Acute Toxicity of Simple Green to the Brine Shrimp *Artemia salina* and the grass shrimp *Palaemonetes pugio*.

A Final Report
by

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ABSTRACT

The acute toxicity of Simple Green to the brine shrimp Artemia salina and the grass shrimp Palaemonetes pugio was evaluated.

For Artemia, the EPA Standard Method for testing the toxicity of oil dispersants was used. The 48-h LC_{50} for Artemia in Simple Green Liquid (SGL) was 609.7 ppm compared with 461 and 153.2 ppm for the oil-in-water dispersion and the 1:10 SGL-oil mixture. The 48-h LC_{50} for the reference toxicant (SDS) was 27 ppm, making it 23 times more toxic than SGL. Unlike most oil dispersants presently in use, SGL-oil mixture was only slightly (3 times) more toxic than the oil alone.

Results from 96-h LC_{50} tests with Palaemonetes also show that SGL is non-toxic. Extracts from Simple Green Sponge was also not toxic to both Artemia and Palaemonetes, although the latter was more susceptible than the former to the extracts.

The results are discussed and comparison made between SGL and other oil dispersants now in use.

INTRODUCTION

The transportation of crude oil and oil products by sea is increasing steadily and this has resulted in a greater risk of oil pollution of coastal waters with a concomitant increase in the danger to marine fauna and flora. There is also the danger of spoiling the beauty of beaches and other waterfront recreational areas. These are risks that any coastal nation must live with because mankind will depend on crude oil and its products for a long time to come for energy and other uses.

With the increase in oil pollution have come various methods of treatment, among the most successful of which have been the "oil-spill removers" or oil dispersants (Postmann and Connor, 1968). The proper treatment of a spill with a dispersant merely aids the sea in accomplishing what would occur naturally but at a very slow rate, since the sea naturally disperses oil spilled in it (Lindblom, 1978). Dispersants help the sea disperse oil spill much more rapidly and safely, thereby preventing the formation of tar balls and oil-in-water emulsions ("Mousee"), and hence removing the fire hazard usually associated with spills. Another advantage in the use of dispersants is that they increase the rate of evaporation, biodegradation and solubilization of the oil slick.

Despite all the advantages of oil dispersants outlined above, their use in cleaning oil slicks have been the subject of much controversy. Most of the fears and opposition to the use of oil spill dispersants stem from the "hard lesson" learned during and after the clean up operations of the "Torry Canyon" spill. The dispersants used then were by themselves more toxic to marine animals than the oil in the water, and when mixed with oil, they were even more toxic (Swedmark et al., 1973; Swedmark, 1976; Johnston, 1984). However in recent years every effort has (and is still) being made

to produce oil dispersants that are equally effective in dispersing oil slicks as the older forms but are less toxic. Simple Green, an all-purpose liquid cleaner manufactured by Sunshine Makers Inc. of Sunset Beach, California, U.S.A., is one such compound.

Simple Green has many qualities that make it a potentially effective oil dispersant. For example, it is biodegradable, non-toxic (to humans), non-flammable, contains no harmful bleach or ammonia and is solvent based. However every newly developed dispersant must be tested for their dispersing effectiveness and toxicity to marine animals before being approved by the Environmental Protection Agency (EPA) for use in the environment.

The present investigation is primarily concerned with determining the acute toxicity of Simple Green Liquid (SGL) to marine crustaceans. A standard acute toxicity for oil dispersants according to the EPA specifications using the brine shrimp Artemia salina, was performed (see Fed. Reg. Vol. 48, No. 245, 1983 and Fed. Reg. Vol. 49, No. 139, 1984). In addition to the standard EPA tests, acute toxicity tests were made using both SGL and Simple Green extracted from biosponge (SGSS) on Palaemonetes pugio and Artemia salina. These data are included to provide a much broader data base for comparative purposes. Emphasis in the discussion will be on the relative toxicity of SGL alone, in a 1:10 mixture of SGL to crude oil and a reference toxicant, sodium dodecyl sulfate (SDS).

MATERIALS AND METHODS

Preparation of solutions - Throughout this study, the different Simple Green formulations and the reference toxicant were prepared as follows:

Simple Green Liquid (SGL): The concentrate, i.e. the undiluted material was used as stock from which a working stock of 0.1% was prepared everyday. From the working stock, various test concentrations were made (see later sections for details). All SGL solutions were prepared using synthetic seawater (20 ppt).

Simple Green Sponge Squeezings (SGSS): Depending on the test animal (*Artemia salina* or *Palaemonetes pugio*) synthetic or natural seawater was used to soak the sponge. In either case, a 100 cm^3 Simple Green Sponge was soaked in 1 L of seawater for 1 hour, after which the sponge was squeezed, immersed and squeezed again. This operation was repeated 10 times before discarding the sponge. The resulting solution or extract was then mixed and used in preparing the test concentrations in percent by volume (see later sections for concentrations used).

Sodium dodecyl sulfate (SDS): This Lauryl sulfate sodium salt was the recommended reference toxicant (See Fed. Reg. Vol. 48, No. 246, 1983). It is recommended because of its rapid nonselective and consistent toxicity to the test species (La Roche et al., 1982). A working stock solution was prepared by weighing 1 g of the salt (reagent grade) and dissolving in 1 L of seawater, this gave a 1 g/L (=1 ppt) sollution. From this working stock, various test solutions in ppm were prepared.

Simple Green Liquid and Crude Oil Mixture (SGL + CO) and Crude Oil in Seawater Dispersion (OWD): The oil used in this study was Prudhoe Bay Crude obtained from the EPA as a Standard Reference oil. The physico-chemical data and the major constituents of this oil are presented (as received from EPA) in the Appendix. One ppt of SGL + CO and OWD mixtures were prepared by blending appropriate volumes of seawater, SGL and crude oil as recommended by EPA (Fed. Reg. Vol. 48, No. 246, 1983 and Vol. 40, No. 139, 1984). From the 1 ppt mixtures, several test concentrations of SGL + CO and OWD were prepared for the toxicity tests.

Acute Toxicity Tests

Artemia bioassay: For details of the materials and methods used in this section of the investigation, see Fed. Reg. Vol. 48, No. 246, 1983 and Vol. 49, No. 139, 1984. These documents were followed as closely as possible throughout the Artemia assay. Artemia cysts were from the San Francisco Bay area and only 24-h old nauplii were used for the tests. Synthetic seawater was prepared according to Fed. Reg. Vol. 48, 1983. Although the method of assay was static without renewal, the bowls were examined every day and dead nauplii removed with as little of the culture medium as possible. All the assays were terminated after 48 h except for those with SGL where many individuals survived through day 4. Salinity of the synthetic seawater was 20 ppt. The pH before and after the assay was recorded using a portable Corning pH Meter model 3D. Dissolved oxygen (DO) determination was by titration, using the Winkler technique. For both pH and DO determinations, water samples were withdrawn from the culture bowls with the aid of an all glass syringe fitted with a hypodermic needle to

avoid the surface film of oil. Care was exercised to ensure that the DO water samples had little or no air bubbles. All the tests were conducted in a culture cabinet set at 20°C and continuously illuminated from below with several fluorescent lamps. Illumination from below the culture dishes kept the Artemia away from the layer of oil film because of their positive phototaxis. The test concentrations in the definitive bioassays with Artemia were 50, 100, 200, 400, 800 and 1000 ppm for both OWD and SGL + CO.

The results obtained from the assays were analysed by both the graphic and the probit analysis technique with the aid of a computer. The computer program for estimating the LC50 using the probit technique was derived from that of Lieberman (1983).

Palaemonetes pugio bioassay: Ovigerous grass shrimp were collected from a laboratory "habitat" and incubated at 25°C until hatching occurred. After hatching, the larvae (usually 12 h old) were exposed to various concentrations of the test solutions. Larvae from different females were pooled prior to distribution to ensure that there was enough for all the experiments.

