 49 µg or organics per plate. This value was essentially on the borderline between high and moderate (H/M) mutagenicity using IERL-EPA Level 1 criteria for the Ames bioassay[1].

Solvent control and positive control values were within acceptable ranges. These results achieved the assay acceptance criteria and provided confidence in the assumption that the recorded data represented typical responses to the test material.

B. Tables

This report is based on the data provided in Tables 1 and 2.

RESULTS TABLE 1

A. NAME OR CODE DESIGNATION OF THE TEST COMPOUND: 424(ADELPHI 1 XAD EXTRACT)
B. SOLVENT: DMSO
C. TEST INITIATION DATES: 08/22/81 08/25/81
D. TEST COMPLETION DATE: 09/01/81
E. S-9 LOT#: S9-11
NOTE: CONCENTRATIONS ARE GIVEN IN MICROLITERS PER PLATE

TEST			SPECIES	TISSUE	REVERTANTS PER PLATE TA-98 1	TA-98 2	TA-98 3	TA-100 1	TA-100 2	TA-100 3
NONACTIVATION										
SOLVENT CONTROL			---	---	43			139		
SOLVENT CONTROL			---	---	44			123		
POSITIVE CONTROL**			---	---	853			1855		
POSITIVE CONTROL**			---	---	732			1748		
TEST COMPOUND										
10.000	UL		---	---	64			162		
50.000	UL		---	---	63			188		
100.000	UL		---	---	70			221		
200.000	UL		---	---	28			128		
ACTIVATION										
SOLVENT CONTROL			RAT	LIVER	40			122		
SOLVENT CONTROL			RAT	LIVER	39			126		
POSITIVE CONTROL***			RAT	LIVER	1772			2271		
POSITIVE CONTROL***			RAT	LIVER	1831			2161		
TEST COMPOUND										
10.000	UL		RAT	LIVER	83			142		
50.000	UL		RAT	LIVER	260			214		
100.000	UL		RAT	LIVER	396			255		
200.000	UL		RAT	LIVER	437			216		

** :
TA-98 2-NITROFLUORENE 10 UG/PLATE
TA-100 SODIUM AZIDE 10 UG/PLATE
SOLVENT 50 UL/PLATE

***
TA-98 2-ANTHRAMINE 2.5 UG/PLATE
TA-100 2-ANTHRAMINE 2.5 UG/PLATE

RESULTS

TABLE 2

A. NAME OR CODE DESIGNATION OF THE TEST COMPOUND: 424 (ADELPHI 1 XAD EXTRACT)
B. SOLVENT: DMSO
C. TEST INITIATION DATES: 09/03/81
D. TEST COMPLETION DATE: 09/07/81
E. S-9 LOT#: S9-11
NOTE: CONCENTRATIONS ARE GIVEN IN MICROLITERS PER PLATE

TEST		SPECIES	TISSUE	REVERTANTS PER PLATE TA-1535 1	2	3	TA-1537 1	2	3
NONACTIVATION									
SOLVENT CONTROL		---	---	18			7		
SOLVENT CONTROL		---	---	28			12		
POSITIVE CONTROL**		---	---	1041			823		
POSITIVE CONTROL**		---	---	954			349		
TEST COMPOUND									
10.000	UL	---	---	-			11		
50.000	UL	---	---	-			17		
100.000	UL	---	---	-			20		
200.000	UL	---	---	41			9		
ACTIVATION									
SOLVENT CONTROL		RAT	LIVER	15			8		
SOLVENT CONTROL		RAT	LIVER	14			10		
POSITIVE CONTROL***		RAT	LIVER	586			297		
POSITIVE CONTROL***		RAT	LIVER	369			173		
TEST COMPOUND									
10.000	UL	RAT	LIVER	-			41		
50.000	UL	RAT	LIVER	-			37		
100.000	UL	RAT	LIVER	-			46		
200.000	UL	RAT	LIVER	15			26		

**,
TA-1535 SODIUM AZIDE 10 UG/PLATE
TA-1537 9-AMINOACRIDINE 50 UG/PLATE
SOLVENT 50 UL/PLATE
- INDICATES TEST WAS NOT DONE

***
TA-1535 2-ANTHRAMINE 2.5 UG/PLATE
TA-1537 2-ANTHRAMINE 2.5 UG/PLATE

VII. ASSAY ACCEPTANCE AND EVALUATION CRITERIA

Statistical methods are not currently used, and evaluation is based on the criteria included in this protocol.

Plate test data consists of direct revertant colony counts obtained from a set of selective agar plates seeded with populations of mutant cells suspended in a semisolid overlay. Because the test material and the cells are incubated in the overlay for approximately 2 days and a few cell divisions occur during the incubation period, the test is semiquantitative in nature. Although these features of the assay reduce the quantitation of results, they provide certain advantages not contained in a quantitative suspension test:

- The small number of cell divisions permits potential mutagens to act on replication DNA, which is often more sensitive than nonreplicating DNA.
- The combined incubation of the test article and the cells in the overlay permits constant exposure of the indicator cells for approximately 2 days.

A. Surviving Populations

Plate test procedures do not permit exact quantitation of the number of cells surviving chemical treatment. At low concentrations of the test material, the surviving population on the treatment plates is essentially the same as that on the negative control plate. At high concentrations, the surviving population is usually reduced by some fraction. Our protocol will normally employ several doses ranging over two or three log concentrations, the highest of these doses being selected to show slight toxicity as determined by subjective criteria.

B. Dose-Response Phenomena

The demonstration of dose-related increased in mutant counts is an important criterion in establishing metagenicity. A factor that might modify dose-response results for a mutagen would be the selection of doses that are too low (usually mutagenicity and toxicity are related). If the highest dose is far lower than a toxic concentration, no increases may be observed over the dose range selected. Conversely, if the lowest dose employed is highly cytotoxic, the test material may kill any mutants that are induced, and the test material will not appear to be mutagenic.

C. Control Tests

Positive and negative control assays were conducted with each experiment and consisted of direct-acting mutagens for nonactivation assays and mutagens that require metabolic biotransformation in activation assays.

Negative controls consisted of the test material solvent in the overlay agar together with the other essential components. The negative control plate for each strain gave a reference point to which the test data was compared. The positive control assay was conducted to demonstrate that the test systems were functional with known mutagens.

The following normal range of revertants for solvent controls are generally considered acceptable.

TA-1535:	8-30
TA-1537:	4-30
TA-98:	20-75
TA-100:	80-250

D. Evaluation Criteria for Ames Assay

Because the procedures to be used to evaluate the mutagenicity of the test material are semiquantitative, the criteria to be used to determine positive effects are inherently subjective and are based primarily on a historical data base. Most data sets will be evaluated using the following criteria.

1. Strains TA-1535 and TA-1537

If the solvent control value is within the normal range, a test material that produces a positive dose response over three concentrations with the highest increase equal to three times the solvent control value will be considered to be mutagenic.

2. Strains TA-98 and TA-100

If the solvent control value is within the normal range, a test material that produces a positive dose response over three concentrations with the highest increase equal to twice the solvent control value for TA-98 and TA-100 will be considered to be mutagenic.

3. Pattern

Because TA-1535 and TA-100 are both derived from the same parental strain (G-46), to some extent there is a built-in redundancy in the microbial assay. In general, the two strains of a set respond to the same mutagen and such a pattern is sought. Generally, if a strain responds to a mutagen in nonactivation tests, it will do so in activation tests.

4. Reproducibility

If a test material produces a response in a single test that cannot be reproduced in additional runs, the initial positive test data lose significance.

The preceding criteria are not absolute, and other extenuating factors may enter into a final evaluation decision. However, these criteria will be applied to the majority of situations and are presented to aid those individuals not familar with this procedure. As the data base is increased, the criteria for evaluation can be more firmly established.

E. Relation Between Mutagenicity and Carcinogenicity

It must be emphasized that the Ames *Salmonella*/Microsome Plate Assay is not a definitive test for chemical carcinogens. It is recognized, however, that correlative and functional relations have been demonstrated between these two endpoints. The results of comparative tests on 300 chemicals by McCann *et al.*[4] show an extremely good correlation between results of microbial mutagenesis tests and *in vivo* rodent carcinogenesis assays.

All evaluations and interpretation of the data to be presented in the final report will be based only on the demonstration, or lack, of mutagenic activity.

F. Criteria for Ranking Samples in the Ames Assay

The goal of EPA Level 1 Ames testing is to rank source streams by relative degree of genetic toxicity (mutagenicity). Samples are first identified as mutagenic or nonmutagenic by the criteria in Section D above and then ranked using the mutagenicity categories presented in the table below. The lowest concentration giving a positive response in any strain, with or without metabolic activation, is identified as the minimum effective concentration (MEC) for that sample. The mutagenicity of the sample is evaluated as high (H), moderate (M), low (L), or nondetectable (ND) according to the evaluation criteria developed in the Level 1 manual[1] and summarized below. Samples with no detectable activity at the maximum applicable dose (MAD) are ranked nondetectable (ND).

Ames Assay Mutagenicity Ranking Criteria[1]

Mutagenic Activity	Solids (MEC in µg/plate)	Liquids[a] (MEC in µl/plate)
High (H)	<50	<2
Moderate (M)	50-500	2-20
Low (L)	500-5000	20-200
Not Detectable (ND)	>5000	>200

[a]Concentration of organic extracts is based upon organic content (µg organics per plate) and not volume (µl extract per plate) of sample tested.

VIII. REFERENCES

1. Brusick, D.J., et al.: IERL-RTP Procedures Manual: Level 1 Environmental Assessment Biological Tests. EPA Contract No. 68-02-2681, Technical Directive No. 501, Litton Bionetics, Inc., Kensington, MD, September 1980, 177 pp. In press.

2. Brusick, D.J.: Level 1 Bioassay Assessment and Data Formatting. EPA-600/7-80-079, Litton Bionetics, Inc., Kensington, MD, April 1980, 100 pp.

3. Brusick, D.J. and Young, R.R.: Level 1 Environmental Assessment: Limits of Bioassay Resolution and Detectability. EPA Contract No. 68-02-2681, Technical Directive No. 301, Litton Bionetics, Inc., Kensington, MD, February 1981, 56 pp. In press.

4. McCann, J., Choi, E., Yamasaki, E. and Ames, B.N.: Detection of carcinogens as mutagens in the Salmonella/microsome test: Assay of 300 chemicals. Proc. Nat. Acad. Sci., USA 72:5135-5139, 1975.

5. Ames, B.N., Gurney, E.G., Miller, J.A. and Bartsch, H.: Carcinogens as frameshift mutagens: Metabolites and derivatives of 2-acetylaminofluorene and other aromatic amine carcinogens. Proc. Nat. Acad. Sci., USA 69:3128-3132, 1972.

6. Ames, B.N., Lee, F.D., and Durston, W.E.: An improved bacterial test system for the detection and classification of mutagens and carcinogens. Proc. Nat. Acad. Sci., USA 70:782-786, 1973.

7. Ames, B.N., Durston, W.E., Yamasaki, E. and Lee, F.D.: Carcinogens are mutagens: A simple test system combining liver homogenates for activation and bacteria for detection. Proc. Nat. Acad. Sci., USA 70:2281-2285, 1973.

