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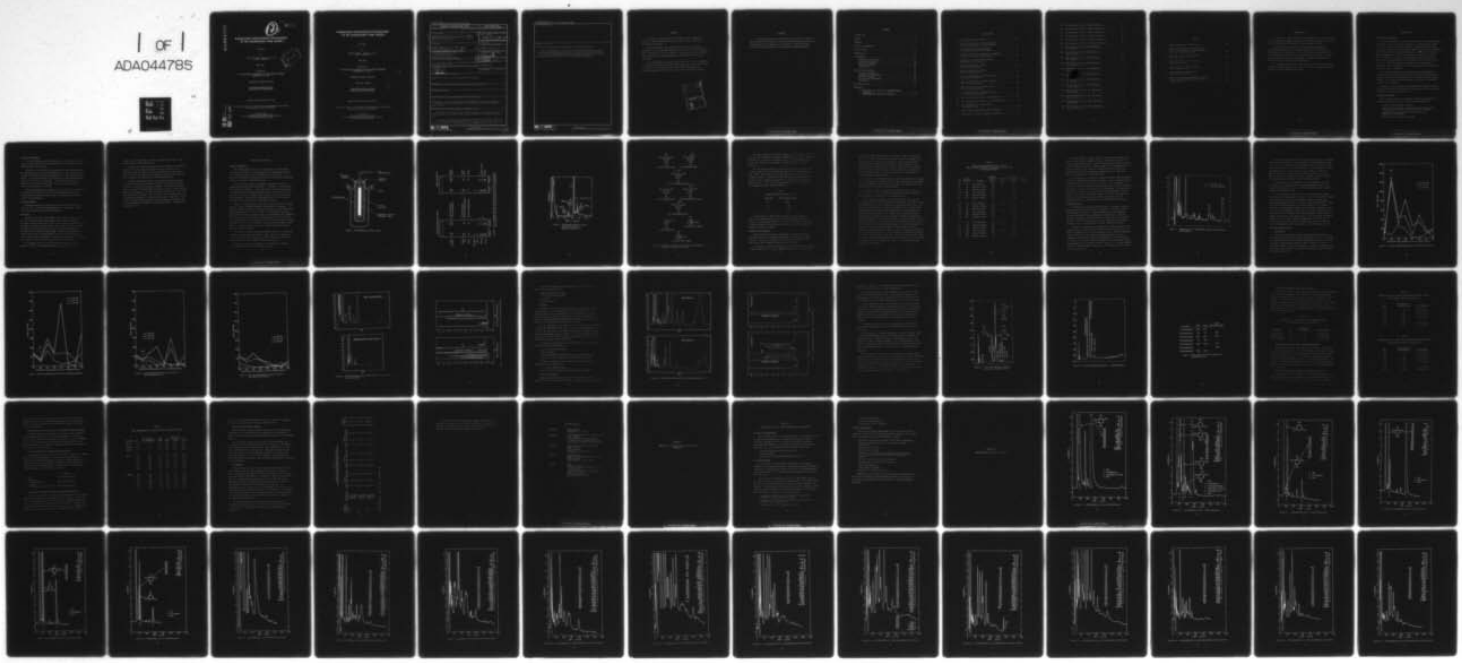

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MAMMALIAN TOXICOLOGICAL EVALUATIONS OF TNT WASTEWATER ("PINK WATER")

Final Report

By

GORDON W. NEWELL, JAMES V. DILLEY, and
RONALD J. SPANGGORD

March 1976

Supported by

U.S. ARMY MEDICAL RESEARCH AND DEVELOPMENT COMMAND
WASHINGTON, D.C. 20314

CONTRACT NO. DAMD 17-74-C-4115

STANFORD RESEARCH INSTITUTE
MENLO PARK, CALIFORNIA 94025

The findings in this report are not to be construed as an official Department of the Army position unless so designated by other authorized documents.

Dr. Jack C. Dacre
Environmental Protection Research Division
U.S. Army Medical Bioengineering Research and Development Laboratory
Fort Detrick, Frederick, Maryland 21701

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20. ABSTRACT (Continue on reverse side if necessary and identify by block number)

The purpose of this work was to determine the acute mammalian toxicity of TNT wastewaters (pink water) under various conditions of pH and irradiation time.

Pink water residues were prepared by irradiation of authentic wastewater to 0, 50, and 100% degradation levels of the major component, TNT, at pH values of 5.0, 7.0, and 9.4. The wastewaters were lyophilized to dry residues,

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The acute mammalian toxicity studies indicated that the unirradiated residue was the most toxic and was unaffected by pH. Toxicity decreased as a function of irradiation time, and the 100% degradation level residues showed one-fourth the toxicity of the nonirradiated residues.

SUMMARY

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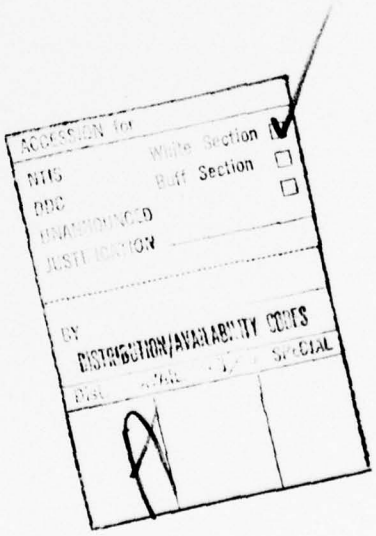

FOREWORD

In conducting the research described in this report, the investigators adhered to the "Guide for Laboratory Animal Facilities and Care," as promulgated by the Committee on the Guide for Laboratory Animal Resources, National Academy of Sciences-National Research Council.

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ILLUSTRATIONS

TABLES

INTRODUCTION

The objective of this research was to determine the acute toxicological effect on mammalian systems of actual and artificial wastewaters obtained from trinitrotoluene (TNT) production and handling facilities.

TNT discharged from munition facilities undergoes photolytic decomposition when exposed to sunlight, producing a complex mixture of nitrobodies and colored species. The resulting color is responsible for the term "pink water" that is used to describe this discharge. Because this discharge can enter the environment, its potential hazard to the health of living systems must be investigated.

The data obtained from this study will aid the Army in establishing effluent standards and will help define the level of wastewater treatment necessary at Army ammunition facilities.

EXPERIMENTAL

Preparation of Residues

The initial studies were performed with aqueous solutions (deionized water, TOC < 1 ppm) of pure TNT (synthetic, K & K Laboratories, purity >99% by glc, tlc, and elemental analysis) to generage baseline data on chemical profiles and toxicological responses of authentic wastewaters. These initial studies were performed at pH 7 with solutions that had been photodegraded to 50 and 100% composition levels of the initial TNT concentrations. Authentic wastewaters were handled similarly, except that photodegradations were performed at pH 5.0 and 9.4 as well as at 7.0. These pH values were selected by the Army and reflect variations in the treatment of the TNT yellow water at TNT production facilities prior to discharge.

