Technical Note N-1195

THE BIODEGRADATION OF OIL IN SEAWATER FOR NAVAL POLLUTION CONTROL

By

T. B. O'Neill, Ph.D.

January 1972

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NAVAL CIVIL ENGINEERING LABORATORY
Port Hueneme, California 93043

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ABSTRACT

Natural oil seeps and accidental spills are common to the coast of Southern California. Field observations and laboratory tests support the view that marine microorganisms have the capacity to oxidize and thus degrade oil and derivatives of oil. This report describes the sampling of beach sand, sediment water, and tar deposits from 15 different sites of Southern California, where oil spills are chronic.

The laboratory techniques of isolating and culturing hydrocarbon-oxidizing microorganisms are also described, as are cell density studies, where Navy fuels are used as the sole source of energy for microbes in the collected samples.

To date, 62 hydrocarbon-oxidizing species, 48 bacteria, 10 fungi and 4 yeasts have been isolated.

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INTRODUCTION

The problem of oil spillage is one of great magnitude and concern to the Navy, particularly in this period of ecological awareness. Oil on the seas is a fire hazard and a menace to the organisms of that environment. It is likewise an offense against aesthetics to those who use the seas for recreation and inspiration.

The problems associated with the spillage of oil on beaches and waters are not new. They are concomitant with the origin and development of propulsion units using fossil fuels. In content of the present publication, it is imperative to state that natural oil seeps from the ocean floor have existed for millenia. Merz (1959) reported an almost universal presence of oil, derived from natural oil seeps on the beaches of Southern California. The amounts were generally small, less than 2 ounces of oily substance per 500 $feet^2$ and not persistent. Coal Oil Point in the Santa Barbara Channel is an extreme example of a beach having continued deposits of large amounts of crude oil and tar. Allen et.al. (1970) determined this seepage to be on the order of 50-70 barrels (8,000 to 11,000 liters) of oil per day into the channel. The deposition on the beach was estimated to the 100 times greater than any other beach in Southern California.

As well known as the appearance of oil and tar on beaches and their adjacent water, is its disappearance after unspecified periods of time. ZoBell (1969) reported that oil-oxidizing bacteria are often abundant in marine sediments and seawater of coastal areas where natural or man-induced oil pollution is chronic. He cites populations of greater than 10^8 per mℓ of mud from San Pedro and Long Beach Harbors. He further states that oil-oxidizing bacteria yeasts and fungi have been demonstrated in water and mud samples in many parts of the world. In 1963, ZoBell estimated that in the sea, oil might be oxidized at rates as high as 100-960 mg/m^3 day or 3[illegible]-350 g/m^3 year.

More recently, Miget (1971) reported the isolation of oil-oxidizing bacteria from Coal Oil Point.

The present report is concerned with the isolation of fungi, yeasts and bacteria from inshore waters and mud of areas where oil spills are chronic.

METHODS

Sampling

The geographical areas selected for sampling, the reasons for selection of sites, and the designation of samples are as follows:

1. Gaviota State Beach Park (31 miles west of Santa Barbara) selected as the westernmost readily accessible land adjacent to the Santa Barbara Channel. Small amounts of oil and tar were found on the beach. Three samples were taken at this point.

   a. Surface sand approximately 20 feet below high tide mark.
   b. Same site as a, but from depth of eight inches.
   c. Ocean bottom at depth of approximately 10 feet, under pier, sample taken with T-type bottom sampler.

2. Getty Oil Company - Gaviota Marine Terminal (24 miles west of Santa Barbara) from intertidal zone in stream bed containing effluent from cooling tanks and at the shore end of loading pipes. The sand had an odor of oil.

   a. From surface of stream bed.
   b. As site a, but from depth of eight inches.

3. Ellwood Oil Fields - Signal Oil Company (approximately 24 miles west of Santa Barbara) wells at edge of water line. Beach contained some oil and tar.

   a. Sand from surface of beach under pier where oil constantly drips to sand underneath.
   b. Sand from beach at base of runoff water from wells. Sample taken at depth of eight inches.

4. Coal Oil Point (approximately 7 miles west of Santa Barbara) well known collecting site for oil from adjacent natural seeps. Approximately 25 percent of the beach was covered with tar and oil.

   a. Sand from surface at edge of beach covered by incoming waves and uncovered by receding waves. Approximately 30 feet from the high tide mark.
   b. As a, but from depth of four inches.
   c. Tar and associated sand from same area as a.
   d. Surface water from depth of approximately two feet and in an oil slick.