8. McCann, J., Springarn, N.E., Kobori, J. and Ames, B.N.: Detection of carcinogens as mutagens: Bacterial tester strains with R factor plasmids. Proc. Nat. Acad. Sci. USA 72:979-983, 1975.

9. Ames, B.N., McCann, J. and Yamasaki, E.: Methods for detecting carcinogens and mutagens with the Salmonella/mammalian-microsome mutagenicity test. Mutation Res., 31:347-364, 1975.

10. Vogel, H.J. and Bonner, D.M.: Acetylornithinase of E. coli partial purification and some properties. J. Biol. Chem., 218:97-106, 1966.

GENETICS ASSAY NO. 5806

LBI SAFETY NO. 7032

CYTOTOXIC EVALUATION OF 424 (ADELPHI 1 XAD EXTRACT) IN THE RODENT CELL (CHO) CLONAL TOXICITY ASSAY

FINAL REPORT

SUBMITTED TO:

ACUREX CORPORATION
485 CLYDE AVENUE
MOUNTAIN VIEW, CALIFORNIA 94092

SUBMITTED BY:

LITTON BIONETICS, INC.
5516 NICHOLSON LANE
KENSINGTON, MARYLAND 20895

LBI PROJECT NO. 22064

REPORT DATE: SEPTEMBER 1981

5-92

Litton BIONETICS

PREFACE

This assay conforms to the standard EPA Level 1 procedure for the Chinese hamster ovary cell (CHO) clonal toxicity assay as described in "IERL-RTP Procedures Manual: Level 1 Environmental Assessment Biological Tests" (1). The data was evaluated and formatted as recommended in "Level 1 Biological Testing Assessment and Data Formatting" (2).

The CHO clonal toxicity assay has been shown to be a sensitive method for detecting cytotoxic activity for a variety of chemicals representing various chemical classes (3). This assay is one of several recommended by EPA to identify, categorize and rank the pollutant potential of influent and effluent streams from industrial and energy-producing processes. This assay has been well validated with a wide range of positive and negative control chemicals and complex environmental samples.

All procedures and documents pertaining to the receipt, storage, preparation, testing and evaluation of the test material shall conform to Litton Bionetics, Inc. standard operating procedures and the Good Laboratory Practices Act of 1979. Deviations from standard procedure shall be fully documented and noted in the report.

All test and control results in this report are supported by fully documented raw data which are permanently maintained in the files of the Department of Molecular Toxicology or in the archives of Litton Bionetics, Inc., 5516 Nicholson Lane, Kensington, Maryland 20895. Copies of raw data will be supplied to the sponsor upon request.

TABLE OF CONTENTS

		Page No.
PREFACE		i
I.	ASSAY SUMMARY	1
II.	OBJECTIVE	2
III.	TEST MATERIAL	3
	A. Description	3
	B. Handling and Preparation	3
IV.	MATERIALS	4
	A. Indicator Cells	4
	B. Media	4
	C. Controls	4
V.	EXPERIMENTAL DESIGN	5
	A. Dose Selection	5
	B. Clonal Toxicity Assay	5
VI.	ASSAY ACCEPTANCE CRITERIA	7
VII.	RESULTS	8
	A. Interpretation	8
	B. Tables and Figures	8
VIII.	ASSAY EVALUATION CRITERIA	11
IX.	REFERENCES	12

I. ASSAY SUMMARY

A. SPONSOR: Acurex Corporation

B. MATERIAL (TEST COMPOUND): GENETICS ASSAY NUMBER: 5806

1. Identification: 424 (Adelphi 1 XAD extract)

2. Date Received: June 28, 1981

3. Physical Description: Transparent liquid with a yellow tint.

C. TYPE OF ASSAY: Rodent Cell (CHO) Clonal Toxicity Assay

D. ASSAY DESIGN NUMBER: 442

E. STUDY DATES:

A. Initiation: August 20, 1981

B. Completion: August 27, 1981

F. SUPERVISORY PERSONNEL:

A. Study Director: Brian C. Myhr, Ph.D.

B. Laboratory Supervisor: Robert Young, M.S.

G. EVALUATION:

The test material caused a slight increase in toxicity with increasing concentrations up to 10 µl/ml. The material was completely lethal to the cells at the maximum applicable dose of 20 µl/ml. The EC_{50} was estimated graphically to be 13 µl/ml which was equivalent to 63.2 µg of organics per ml. This sample was therefore evaluated to be in the moderate (M) toxicity category defined for the IERL-EPA Level 1 CHO clonal toxicity bioassay.[1]

Submitted by:

Study Director

Brian Myhr 9/9/81

Brian Myhr, Ph.D. Date

Associate Director,
Department of Molecular Toxicology

Reviewed by:

James L. Lewerman PhD for VPASD

David J. Brusick, Ph.D. Date 9/9/87

Director,
Department of Molecular Toxicology

II. OBJECTIVE

The objective of this study was to determine and rank the cytotoxicity of sample 424 (Adelphi 1 XAD extract) to cultured Chinese hamster cells (CHO-K1 cell line). The measure of cytotoxicity was the reduction in colony-forming ability after a 24-hour exposure to the test material. After a period of recovery and growth, the number of colonies that developed in the treated cultures was compared to the colony number in unexposed vehicle control cultures. The concentration of test material that reduced the colony number by 50% was estimated graphically and referred to as the EC50 value. Standard EPA Level 1 toxicity evaluation criteria for the CHO clonal toxicity assay were used to rank the toxicity potential of the test material.

III. TEST MATERIAL

A. Description

The test material was received as a clear yellow tinted solution in methylene chloride. The sample contained 17.0 milligrams of organic material in an undetermined volume of methylene chloride. No information on the sampling parameters (such as the equivalent volume of stack gas represented by the sample) was provided.

B. Handling and Preparation

The test material was received at LBI on June 28, 1981. The sample was assigned LBI safety number 7032 and LBI assay number 5806. The sample was stored at +4°C in the dark.

Pretest sample preparation consisted of solvent-exchanging the sample into dimethylsulfoxide (DMSO). The sample was transferred with methylene chloride rinses into a graduated conical tube. The methylene chloride was gradually evaporated (50°C under a stream of nitrogen) and DMSO was sequentially added. The sample was brought to volume in 3.5 ml of DMSO, giving a sample concentration of 4.86 mg organics per ml DMSO. The sample was then transferred to a glass vial and sealed with a teflon-coated rubber septum.

A total volume of 0.45 ml of test sample was used in the CHO assay. The maximum concentration of 20 µl/ml was obtained by adding 0.12 ml of sample to 5.88 ml of F12 medium; this resulted in 2% (v/v) DMSO in the medium and effectively limited the concentration of test material that could be assayed. Only two plates were dosed at the top dose in order to conserve sample. Another 0.12 ml aliquot of sample was used to prepare the 10 µl/ml test concentration. An additional 0.21 ml of test sample was used to prepare a series of dilutions in DMSO from which 1:100 dilutions into growth medium were performed to obtain the lower assayed concentrations. Thus, except for the 20 µl/ml test concentration, the final DMSO concentration was constant at 1% (v/v).

IV. MATERIALS

A. Indicator Cells

The indicator cells for this study were Chinese hamster CHO-K1 cells (ATCC No. CCL 61) obtained from Flow Laboratories, Inc., Rockville, MD. This cell type was derived from ovarian tissue and has spontaneously transformed to a stable, hypodiploid line of rounded, fibroblastic cells with unlimited growth potential. Monolayer cultures have a fast doubling time of 11 to 14 hours, and untreated cells can normally be cloned with an efficiency of 80% or greater. Laboratory stock are maintained by routine serial subpassage. Cells are cultivated in Ham's F-12 nutrient medium at 37°C in 5 percent CO_2 with saturated humidity. Stocks are continually observed macroscopically and microscopically for possible microbial contamination. Laboratory cultures are periodically checked by culturing and staining methods for the absence of mycoplasma contamination. Laboratory cultures are discarded every three months and new cultures started from mycoplasma-free, long-term frozen cultures.

B. Media

The CHO-K1 cell line has an absolute requirement for proline and therefore must be maintained in culture medium containing sufficient amounts of this amino acid. Ham's F12 medium, which contains 3×10^{-4} M L-proline was used, supplemented with 10% fetal bovine serum, 2mM L-glutamine, 100 units/ml of penicillin, 100 µg/ml of streptomycin, and 0.9 µl/ml of amphotericin B. A 10X formulation of Ham's F10 is available commercially and was used for the testing of aqueous test samples in order to avoid the dilution of medium components. This medium contains 1×10^{-4} L-proline and was supplemented in the same manner as F12, except that kanamycin at 40 µg/ml is included for additional protection against bacterial contamination. Both media formulations support the growth and cloning of CHO cells equally well.

C. Controls

The negative control consists of three untreated cultures carried through the same experimental time period as the treated cells. The test material was supplied as a solution in an organic vehicle (usually DMSO) and was diluted into the medium to provide each test concentration, three vehicle control cultures containing the organic solvent at 1% or 2% by volume are prepared. Particulate test samples were suspended in small amounts of DMSO, but DMSO was not included in the negative control because the maximum content in the treated cultures is only 0.42%. The average number of colonies in the negative control establishes the cloning efficiency of the CHO cells used in the assay, and the appropriate negative or vehicle controls provide the reference points for determining the effects of different concentrations of the test material on cell survival.

V. EXPERIMENTAL DESIGN

A. Dose Selection

Unless the approximate toxicity is already known or the sample size is limiting, the following dose ranges are usually tested for different sample forms. Aqueous samples, suspensions, or slurries are tested from 600 µl/ml to 3 µl/ml, usually in six dose steps. Eight doses are often used when the amount of test sample is limited to provide a more precise description of toxicity in the event of sharp dose-response curves. Dry, particulate material is dissolved or suspended in DMSO, diluted into growth medium, and tested at six dose levels from 1000 µg/ml to 3 µg/ml. Samples that are solvent-exchanged into DMSO are tested from 20 µl/ml (2% DMSO in growth medium) to 0.2 µl/ml, also in six dose steps. A second dose study is performed with an adjusted dose range if the EC50 was not located properly in the initial test. However, EC50 values greater than 1000 µg/ml for particulate material, 600 µl/ml for aqueous samples, or 20 µl/ml for organic solutions will not be determined.

This sample, number 424 (Adelphi 1 XAD extract) was tested at eight dose levels. The concentrations started with the maximum applicable dose (MAD) of 20 µl/ml and included 10, 6, 3, 1, 0.6, 0.3, and 0.1 µl of test material per ml of culture medium. The corresponding concentration of organics at the MAD level was 97.2 µg/ml; the lower doses were equivalent to 48.6, 29.2, 14.6, 4.9, 2.9, 1.5, and 0.5 µg/ml.

B. Clonal Toxicity Assay

Cells from monolayer stock cultures in logarithmic growth phase were trypsinized with 0.1% trypsin plus 0.01% versene for 4 minutes and the density of the resulting cell suspension determined by hemocytometer. A number of 60-mm culture dishes were then seeded with 200 cells and 4 ml of culture medium per dish. The cultures were incubated for approximately 6 hours at 37°C in a humidified atmosphere containing 5% CO_2 to allow attachment of the cells. The 6-hour attachment period was used in order to avoid cell division and the subsequent formation of two-cell colonies prior to treatment.