For authentic wastewaters, both irradiated and nonirradiated solutions were partitioned with benzene to form two extracts (benzene and aqueous) that were lyophilized to solid residues for toxicological testing. If no extraction was performed, the residue is referred to herein as "neat residue."

Wastewater lyophilizations were performed at Hirschberg Freeze-Dry in South San Francisco, California, and at SRI with the aid of a Welsh Scientific Co. Model 1402 lyophilizer.

Systems Investigated

The following synthetic and authentic wastewater solutions were prepared and/or characterized for toxicological evaluation:

- Synthetic TNT wastewater solutions.
- Authentic TNT wastewater solutions obtained from the Joliet Army Ammunitions' (JAAP) load and pack (LAP) operation.
- Condensate wastewater from JAAP and Volunteer Army Ammunitions Plant (VAAP).
- Red Water and yellow water from JAAP.

Photolytic Conditions

All photoirradiations were performed in a water-jacketed 3.2-liter Pyrex reaction vessel with an immersion well and a 1200-watt Hanovia medium-pressure mercury lamp.

Solutions of pure chemicals and wastewaters were irradiated until the concentration of the major component (TNT for LAP, red water and 2,4-DNT for condensate water) had decreased to 50% and 100% degradation levels. For saturated TNT and 2,4-DNT solutions, 100% degradation was achieved after 24 hr of irradiation. A 50% degradation level was achieved for TNT between 30 min and 2 hr, and 2 hr was required to achieve the same level for 2,4-DNT.

When pH adjustments were necessary to bring solutions to neutrality, 0.1N and 6N HCl solutions or 0.1N and 5N NaOH solutions were used. An Orion Model 610 digital pH meter with a combination electrode was used for pH measurements.

Profiling Methods

Wastewater samples and extracts were profiled using thin-layer (tlc), gas (gc), and liquid chromatographic (lc) techniques. Details of these techniques are presented in Appendix A.

Toxicology

Adult male and female Swiss-Webster mice weighing between 15 and 20 g were obtained from Simonsen Laboratories, Gilroy, California. The animals were isolated for at least 1 week after their arrival in the laboratory to ensure that only healthy animals were used for the study. The animals were housed in plastic cages on hardwood bedding and provided with food and water ad libitum. Adult male and female New Zealand white rabbits weighing between 2 and 3 kg were obtained from L.I.T. Rabbitry, Aptos, California. The rabbits were housed in stainless-steel cages with wire bottoms and provided with food and water.

The compounds used for the oral toxicity testing in mice were prepared as corn oil suspensions/solutions just before use. The

material used for skin and eye irritation studies was used as a dry powder without further modification.

All mice were fasted overnight before oral dosing. After administration of the test substance by gastric tube, the animals were observed closely for signs of toxicity or mortality for the next 14 days. The oral LD_{50} and the 95% confidence limits for each compound was calculated by the method of Litchfield and Wilcoxon.

The Draize method was used for skin and eye irritation studies. Test material (0.1 g) was applied to the eyes of rabbits and washed out 30 sec, 5 min, or 24 hr after treatment. The eyes were examined daily for 7 days for the presence and degree of iritis, conjunctivitis, and/or corneal opacity. An area of skin was clipped free of hair over the dorsal area of rabbits. Half the skin area was abraded, and 0.5 g of material was applied to the skin surface under a rubber dam. After 24 hr, the rubber dam was removed, and the abraded and unabraded skin area was graded for the degree of erythema and edema. The grading was repeated at 72 hr.

RESULTS AND DISCUSSION

Synthetic Wastewater

TNT (>99%), obtained from K & K Laboratories, was dried under vacuum and was shown to be chromatographically homogeneous (tlc, gc) and to possess elemental data consistent with its structure. This material was dissolved in distilled water that contained no extractable organics that could be observed by gc/ms.

In the initial studies, we prepared a synthetic wastewater by irradiating pure TNT in distilled water. A schematic of the reaction vessel appears in Figure 1. This solution was extracted with benzene to produce benzene and aqueous extracts, which were profiled by tlc, glc, and lc. Later, we compared these profiles with those authentic LAP wastewaters. We used the lyophilized residues of the synthetic wastewater for initial toxicity range-finding studies.

Figure 2 is representative thin-layer chromatograms of the benzene extracts of both irradiated authentic and synthetic wastewaters. It should be pointed out that the authentic wastewater contains RDX, which did not migrate well in the tlc system. The components in Figure 2 were identified based on characteristic colors developed with a DMSO/EDA (10/2) spray and a fluorescamine/triethylamine (0.01% in acetone/10% in dichloromethane) spray as compared with colors of authentic standards provided to us by Dr. Lloyd Kaplan of the Naval Surface Weapons Center, White Oak, Maryland. We could not successfully profile the aqueous fraction using tlc methods.

Glc analysis showed only minor degradation products, as indicated in Figure 3, which were identified by gc/ms. Again, the components of the aqueous fraction could not be profiled by this technique, even when derivatization was attempted (trimethylsilylation).

Figure 4 presents the structures of the identified components. These components were later confirmed by lc data.