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Natural oil seeps and accidental spills are common to the coast of Southern California. Field observations and laboratory tests support the view that marine microorganisms have the capacity to oxidize and thus degrade oil and derivatives of oil. This report describes the sampling of beach sand, sediment water, and tar deposits from 15 different sites of Southern California, where oil spills are chronic.

The laboratory techniques of isolating and culturing hydrocarbon-oxidizing microorganisms are also desribed, as are cell density studies, where Navy fuels are used as the sole source of energy for microbes in the collected samples.

To date, 62 hydrocarbon-oxidizing species, 48 bacteria, 10 fungi, and 4 yeasts have been isolated.

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Oceans						
Shores						
Marine microorganisms						
Oxidizers						
Degradation						
Sampling						

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5. Goleta Beach State Park (5 miles west of Santa Barbara). Beach containing small amounts of oil and tar.

a. Sand from surface of beach in intertidal zone.

6. Summerland Oil Fields (4 miles east of Santa Barbara) beach at Lookout Park, without oil or tar on the beach.

a. Sand from beach at depth of four inches.

7. Carpinteria Beach (12 miles east of Santa Barbara). Adjacent to Standard Oil Company Pier. At this site, accessible at low tide, oil seeps from rocky cliffs and flows to the beach below.

a. Sand from surface at base of rocky outcrop and adjacent to flowing oil.
b. As a, but from depth of four inches.
c. As a, but from depth of six inches.
d. Crude oil seepage from rocky outcrop.

8. McGrath Lake (3 miles south of Ventura). This is a fresh water lake with considerable salt water intrusion. The lake lies roughly parallel to the beach lines and during periods of extremely high tides one end of the lake is open to the ocean. In the vicinity of the lake are several oil wells.

a. Sample secured from bottom of lake with T-type sampler.

9. Phillips Oil Terminal. Part of Los Angeles west end of basin, across from Todd Shipyard. Oil Slicks on water surface.

a. Sample from surface of water in midst of oil slick.
b. Sample from floor of basin at depth of 30 feet.

10. Standard Oil Company Terminal, west side of Turning Basin. Oil slicks on water surface.

a. Sample from surface of water in midst of oil slick.
b. Sample from floor of basin at 20 foot depth.

11. Shell Oil Company Terminal, east edge of Turning Basin, south part of East Basin Channel. Oil slicks on water surface.

a. Sample from water surface in midst of oil slick and including a chip from oil-soaked piling.
b. Sample from floor of basin at 12 foot depth.

12. Vegetable Products Plant, north end of Slip No. 4. Oil slick on water surface.

a. Sample from floor of slip at depth of 21 feet.

13. Los Angeles County Storage Shed, west side of entrance to Dominguez Channel. Oil slicks on water surface.

a. Sample from channel bottom.

14. Anchorage, north end of Dominguez Channel. Oil slicks on surface.

a. Sample from channel floor at depth of 9 feet.

15. City of Los Angeles Storage Shed, west side of central portion of Slip No. 1. Oil slicks on water surface.

a. Sample from surface in midst of oil slick.
b. Sample from bottom at depth of 32 feet.

All samples taken have a single characteristic in common: they are from areas constantly subject to man-made and/or natural oil spills.

An attempt was made to maintain aseptic conditions while securing the samples. Thus, collecting spatulas were flamed with a portable propane burner. The samples were placed in sterile containers and the period of time between collection and transfer to microbiological media for subsequent studies was minimal. Samples from the Santa Barbara and Ventura areas (1 to 8) were placed in media at the collection site. Samples (9 to 15) from Los Angeles Harbor were taken from a boat and the placement into media occurred when the boat docked, a maximum period of 2 hours.