Fifty larvae were transferred into 9 cm Carolina culture dishes containing 200 cm^3 of the test solution. The larvae were fed freshly hatched Artemia salina nauplii whose cysts were from the Great Salt Lakes area. The bowls were kept in a culture cabinet set at 25°C and 12 h photoperiod. All solutions were made with 20 ppt filtered natural seawater. The different concentrations used in the definitive bioassays were:

SGL = 100,200,240,280,300 and 500 ppm

SGSS = 1,2,3,4,5 and 6%V/V

SDS = 10,25,50,100,200 ppm.

The bowls were examined daily and the number of dead larvae noted. This was followed by a change of the culture medium which was prepared fresh daily. Few drops of 18-24 h old Artemia nauplii were then added to each bowl before returning them to the culture cabinet. This procedure was repeated daily for four days after which the experiments were terminated.

RESULTS

Artemia salina bioassay:

The physico-chemical characteristics of the various test concentrations of SGL, SGL + CO and OWD at the beginning of the tests and after 48 h are presented in Table 1, Table 2 and Table 3, respectively. One-way ANOVA tests on the replicate values for both the pH and DO indicated that there was no significant ($P < 0.01$) difference between the values at the beginning and after 48 h. Also there was no significant difference ($P < 0.01$) in the pH and DO values between test concentrations within any test duration.

Table 4 is the data summary for *Artemia* bioassay using SGL; the percent survival at the end of each 24 h is shown. The results of the probit analysis using a computer program for SGL assays are shown in Table 5. The graphic technique was also employed and the regression equations generated together with the LC50's are given below. For 48-h data, the equation is:

$$Y = -436.76 + 76.09 \log_{10} X$$

($r = 0.942$ significant at $p = 0.01$)

where y = concentration (ppm) and x = percent mortality.

48-h LC50 = 600.14 ppm

For 96-h data, the equation is

$$Y = -523.57 + 95.55 \log_{10} X$$

($r = 0.927$ significant at $p = 0.01$)

96-h LC50 = 404.57 ppm

It is evident that the LC50's are similar regardless of the method used in their computation.

The results for Artemia bioassay using Simple Green sponge squeezings (SGSS) are given in Table 6. This table includes (a) Data summary for each 24 h and (b) Results of the probit analysis. As with the SGL data, the graphic technique was also employed and the regression equation and the derived 48-h LC50 are shown below.

Equation is $Y = -116.78 + 57.17 \log_{10} X$ where Y is the percent mortality and X the concentration. The correlation coefficient was 0.957 (significant, $p = 0.05$). 48-h LC50 = 18.49% (V/V). This value compares favorably with 17.83% (V/V) obtained by the probit method.

Table 7 contains the survivorship for each 24 h and the results of the probit analysis for Artemia bioassay using SDS solution. For comparison, the regression equation obtained by graphic analysis of the data and the 48-h LC50 obtained thereof are presented below:

Equation: $Y = -194.24 + 73.62 \log X$

correlation coefficient = 0.972 (significant $p = 0.01$).

The 48-H LC50 determined from regression is 27.59 ppm which is not significantly different from 27.21 ppm obtained by probit analysis.

The results of the Artemia bioassays with SGL + CO and OWD are presented in Table 8 and Table 9, respectively. The percent survival at the end of every 24 h and the data from the 48-h LC_{50} determinations using a computer program are shown in these tables.

Grass shrimp bioassay using natural seawater:

Results of bioassays with the grass shrimp Palaemonetes pugio together with the results of the probit analyses are presented in Tables 10 through

15. Table 16 is a summary of the 48-h and 96-h LC50's determined for the two formulations of Simple Green and the reference toxicant. The 48-h LC_{50} for all tests conducted with Artemia only are presented in Table 17. This table makes it easy to compare the toxicity of SGL relative to the other solutions and when mixed with crude oil. It is obvious that the reference toxicant (SDS) is the most toxic and the dispersant (SGL) the least toxic, in fact SGL is about 23 times less toxic than the reference toxicant. This also holds true for the results of the 48- and 96-h tests with Palaemonetes pugio larvae (Table 16). Here, SDS is 6 and 5 times more toxic than SGL in the 48- and 96-h tests respectively. Tests with the Simple Green Sponge Squeezings (SGSS) on both Artemia and Palaemonetes for 48-h, show that SGSS is less toxic to these crustaceans than SDS (Table 16).

Table 1. Physico-chemical characteristics of various concentrations of Simple Green Liquid (SGL) during 48 h acute toxicity tests with Artemia salina nauplii. Synthetic seawater was used throughout the test. The mean ($\bar{X}$) ± standard deviation (s.d.) of three determinations per concentration is presented.

Duration (h)	Concentration (ppm)	pH		Dissolved Oxygen	
		$\bar{X}$	s.d.	$\bar{X}$	s.d.
0	control	7.03	0.07	8.55	0.28
	100	6.92	0.13	8.65	0.49
	200	7.07	0.09	8.45	0.21
	260	7.38	0.55	8.83	0.1
	280	7.12	0.19	7.8	0.07
	300	7.16	0.13	8.2	0.69
48	control	7.21	0.45	8.4	0.21
	100	7.39	0.28	8.2	0.21
	200	7.27	0.4	8.38	0.32
	240	7.16	0.3	8.53	0.1
	280	7.1	0.12	7.5	0.78
	300	7.12	0.09	7.58	0.67

Table 2. Physico-chemical characteristics of various concentrations of Simple Green Liquid plus Crude Oil Mixture (SGL + CO) at the beginning and after 48-h acute toxicity tests with Artemia salina nauplii. Synthetic seawater was used throughout the test. The mean ($\bar{X}$) ± standard deviation (s.d.) of three determinations per concentration is presented.

Duration (h)	Concentration (ppm)	pH		Dissolved Oxygen	
		$\bar{X}$	s.d.	$\bar{X}$	s.d.
0	control	7.07	0.17	8.55	0.28
	50	7.06	0.36	8.45	0.69
	200	6.92	0.44	8.45	0.21
	400	7.14	0.17	8.83	0.1
	800	6.82	0.37	7.8	0.07
	1000	7.07	0.12	8.2	0.49
48	control	7.09	0.14	8.4	0.21
	50	7.03	0.03	8.2	0.21
	200	7.29	0.4	8.38	0.32
	400	7.00	0.13	8.42	0.1
	800	7.03	0.1	7.5	0.78
	1000	7.05	0.3	7.58	0.67

Table 3. Physico-chemical characteristics of various concentrations of Crude oil-in-seawater dispersion (OWD) at the beginning and after 48-h acute toxicity tests with Artemia salina nauplii. Synthetic seawater was used throughout the test. The mean (X) ± standard deviation (s.d.) of three determinations per concentration is presented.

Duration (h)	Concentration (ppm)	pH		Dissolved Oxygen	
		X	s.d.	X	s.d.
0	control	7.13	0.09	9.05	0.21
	50	7.16	0.21	8.63	0.25
	200	7.28	0.13	8.35	0.07
	400	7.16	0.13	8.15	0.07
	800	7.2	0.09	8.13	0.25
48	control	7.18	0.26	8.4	0.7
	50	7.32	0.33	7.85	0.57
	200	7.13	0.06	8.0	0.28
	400	7.11	0.09	7.55	0.42
	800	7.13	0.15	7.45	0.14

Table 4. Data summary for *Artemia* bioassay using Simple Green liquid. Experiments were conducted in duplicate with 100 nauplii per replicate for each test concentration.

Concentration (ppm)	Percent survival at end of: 24 h	48 h	72 h	96 h
Seawater	93	88	84	84
control	98	95	92	92
300	97	93	91	91
	99	97	96	96
400	90	74	66	40
	92	83	72	56
500	85	69	48	12
	88	67	45	8
600	84	62	36	2
	79	60	35	2
700	85	55	30	3
	80	58	24	3
800	77	20	1	0
	75	17	1	0
1000	17	0	0	0
	14	1	0	0

Table 5. Computer printout for (a) 48-h LC50 and (b) 96-h LC50 determinations for Artemia salina in Simple Green Liquid by probit analysis. Concentration (dose) is in ppm, and ED50 is equivalent to LC50.