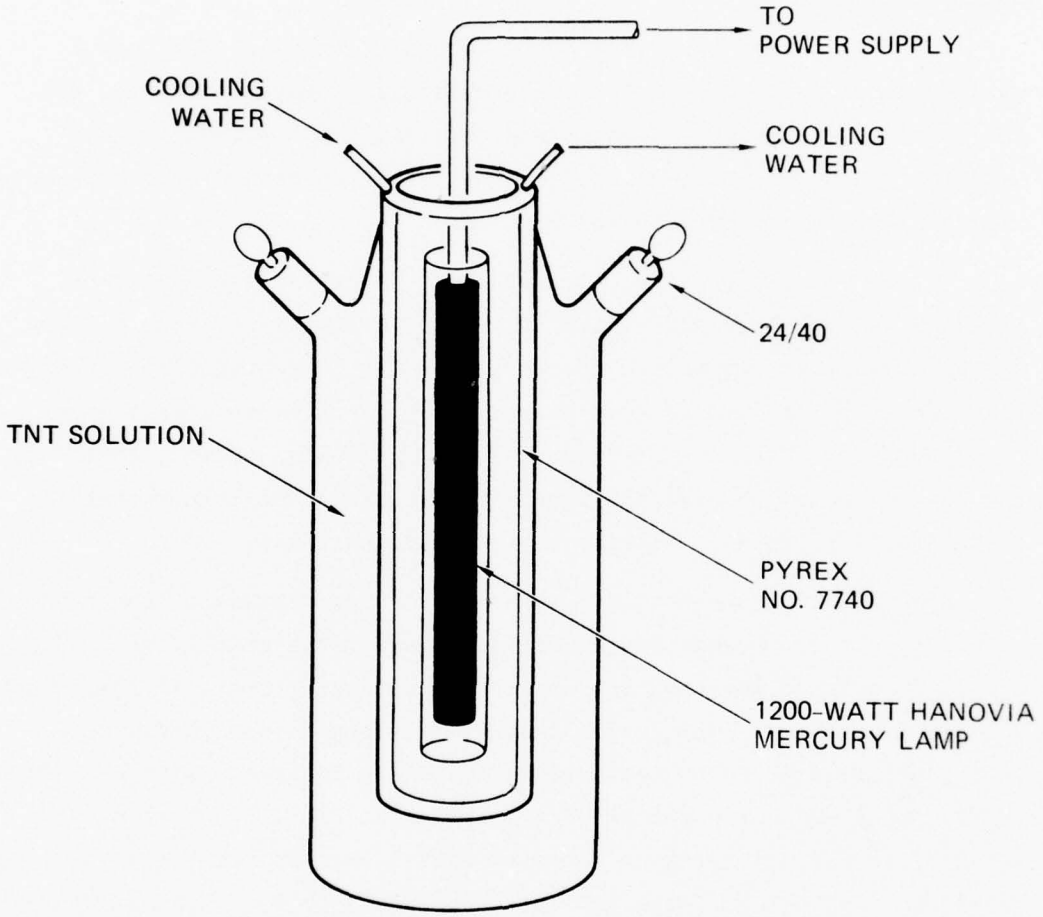


FIGURE 1 PHOTOCHEMICAL REACTION VESSEL

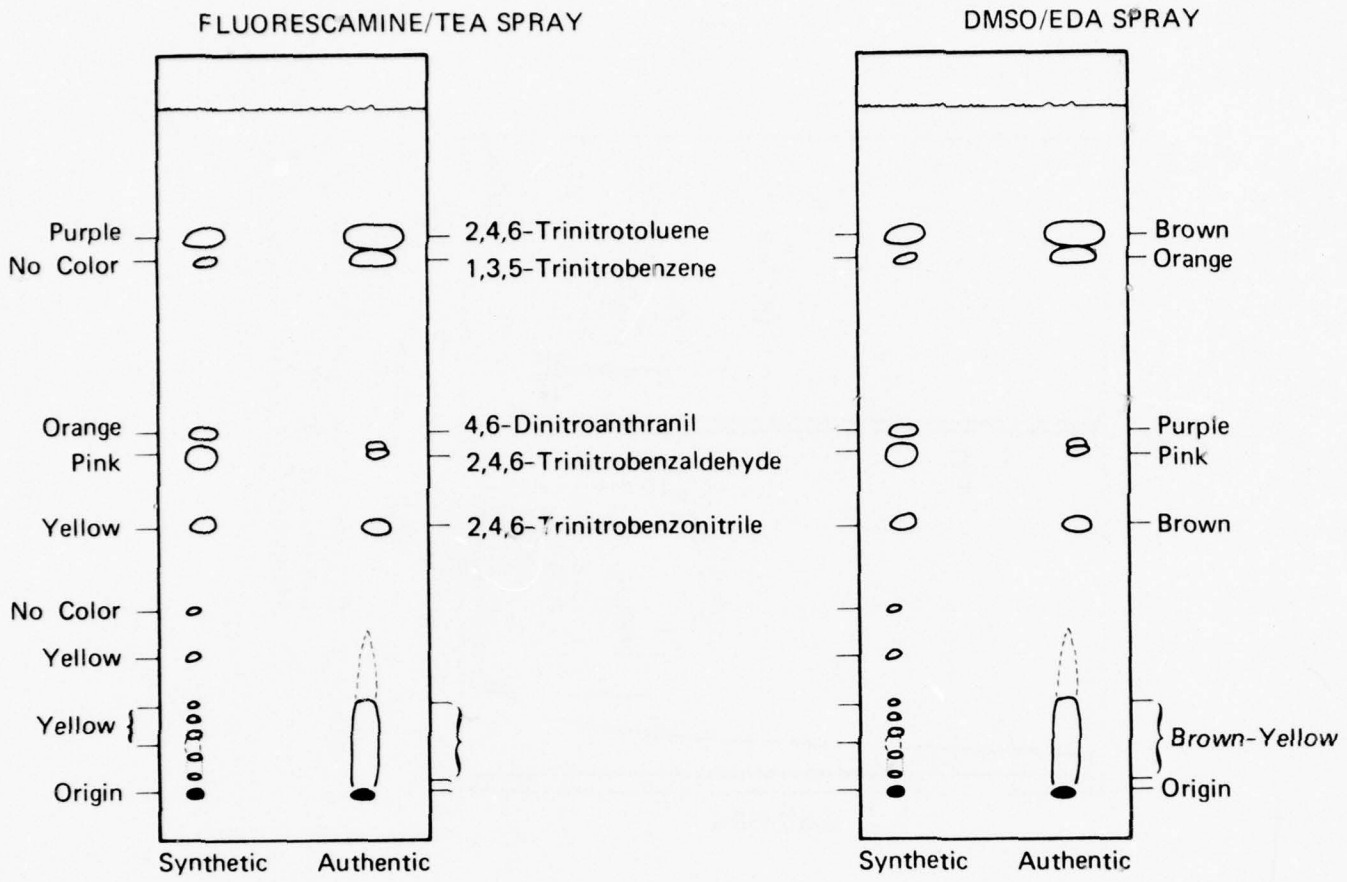


FIGURE 2 TLC OF BENZENE EXTRACT OF 50% IRRADIATED SYNTHETIC (TNT ALONE) AND AUTHENTIC (TNT, RDX, AND INORGANIC SALTS) LAP WASTEWATERS