If the medium was aspirated from the cultures and 4 ml medium containing the test material applied. Three cultures were exposed to each test concentration. After an exposure time of 24 hours at 37°C, the medium was removed by aspiration and each culture washed three times with approximately 4 ml aliquots of Dulbecco's phosphate buffered saline (pre-warmed to 37°C). Fresh culture medium (5 ml) was placed in each dish and incubation at 37°C is continued for an additional 6 days to allow colony development.

If the test material caused a color change in the culture medium, the pH of the medium containing the high dose would be determined at the time of treatment. The pH at the lowest dose that results in a slight color change

would also be recorded. At the end of the treatment period, the pH values of the discarded media from the two described treatments would be recorded again. No sample related pH effects were noted.

After the incubation period, the medium was drained from the cultures and the surviving colonies fixed with 100% ethanol and stained with Giemsa. Colonies were counted by eye; tiny colonies of approximately 50 cells or less were arbitrarily excluded from the counts.

VI. ASSAY ACCEPTANCE CRITERIA

The assay is considered acceptable for evaluation of the test results if the following criteria are met:

- The average cloning efficiency of the CHO-K1 cells in the negative controls is 70% or greater, but not exceeding 115%.
- The distribution of colonies in the treated cultures is generally uniform over the surface of the culture dish.
- The data points for each test concentration critical to the location of the EC50 are the averages of at least two treated cultures.
- A sufficient number of test concentrations are available to clearly locate the EC50 within a toxicity region as defined under Assay Evaluation Criteria.
- If the EC50 value is greater than 1000 µg/ml, 600 µliters of aqueous sample/ml, or 20 µliters of nonaqueous sample/ml, the plotted curve does not exceed 110% of the negative control.

VII. RESULTS

A. Interpretation

The application of sample 424 (Adelphi 1 XAD extract) to the CHO cell cultures caused a rapid lowering of the number of cells able to form colonies as the concentration increased above 10 µl/ml. Relative survival values were calculated as the ratio of colonies formed in treated cultures to the colonies formed in the appropriate vehicle control, and these relative survival values were plotted against the concentration of test material. As shown in Figure 1, the relative survival decreased gradually in the 0.1 to 10 µl/ml range and dropped to zero at the 20 µl/ml dose level.

The concentration expected to kill 50 percent of the cells (EC_{50}) was found to be 13 µl of test material per ml of culture medium. This concentration was equivalent to 63.2 µg of organic material per ml of culture medium. This value placed the test material in the moderate (M) toxicity range defined for the IERL-EPA CHO clonal toxicity bioassay.

The cells used for the assay were in logarithmic growth phase and were 100% viable. About 77% of the seeded cells formed colonies and in both the 1% and 2% DMSO vehicle control, slightly less than 72% of the cells formed colonies. Colony growth was normal and well distributed on the culture dishes. These results achieved the assay acceptance criteria and provided confidence in the assumption that the recorded data represented typical responses to the test material.

B. Tables and Figures

This report is based on the data provided in Table 1 and Figure 1.

TABLE 1
RODENT CELL (CHO) CLONAL TOXICITY ASSAY

Sample Identity: 424 (Adelphi 1 XAD Extract

Description of Sample: Transparent liquid with a yellow tint

LBI Assay No.: 5806

Date Received: June 28, 1981

Test Date: August 20, 1981

Vehicle: DMSO

Cell Type: CHO-K1

Cells Seeded per Dish: 200

EC50 Value: 63.2 µg organics/ml (13.0 µl/ml)

Toxicity Classification: Moderate (M)

pH Alterations: None

Comments on Treatment: Sample prepared in DMSO at a concentration of 4.86 µg organics/µl

COLONY COUNTS

Sample	Applied Concentration µl/ml	Dish #1	Dish #2	Dish #3	Average Count	Relative Survival[a] %	Cloning Efficiency %
NC[b]	--	157	160	144	153.7	---	76.9
VC,1%[c]	10	154	131	145	143.3	100.0	71.7
VC,2%	20	145	140	144	143.0	100.0	71.5
Test	0.1	145	130	133	136.0	94.9	
Test	0.3	119	131	134	128.0	89.3	
Test	0.6	122	137	130	129.7	90.5	
Test	1.0	132	130	110	124.0	86.5	
Test	3.0	123	119	120	120.7	84.2	
Test	6.0	129	121	115	121.7	84.9	
Test	10.0	117	106	121	114.7	80.0	
Test[d]	20.0	0	0	---	0	0	

[a]Relative to 2% VC for 20 µl/ml treatment and to 1% VC for other treatments.

[b]NC = Negative Control, F_{12} medium.

[c]VC = Vehicle Control, percent DMSO given.

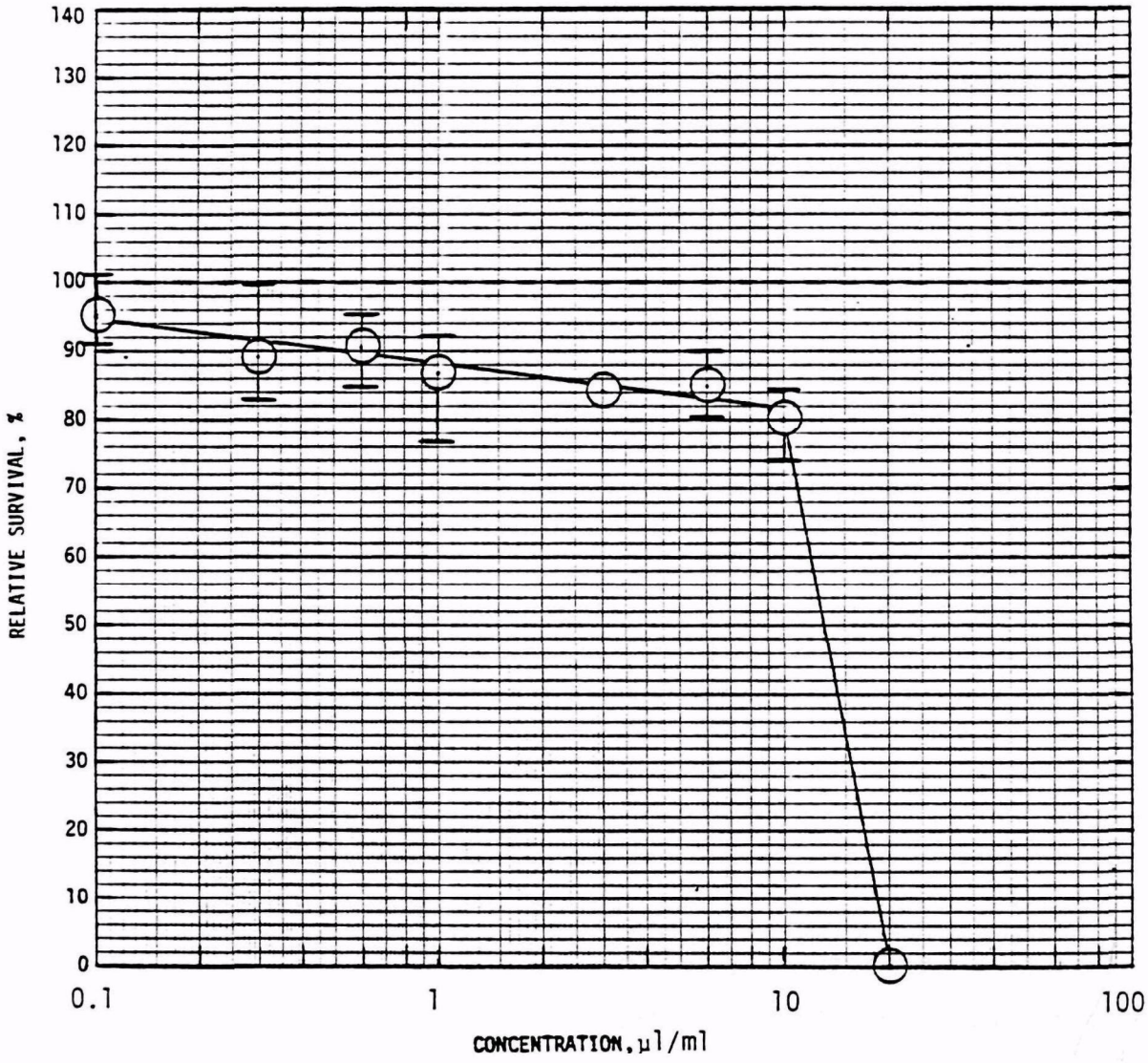
[d]Only two plates dosed to conserve limited test material.

FIGURE 1

RODENT CELL (CHO) CLONAL TOXICITY ASSAY

EC_{50} DETERMINATION

424 (ADELPHI 1 XAD EXTRACT)



VIII. ASSAY EVALUATION CRITERIA

The EC50 value represents the concentrations of test material that reduces the colony-forming ability of CHO cells to 50% of the vehicle or negative control value. EC50 values are determined graphically by fitting a curve by eye through relative survival data plotted as a function of the logarithm of the applied concentration. Each data point normally represents the average of three culture dishes. In order to indicate the variability of the data, the high and low colony counts for each concentration are used to calculate the relative survivals, and the range is shown by a bar at the position of the plotted average. If no bar is shown, the variability was within the size of the symbol. Statistical analysis is unnecessary in most cases for evaluation.

The toxicity of the test material is evaluated as high, moderate, low, or nondetectable according to the range of EC50 values defined in the following table.

Toxicity[a]	Solids (EC_{50} in µg/ml)	Aqueous Liquids (EC_{50} in µl/ml)	Nonaqueous Liquids[b] (EC_{50} in µl/ml)
High	<10	<6	<.2
Moderate	10 to 100	6 to 60	.2-2
Low	100 to 1000	60 to 600	2-20
Not Detectable	>1000	>600	>20

[a]Evaluation criteria formulated by Litton Bionetics, Inc. for IERL-RTP Procedures Manual: Level Environmental Assessment Biological Tests.

[b]Criteria for nonaqueous liquids are tentative and under evaluation. If the organic or solids content is known, the sample is evaluated under the solids criteria.

Another evaluation scheme is proposed for extracts obtained from SASS train gas volumes. The proportion of the total gas volume corresponding to the volume of extract used in the bioassay is calculated and expressed as L/ml of culture medium (or DSCF/ml of culture medium). A criterion of 1000 L/ml is set as the limit for nondetectable toxicity. This gas volume corresponds to the average volume breathed by humans over a 2-hour period. The subsequent toxicity ranges are defined by 10-fold dilution steps to conform to standard procedure. The toxicity ranges are defined in the following table for liter and dry standard cubic feet units:

Toxicity	EC_{50} In Liters/ml (L/ml)	EC_{50} In Dry Standard Cubic Feet/ml (DSCF/ml)
High	<10	<0.35 DSCF
Moderate	10-100	0.35-3.5
Low	100-1000	3.5-35
Nondetectable	>1000	>35

IX. REFERENCES

1. Brusick, D.J., et al.: IERL-RTP Procedures Manual: Environmental Assessment Biological Tests. EPA Contract No. 68-02-2681, Technical Directive No. 501, Litton Bionetics, Inc., Kensington, MD, September 1980, 177 pp. In press.