(a)

DATA AS INPUT

DOSE	NO. TESTED	NO. RESPONDING
300	200	10
400	200	43
500	200	64
600	200	78
700	200	87
800	200	164
1000	200	199

PROBIT ANALYSIS:ASSAY=SGL At SS 48-h LC 50

SLOPE= 5.83935

INTERCEPT =-11.2631

VARIANCE SLOPE= .0814411

CHI 2 = 78.0076 DF= 5

LOG.ED50= 2.78509
95% CONFIDENCE INTERVAL= 2.79825 - 2.77198

VARIANCE LOG.ED50= 4.43204E-05

ED50= 609.663
95% CONFIDENCE INTERVAL = 628.421 - 591.539

(b)

DATA AS INPUT

DOSE	NO. TESTED	NO. RESPONDING
300	200	13
400	200	104
500	200	180
600	200	196
700	200	194
800	200	200
1000	200	200

PROBIT ANALYSIS:ASSAY=SGL At SS 96-h LC 50

SLOPE= 10.7719

INTERCEPT =-23.0226

VARIANCE SLOPE= .319675

CHI 2 = 37.1461 DF= 5

LOG.ED50= 2.60146
95% CONFIDENCE INTERVAL= 2.61102 - 2.59056

VARIANCE LOG.ED50= 2.91021E-05

ED50= 399.452

Table 6. Results of *Artemia* bioassay using Simple Green sponge squeezings; (a) percent survival at end of every 24 h; (b) computer printout for 48-h LC50 determination by probit analysis. Experiment was run in duplicate with 100 nauplii per test concentration per replicate.

(a)

Concentration % (V/V)	Percent survival at end of: 24 h	48 h
Seawater control	97	92
	100	95
10	89	80
	93	83
20	77	62
	73	59
30	45	4
	41	4
40	39	2
	26	0
50	13	0
	6	0

(b)

DATA AS INPUT

DOSE	NO. TESTED	NO. RESPONDING
10	200	37
20	200	79
30	200	182
40	200	198
50	200	200

PROBIT ANALYSIS:ASSAY=SGS At SS 48-h LC 50

SLOPE= 4.98847

INTERCEPT =-1.24135

VARIANCE SLOPE= .0679851

CHI 2 = 57.9746 DF= 3

LOG.ED50= 1.25115
95% CONFIDENCE INTERVAL= 1.27371 - 1.22682

VARIANCE LOG.ED50= 1.41079E-04

ED50= 17.8301
95% CONFIDENCE INTERVAL = 18.7805 - 16.8585

Table 7. Results of Artemia bioassay using Sodium dodecyl sulfate as the reference toxicant; (a) percent survival at end of every 24 h; (b) computer printout for 48-h LC50 determination by probit analysis. The experiment was run in duplicate with 100 nauplii per test concentration per replicate.

(a)

Concentration ppm	Percent survival at end of: 24 h	48 h
Seawater control	100	98
	99	97
20	91	71
	89	73
30	84	50
	83	56
40	55	23
	49	5
50	18	1
	15	0
60	8	0
	14	0

(b)

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        DATA AS INPUT

    DOSE          NO. TESTED   NO. RESPONDING

     20             200            56
     30             200            94
     40             200            172
     50             200            199
     60             200            200
. PROBIT ANALYSIS:ASSAY=SDS At SS 48-h LC 50

    SLOPE= 6.51611

    INTERCEPT =-4.32697

    VARIANCE SLOPE= .131218

    CHI 2 = 35.6499    DF= 3

    LOG.ED50= 1.43137
    95% CONFIDENCE INTERVAL= 1.44819 - 1.413

    VARIANCE LOG.ED50= 7.87377E-05

    ED50= 27.0005
    95% CONFIDENCE INTERVAL = 28.0667 - 25.8822
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Table 8. Results of *Artemia* bioassay using Simple Green and Oil Mixture; (a) Percent survival at the end of every 24 h; (b) computer printout for 48-h LC_{50} determination by probit analysis. Experiment was conducted in duplicate with 60 nauplii per test concentration per replicate.

(a)

Concentration (ppm)	Percent Survival at end of: 24 h	48 h
control	0	0
50	94	80
100	97	75
200	94	34
400	85	18
800	87	8
1000	83	0

(b)

DATA AS INPUT

DOSE	NO. TESTED	NO. RESPONDING
50	120	84
100	120	77
200	120	72
400	120	58
800	120	110
1000	120	120

PROBIT ANALYSIS;ASSAY=48-H - LC 50 FOR ARTEMIA IN OIL AND S S

SLOPE= 8.18703

INTERCEPT = .421181

VARIANCE SLOPE= .0194875

CHI S = 14.5584 DF= 4

LOG ED50= 8.18511

95% CONFIDENCE INTERVAL= 8.03721 - 8.13333

VARIANCE LOG ED50= 7.367638E-04

ED50= 153.148

95% CONFIDENCE INTERVAL = 148.38 - 134.78

Table 9. Results of Artemia bioassay using Crude Oil in water dispersion; (a) Percent survival at the end of every 24 h; (b) computer printout for 48-h LC_{50} determination by probit analysis. Experiment was conducted in duplicate with 60 nauplii per test concentration per replicate.

(a)

Concentration (ppm)	Percent Survival at end of: 24 h	48 h
control	0	0
50	97	95
100	97	83
200	91	63
400	88	60
800	83	36
1000	84	30

(b)