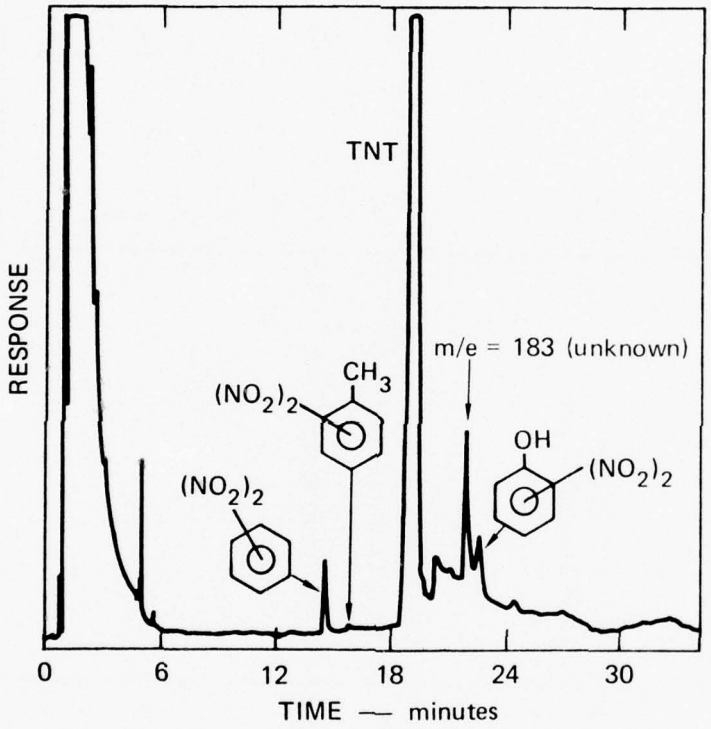


FIGURE 3 GAS CHROMATOGRAM OF BENZENE EXTRACT OF ARTIFICALLY PRODUCED PINK WATER

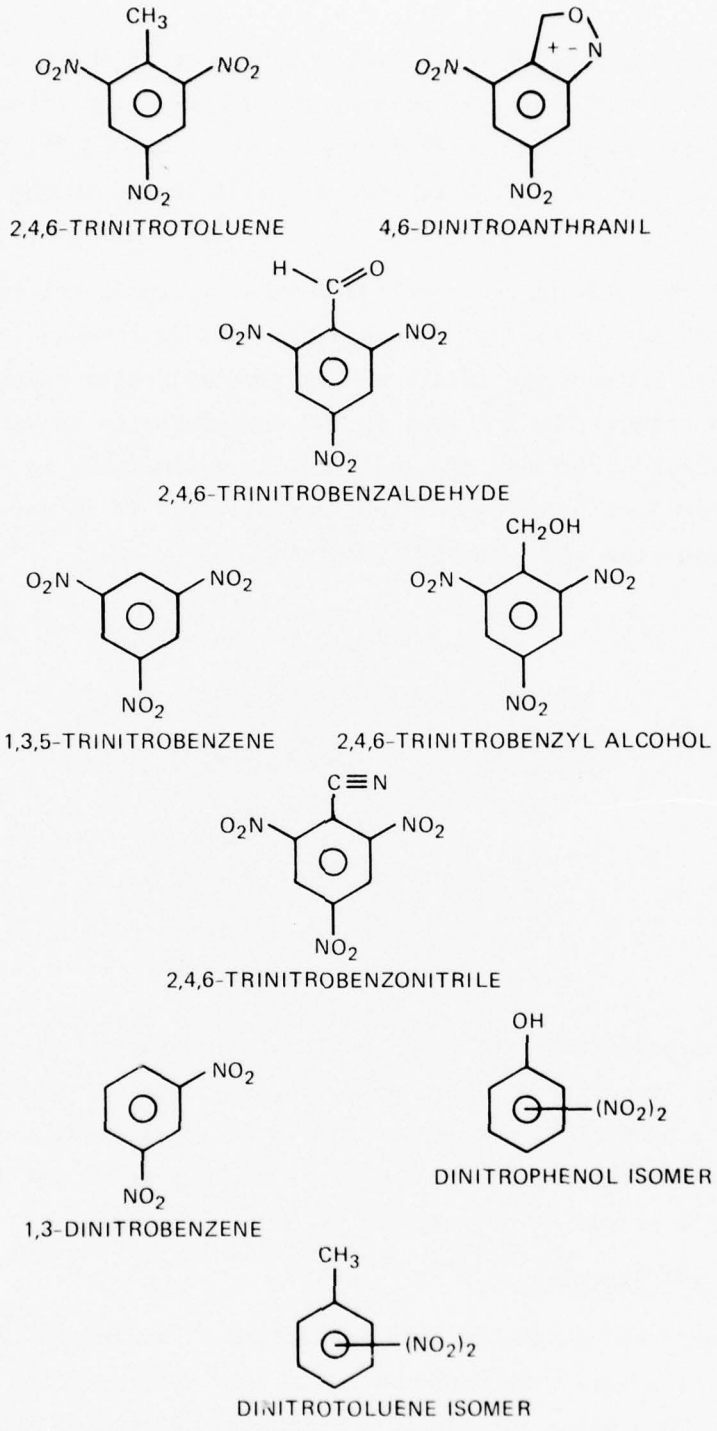


FIGURE 4 COMPOUNDS TENTATIVELY IDENTIFIED AS COMPONENTS OF ARTIFICIALLY PRODUCED PINK WATER

TNT always appeared as the major component of the benzene fraction. This fraction, consisting of primary photodegradation products, decreased by weight as a function of irradiation time. At 100% TNT degradation levels, the majority of the residue was distributed in the aqueous fraction.

The term "100% degradation" is defined as the level in which there is little change in the TNT concentration with increasing irradiation time. Table 1 shows the levels of TNT remaining with respect to irradiation time. The degrease in TNT concentration is rapid within the first hour of irradiation and there is essentially no significant change after 4 to 5 hr. Thus "100% degradation" is in actuality 98.5-99.5% degradation with some TNT remaining.

Table 1

PHOTODECOMPOSITION OF TNT AT pH 9.4

Time (hr)	TNT Remaining (ppm)
0	193
1	27.9
2	5.4
3	4.3
4	2.4
5	2.1

The photolysis of TNT resulted in significant pH changes as a function of irradiation time. At 0, 50, and 100% TNT degradation levels, the pH decreased from 6.6 to 4.8 at 50% degradation and to 3.8 at 100% degradation, indicating that at least some of the degradation products are acidic in nature.

Authentic TNT Wastewater

We performed toxicological evaluations of wastewater residues resulting from waters obtained from the JAAP LAP operation in Joliet, Illinois. This water contained 125.5 ppm of TNT and 30 ppm of RDX plus several minor components that were not identified.

The lyophilization of LAP wastewater was demonstrated to be feasible in our laboratory and in small commercial lyophilizer at E. Herschberg

Freeze-Dry in South San Francisco, California, using a 2.2-mm vacuum for 15 hr at 49°C. However, when a larger commercial lyophilizer was used to lyophilize 500 gal. of wastewater, a higher vacuum, higher temperature, and longer chamber time were used to produce a dried residue (0.19-mm vacuum, 60°C for 24 hr). These conditions resulted in loss of TNT from the residue. By back calculation, we added TNT to the residue to give the expected level (9%), as determined in laboratory-scale lyophilizations.

The bulk of the lyophilized residue (91%) was found to be inorganic salts. An emission spectrum analysis of the residue showed that sodium was the predominant cation (30%) and calcium (4%), magnesium (4%) and potassium (3%) were found in lesser amounts.

Qualitative tests for anions were positive for $CO_3^{=}$, $SO_4^{=}$, Cl^{-}, NO_3^{-}, and NO_2^{-}. Nitrite ion was determined quantitatively through a Griess reagent to be present in the residue at 0.5%.

The lyophilized residue was reconstituted with water, and TNT was added until the final concentration was 200 ppm. From this solution, we prepared three 2.3-liter solutions by adjusting the pH to 9.4, 7.0, and 5.0 with the addition of 6N HCl or 6N NaOH. These solutions were photolyzed until the initial TNT concentrations had decreased by 50% (10 min) and nearly 100% (5 hr). Samples of the aqueous solutions (200 ml) were lyophilized to solid residues (labeled "neat-aqueous"). One-liter samples of the aqueous solutions were extracted with 2 x 400 ml portions of benzene. The benzene layers were combined and lyophilized to solid residues (labeled "benzene extract"). We also lyophilized to dryness 200 ml of the aqueous residue resulting from the benzene extraction (labeled "aqueous residue"). These fractions were submitted for toxicological evaluation. Table 2 presents the weight distribution of solids obtained from each fraction. The table also shows the weights of solids obtained from pilot runs of selected fractions as an indicator of material loss. All samples for toxicological investigation were prepared in this manner.