2. Brusick, D.J.: Level 1 Bioassay Assessment and Data Formatting. EPA-600/7-80-079, Litton Bionetics, Inc., Kensington, MD, April 1980, 100 pp.

3. Brusick, D.J. and Young, R.R.: Level 1 Environmental Assessment: Limits of Bioassay Resolution and Detectability. EPA Contract No. 68-02-268, Technical Directive No. 301, Litton Bionetics, Inc., Kensington, MD, February 1981, 56 pp. In press.

GENETICS ASSAY NO. 5809
LBI SAFETY NO. 7033

MUTAGENICITY EVALUATION OF 436 (ADELPHI 2 XAD EXTRACT) IN THE EPA LEVEL 1 AMES SALMONELLA/MICROSOME PLATE TEST

FINAL REPORT

SUBMITTED TO:

ACUREX CORPORATION
485 CLYDE AVENUE
MOUNTAIN VIEW, CALIFORNIA 94042

SUBMITTED BY:

LITTON BIONETICS, INC.
5516 NICHOLSON LANE
KENSINGTON, MARYLAND 20895

LBI PROJECT NO. 22064

REPORT DATE: SEPTEMBER 1981

PREFACE

This assay conforms to the standard EPA Level 1 procedure for the Ames Salmonella/microsome mutagenesis assay as described in "IERL-RTP Procedures Manual: Level 1 Environmental Assessment Biological Tests"[1]. The data was evaluated and formatted as recommended in "Level 1 Biological Testing Assessment and Data Formatting"[2].

The Ames Salmonella/microsome mutagenesis assay has been shown to be a sensitive method for detecting mutagenic activity for a variety of chemicals representing various chemical classes[3]. This assay is one of several recommended by EPA to identify, categorize and rank the pollutant potential of influent and effluent streams from industrial and energy-producing processes. This assay has been well validated with a wide range of positive and negative control chemicals and complex environmental samples.

All procedures and documents pertaining to the receipt, storage, preparation, testing and evaluation of the test material shall conform to Litton Bionetics, Inc. standard operating procedures and the Good Laboratory Practices Act of 1979. Deviations from standard procedure shall be fully documented and noted in the report.

All test and control results in this report are supported by fully documented raw data which are permanently maintained in the files of the Department of Molecular Toxicology or in the archives of Litton Bionetics, Inc., 5516 Nicholson Lane, Kensington, Maryland 20895. Copies of raw data will be supplied to the sponsor upon request.

TABLE OF CONTENTS

		Page No.
	PREFACE	i
I.	ASSAY SUMMARY	1
II.	OBJECTIVE	2
III.	TEST MATERIAL	3
	A. Description	3
	B. Handling and Preparation	3
IV.	MATERIALS	4
	A. Indicator Microorganisms	4
	B. Media	4
	C. Activation System	5
	1. S9 Homogenate	5
	2. S9 Mix	5
V.	EXPERIMENTAL DESIGN	6
	A. Dose Selection	6
	B. Mutagenicity Test	6
	1. Nonactivation Assay	6
	2. Activation Assay	6
	C. Control Compounds	7
	D. Recording and Presenting Data	7
VI.	RESULTS	9
	A. Interpretation	9
	B. Tables	9
VII.	ASSAY ACCEPTANCE AND EVALUATION CRITERIA	12
	A. Surviving Populations	12
	B. Dose-Response Phenomena	12
	C. Control Tests	12
	D. Evaluation Criteria for Ames Assay	13
	1. Strains TA-1535 and TA-1537	13
	2. Strains TA-98 and TA-100	13
	3. Pattern	13
	4. Reproducibility	13
	E. Relation Between Mutagenicity and Carcinogenicity	14
	F. Criteria for Ranking Samples in the Ames Assay	14
VIII.	REFERENCES	15

I. ASSAY SUMMARY

A. Sponsor: Acurex Corporation

B. Material (Test Compound): Genetics Assay Number: 5809

1. Identification: 436 (Adelphi 2 XAD Extract)

2. Date Received: June 28, 1981

3. Physical Description: Clear, colorless liquid

C. Type of Assay: EPA Level 1 Ames Salmonella/Microsome Plate Test

D. Assay Design Number: 401 (EPA Level 1)

E. Study Dates:

1. Initiation: August 22, 1981

2. Completion: September 7, 1981

F. Supervisory Personnel:

A. Study Director: D.R. Jagannath, Ph.D.

G. Evaluation:

The test sample, 436, was mutagenic in the Ames Salmonella/microsome assay. The sample was mutagenic for frameshift tester strains TA-1537 and TA-98 under activation (+S9) conditions. Strain TA-100 was reverted by the sample but the response did not meet the established criteria for a positive effort. Strain TA-1535 was not affected by sample 436 at the single concentration evaluated. The minimum effective concentration (MEC) was 10 µl/plate with strain TA-98. This was equivalent to 13 µg organics/plate. This response was evaluated to be in the high (H) mutagenicity range defined for the IERL-EPA Level 1 Ames bioassay[1].

Submitted by:

Study Director

for: D.R. Jagannath, Ph.D. 9/11/81 Date
Section Chief,
Submammalian Genetics,
Department of Molecular Toxicology

Reviewed by:

David J. Brusick, Ph.D. 9/11/81 Date
Director,
Department of Molecular Toxicology

II. OBJECTIVE

The objective of this study was to determine the genetic activity of sample 436 (Adelphi 2 XAD extract) in the Salmonella/microsome assay with and without the addition of mammalian metabolic activation preparations. The genetic activity of a sample is measured in these assays by its ability to revert the Salmonella indicator strains from histidine dependence to histidine independence. The degree of genetic activity of a sample is reflected in the number of revertants that are observed on the histidine-free medium.

III. TEST MATERIAL

A. Description

The test material was received as a clear, colorless solution in methylene chloride. The sample contained 4.7 milligrams of organic material in an undetermined volume of methylene chloride. No information on the sampling parameters (such as the equivalent volume of stack gas represented by the sample) was provided.

B. Handling and Preparation

The test material was received at LBI on June 28, 1981. The sample was assigned LBI safety number 7033 and LBI assay number 5809. The sample was stored at +4°C in the dark.

Pretest sample preparation consisted of solvent exchanging the sample into dimethylsulfoxide (DMSO). The sample was transferred with methylene chloride rinses into a graduated conical tube. The methylene chloride was gradually evaporated (50°C under a stream of nitrogen) and DMSO was sequentially added. The sample was brought to volume in 3.5 ml of DMSO, giving a sample concentration of 1.3 mg organics per ml DMSO. The sample was transferred to a glass vial and sealed with a teflon-coated rubber septum.

A total volume of 2.56 ml of test material was used between three trials. In the trials, varying aliquots of the test material were added directly to the test mixtures to give the desired concentration. The amount of sample used was 0.36 ml for Trial 1, 1.08 ml for Trial 2 and 1.12 ml for Trial 3.

IV. MATERIALS

A. Indicator Microorganisms

The *Salmonella typhimurium* strains used in this assay were obtained from Dr. Bruce Ames, University of California at Berkeley.[4-8] The following four strains were used.

Strain Designation	Gene Affected	Additional Mutations			Mutation Type Detected
		Repair	LPS	R Factor	
TA-1535	*his* G	Δ *uvr* B	*rfa*	-	Base-pair substitution
TA-1537	*his* C	Δ *uvr* B	*rfa*	-	Frameshift
TA-98	*his* D	Δ *uvr* B	*rfa*	pKM101	Frameshift
TA-100	*his* G	Δ *uvr* B	*rfa*	pKM101	Base-pair substitution

All the above strains have, in addition to the mutation in the histidine operon, mutation (*rfa*-) that leads to defective lipopolysaccharide coat, a deletion that covers genes involved in the synthesis of vitamin biotin (*bio*-) and in the repair of ultraviolet (uv) - induced DNA damage (*uvr*B-). The *rfa*- mutation makes the strains more permeable to many large molecules. The *uvr*B- mutation decreases repair of some types of chemically or physically damaged DNA and thereby enhances the strain's sensitivity to some mutagenic agents. The resistant transfer factor plasmid (R factor) pKM101 in TA-98 and TA-100 is believed to cause an increase in error-prone DNA repair that leads to many more mutations for a given dose of most mutagens.[8] In addition, plasmid pKM101 confers resistance to the antibiotic ampicillin, which is a convenient marker to detect the presence of plasmid in the cells.

All indicator strains are kept at 4°C on minimal medium plates supplemented with a trace of biotin and an excess of histidine. In addition, the plates with plasmid-carrying strains contain ampicillin (25 μg/ml) to ensure stable maintenance of plasmid pKM101. New stock culture plates are made as often as necessary from the frozen master cultures or from single colony reisolates that were checked for their genotypic characteristics (*his*, *rfa* *uvr*B, *bio*) and for the presence of plasmid. For each experiment, an inoculum from the stock culture plates is grown overnight at 37°C in nutrient broth (Oxoid CM67) and used.

B. Media

The bacterial strains were cultured in Oxoid Media #2 (Nutrient Broth). The selective medium was Vogen Bonner Medium E with 2% glucose.[10] The

overlay agar consisted of 0.6% purified agar with 0.05 mM histidine, 0.05 mM biotin and 0.1M NaCl according to the methods of Ames *et al.*[9]

C. Activation System

1. S9 Homogenate

A 9,000 x *g* supernatant prepared from Sprague-Dawley adult male rat liver induced by Aroclor 1254 (Ames *et al.*[9]) was purchased from Bionetics Laboratory Products, Litton Bionetics, Inc. and used in these assays.

2. S9 Mix

S9 mix used in these assays consisted of the following components:

Components	Concentration per Milliliter S9 Mix
NADP (sodium salt)	4 µmoles
D-glucose-6-phosphate	5 µmoles
$MgCl_2$	8 µmoles
KCl	33 µmoles
Sodium phosphate buffer pH 7.4	100 µmoles
Organ homogenate from rat liver (S9 fraction)	100 µliters

V. EXPERIMENTAL DESIGN

A. Dosage Selection

Test strategy and dose selection depend upon sample type and sample availability. The Level 1 manual[1] recommends solids to be initially tested at the maximum applicable dose (MAD) of 5 mg per plate and at lower concentrations of 2.5, 1, 0.5, 0.1 and 0.05 mg per plate. Liquids are tested initially at the MAD of 200 µl per plate, and at lower concentrations of 100, 50 and 10 µl per plate. Samples are retested over a narrower range of concentrations with strains showing positive results initially. Alternate dose are employed if sample size is limiting or at the direction of the sponsor.

A preliminary range-finding test was run with strain TA-98 using the recommended doses of 200, 100, 50, and 10 µl per plate. Subsequent testing with the other strains utilized these dose levels. These doses were equivalent to 260, 130, 65, and 13 µg of organics per plate.

B. Mutagenicity Testing

The procedure used was based on the paper published by Ames et. al.[9] and was performed as follows:

1. Nonactivation Assay

To a sterile 13 x 100 mm test tube placed in a 43°C water bath the following was added in order:

- 2.00 ml of 0.6% agar containing 0.05 mM histidine and 0.05 mM biotin.
- 0.01 ml to 0.2 ml of a solution of the test chemical to give the appropriate dose.
- 0.1 ml to 0.2 ml of indicator organism(s).
- 0.50 ml of 0.2M phosphate buffer, pH 7.4.