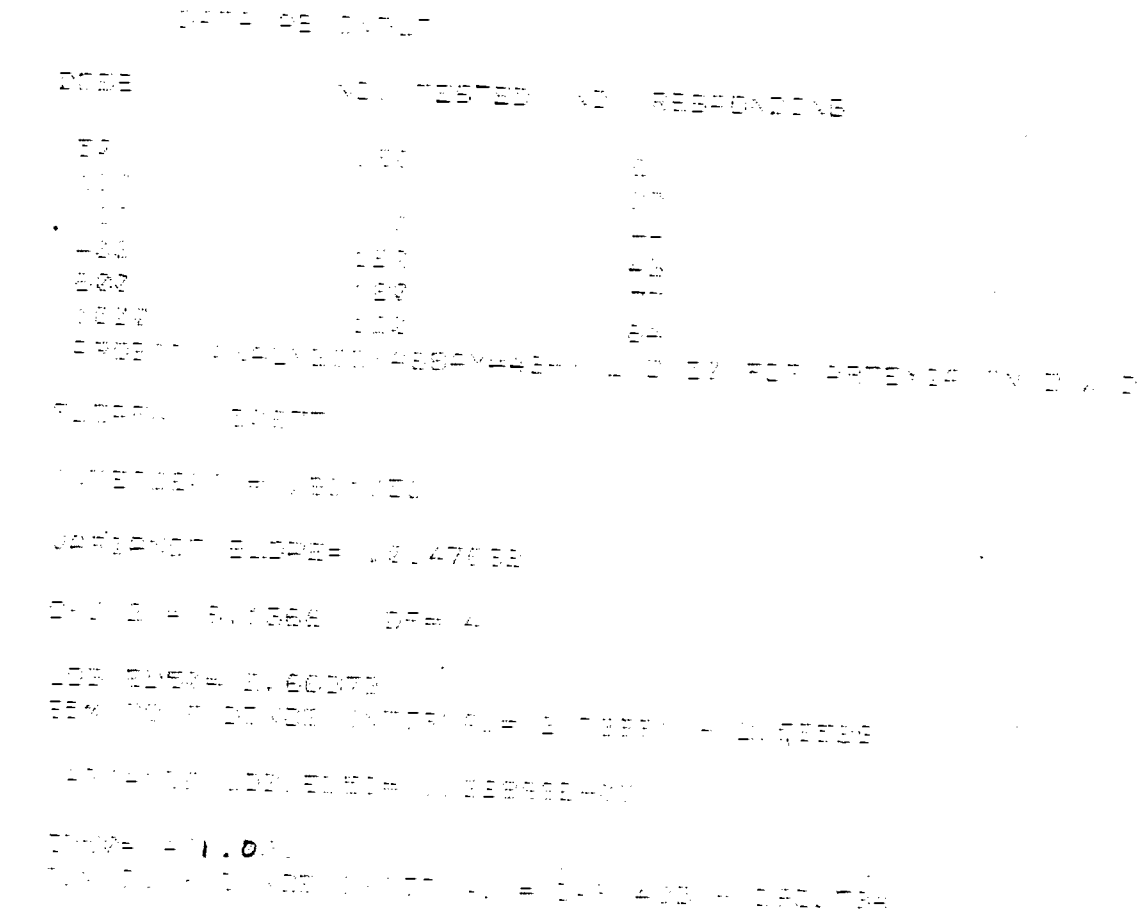

Table 10. Palaemonetes pugio. Survivorship data for bioassay using Simple Green Liquid in natural seawater. $\bar{X}$ = mean; s.d. = standard deviation; N = total number of larvae used.

Concentration (ppm)	No. of replicates	Percent survival after: 24 h $\bar{X}$	24 h s.d.	48 h $\bar{X}$	48 h s.d.	72 h $\bar{X}$	72 h s.d.	96 h $\bar{X}$	96 h s.d.	N
Seawater control	5	99.6	0.89	99.2	1.09	99.2	1.09	99.2	1.09	250
100	2	96.0	0.0	96.0	0.0	93.0	1.41	92.0	0.0	100
200	5	98.4	3.58	94.0	5.09	90.0	5.83	84.8	9.44	250
240	4	98.0	2.83	82.0	11.55	30.0	8.16	15.0	8.25	200
280	4	99.0	1.15	40.0	22.8	7.3	7.4	2.5	1.73	200
300	5	99.2	1.09	18.4	18.4	1.4	0.5	0.0	0.0	250
500	3	80.7	17.47	0.0	0.0	0.0	0.0	0.0	0.0	150

Table 11. Results of probit analysis using computer program; (a) for 48-h LC50 and (b) 96-h LC50. Larvae of Palaemonetes pugio were used in natural seawater containing Simple Green sponge squeezings.

(a)

DATA AS INPUT

DOSE	NO. TESTED	NO. RESPONDING
100	100	4
200	250	15
240	200	36
280	200	120
300	250	204
500	150	150

PROBIT ANALYSIS:ASSAY=SGL P.pugio 48-h LC 50

SLOPE= 10.1601

INTERCEPT =-19.6409

VARIANCE SLOPE= .437794

CHI 2 = 19206.8 DF= 4

LOG.ED50= 2.42526
95% CONFIDENCE INTERVAL= 2.43443 - 2.41642

VARIANCE LOG.ED50= 2.06636E-05

ED50= 266.231
95% CONFIDENCE INTERVAL = 271.912 - 260.866

(b)

DATA AS INPUT

DOSE	NO. TESTED	NO. RESPONDING
100	100	8
200	250	38
240	200	170
280	200	196
300	250	250
500	150	150

PROBIT ANALYSIS:ASSAY=SGL P.pugio 96-h LC 50

SLOPE= 11.4275

INTERCEPT =-21.516

VARIANCE SLOPE= .607535

CHI 2 = 4787.2 DF= 4

LOG.ED50= 2.32037
95% CONFIDENCE INTERVAL= 2.33074 - 2.30823

VARIANCE LOG.ED50= 3.19759E-05

ED50= 209.109
95% CONFIDENCE INTERVAL = 214.162 - 203.218

(a) for 48-h
ed in natural

2 0

monetes pugio. Survivorship data for bioassay using Simple
eezings in natural seawater (20 ppt) at 25°C. Total number
st concentration was 200. $\bar{X}$ = mean; s.d. = standard

Percent survival after:							
24 h		48 h		72 h		96 h	
$\bar{X}$	s.d.	$\bar{X}$	s.d.	$\bar{X}$	s.d.	$\bar{X}$	s.d.
100.0	0.0	99.5	1.0	99.0	1.15	99.0	1.15
99.5	1.0	98.0	2.8	98.0	2.8	98.2	2.8
99.0	2.0	98.5	3.0	98.0	2.83	98.0	2.83
99.0	2.0	94.5	2.52	94.5	2.52	94.5	2.52
98.0	2.3	76.0	6.32	69.0	3.46	65.0	2.58
90.0	10.46	10.0	9.79	5.0	5.23	3.0	2.44
94.0	3.65	17.5	5.74	1.8	1.5	0.0	0.0

(a) for 48-h
ed in natural

20

lts of probit analysis for the effect of Simple Green sponge
<u>laemonetes pugio</u> larvae; (a) for 48-h LC50, (b) for 96-h
seawater (20 ppt) was used as the diluent.

TA AS INPUT

NO. TESTED	NO. RESPONDING
200	4
200	3
200	11
200	48
200	180
200	200

ANALYSIS:ASSAY=SGSS P.pugio 48-h LC 50

.03205

PT = .5807

E SLOPE= .199781

15866.2 DF= 4

0= .628451
FIDENCE INTERVAL= .644374 - .613415

E LOG.ED50= 6.12105E-05

4.25061
NFIDENCE INTERVAL = 4.40934 - 4.10596

DATA AS INPUT

NO. TESTED	NO. RESPONDING
200	4
200	4
200	11
200	70
200	155
200	200
200	200

T ANALYSIS:ASSAY=SGSS P.pugio 96-h LC 50

= 8.34993

CEPT = .0072784

NCE SLOPE= .197373

= 191829 DF= 5

ED50= .588355
CONFIDENCE INTERVAL= .600558 - .57566

NCE LOG.ED50= 3.96347E-05

= 3.87574

(a) for 48-h
in natural 20

alaemonetes pugio. Survivorship data for bioassay using Sodium
ate in natural seawater (20 ppt) at 25°C. Total number of
est concentration was 250. $\bar{X}$ = mean; s.d. = standard

on	Percent survival after:							
	24 h		48 h		72 h		96 h	
	$\bar{X}$	s.d.	$\bar{X}$	s.d.	$\bar{X}$	s.d.	$\bar{X}$	s.d.
	100.0	0.0	99.6	0.89	99.6	0.89	99.6	0.89
	98.4	1.67	98.0	1.41	97.6	0.89	97.6	0.89
	98.8	1.09	97.6	0.89	94.8	1.79	92.4	3.58
	98.0	1.41	35.6	16.88	30.8	13.24	20.8	6.72
	18.8	10.06	0.0	0.0	0.0	0.0	0.0	0.0

=) for 48-h
in natural

sults of probit analysis for the effect of SDS on Palaemonetes (a) for 48-h LC50, (b) for 96-h LC50.

DATA AS INPUT

NO. TESTED	NO. RESPONDING
250	5
250	6
250	161
250	250

BIT ANALYSIS:ASSAY=SDS P.pugio 48-h LC 50

E= 5.66851

RCEPT =-4.27216

IANCE SLOPE= .108053

2 = 649.386 DF= 2

ED50= 1.63573
CONFIDENCE INTERVAL= 1.6572 - 1.61415

IANCE LOG.ED50= 1.18987E-04

0= 43.2246
CONFIDENCE INTERVAL = 45.4152 - 41.1293

DATA AS INPUT

SE	NO. TESTED	NO. RESPONDING
0	250	6
5	250	19
0	250	198
00	250	250

ROBIT ANALYSIS:ASSAY=SDS P.pugio 96-h LC 50

LOPE= 5.54639

NTERCEPT =-3.725

ARIANCE SLOPE= .108405

HI 2 = 202.312 DF= 2

OG.ED50= 1.5731
5% CONFIDENCE INTERVAL= 1.59489 - 1.551

VARIANCE LOG.ED50= 1.23781E-04

ED50= 37.4193
95% CONFIDENCE INTERVAL = 39.3447 - 35.563

for 48-h
n natural 20

Comparing the 48-h and 96-h LC_{50}'s for the toxicity of Simple
id (SGL), Simple Green Sponge Squeezings (SGSS) and sodium
lfate (SDS) to Artemia salina and Palaemonetes pugio.