Table 2

WEIGHT DISTRIBUTION OF SOLIDS OBTAINED
FROM THE LYOPHILIZATION OF PHOTOIRRADIATED SOLUTIONS
AT VARIOUS pH LEVELS

pH	Irradiation (%)	Fraction	Weight Recovered (mg)	Wt./10 ml x 20	Ratio of Wt. to Vol. (mg/ml)	Theo. Wt./Vol.
9.4	50	Neat (aqueous)	319	450	1.6	2.5
9.4	50	Aqueous residue	280		1.4	
9.4	50	Benzene extract	210		0.21	
9.4	100	Neat (aqueous)	450		2.3	2.5
9.4	100	Aqueous residue	417		2.1	
9.4	100	Benzene extract	81		0.08	
7.0	50	Neat (aqueous)	246	360	1.2	2.5
7.0	50	Aqueous residue	330		1.7	
7.0	50	Benzene extract	233		0.23	
7.0	100	Neat (aqueous)	343	352	1.7	2.5
7.0	100	Aqueous residue	340		1.7	
7.0	100	Benzene extract	62		0.06	
5.1	50	Neat (aqueous)	424	482	2.1	2.5
5.1	50	Aqueous residue	327		1.6	
5.1	50	Benzene extract	159		0.16	
5.1	100	Neat (aqueous)	394	480	2.0	2.5
5.1	100	Aqueous residue	291		1.5	
5.1	100	Benzene extract	53		0.05	

The data in Table 2 indicate that the longer the irradiation time, the more water-soluble components formed. This finding accounts for the low weight distribution of solids obtained from the benzene extracts of 100% irradiated solutions. High-pressure liquid chromatograms of the benzene fractions before and after lyophilization are qualitatively and quantitatively alike, indicating components are not being lost during lyophilization of benzene solutions.

To demonstrate that wastewater prepared by mercury lamp irradiation approximated wastewater generated by sunlight, we put a 100-ppm solution of TNT on an SRI roof and left it there for 2 clear, sunny days. At the end of this time, the water had turned pink, and the TNT concentration had dropped to 50 ppm. The same level of degradation of LAP water was achieved in less than 30 min in the laboratory irradiation. Figure 5 presents hplc profiles of benzene extracts of the two solutions; the profiles are qualitatively alike, indicating that processes for preparing samples are similar and approach environmental conditions.

Solution Profiles

Tlc methods gave positive identifications of components found in benzene extracts of LAP solutions (see Figure 2). However, we used hplc methods extensively to confirm identifications (by comparing retention times with those of authentic standards) and to provide solution fingerprints that were much more complex than those observed in the tlc system. Also, aqueous fraction components were chromatographed by hplc as ion-pairs with tetrabutyl ammonium hydroxide.

Figures B-1 through B-6 (see Appendix B) are hplc profiles of the benzene extracts of pH 5 and 9.4 solutions (pH 7 was similar to pH 5) through irradiations to the 100% completion level. These chromatograms (which represent only uv-absorbing compounds) indicate that the components of the benzene fraction photodegrade to a complex mixture of products in the early stages of irradiation and then undergo further photodegradation as a function of time to produce a less complex profile. When TNT is degraded almost completely, little material can be removed by benzene extraction.

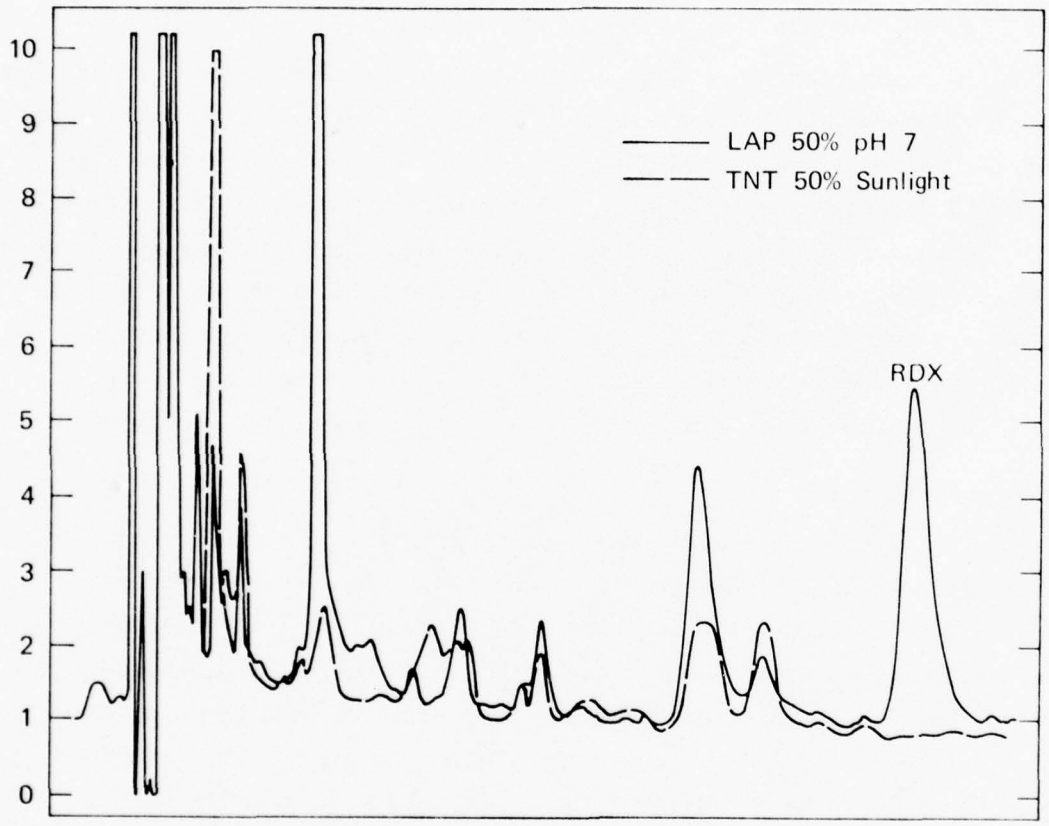


FIGURE 5 COMPARISON OF UV LAMP VERSUS SUNLIGHT DEGRADATION OF TNT SOLUTIONS

The components identified in the benzene fraction appear as primary photodegradation products of TNT and undergo further degradation with time. They may eventually be consumed when no TNT remains as a source for their production. 1,3,5-Trinitrobenzene is an exception to further photodegradation and may accumulate in the solution. The pH 9.4 extracts were devoid of many of the components found in pH 5.0 and pH 7.0 solutions, including the nitrile, aldehyde, and benzyl alcohol. The trinitrobenzene component was still present, however.