This mixture was swirled gently and then poured onto minimal agar plates (see IV B, Media). After the top agar had set, the plates were incubated at 37°C for approximately 2 days. The number of his+ revertant colonies growing on the plates were counted with an automatic colony counter and recorded.

2. Activation Assay

The activation assay was run concurrently with the nonactivation assay. The only difference was the addition of 0.5 ml of S9 mix (see IV C, Activation System) to the tubes in place of 0.5 ml of phosphate buffer which was added in nonactivation assays. All other details were similar to the procedure for nonactivation assays.

A detailed flow diagram for the plate incorporation assay is provided in Figure 1.

C. Control Compounds

A negative control consisting of the solvent used for the test material was also assayed concurrently with the test material. For negative controls, step 'b' of Nonactivation Assays was replaced by 0.05 ml of the solvent. The negative controls were employed for each indicator strain and were performed in the absence and presence of S9 mix. The solvent used to prepare the stock solution of the test material is given in the Results section of this report. All dilutions of the test material were made using this solvent. The amount of solvent used was equal to the maximum volume used to give the appropriate test dose.

Specific positive control compounds known to revert each strain were also used and assayed concurrently with the test material. The concentrations and specificities of these compounds to specific strains are given in the following table:

Assay	Chemical	Solvent	Concentration per plate (µg)	Salmonella Strains
Nonactivation	Sodium azide	Water	10.0	TA-1535, TA-100
	2-Nitrofluorene (NF)	Dimethyl-sulfoxide	10.0	TA-98
	9-aminoacridine (9AA)	Ethanol	50.0	TA-1537
Activation	2-anthramine (ANTH)	Dimethyl-sulfoxide	2.5	For all strains

D. Recording and Presenting Data

The number of colonies on each plate were counted and recorded on printed forms. These raw data were analyzed in a computer program and reported on a printout. The results are presented as revertants per plate for each indicator strain employed in the assay. The positive and solvent controls are provided as reference points.

AMES ASSAY [PLATE INCORPORATION METHOD]

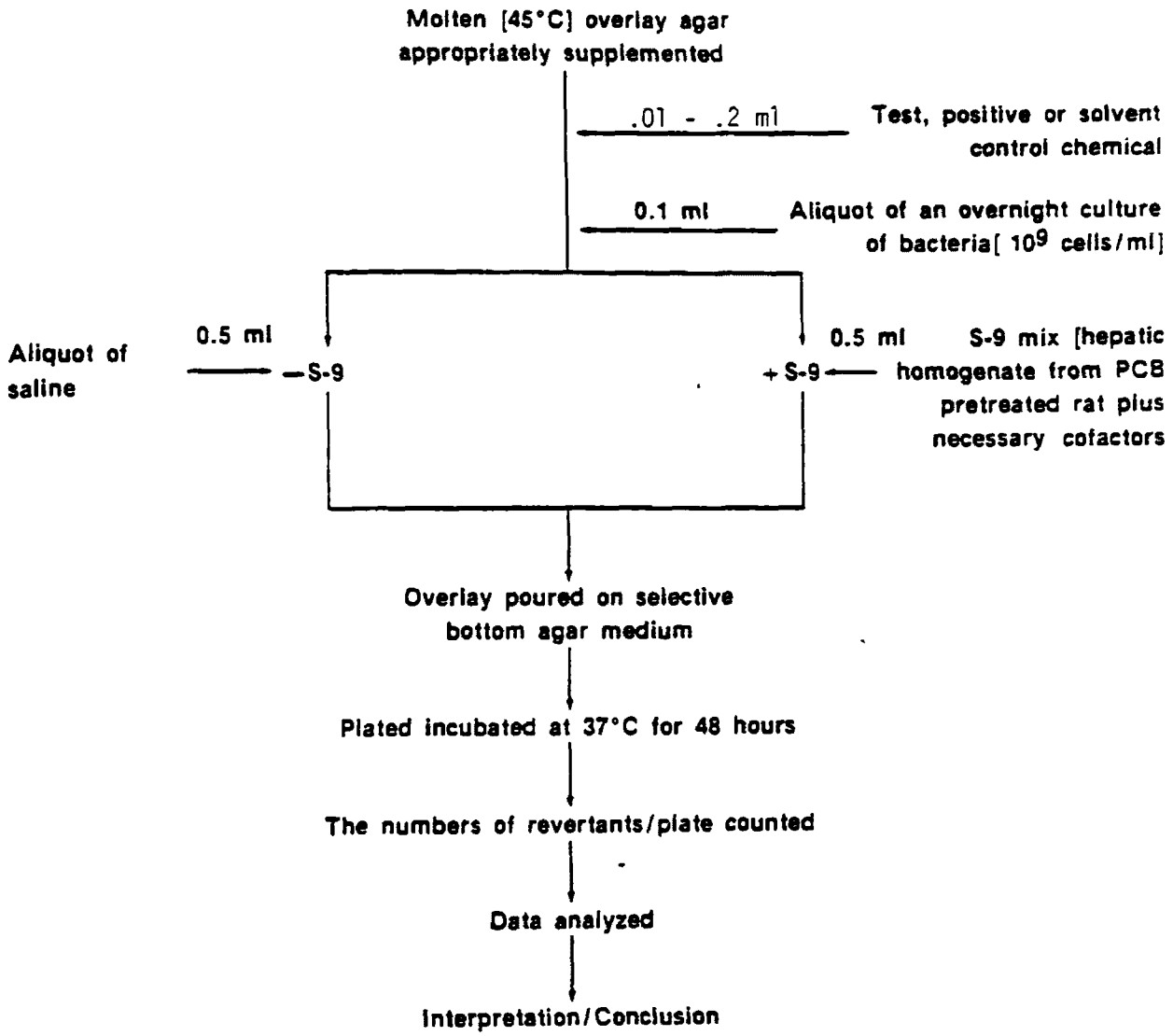


Figure 1 AMES SALMONELLA/MICROSOME MUTAGENESIS ASSAY

VI. RESULTS

A. Interpretations

The test material, sample 436, was tested in strains TA-98, TA-100, TA-1535 and TA-1537 both with and without the addition of metabolic activation. The tests were conducted at concentrations of 10, 50, 100 and 200 µl/plate except for strain TA-1535 which was tested only at 200 µl/plate. The assay was conducted in three steps. Trial 1 was a range-finding assay using strain TA-98 without activation. Trial 2 utilized strain TA-98 with activation and strain TA-100 both with and without activation. Trial 3 was with strains TA-1535 and TA-1537 both with and without activation.

The response in all strains without metabolic activation was negative. With activation, strains TA-98 and TA-1537 were mutagenic under the assay conditions and both showed a dose-related increase in the number of revertants. Strain TA-100 was reverted by the test material but the response did not meet the criteria for a positive effect. Strain TA-1535 was negative at the single dose tested. There was indication of sample related toxicity at the maximum applicable dose of 200 µl/plate.

The minimum effective concentration (MEC) was observed to be 10 µl/plate in TA-98 with activation. This was equivalent to 13 µg of organic material per plate. This response was categorized as high (H) mutagenic activity using the IERL-EPA Level 1 criteria for the Ames assay[1].

Solvent and positive control values were within acceptable ranges. These results achieve the assay acceptance criteria and provide confidence in the assumptions that the recorded data represented typical responses to the test material.

B. Tables

This report is based on the data provided in Tables 1 and 2.

RESULTS

TABLE 1

A. NAME OR CODE DESIGNATION OF THE TEST COMPOUND: 436(ADELPHI 2 XAD EXTRACT)
B. SOLVENT: DMSO
C. TEST INITIATION DATES: 08/22/81 08/25/81
D. TEST COMPLETION DATE: 09/01/81
E. S-9 LOT#: S9-11
NOTE: CONCENTRATIONS ARE GIVEN IN MICROLITERS PER PLATE

TEST	SPECIES	TISSUE	REVERTANTS PER PLATE TA-98 1	TA-98 2	TA-98 3	TA-100 1	TA-100 2	TA-100 3
NONACTIVATION								
SOLVENT CONTROL	---	---	43			123		
SOLVENT CONTROL	---	---	44			139		
POSITIVE CONTROL**	---	---	853			1748		
POSITIVE CONTROL**	---	---	732			1855		
TEST COMPOUND								
10.000 UL	---	---	55			125		
50.000 UL	---	---	50			200		
100.000 UL	---	---	59			198		
200.000 UL	---	---	64			127		
ACTIVATION								
SOLVENT CONTROL	RAT	LIVER	40			122		
SOLVENT CONTROL	RAT	LIVER	39			126		
POSITIVE CONTROL***	RAT	LIVER	1772			2161		
POSITIVE CONTROL***	RAT	LIVER	1831			2271		
TEST COMPOUND								
10.000 UL	RAT	LIVER	80			148		
50.000 UL	RAT	LIVER	237			187		
100.000 UL	RAT	LIVER	424			208		
200.000 UL	RAT	LIVER	333			109		

**.
TA-98 2-NITROFLUORENE 10 UG/PLATE
TA-100 SODIUM AZIDE 10 UG/PLATE
SOLVENT 50 UL/PLATE

***
TA-98 2-ANTHRAMINE 2.5 UG/PLATE
TA-100 2-ANTHRAMINE 2.5 UG/PLATE

RESULTS

TABLE 2

A. NAME OR CODE DESIGNATION OF THE TEST COMPOUND: 436(ADELPHI 2 XAD EXTRACT)
B. SOLVENT: DMSO
C. TEST INITIATION DATES: 09/03/81
D. TEST COMPLETION DATE: 09/07/81
E. S-9 LOT#: S9-11
NOTE: CONCENTRATIONS ARE GIVEN IN MICROLITERS PER PLATE

TEST		SPECIES	TISSUE	REVERTANTS PER PLATE TA-1535 1	TA-1535 2	TA-1535 3	TA-1537 1	TA-1537 2	TA-1537 3
NONACTIVATION									
SOLVENT CONTROL		---	---	18			7		
SOLVENT CONTROL		---	---	28			12		
POSITIVE CONTROL**		---	---	1041			823		
POSITIVE CONTROL**		---	---	954			349		
TEST COMPOUND									
10.000	UL	---	---	-			13		
50.000	UL	---	---	-			19		
100.000	UL	---	---	-			23		
200.000	UL	---	---	31			25		
ACTIVATION									
SOLVENT CONTROL		RAT	LIVER	15			8		
SOLVENT CONTROL		RAT	LIVER	14			10		
POSITIVE CONTROL***		RAT	LIVER	586			297		
POSITIVE CONTROL***		RAT	LIVER	369			173		
TEST COMPOUND									
10.000	UL	RAT	LIVER	-			28		
50.000	UL	RAT	LIVER	-			35		
100.000	UL	RAT	LIVER	-			38		
200.000	UL	RAT	LIVER	15			48		

** TA-1535 SODIUM AZIDE 10 UG/PLATE
TA-1537 9-AMINOACRIDINE 50 UG/PLATE
SOLVENT 50 UL/PLATE

*** TA-1535 2-ANTHRAMINE 2.5 UG/PLATE
TA-1537 2-ANTHRAMINE 2.5 UG/PLATE

- INDICATES TEST WAS NOT DONE

VII. ASSAY ACCEPTANCE AND EVALUATION CRITERIA

Statistical methods are not currently used, and evaluation is based on the criteria included in this protocol.