LC50 and 95% confidence interval	Artemia salina	Palaemonetes pugio
48-h	609.66	266.23
95% interval	628.42 -591.54	271.91 - 260.87
96-h	399.45	209.1
95% interval	409.09 - 389.55	214.16 - 203.34
48-h	356.6	85
95% interval	375.6 - 337.2	88 - 82
96-h	no data	77.6
95% interval	no data	79.8 - 75.2
48-h	27.0	43.22
95% interval	28.07 - 25.88	45.42 - 41.13
96-h	no data	37.42
95% interval	no data	39.34 - 35.56

al test concentrations of SGSS were in % V/V. These were converted
for presentation in this table by multiplying by a conversion
of 20 (see Work Sheet in Appendix for derivation of conversion
).

for 48-h
n natural

Table 17. Toxicity (48-h LC_{50}) data for Artemia salina using SGL, OWD, SDS and SGL-oil mixture. All concentrations are in ppm.

Test Solution	48-h LC_{50}	Fiducial Limits	
		Upper	Lower
SGL*	609.66	628.42	591.54
OWD**	461.03	549.42	393.78
SGL and Oil	153.15	172.59	134.79
SDS***	27.0	28.07	25.88

*SGL = Simple Green Liquid
**OWD = Crude oil-in-water dispersion
***SDS = Sodium dodecyl sulphate (reference toxicant)

for 48-h
natural

18. Comparison of toxicity data for various oil dispersants to
ed invertebrates using the 48-h LC_{50}.

sant	Species	LC_{50} (ppm)	Reference
2	barnacle	< 10	Corner et al. 1968
2	European brown shrimp	3.3 - 10	Postmann 19
one	cockle	10 - 33	Postmann 19
ene	European brown shrimp	100-300	Postmann 19
ple Green	grass shrimp	266	this study
ple Green	brine shrimp	609	this study
n CW	prink shrimp	14.6	Postmann and Connor 1968
mol	pink shrimp	148	Postmann and Conner 1968
mol	brown shrimp	156	Postmann and Conner 1968
mol	shore crabs	435	

mol was the least toxic in a group of 12 oil dispersants tested.
toxic was Slickgone 2 with a 48-h LC_{50} of 3.5 - 21.3 ppm for the
e groups of crustaceans tested.

irst stage larvae of grass shrimp and 24-h old nauplii of the
e shrimp (most sensitive life stages) were used in this study
f. adults used in studies with the other dispersants). Using
lts in SGL may further increase the LC_{50} making SGL even less
ic than portrayed here.

Table 19. Comparison of 96-h LC_{50} data for toxicity of various oil dispersants to fish.

Dispersant	Test Animal	LC_{50} (ppm)	Reference
BP1100X	rainbow trout fingerling	510-830	Doe and Wells, 1978
Sugee #2	rainbow trout fingerling	1150-1900	Doe and Wells, 1978
BP1100X	Mummichog	3600-5600	Doe and Wells, 1978
Sugee #2	Mummichog	6400-13,600	Doe and Wells, 1978
Simple Green	Mummichog	1574	Connelly, 1984
Corexit	Cod	130	Swedmark, 1974
BP1100X	Cod	> 688	Swedmark, 1974
BP1100X	Cod	120	Swedmark, 1974

DISCUSSION

The Environmental Protection Agency (EPA) guideline for toxicity testing of oil dispersants does not give any minimum or 'standard' concentration that must be met for a dispersant to be considered safe for use. In Canada, an oil dispersant must have a 96-h LC_{50} of 1000 ppm or greater and when mixed with oil, 100 ppm or greater (in toxicity tests with rainbow trout) before it can be approved for use in oil spill cleanup operations (Doe and Wells, 1978). In the absence of such 'acceptability criteria' or baseline, especially for marine invertebrates with which this report is mainly concerned, I am going to base my conclusions on the results of comparing the toxicity of dispersants currently in use with that of SGL and with the reference toxicant.

Surprisingly, there is limited information in the open literature on the acute toxicity of oil dispersants despite the large numbers of new ones being produced every year. This is probably due to the fact that the majority of the data on these chemicals is proprietary in nature. Thus I have unavoidably drawn heavily from the few data that I can find in the literature so as to make a reasonable comparison between SGL and other oil dispersants.

The results of these toxicity tests show that SGL alone and the crude oil alone (OWD) are relatively nontoxic to Artemia salina when compared with the reference toxicant (SDS) and the SGL-oil mixture (Table 17). The 48-h LC_{50} values indicate that SDS is approximately 23 and 17 times as toxic to Artemia as SGL and OWD respectively. When SGL is mixed with the crude oil in a 1:10 ratio, the resulting mixture is about 4 and 3 times more toxic than SGL and OWD, respectively. These findings are similar to what has been reported in the literature that crude oils generally have

very little toxic effects on both vertebrates and invertebrates. However when mixed with oil dispersants, the effects are much more drastic (see Review by Johnston, 1984). Also Swedmark et al. (1973) studying the toxicity of nine oil dispersants to marine animals, found that all the oil-dispersant mixtures were more toxic than either dispersants alone or crude oil alone.

One possible explanation for the above findings is that the addition of dispersant may enhance the contact between the oil and the cuticle of arthropods (notably crustaceans) or the body surface of fish thereby increasing the penetration of the water soluble fractions (WSF). This together with the dispersing effectiveness of the dispersant (which will obviously increase the concentration of WSF of the oil) could account for the increased toxicity generally observed for oil-dispersant mixtures compared with either oil or dispersant alone. There are, however, many exceptions to the above trend, i.e. some dispersants are by themselves more toxic than when in a mixture with crude oil. Essolvene for example was shown to be 3 and 4 times more toxic to amphipods and gastropods respectively when used alone than when mixed with Kuwait crude (Logan and Perkins, 1972). Simple Green as shown above is by itself not toxic and when mixed with crude oil, increased the toxicity only slightly compared with what has been reported for other dispersants.

The relative toxicity of SGL to other dispersants (both old and new) are presented in Tables 18 and 19. Among the 48-h tests with invertebrates (Table 18), it can be seen that of all the dispersants listed, SGL is the least toxic, comparable only to Dermol which itself was the least toxic in a test of 12 dispersants (see Portmann and Connor, 1968). It should be pointed out that the above comparison is not quite fair because the

toxicity tests with SGL were conducted using one of the most sensitive life stages (larvae), while the other dispersants were tested on adults which are generally more resistant to toxicants. Had the SGL tests been conducted on adults of the test species, the outcome would have been different and it is possible that the 48-h LC_{50} would have been even higher. In which case SGL would have been more distinctively non-toxic.

The results of 96-h tests with fish also show that the toxicity of Simple Green is well within the range of other dispersants approved for use in oil spills (Table 19). For example, BP1100X and Sugree #2 were placed on the Canadian EPS (Counterpart of U.S. EPA) list of acceptable dispersants in 1974. The Canadian toxicity tests for dispersants is much harder to pass than that of the United Kingdom (Doe and Wells, 1978). Many dispersants that had been approved by U.K. failed the Canadian test. Thus for SGL to range among those dispersants that pass the Canadian test is commendable indeed.