The aqueous fraction components could be fingerprinted by hplc when converted to ion-pairs with tetrabutylammonium hydroxide. The ion-pairs are extracted from water with dichloromethane and chromatographed under conditions (B).

The chromatographic profiles in each case were highly complex. Equal weights of each residue (at pH 5, 7, and 9.4, 50 and 100% irradiated neat and aqueous extracts) were suspended in water, treated with tetrabutylammonium hydroxide, and extracted with dichloromethane. Each extract was dried and reduced to a common volume (1 ml). From the resulting 12 chromatograms, Figures B-7 through B-18 (Appendix B), the intensities (area integral) of the eight most common components to all extracts were plotted against their chromatographic retention times. Figures 6 through 9 present these results. The pH 9.4 neat and aqueous residues showed the least complex chromatograms. Possibly, in the basic solution, anionic reactions compete with photolytic reactions for the degradation of TNT, accounting for the less complex spectra.

Miscellaneous Wastewaters

Yellow Water

Yellow water comes from the TNT continuous-processing plant to a neutralization plant, where the pH is adjusted from 1.1 to 7.6. During the neutralization, the TNT concentration changes from 103.5 to 85 ppm. The liquid chromatographic profiles, shown in Figure 10, reflect a significant change in chemical components as a result of neutralization. The change also is apparent in the gas chromatograms of both solutions, presented in Figure 11.