Laboratory Studies

Aliquot portions of each sample (1.0 mℓ of liquid, 0.1 gm solid) were placed in a 250 mℓ Erlenmeyer flask containing 40 mℓ of an oil-mineral salt medium designated O.MS (Pelczar, 1965). This medium consists of three basic solutions:

$$\begin{aligned} (NH_4)_2SO_4 &\ldots\ldots\ldots\ldots\ 4.0 \text{ gm} \\ K_2HPO_4 &\ldots\ldots\ldots\ldots\ 4.0 \text{ gm} \\ KH_2PO_4 &\ldots\ldots\ldots\ldots\ 2.0 \text{ gm} \\ H_2O &\ldots\ldots\ldots\ldots\ 2800 \text{ m}\ell \end{aligned} \qquad (1)$$

$CaCl_2$ . . . . 0.04 gm		
$FeSO_4\ 7H_2O$. . . . . 0.02 gm		(2)
H_2O. . . . . 400 mℓ		

$MgSO_4\ 7H_2O$. . . . . 0.8 gm		
$MnCl_2\ 4H_2O$. . . . . 0.4 gm		(3)
H_2O. . . . . 400 mℓ		

The water used in the three solutions was aged seawater filtered through a 0.45μ membrane filter. The three solutions were then combined as follows: solution (1) - 7 parts, solution (2) - 1 part and solution (3) - 1 part and 49 mℓ aliquots were placed in flasks. Diatomaceous earth (100 mg), sterilized by hot air, was added to increase the surface area of solids in the media, thereby facilitating microbial attachment and activity. All flasks were autoclaved, and upon cooling, specific hydrocarbon sources were added as the sole source of carbon. The three oils used; crude oil, marine diesel, and bunker C; were selected on the basis of ready availability and as representatives of fuels extensively transported and used by the Navy. To each flask was added 0.3 gms of hydrocarbon so that their final concentration was 0.75%. The hydrocarbons were checked for sterility prior to utilization in the media.

Each of the samples of soil (0.1 gm/flask) and water (1.0 mℓ/flask) to be studied were added to each of the three different O.MS media, that is, media containing crude oil, marine diesel and bunker C. A fourth series of flasks was used as controls. These contained mineral salts but no hydrocarbons, thus no source of organic carbon.

The flasks were placed on a rotary shaker and incubated at room temperature for one week. During this period the flasks were rated positive or negative on the basis of the ability of microorganisms from the samples to utilize the hydrocarbon fuels as an energy source. The criterion used in evaluation is cell density in experimental flasks as compared to controls.

Cell density was determined by dilution series and plate counts on trypticase soy agar. The plates were prepared in duplicate. This quantitative technique was used with full awareness of inherent shortcomings in the procedure, namely, that no one medium or conditions of incubation will provide conditions suitable for the growth of all microorganisms in the original sample.

At daily intervals during the weekly period of incubation, loopfuls of flask contents were transferred to plates of trypticase soy agar for isolation of bacteria and to Mycophil* agar and Eugonagar* for the

*Trademark, Baltimore Biological Laboratory isolation of yeasts and molds. Pure cultures of bacteria, yeasts, and molds were derived by the streak method and presently are maintained on slants with the appropriate media.

Literature Survey

The author perused several classical texts and journal articles containing extensive references to microbial oxidation of hydrocarbons (ZoBell, Grant and Haas, 1949; ZoBell, 1946, 1950, 1963, 1969; Beerstecher, 1954; Senez and Konovaltschikoff-Mazoyer, 1956; Fuhs, 1961; Sharpley, 1964; and Davis, 1966). A listing of microorganisms reputed to have an ability to oxidize hydrocarbons has been compiled and compared with the most recent catalogue of the American Type Culture Collection (Anon, 1970). From this catalogue, 45 pure cultures of hydrocarbon-oxidizing bacteria have been ordered for future experiments.

RESULTS

Isolation Techniques

From all samples, microorganisms having the ability to oxidize hydrocarbons have been isolated. To date, 62 species have been isolated and are being maintained in the laboratory. Of these cultures, 48 have been identified as bacteria, and of these, 42 are rod-forms and 6 are coccoid. Ten hydrocarbon-oxidizing fungi have also been isolated as have 4 yeasts.

Cell Density Studies

Controls. As previously stated, an increase in cell density is accepted as evidence of microbial utilization of the sole source of carbon, the hydrocarbons. The densities cited in Table 1 are used towards this end and a review of the densities does lead to a recognition of certain tendencies.

Cell densities of controls vary greatly according to the sampling site. Thus, the bacterial population of the surface of a sandy beach is minimal, undoubtedly due to several factors such as the lack of organic matter as a nutrient, the lack of moisture, and the harmful effects of sunlight and moving sand. The populations do increase in the controls to the extent that there is some organic matter transferred in the sand and peaks during the first 36 hours of incubation.