When compared with BP1100X for Mumichog, SGL may appear slightly more toxic. However this could be the result of differences in temperature and other experimental conditions or the susceptibility of the fish population used. Even if the difference between SGL and BP1100X was real, a small dilution of SGL should bring it at par with BP1100X. It is worth noting that Corexit 9527 failed the Canadian toxicity test but after a 10% dilution it was approved. Similar steps could be taken for SGL if deemed necessary on the basis of the fish toxicity data. However the results of the crustacean toxicity studies clearly show that SGL is a non-toxic liquid cleaner that can safely be used as an oil dispersant in the marine and estuarine environment if it meets the "effectiveness tests."

In the absence of the results for the oil dispersing effectiveness tests with SGL, the following personal observations are worth noting. All glassware used in oil toxicity tests were cleaned with dilute solutions (about 20% V/V) of SGL after rinsing with few mls of n Hexane. Due to the oil dispersing capabilities of SGL in very dilute solutions, cleaning oil-contaminated glassware was fast and very easy. Since it has been proven that SGL is nontoxic to Artemia nauplii and grass shrimp larvae (this report) and also to fish (report by Connelly) it can safely be used in cleaning laboratory glassware used for toxicity studies.

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APPENDICES

APPENDIX A - Physico-chemical characteristics and major composition of Standard Reference oil (Prudhoe Bay Crude) as received from EPA.

APPENDIX B - Work Sheet for determining the Conversion Factor for SGSS.

APPENDIX A

U.S. Environmental Protection Agency
Environmental Monitoring and Support Laboratory - Cincinnati

American Petroleum Institute
Department of Environmental Affairs

STANDARD REFERENCE OIL SAMPLE

PRUDHOE BAY CRUDE OIL

This sample is made available for the sole purpose of providing a reference oil for research and laboratory testing purposes.

Storage and Handling

Store reference oil samples at a temperature of no more than 20°C, preferably in a dark area.

Ampuls, 20 mL - open the ampul by snapping off the top at the break area on the neck.

To retain a portion of the ampul contents, immediately transfer the oil to a clean, dry glass flask or vial, and seal. Do not use a plastic container. Non-glass stoppers must contain a Teflon insert to prevent oil contact with plastic or metal.

Bottles, one-pint - bottles of reference oils are closed with a plastic screw cap containing a Teflon insert. If bottle is used to store a portion of the oil contents after opening, be sure that the Teflon insert remains in the cap.

ASTM Standard Methods for Waterborne Oil Samples

Analyte	ASTM Method*
Specific and API gravity	D1298-80 (Part 23)
Nitrogen, sulfur, nickel and vanadium	D3327-79 (Part 31)
Sulfur compounds, profile	D3328-78 (Part 31)
Simulated distillation profile	D2887-73 (Part 24)
Infrared spectrum	D3414-79 (Part 31)
UV fluorescence spectrum	D3650-78 (Part 31)

*ASTM series available from: American Society for Testing and Materials, 1916 Race Street, Philadelphia, PA 19103.

REFERENCE VALUES

Prudhoe Bay Crude Oil

This oil has been analyzed by skilled oil testing and research laboratories to characterize it and to ensure that substantial compositional changes have not occurred during storage and sample preparation. Results for various selected parameters were as follows:

Analyte	Result
Specific gravity*	0.894 kg/L
API gravity*	26.8 degrees
Sulfur	1.03 weight %
Sulfur compounds, profile	See Fig. 1
Nitrogen	0.20 weight %
Vanadium	21 mg/L
Nickel	11 mg/L
Simulated distillation profile	See Fig. 2 and Table 1
Infrared spectrum	See Fig. 3
UV fluorescence spectrum	See Fig. 4
Pour point	+25°F
Viscosity,	
at 40°C	14.09 cSt
at 100°C	4.059 cSt
Index	210

*at 15/15°C

Table 1
Boiling Range Distribution for
Prudhoe Bay Crude

Percent Recovered	Temperature Degrees F	Percent Recovered	Temperature Degrees F	Percent Recovered	Temperature Degrees F
IBP	--	36	512	72	766
1	132	37	519	73	773
2	159	38	526	74	780
3	178	39	533	75	787
4	192	40	539	76	794
5	208	41	545	77	801
6	214	42	553	78	809
7	233	43	562	79	816
8	240	44	569	80	823
9	254	45	574	81	830
10	267	46	583	82	838
11	279	47	592	83	845
12	287	48	599	84	853
13	299	49	605	85	861
14	311	50	613	86	869
15	322	51	620	87	877
16	332	52	626	88	886
17	341	53	633	89	894
18	353	54	641	90	903
19	367	55	648	91	912
20	379	56	654	92	921
21	389	57	661	93	931
22	398	58	668	94	941
23	407	59	675	95	951
24	413	60	681	96	962
25	421	61	689	97	973
26	432	62	695	98	985
27	439	63	702	99	997
28	446	64	709	FBP	1003
29	455	65	716		
30	466	66	723		
31	475	67	730		
32	479	68	737		
33	486	69	744		
34	496	70	752		
35	504	71	758		

IBP - initial boiling point; FBP-final boiling point

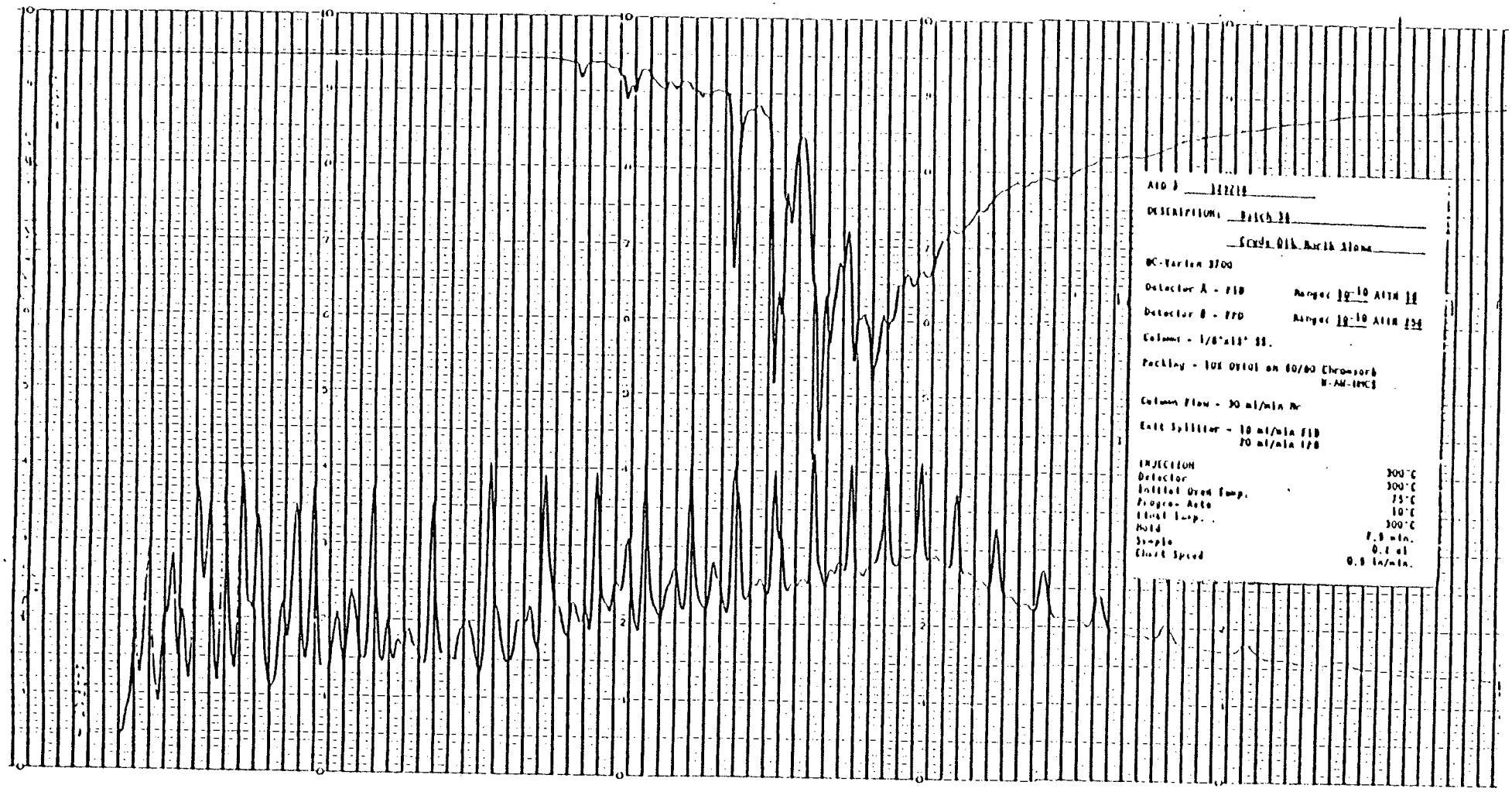

Fig. 1. Gas Chromatogram of Prudhoe Bay Crude Oil; Upper Trace - Flame Photometric, Lower Trace - Flame Ionization.