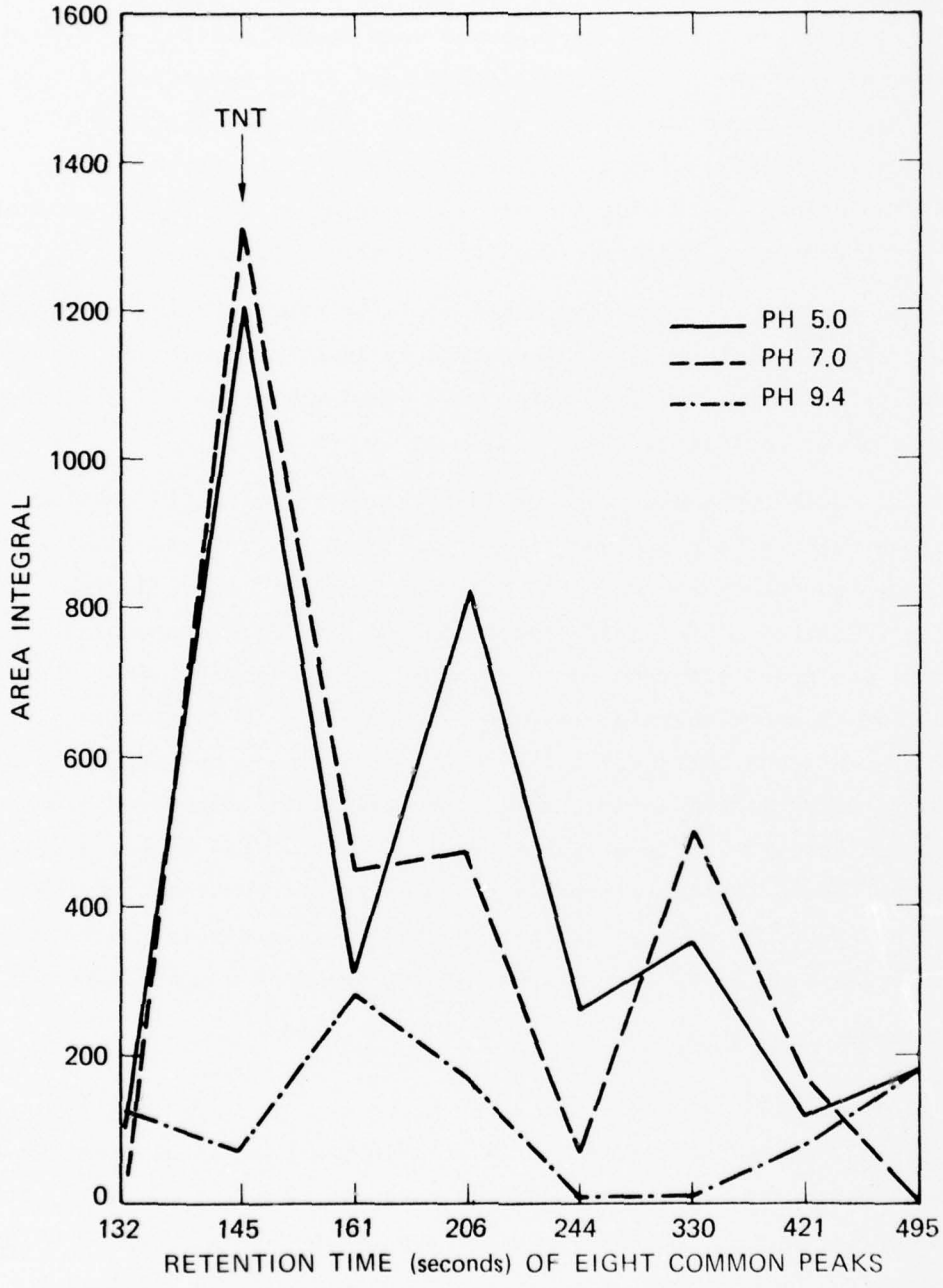


FIGURE 6 RELATIVE COMPOSITION OF NEAT, 50% IRRADIATED RESIDUES

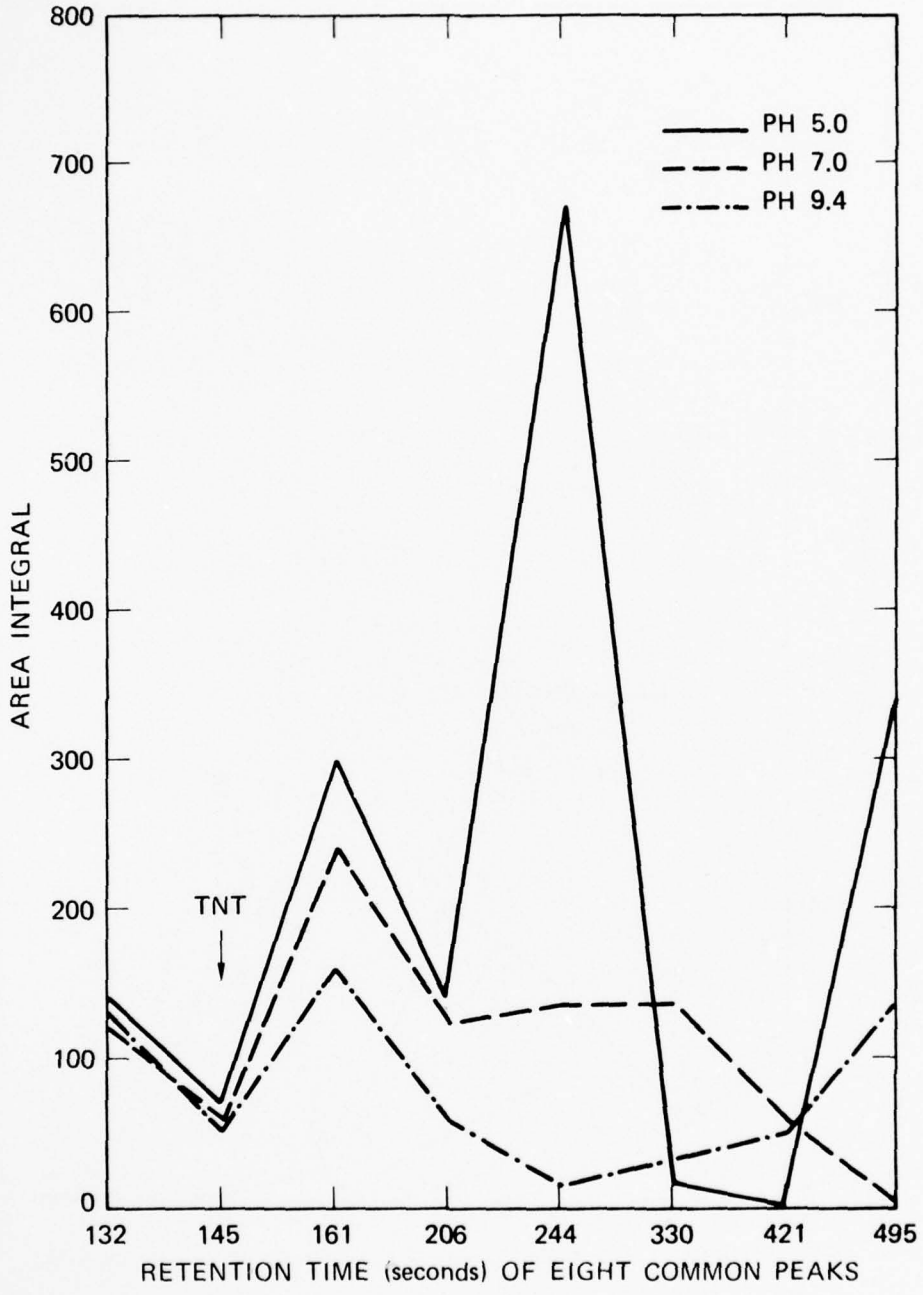


FIGURE 7 RELATIVE COMPOSITION OF NEAT, 100% IRRADIATED RESIDUES

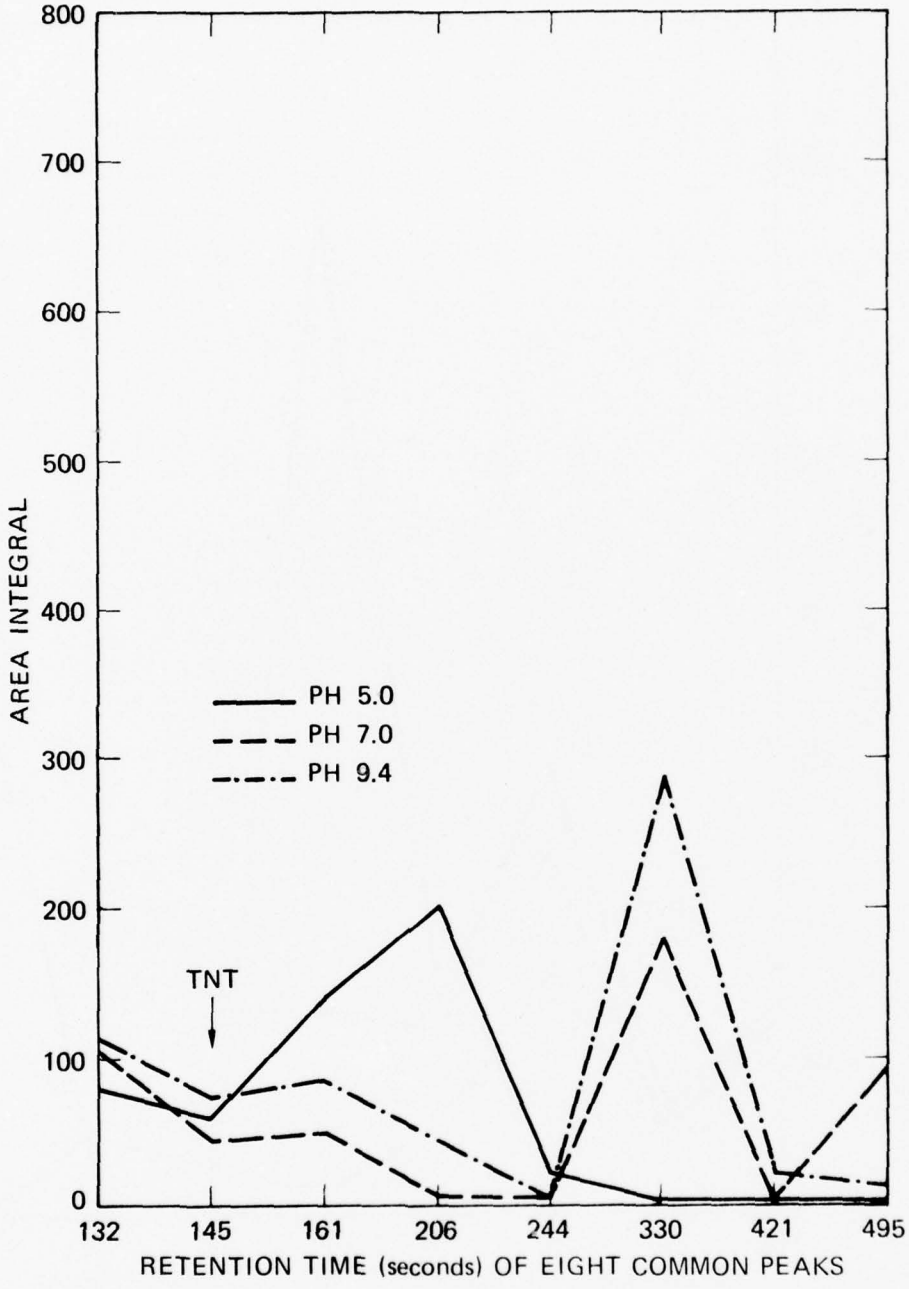


FIGURE 8 RELATIVE COMPOSITION OF AQUEOUS EXTRACT, 50% IRRADIATED RESIDUES