Cell density in soil samples from the ocean floor as contrasted to that of beach sand, is extremely high. The amount of available moisture is not a problem nor is the amount of organic matter which is available as an energy source. The adverse effects of the sun's ultraviolet rays are nil at these sites. Not only is the initial population greater, but the increase within the flasks is more pronounced due to the greater amount of organic matter in the original sample though the peak period again appears to occur in approximately 36 hours after incubation.

Samples of surface water appear to have even lesser cell densities than those of surface sand. This is the result of the presence of extremely small amounts of organic matter on the surface of the water and the presence of ultraviolet radiation from the sun. In the enclosed areas of Los Angeles Harbor, with great commercial activity, the cell densities are greater than those open waters of isolated beaches such as Carpinteria.

The cell density appears to decrease with depth, again a manifestation of the lack of organic matter.

Microbial population on tar of the beach is minimal, even when compared to those of the adjacent sand. Thus, at Coal Oil Point, the surface sand registered 46,000 cells/gm and the tar spot adjacent to the sand, 540 cells/gm and at Carpinteria, 11,500 from sand and 56 from tar, respectively. Perhaps the number on the tar is less because most bacteria are either harmed by the hydrocarbons of the tar or they cannot utilize the hydrocarbon as a source of energy. In this comparison, a most important consideration is not the initial density of the organisms, the quantitative determination, but rather the quality of these organisms. Thus, for example, while the tar sample has an initial low cell density as contrasted to that sand, at 36 hours the cell density of the tar is 14,300,000 and that of sand is 1,280,000. Using the comparison, the tar samples from the Coal Oil Point showed a much greater population increase in 36 hours (540 cells/gm to 17,610,000) than that from the sand (46,000 cells/gm to 8,000,000). The 0.1 gm of tar sample represents a much greater amount of nutrients for hydrocarbon-oxidizing bacteria than does a similar mass of sand.

Experimental Flasks. After 24 hours of incubation, microbial populations were determined for experimental flasks and compared with controls. One hundred and fourteen determinations were made and of these, all but nine had greater cell densities in the controls. This again suggests that many microbes in the original sample did not survive the presence of hydrocarbons. Those organisms that did survive derived energy either from the organic matter of the sample or from the added hydrocarbon, or from both. Of the ten experimental flasks containing cell densities greater than the controls, six of them contained tar on the original sample. This suggests, as previously stated, that the bacteria on the tar sample are, for the most part, hydrocarbon-oxidizing forms.

There appears to be no significant differences in cell density in experimental flasks having different hydrocarbon sources when the time period is constant, though cell densities appear to be slightly greater in marine diesel than crude oil and bunker C and least in crude oil.

After 36 hours of incubation, all of the experimental flasks had greater cell densities than the controls, except for the bottom samples. It is believed that the relatively high content of organic matter in the sediment contributed to the high cell density.

The peak of cell density appears to occur between the 36th and 72nd hour after incubation. In the 72nd hour, again the populations of experimental flasks were greater than the controls. Once more the exception was controls containing bottom samples.

After a week of incubation, all cell densities were minimal. Most probably due to the accumulation of waste products and/or the exhaustion of one or more media components.

CONCLUSION

The observations that oil from natural seeps or man-made disasters ultimately disappear from beaches and water, coupled with laboratory results, does support the view that hydrocarbon-oxidizing microorganisms occur in that environment. Results of the present study indicate that such microorganisms are not uncommon and not difficult to isolate and grow.

Future research will emphasize the combination of pure cultures to enhance the biodegradable activity of the organism and the analyses of media and environmental conditions that also increase their ability to oxidize Navy fuels.