Plate test data consists of direct revertant colony counts obtained from a set of selective agar plates seeded with populations of mutant cells suspended in a semisolid overlay. Because the test material and the cells are incubated in the overlay for approximately 2 days and a few cell divisions occur during the incubation period, the test is semiquantitative in nature. Although these features of the assay reduce the quantitation of results, they provide certain advantages not contained in a quantitative suspension test:

- The small number of cell divisions permits potential mutagens to act on replication DNA, which is often more sensitive than nonreplicating DNA.
- The combined incubation of the test article and the cells in the overlay permits constant exposure of the indicator cells for approximately 2 days.

A. Surviving Populations

Plate test procedures do not permit exact quantitation of the number of cells surviving chemical treatment. At low concentrations of the test material, the surviving population on the treatment plates is essentially the same as that on the negative control plate. At high concentrations, the surviving population is usually reduced by some fraction. Our protocol will normally employ several doses ranging over two or three log concentrations, the highest of these doses being selected to show slight toxicity as determined by subjective criteria.

B. Dose-Response Phenomena

The demonstration of dose-related increased in mutant counts is an important criterion in establishing metagenicity. A factor that might modify dose-response results for a mutagen would be the selection of doses that are too low (usually mutagenicity and toxicity are related). If the highest dose is far lower than a toxic concentration, no increases may be observed over the dose range selected. Conversely, if the lowest dose employed is highly cytotoxic, the test material may kill any mutants that are induced, and the test material will not appear to be mutagenic.

C. Control Tests

Positive and negative control assays were conducted with each experiment and consisted of direct-acting mutagens for nonactivation assays and mutagens that require metabolic biotransformation in activation assays.

Negative controls consisted of the test material solvent in the overlay agar together with the other essential components. The negative control plate for each strain gave a reference point to which the test data was compared. The positive control assay was conducted to demonstrate that the test systems were functional with known mutagens.

The following normal range of revertants for solvent controls are generally considered acceptable.

TA-1535:	8-30
TA-1537:	4-30
TA-98:	20-75
TA-100:	80-250

D. Evaluation Criteria for Ames Assay

Because the procedures to be used to evaluate the mutagenicity of the test material are semiquantitative, the criteria to be used to determine positive effects are inherently subjective and are based primarily on a historical data base. Most data sets will be evaluated using the following criteria.

1. Strains TA-1535 and TA-1537

If the solvent control value is within the normal range, a test material that produces a positive dose response over three concentrations with the highest increase equal to three times the solvent control value will be considered to be mutagenic.

2. Strains TA-98 and TA-100

If the solvent control value is within the normal range, a test material that produces a positive dose response over three concentrations with the highest increase equal to twice the solvent control value for TA-98 and TA-100 will be considered to be mutagenic.

3. Pattern

Because TA-1535 and TA-100 are both derived from the same parental strain (G-46), to some extent there is a built-in redundancy in the microbial assay. In general, the two strains of a set respond to the same mutagen and such a pattern is sought. Generally, if a strain responds to a mutagen in nonactivation tests, it will do so in activation tests.

4. Reproducibility

If a test material produces a response in a single test that cannot be reproduced in additional runs, the initial positive test data lose significance.

The preceding criteria are not absolute, and other extenuating factors may enter into a final evaluation decision. However, these criteria will be applied to the majority of situations and are presented to aid those individuals not familar with this procedure. As the data base is increased, the criteria for evaluation can be more firmly established.

E. Relation Between Mutagenicity and Carcinogenicity

It must be emphasized that the Ames *Salmonella*/Microsome Plate Assay is not a definitive test for chemical carcinogens. It is recognized, however, that correlative and functional relations have been demonstrated between these two endpoints. The results of comparative tests on 300 chemicals by McCann *et al.*[4] show an extremely good correlation between results of microbial mutagenesis tests and *in vivo* rodent carcinogenesis assays.

All evaluations and interpretation of the data to be presented in the final report will be based only on the demonstration, or lack, of mutagenic activity.

F. Criteria for Ranking Samples in the Ames Assay

The goal of EPA Level 1 Ames testing is to rank source streams by relative degree of genetic toxicity (mutagenicity). Samples are first identified as mutagenic or nonmutagenic by the criteria in Section D above and then ranked using the mutagenicity categories presented in the table below. The lowest concentration giving a positive response in any strain, with or without metabolic activation, is identified as the minimum effective concentration (MEC) for that sample. The mutagenicity of the sample is evaluated as high (H), moderate (M), low (L), or nondetectable (ND) according to the evaluation criteria developed in the Level 1 manual[1] and summarized below. Samples with no detectable activity at the maximum applicable dose (MAD) are ranked nondetectable (ND).

Ames Assay Mutagenicity Ranking Criteria[1]

Mutagenic Activity	Solids (MEC in µg/plate)	Liquids[a] (MEC in µl/plate)
High (H)	<50	<2
Moderate (M)	50-500	2-20
Low (L)	500-5000	20-200
Not Detectable (ND)	>5000	>200

[a]Concentration of organic extracts is based upon organic content (µg organics per plate) and not volume (µl extract per plate) of sample tested.

VIII. REFERENCES

1. Brusick, D.J., et al.: IERL-RTP Procedures Manual: Level 1 Environmental Assessment Biological Tests. EPA Contract No. 68-02-2681, Technical Directive No. 501, Litton Bionetics, Inc., Kensington, MD, September 1980, 177 pp. In press.

2. Brusick, D.J.: Level 1 Bioassay Assessment and Data Formatting. EPA-600/7-80-079, Litton Bionetics, Inc., Kensington, MD, April 1980, 100 pp.

3. Brusick, D.J. and Young, R.R.: Level 1 Environmental Assessment: Limits of Bioassay Resolution and Detectability. EPA Contract No. 68-02-2681, Technical Directive No. 301, Litton Bionetics, Inc., Kensington, MD, February 1981, 56 pp. In press.

4. McCann, J., Choi, E., Yamasaki, E. and Ames, B.N.: Detection of carcinogens as mutagens in the Salmonella/microsome test: Assay of 300 chemicals. Proc. Nat. Acad. Sci., USA 72:5135-5139, 1975.

5. Ames, B.N., Gurney, E.G., Miller, J.A. and Bartsch, H.: Carcinogens as frameshift mutagens: Metabolites and derivatives of 2-acetylaminofluorene and other aromatic amine carcinogens. Proc. Nat. Acad. Sci., USA 69:3128-3132, 1972.

6. Ames, B.N., Lee, F.D., and Durston, W.E.: An improved bacterial test system for the detection and classification of mutagens and carcinogens. Proc. Nat. Acad. Sci., USA 70:782-786, 1973.

7. Ames, B.N., Durston, W.E., Yamasaki, E. and Lee, F.D.: Carcinogens are mutagens: A simple test system combining liver homogenates for activation and bacteria for detection. Proc. Nat. Acad. Sci., USA 70:2281-2285, 1973.

8. McCann, J., Springarn, N.E., Kobori, J. and Ames, B.N.: Detection of carcinogens as mutagens: Bacterial tester strains with R factor plasmids. Proc. Nat. Acad. Sci. USA 72:979-983, 1975.

9. Ames, B.N., McCann, J. and Yamasaki, E.: Methods for detecting carcinogens and mutagens with the Salmonella/mammalian-microsome mutagenicity test. Mutation Res., 31:347-364, 1975.

10. Vogel, H.J. and Bonner, D.M.: Acetylornithinase of E. coli partial purification and some properties. J. Biol. Chem., 218:97-106, 1966.

GENETICS ASSAY NO. 5809

LBI SAFETY NO. 7933

CYTOTOXIC EVALUATION OF 436 (ADELPHI 2 XAD EXTRACT) IN THE RODENT CELL (CHO) CLONAL TOXICITY ASSAY

FINAL REPORT

SUBMITTED TO:

ACUREX CORPORATION
485 CLYDE AVENUE
MOUNTAIN VIEW, CALIFORNIA 94092

SUBMITTED BY:

LITTON BIONETICS, INC.
5516 NICHOLSON LANE
KENSINGTON, MARYLAND 20895

LBI PROJECT NO. 22064

REPORT DATE: SEPTEMBER 1981

Litton BIONETICS

PREFACE

This assay conforms to the standard EPA Level 1 procedure for the Chinese hamster ovary cell (CHO) clonal toxicity assay as described in "IERL-RTP Procedures Manual: Level 1 Environmental Assessment Biological Tests" (1). The data was evaluated and formatted as recommended in "Level 1 Biological Testing Assessment and Data Formatting" (2).

The CHO clonal toxicity assay has been shown to be a sensitive method for detecting cytotoxic activity for a variety of chemicals representing various chemical classes (3). This assay is one of several recommended by EPA to identify, categorize and rank the pollutant potential of influent and effluent streams from industrial and energy-producing processes. This assay has been well validated with a wide range of positive and negative control chemicals and complex environmental samples.

All procedures and documents pertaining to the receipt, storage, preparation, testing and evaluation of the test material shall conform to Litton Bionetics, Inc. standard operating procedures and the Good Laboratory Practices Act of 1979. Deviations from standard procedure shall be fully documented and noted in the report.

All test and control results in this report are supported by fully documented raw data which are permanently maintained in the files of the Department of Molecular Toxicology or in the archives of Litton Bionetics, Inc., 5516 Nicholson Lane, Kensington, Maryland 20895. Copies of raw data will be supplied to the sponsor upon request.

TABLE OF CONTENTS

		Page No.
	PREFACE	i
I.	ASSAY SUMMARY	1
II.	OBJECTIVE	2
III.	TEST MATERIAL	3
	A. Description	3
	B. Handling and Preparation	3
IV.	MATERIALS	4
	A. Indicator Cells	4
	B. Media	4
	C. Controls	4
V.	EXPERIMENTAL DESIGN	5
	A. Dose Selection	5
	B. Clonal Toxicity Assay	5
VI.	ASSAY ACCEPTANCE CRITERIA	7
VII.	RESULTS	8
	A. Interpretation	
	B. Tables and Figures	
VIII.	ASSAY EVALUATION CRITERIA	11
IX.	REFERENCES	12

I. ASSAY SUMMARY

A. SPONSOR: Acurex Corporation

B. MATERIAL (TEST COMPOUND): GENETICS ASSAY NUMBER: 5809

1. Identification: 436 (Adelphi 2 XAD extract)

2. Date Received: June 28, 1981

3. Physical Description: Clear, transparent liquid

C. TYPE OF ASSAY: Rodent Cell (CHO) Clonal Toxicity Assay

D. ASSAY DESIGN NUMBER: 442

E. STUDY DATES:

1. Initiation: August 19, 1981

2. Completion: August 26, 1981

F. SUPERVISORY PERSONNEL:

A. Study Director: Brian C. Myhr, Ph.D.

B. Laboratory Supervisor: Robert Young, M.S.

G. EVALUATION:

The test material caused only a slight increase in toxicity at the maximum applicable dose (MAD) of 20 µl/ml (26.8 µg of organics per ml). Since no tested dose produced 50 percent killing of the cells, the EC_{50} could not be calculated. However, the sample was ranked by IERL-EPA evaluation criteria as being excluded from the high toxicity category and as having moderate (M) toxicity or less. The inability to test higher concentrations was attributable to the extremely small quantity of test material supplied (4.7 mg).