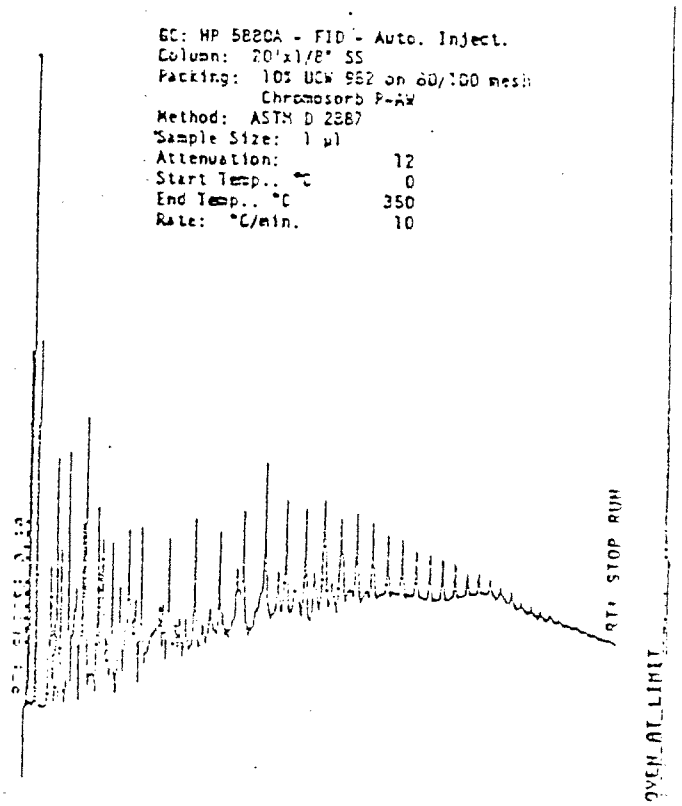

Fig. 2. FID Gas Chromatogram from Determination of Boiling Range Distribution of Prudhoe Bay Crude Oil (Table 1).

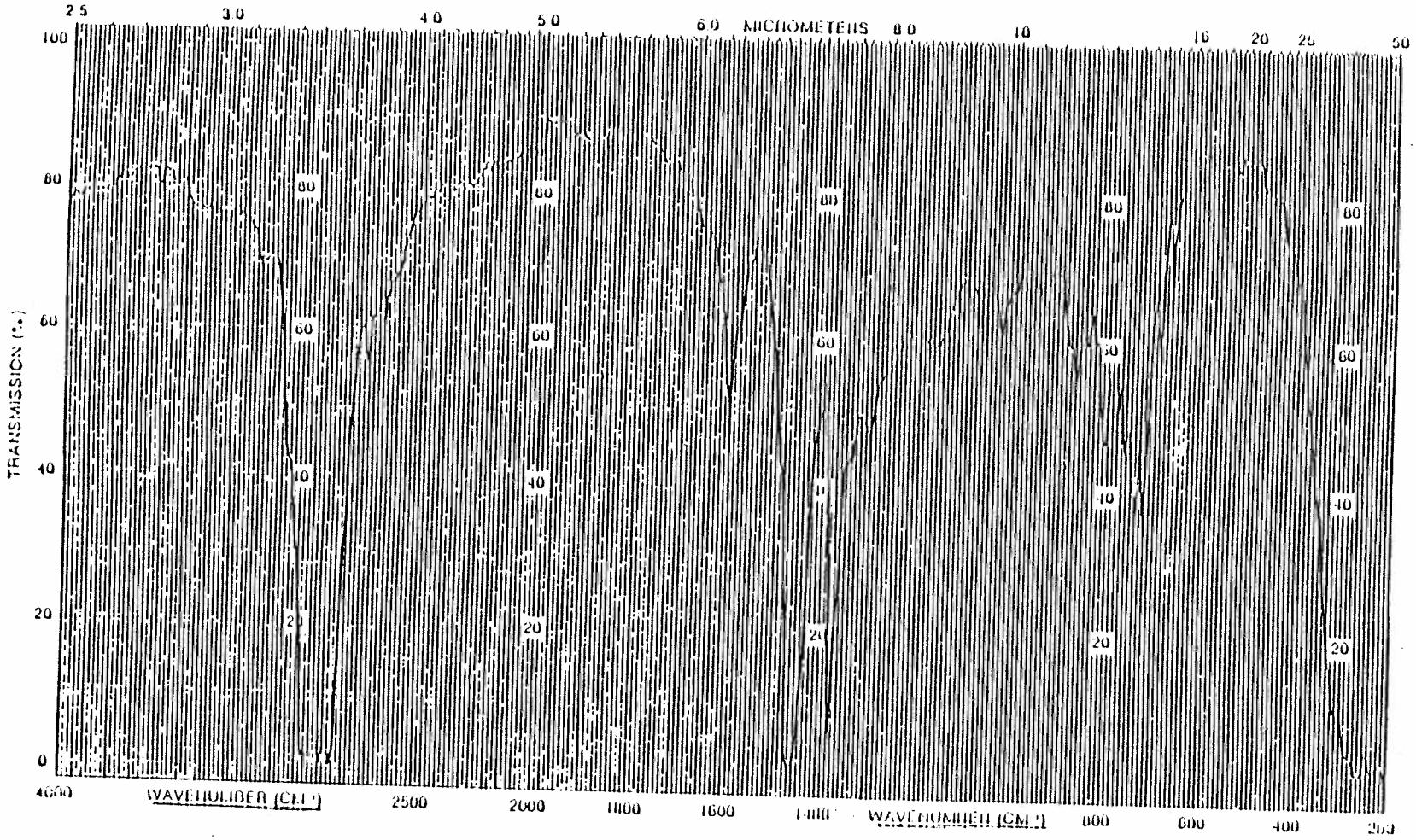

Fig. 3a. Infrared Scan of Prudhoe Bay Crude Oil, Thick Film.