Table 1. Cell Density (Plate Count/mℓ) in Hydrocarbon Media

	0 HOURS	24 HOURS			
SAMPLE DESIGNATION	CONTROL	CONTROL	MARINE DIESEL	CRUDE OIL	BUNKER C
1a	28,100	560,000	710,000	920,000	1,320,000
1b	14,700	320,000	78,000	52,000	106,000
1c	156,000	1,410,000	650,000	730,000	325,000
2a	76,000	830,000	427,000	960,000	530,000
2b	22,300	417,000	112,000	312,000	78,000
3a	35,000	740,000	560,000	191,000	218,000
3b	16,500	510,000	212,000	27,300	145,000
4a	46,000	2,060,000	970,000	3,260,000	5,100,000
4b	18,700	650,000	430,000	2,110,000	2,780,000
4c	540	320,000	7,100,000	5,400,000	3,900,000
4d	1,650	39,000	3,500,000	6,300,000	2,150,000
5a	12,100	730,000	1,020,000	860,000	1,210,000
6a	8,900	540,000	1,890,000	2,320,000	1,470,000
7a	46,000	2,060,000	960,000	3,260,000	5,100,000
7b	18,700	650,000	410,000	2,110,000	2,780,000
7c	540	320,000	7,100,000	5,400,000	3,900,000
7d	1,650	49,000	3,500,000	6,300,000	2,150,000
8a	2,690,000	11,600,000	730,000	213,000	690,000
9a	26,500	109,000	78,000	54,000	38,000
9b	5,400,000	26,100,000	1,790,000	6,700,000	8,100,000
10a	14,300	234,000	126,000	43,000	28,500
10b	3,190,000	27,800,000	14,700,000	7,800,000	9,800,000
11a	34,000	1,270,000	540,000	228,000	410,000
11b	2,180,000	43,000,000	13,600,000	9,200,000	11,700,000
12a	2,780,000	68,000,000	7,100,000	21,800,000	33,000,000
13b	5,100,000	82,000,000	108,000,000	46,000,000	22,700,000
14a	6,400,000	97,000,000	19,100,000	14,600,000	12,300,000
15a	18,700	570,000	178,000	312,000	560,000
15b	3,060,000	73,000,000	11,500,000	49,000,000	68,000,000

Table 1. Cell Density (Plate Count/mℓ) in Hydrocarbon Media (Cont)

	0 HOURS	36 HOURS			
SAMPLE DESIGNATION	CONTROL	CONTROL	MARINE DIESEL	CRUDE OIL	BUNKER C
1a	28,100	740,000	14,700,000	31,000,000	17,200,000
1b	14,700	115,000	2,150,000	1,230,000	1,060,000
1c	156,000	3,180,000	106,000,000	18,900,000	12,900,000
2a	76,000	1,260,000	3,100,000	12,800,000	4,400,000
2b	22,300	610,000	1,870,000	2,790,000	1,430,000
3a	35,000	1,410,000	2,780,000	1,740,000	1,520,000
3b	16,500	880,000	1,170,000	3,020,000	4,100,000
4a	46,000	8,600,000	241,000,000	189,000,000	76,000,000
4b	18,700	2,900,000	74,000,000	21,400,000	15,700,000
4c	540	17,610,000	113,000,000	51,000,000	43,000,000
4d	1,650	630,000	82,000,000	105,000,000	54,000,000
5a	12,100	9,100,000	12,900,000	8,600,000	11,300,000
6a	8,900	7,300,000	10,400,000	6,700,000	9,100,000
7a	46,000	8,600,000	24,300,000	18,900,000	7,600,000
7b	18,700	2,910,000	31,000,000	21,400,000	15,700,000
7c	540	11,610,000	113,000,000	57,000,000	43,000,000
7d	1,650	630,000	78,000,000	104,000,000	54,000,000
8a	2,690,000	138,000,000	470,000,000	320,000,000	212,000,000
9a	26,500	212,000	18,700,000	16,900,000	95,000,000
9b	5,400,000	185,000,000	308,000,000	175,000,000	202,000,000
10a	14,300	1,130,000	7,800,000	15,100,000	23,000,000
10b	3,190,000	24,700,000	1,560,000,000	650,000,000	930,000,000
11a	34,000	4,100,000	12,800,000	7,500,000	5,600,000
11b	2,180,000	117,000,000	17,100,000	11,900,000	14,800,000
12a	2,780,000	306,000,000	108,000,000	970,000,000	144,000,000
13b	5,100,000	70,000,000	138,000,000	85,000,000	91,000,000
14a	6,400,000	206,000,000	370,000,000	250,000,000	317,000,000
15a	18,700	3,300,000	15,900,000	21,700,000	7,600,000
15b	3,060,000	187,000,000	212,000,000	161,000,000	189,000,000

Table 1. Cell Density (Plate Count/mL) in Hydrocarbon Media (Cont)