Submitted by:

Study Director

Brian Myhr 9/9/81

Brian Myhr, PhD. Date

Associate Director,
Department of Molecular
Toxicology

Reviewed by:

James C. Liverman PhD
VPASD for 9/9/81

David J. Brusick, Ph.D. Date

Director,
Department of Molecular
Toxicology

II. OBJECTIVE

The objective of this study was to determine and rank the cytotoxicity of sample 436 (Adelphi 2 XAD Extract) to cultured Chinese hamster cells (CHO-K1 cell line). The measure of cytotoxicity was the reduction in colony-forming ability after a 24-hour exposure to the test material. After a period of recovery and growth, the number of colonies that developed in the treated cultures was compared to the colony number in unexposed vehicle control cultures. The concentration of test material that reduced the colony number by 50% was estimated graphically and referred to as the EC50 value. Standard EPA Level 1 toxicity evaluation criteria for the CHO clonal toxicity assay were used to rank the toxicity potential of the test material.

III. TEST MATERIAL

A. Description

The test material was received as a clear, transparent solution in methylene chloride. The sample contained 4.7 milligrams of organic material in an undetermined volume of methylene chloride. No information on the sampling parameters (such as the equivalent volume of stack gas represented by the sample) was provided.

B. Handling and Preparation

The test material was received at LBI on June 28, 1981. The sample was assigned LBI safety number 7033 and LBI assay number 5809. The sample was stored at +4°C in the dark.

Pretest sample preparation consisted of solvent-exchanging the sample into dimethylsulfoxide (DMSO). The sample was transferred with methylene chloride rinses into a graduated conical tube. The methylene chloride was gradually evaporated (50°C under stream of nitrogen) and DMSO was sequentially added. The sample was brought to volume in 3.5 ml of DMSO, giving a sample concentration of 1.34 mg organics per ml DMSO. The sample was then transferred to a glass vial and sealed with a teflon-coated rubber septum.

A total volume of 0.45 ml of test sample was used in the CHO assay. The maximum concentration of 20 µl/ml was obtained by adding 0.12 ml of sample to 5.88 ml of F12 medium; this resulted in 2% (v/v) DMSO in the medium and effectively limited the concentration of test material that could be assayed. Only two plates were dosed at the top dose in order to conserve sample. Another 0.12 ml aliquot of sample was used to prepare the 10 µl/ml test concentration. An additional 0.21 ml of test sample was used to prepare a series of dilutions in DMSO from which 1:100 dilutions into growth medium were performed to obtain the lower assayed concentrations. Thus, except for the 20 µl/ml test concentration, the final DMSO concentration was constant at 1% (v/v).

IV. MATERIALS

A. Indicator Cells

The indicator cells for this study were Chinese hamster CHO-K1 cells (ATCC No. CCL 61) obtained from Flow Laboratories, Inc., Rockville, MD. This cell type was derived from ovarian tissue and has spontaneously transformed to a stable, hypodiploid line of rounded, fibroblastic cells with unlimited growth potential. Monolayer cultures have a fast doubling time of 11 to 14 hours, and untreated cells can normally be cloned with an efficiency of 80% or greater. Laboratory stock are maintained by routine serial subpassage. Cells are cultivated in Ham's F-12 nutrient medium at 37°C in 5 percent CO_2 with saturated humidity. Stocks are continually observed macroscopically and microscopically for possible microbial contamination. Laboratory cultures are periodically checked by culturing and staining methods for the absence of mycoplasma contamination. Laboratory cultures are discarded every three months and new cultures started from mycoplasma-free, long-term frozen cultures.

B. Media

The CHO-K1 cell line has an absolute requirement for proline and therefore must be maintained in culture medium containing sufficient amounts of this amino acid. Ham's F12 medium, which contains 3×10^{-4} M L-proline was used, supplemented with 10% fetal bovine serum, 2mM L-glutamine, 100 units/ml of penicillin, 100 µg/ml of streptomycin, and 0.9 µl/ml of amphotericin B. A 10X formulation of Ham's F10 is available commercially and was used for the testing of aqueous test samples in order to avoid the dilution of medium components. This medium contains 1×10^{-4} L-proline and was supplemented in the same manner as F12, except that kanamycin at 40 µg/ml is included for additional protection against bacterial contamination. Both media formulations support the growth and cloning of CHO cells equally well.

C. Controls

The negative control consists of three untreated cultures carried through the same experimental time period as the treated cells. The test material was supplied as a solution in an organic vehicle (usually DMSO) and was diluted into the medium to provide each test concentration, three vehicle control cultures containing the organic solvent at 1% or 2% by volume are prepared. Particulate test samples were suspended in small amounts of DMSO, but DMSO was not included in the negative control because the maximum content in the treated cultures is only 0.42%. The average number of colonies in the negative control establishes the cloning efficiency of the CHO cells used in the assay, and the appropriate negative or vehicle controls provide the reference points for determining the effects of different concentrations of the test material on cell survival.

V. EXPERIMENTAL DESIGN

A. Dose Selection

Unless the approximate toxicity is already known or the sample size is limiting, the following dose ranges are usually tested for different sample forms. Aqueous samples, suspensions, or slurries are tested from 600 µl/ml to 3 µl/ml, usually in six dose steps. Eight doses are often used when the amount of test sample is limited to provide a more precise description of toxicity in the event of sharp dose-response curves. Dry, particulate material is dissolved or suspended in DMSO, diluted into growth medium, and tested at six dose levels from 1000 µg/ml to 3 µg/ml. Samples that are solvent-exchanged into DMSO are tested from 20 µl/ml (2% DMSO in growth medium) to 0.2 µl/ml, also in six dose steps. A second dose study is performed with an adjusted dose range if the EC50 was not located properly in the initial test. However, EC50 values greater than 1000 µg/ml for particulate material, 600 µl/ml for aqueous samples, or 20 µl/ml for organic solutions will not be determined.

Sample 436 (Adelphi 2 XAD extract) was tested at eight concentrations. The concentrations used started with the maximum applicable dose (MAD) of 20 µl/ml and included 10, 6, 3, 1, 0.6, 0.3, and 0.1 µl of test material per ml of culture medium. The MAD of 20 µl/ml was equivalent to 26.8 µg of organics per ml of culture medium. The lower dose levels were equivalent to 13.4, 8.04, 4.02, 1.34, 0.80, 0.40 and 0.13 µg of organics per ml.

B. Clonal Toxicity Assay

Cells from monolayer stock cultures in logarithmic growth phase were trypsinized with 0.1% trypsin plus 0.01% versene for 4 minutes and the density of the resulting cell suspension determined by hemocytometer. A number of 60-mm culture dishes were then seeded with 200 cells and 4 ml of culture medium per dish. The cultures were incubated for approximately 6 hours at 37°C in a humidified atmosphere containing 5% CO_2 to allow attachment of the cells. The 6-hour attachment period was used in order to avoid cell division and the subsequent formation of two-cell colonies prior to treatment.

The medium was aspirated from the cultures and 4 ml medium containing the test material applied. Three cultures were exposed to each test concentration. After an exposure time of 24 hours at 37°C, the medium was removed by aspiration and each culture washed three times with approximately 4 ml aliquots of Dulbecco's phosphate buffered saline (pre-warmed to 37°C). Fresh culture medium (5 ml) was placed in each dish and incubation at 37°C is continued for an additional 6 days to allow colony development.

If the test material caused a color change in the culture medium, the pH of the medium containing the high dose would be determined at the time of treatment. The pH at the lowest dose that results in a slight color

change would also be recorded. At the end of the treatment period, the pH values of the discarded media from the two described treatments would be recorded again. No sample related pH effects were noted.

After the incubation period, the medium was drained from the cultures and the surviving colonies fixed with 100% ethanol and stained with Giemsa. Colonies were counted by eye; tiny colonies of approximately 50 cells or less were arbitrarily excluded from the counts.

VI. ASSAY ACCEPTANCE CRITERIA

The assay is considered acceptable for evaluation of the test results if the following criteria are met:

- The average cloning efficiency of the CHO-K1 cells in the negative controls is 70% or greater, but not exceeding 115%.
- The distribution of colonies in the treated cultures is generally uniform over the surface of the culture dish.
- The data points for each test concentration critical to the location of the EC50 are the averages of at least two treated cultures.
- A sufficient number of test concentrations are available to clearly locate the EC50 within a toxicity region as defined under Assay Evaluation Criteria.
- If the EC50 value is greater than 1000 µg/ml, 600 µliters of aqueous sample/ml, or 20 µliters of nonaqueous sample/ml, the plotted curve does not exceed 110% of the negative control.

VII. RESULTS

A. Interpretation

The application of sample 436 (Adelphi 2 XAD extract) to the CHO cell cultures caused only a small decrease in the number of cells able to form colonies as the concentration increased to the maximum applicable dose (MAD) of 20 µl/ml. Relative survival values were calculated as the ratio of colonies formed in treated cultures to the colonies formed in the appropriate vehicle control, and these relative survival values were plotted against the concentration of test material. As shown in Figure 1, the relative survival decreased only to about 88% for the 20 µl/ml treatment.

Since none of the tested doses caused killing approaching 50% of the cells, an EC_{50} could not be calculated. However, a value greater than 20 µl/ml (26.8 µg organics/ml) excluded the sample from the high toxicity category and placed it in the moderate range or even less toxicity, based on the IERL-RTP evaluation criteria.[1] The plotted results suggested the possibility of an EC_{50} less than 100 µl/ml (134 µg organics/ml), so the sample could have been evaluated as moderately toxic or being on the moderate/low toxicity borderline had sufficient sample been available. The toxicity of the sample was therefore evaluated as undetermined but moderate (M) or less.

Testing and evaluating materials such as this sample indicate the need to supply sufficient quantities of test material to ensure adequate testing. While minimum testable sample size is often a function of the biological activity of the sample, efforts should be made to supply at least 100 mg of SASS train organics for combined Ames and CHO testing.

The cells used for the assay were in logarithmic growth phase and were 99.4% viable. About 99% of the cells seeded and cultured in F12 medium formed colonies. The cloning efficiency of the 1% vehicle control was 82.9% and the 2% vehicle control, 75.4%. Colony growth was normal and well distributed on the culture dishes. These results achieved the assay acceptance criteria and provided confidence in the assumption that the recorded data represented typical responses to the test material.

B. Tables and Figures

This report is based on the data provided in Table 1 and Figure 1.

TABLE 1
RODENT CELL (CHO) CLONAL TOXICITY ASSAY

Sample Identity: 436 (Adelphi 2 XAD Extract

Description of Sample: Clear, transparent liquid

LBI Assay No.: 5809

Date Received: June 28, 1981

Test Date: August 19, 1981

Vehicle: DMSO

Cell Type: CHO-K1

Cells Seeded per Dish: 200

EC50 Value: >26.8 µg organics/ml (>20 µl/ml)

Toxicity Classification: Undetermined, but Moderate (M) or less

pH Alterations: None

Comments on Treatment: Sample prepared in DMSO at a concentration of 1.34 µg organics/µl.