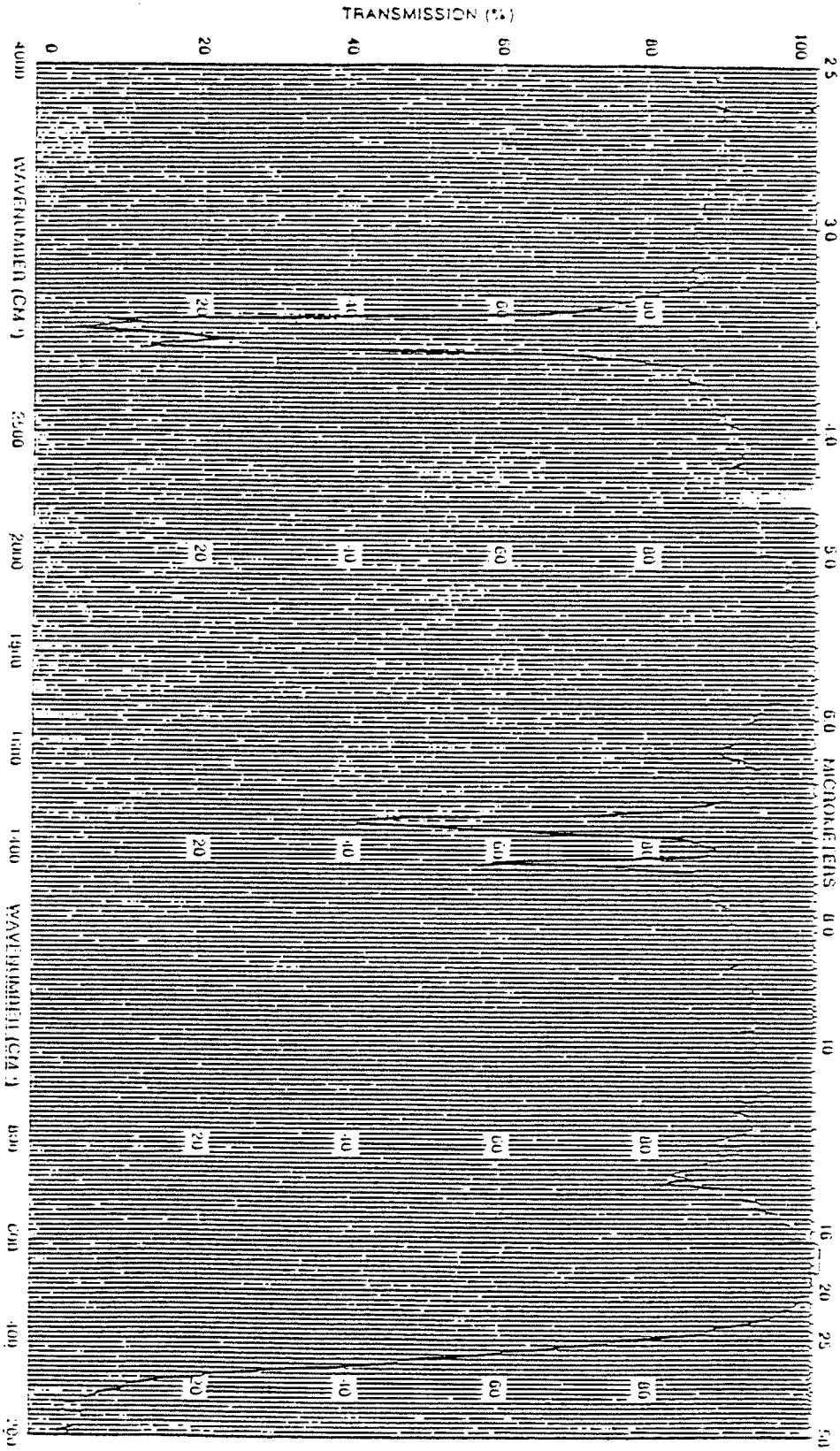

Fig. 3b. Infrared Scan of Prudhoe Bay Crude Oil, Thin Film.

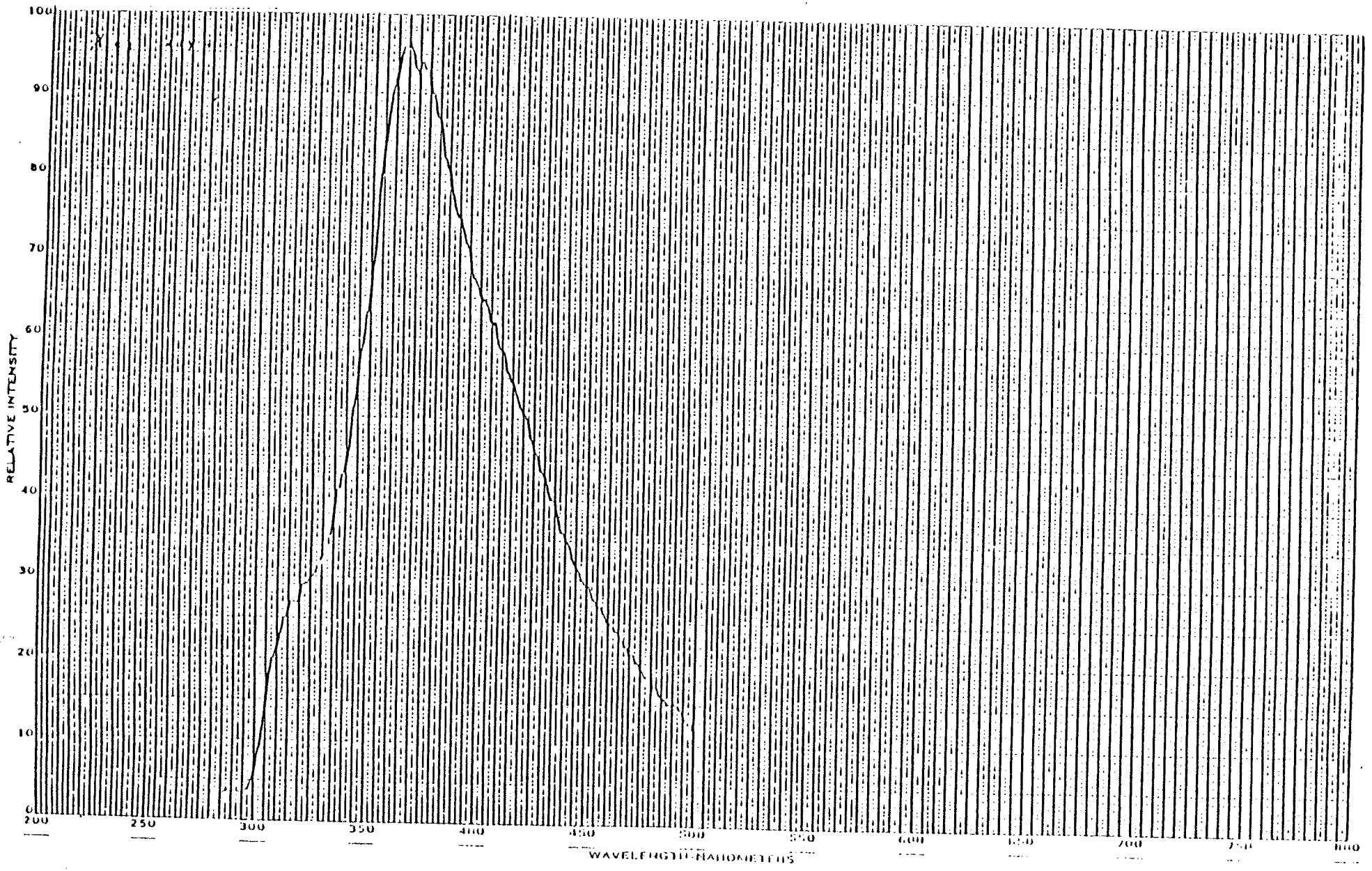

Fig. 4. Ultraviolet Spectrum of Prudhoe Bay Crude Oil.

APPENDIX B

Work Sheet for Deriving Conversion Factor for SGSS (% V/V to ppm)

1. Volume of each loaf of sponge = 500 cm^3
2. Volume of SGL (concentrated) per 500 cm^3 sponge = 20 ml
3. Assuming even distribution and immobilization of SGL in sponge, from 1 and 2 above, each cm^3 of sponge should contain 0.04 ml of SGL.
4. Volume of sponge used in preparing 1L of SGSS (test sponge) = 100 cm^3.
5. Concentration of SGL per L of sponge 'extract' (from 3 and 4 above) = 4 ppt.
6. Because of the brief soaking time and few squeezings of the test sponge, it is reasonable to assume that only 50% of the immobilized SGL was removed. Thus, the concentration of the 'working solution' was 2 ppt.
7. From 6, 1% V/V of the 'working solution' is equivalent to 20 ppm. Therefore the Conversion Factor = 20.