	0 HOURS	72 HOURS			
SAMPLE DESIGNATION	CONTROL	CONTROL	MARINE DIESEL	CRUDE OIL	BUNKER C
1a	28,100	14,300	35,000,000	3,200,000	980,000
1b	14,700	11,800	610,000	750,000	25,500
1c	156,000	198,000	2,680,000	1,370,000	7,300,000
2a	76,000	730,000	2,970,000	1,730,000	2,120,000
2b	22,300	254,000	207,000	1,160,000	670,000
3a	35,000	610,000	1,640,000	970,000	1,270,000
3b	16,500	93,000	520,000	1,320,000	2,110,000
4a	46,000	7,600,000	5,200,000	2,160,000	2,180,000
4b	18,700	1,510,000	2,700,000	1,740,000	12,900,000
4c	540	1,830,000	12,300,000	2,780,000	18,300,000
4d	1,650	420,000	9,200,000	3,100,000	5,400,000
5a	12,100	3,500,000	10,500,000	7,700,000	8,800,000
6a	8,900	1,370,000	3,600,000	11,200,000	12,900,000
7a	46,000	7,600,000	52,000,000	31,300,000	14,700,000
7b	18,700	1,510,000	21,000,000	71,400,000	10,200,000
7c	540	1,830,000	12,900,000	29,800,000	16,200,000
7d	1,650	420,000	89,000,000	38,000,000	54,000,000
8a	2,690,000	76,000,000	138,000,000	104,000,000	92,000,000
9a	26,500	323,000	23,800,000	11,900,000	15,600,000
9b	5,400,000	27,100,000	188,000,000	312,000,000	142,000,000
10a	14,300	430,000	5,700,000	11,600,000	4,500,000
10b	3,190,000	89,000,000	125,000,000	73,000,000	107,000,000
11a	34,000	3,160,000	9,700,000	4,300,000	7,200,000
11b	2,180,000	105,000,000	10,900,000	3,210,000	6,700,000
12a	2,780,000	279,000,000	41,000,000	17,900,000	28,600,000
13b	5,100,000	32,000,000	63,000,000	54,000,000	51,000,000
14a	6,400,000	314,000,000	213,000,000	77,000,000	91,000,000
15a	18,700	1,970,000	7,900,000	4,300,000	1,390,000
15b	3,060,000	126,000,000	201,000,000	158,000,000	16,200,000

Table 1. Cell Density (Plate Count/ml) in Hydrocarbon Media (Cont)

	0 HOURS	1 WEEK			
ESIGNATION	CONTROL	CONTROL	MARINE DIESEL	CRUDE OIL	BUNKER C
1a	28,100	3,070	74,000	126,000	11,500
1b	14,700	17,500	29,400	2,340	20,700
1c	156,000	4,300	710,000	36,000	218,000
2a	76,000	12,300	129,000	174,000	137,000
2b	22,300	109,000	165,000	275,000	450,000
3a	35,000	28,500	450,000	23,700	92,000
3b	16,500	13,600	106,000	301,000	490,000
4a	46,000	980,000	740,000	231,000	189,000
4b	18,700	630,000	810,000	2,160,000	1,630,000
4c	540	79,000	320,000	4,700,000	2,180,000
4d	1,650	17,200	78,000	306,000	5,300,000
5a	12,100	740,000	2,710,000	1,310,000	1,860,000
6a	8,900	175,000	216,000	980,000	710,000
7a	46,000	26,300	790,000	1,130,000	1,060,000
7b	18,700	1,400	278,000	56,000	206,000
7c	540	3,600	182,000	72,000	41,000
7d	1,650	21,300	2,670,000	1,520,000	990,000
8a	2,690,000	2,140,000	7,900,000	2,350,000	3,800,000
9a	26,500	76,000	1,750,000	970,000	1,230,000
9b	5,400,000	3,110,000	2,120,000	780,000	1,650,000
10a	14,300	19,800	1,320,000	4,300,000	7,600,000
10b	3,190,000	2,810,000	6,400,000	3,500,000	5,400,000
11a	34,000	98,000	1,560,000	780,000	63,000
11b	2,180,000	7,400,000	3,020,000	10,900,000	12,200,000
12a	2,780,000	6,500,000	11,700,000	32,000,000	7,800,000
13b	5,100,000	2,780,000	19,200,000	7,900,000	4,800,000
14a	6,400,000	22,500,000	36,000,000	21,700,000	17,400,000
15a	18,700	620,000	12,700,000	3,900,000	2,160,000
15b	3,060,000	19,300,000	27,800,000	32,000,000	18,100,000

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