COLONY COUNTS

Sample	Applied Concentration µl/ml	Dish #1	Dish #2	Dish #3	Average Count	Relative Survival[a] %	Cloning Efficiency %
NC[b]	--	184	187	182	184.3	---	92.2
VC,1%[c]	10	164	175	158	165.7	100.0	82.9
VC,2%	20	157	148	147	150.7	100.0	75.4
Test	0.1	160	166	158	161.3	97.3	
Test	0.3	155	179	145	159.7	96.4	
Test	0.6	166	169	150	161.7	97.6	
Test	1.0	160	166	156	160.7	97.0	
Test	3.0	147	162	171	160.0	96.6	
Test	6.0	154	164	143	153.7	92.8	
Test	10.0	166	160	153	159.7	96.4	
Test[d]	20.0	135	130	---	132.5	87.9	

[a]Relative to 2% VC for 20 µl/ml treatment and to 1% VC for other treatments.

[b]NC = Negative Control, F_{12} medium.

[c]VC = Vehicle Control, percent DMSO given.

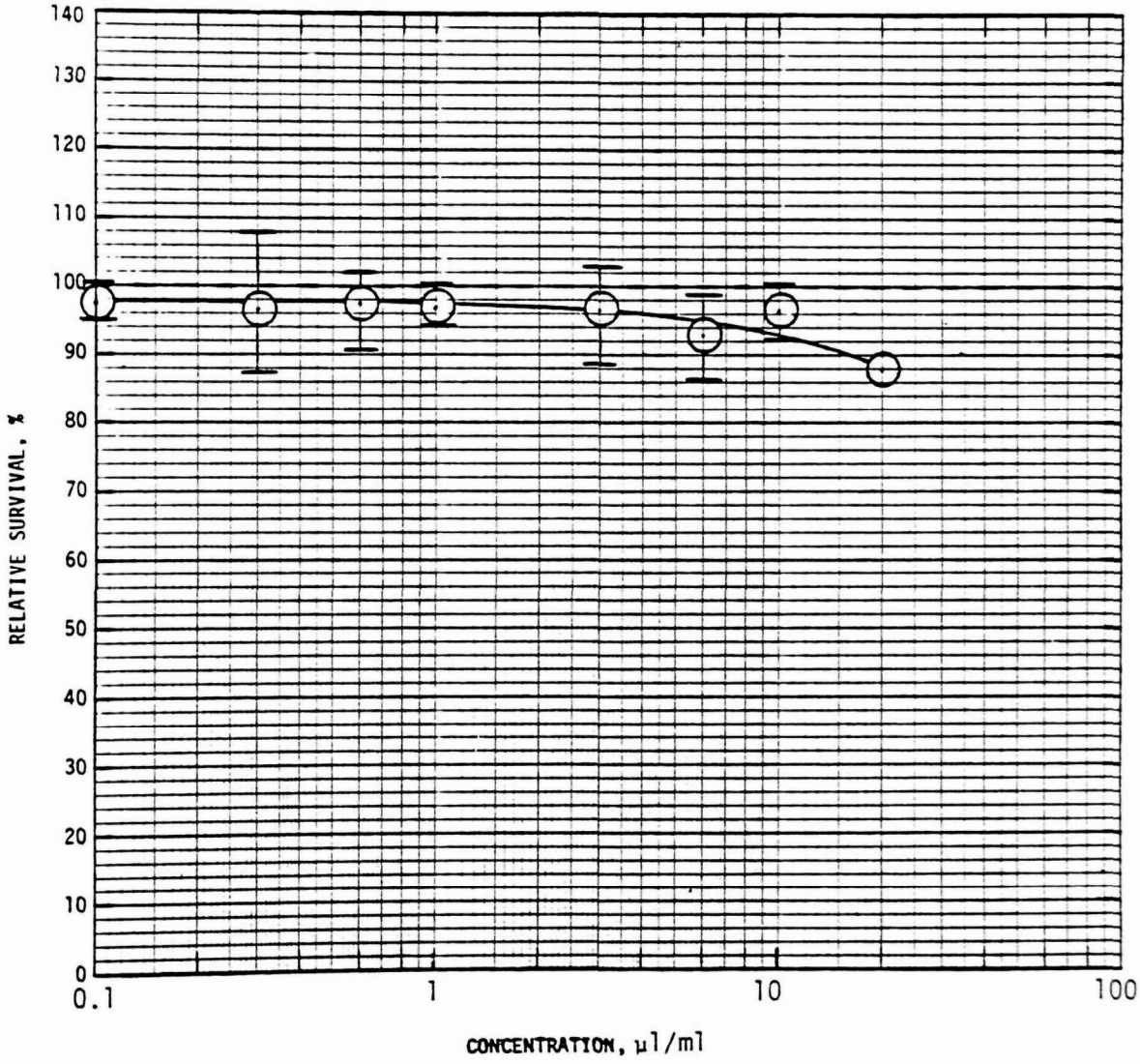
[d]Only two plates dosed to conserve limited test material.

FIGURE 1

RODENT CELL (CHO) CLONAL TOXICITY ASSAY

EC_{50} DETERMINATION

436 (ADELPHI 2 XAD EXTRACT)



VIII. ASSAY EVALUATION CRITERIA

The EC50 value represents the concentrations of test material that reduces the colony-forming ability of CHO cells to 50% of the vehicle or negative control value. EC50 values are determined graphically by fitting a curve by eye through relative survival data plotted as a function of the logarithm of the applied concentration. Each data point normally represents the average of three culture dishes. In order to indicate the variability of the data, the high and low colony counts for each concentration are used to calculate the relative survivals, and the range is shown by a bar at the position of the plotted average. If no bar is shown, the variability was within the size of the symbol. Statistical analysis is unnecessary in most cases for evaluation.

The toxicity of the test material is evaluated as high, moderate, low, or nondetectable according to the range of EC50 values defined in the following table.

Toxicity[a]	Solids (EC_{50} in μg/ml)	Aqueous Liquids (EC_{50} in μl/ml)	Nonaqueous Liquids[b] (EC_{50} in μl/ml)
High	<10	<6	<.2
Moderate	10 to 100	6 to 60	.2-2
Low	100 to 1000	60 to 600	2-20
Not Detectable	>1000	>600	>20

[a]Evaluation criteria formulated by Litton Bionetics, Inc. for IERL-RTP Procedures Manual: Level Environmental Assessment Biological Tests.[1]

[b]Criteria for nonaqueous liquids are tentative and under evaluation. If the organic or solids content is known, the sample is evaluated under the solids criteria.

Another evaluation scheme is proposed for extracts obtained from SASS train gas volumes. The proportion of the total gas volume corresponding to the volume of extract used in the bioassay is calculated and expressed as L/ml of culture medium (or DSCF/ml of culture medium). A criterion of 1000 L/ml is set as the limit for nondetectable toxicity. This gas volume corresponds to the average volume breathed by humans over a 2-hour period. The subsequent toxicity ranges are defined by 10-fold dilution steps to conform to standard procedure. The toxicity ranges are defined in the following table for liter and dry standard cubic feet units:

Toxicity	EC_{50} In Liters/ml (L/ml)	EC_{50} In Dry Standard Cubic Feet/ml (DSCF/ml)
High	<10	<0.35 DSCF
Moderate	10-100	0.35-3.5
Low	100-1000	3.5-35
Nondetectable	>1000	>35

IX. REFERENCES

1. Brusick, D.J., et al.: IERL-RTP Procedures Manual: Environmental Assessment Biological Tests. EPA Contract No. 68-02-2681, Technical Directive No. 501, Litton Bionetics, Inc., Kensington, MD, September 1980, 177 pp. In press.

2. Brusick, D.J.: Level 1 Bioassay Assessment and Data Formatting. EPA-600/7-80-079, Litton Bionetics, Inc., Kensington, MD, April 1980, 100 pp.

3. Brusick, D.J. and Young, R.R.: Level 1 Environmental Assessment: Limits of Bioassay Resolution and Detectability. EPA Contract No. 68-02-268, Technical Directive No. 301, Litton Bionetics, Inc., Kensington, MD, February 1981, 56 pp. In press.

TECHNICAL REPORT DATA
(Please read Instructions on the reverse before completing)

1. REPORT NO. EPA-600/7-84-095b	2.	3. RECIPIENT'S ACCESSION NO.
4. TITLE AND SUBTITLE Environmental Assessment of a Firetube Boiler Firing Coal/Oil/Water mixtures; Volume II. Data Supplement		5. REPORT DATE September 1984
		6. PERFORMING ORGANIZATION CODE
7. AUTHOR(S) R. DeRosier		8. PERFORMING ORGANIZATION REPORT NO. TR-81-89/EE
9. PERFORMING ORGANIZATION NAME AND ADDRESS Acurex Corporation, Energy and Environmental Division, P.O. Box 7555, Mountain View, California 94039		10. PROGRAM ELEMENT NO.
		11. CONTRACT/GRANT NO. 68-02-3188
12. SPONSORING AGENCY NAME AND ADDRESS EPA, Office of Research and Development, Industrial Environmental Research Laboratory, Research Triangle Park, NC 27711		13. TYPE OF REPORT AND PERIOD COVERED Final; 2/81 - 11/83
		14. SPONSORING AGENCY CODE EPA/600/13

15. SUPPLEMENTARY NOTES IERL-RTP project officer is Robert E. Hall, Mail Drop 65, 919/541-2477. Volume I is the Technical Results.

16. ABSTRACT This volume is a compendium of detailed emission and test data from field tests of a firetube industrial boiler burning a coal/oil/water (COW) mixture. The boiler was tested while burning COW fuel, and COW with soda ash added (COW+SA) to serve as an SO2 sorbent. The test data include: preliminary equipment calibration data, boiler operating data for both tests, fuel analysis results, and complete flue gas emission measurement and laboratory analysis results. Flue gas emission measurements included: continuous monitoring for criteria gas pollutants; gas chromatography (GC) of gas grab samples for volatile organics (C1-C6); EPA Method 5 for particulate; controlled condensation system for SO2 emissions; and source assessment sampling system (SASS) for total organics in two boiling point ranges (100 to 300 C and >300 C), organic compound category information using infrared spectrometry (IR) and low resolution mass spectrometry (LRMS), specific quantitation of the semivolatile organic priority pollutants using gas chromatography/mass spectrometry (GC/MS), liquid chromatography (LC) separation of organic extracts into seven polarity fractions with total organic and IR analyses of eluted fractions, flue gas concentrations of trace elements by spark source mass spectrometry (SSMS) and atomic absorption spectroscopy (AAS), and biological assays of organic extracts.

17. KEY WORDS AND DOCUMENT ANALYSIS

a. DESCRIPTORS		b. IDENTIFIERS/OPEN ENDED TERMS	c. COSATI Field/Group	
Pollution	Assessments	Pollution Control	13B	14B
Fire Tube Boilers		Stationary Sources	13A	
Coal	Flue Gases	Coal/Oil/Water Mixture	21D	21B
Fuel Oil		Environmental Assessment	07B	
Water				
Sodium Carbonates				

18. DISTRIBUTION STATEMENT Release to Public	19. SECURITY CLASS (This Report) Unclassified	21. NO. OF PAGES 178
	20. SECURITY CLASS (This page) Unclassified	22. PRICE

EPA Form 2220-1 (9-73)