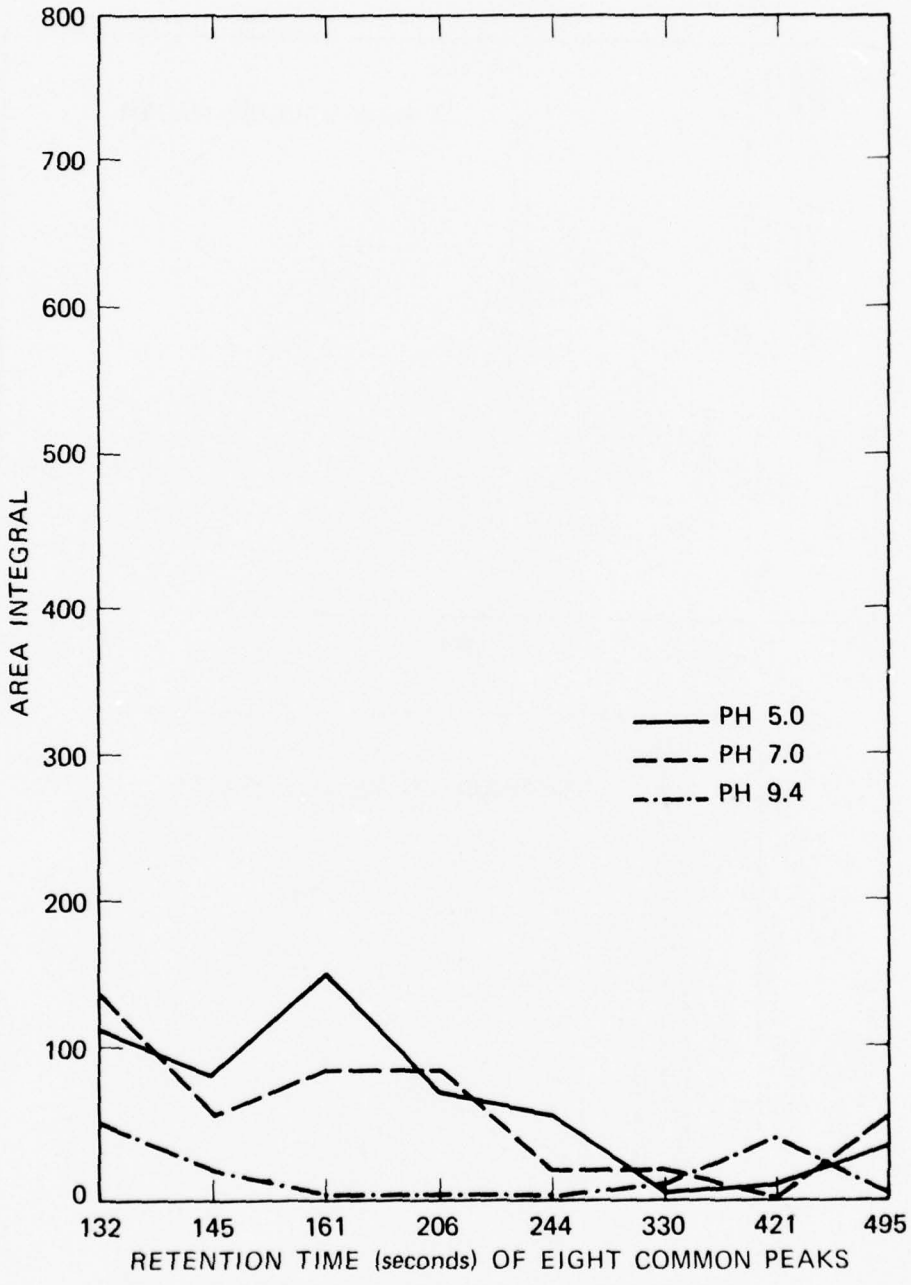
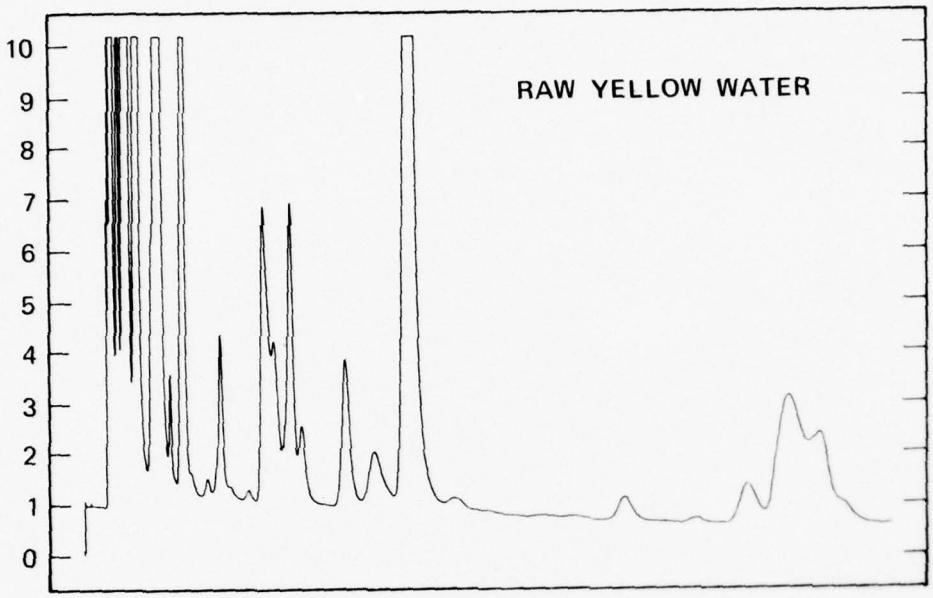
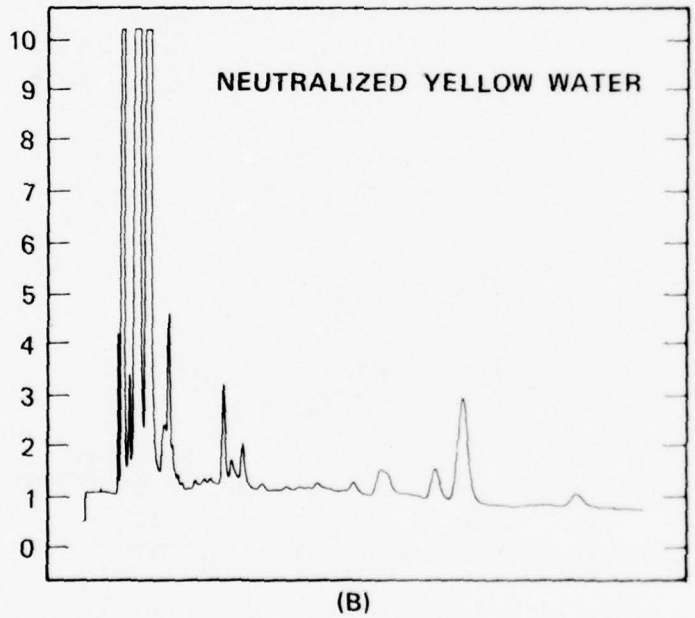


FIGURE 9 RELATIVE COMPOSITION OF AQUEOUS EXTRACT, 100% IRRADIATED RESIDUES



(A)



(B)

FIGURE 10 HPLC PROFILES OF YELLOW WATER PRIOR TO (A) AND AFTER NEUTRALIZATION (B)

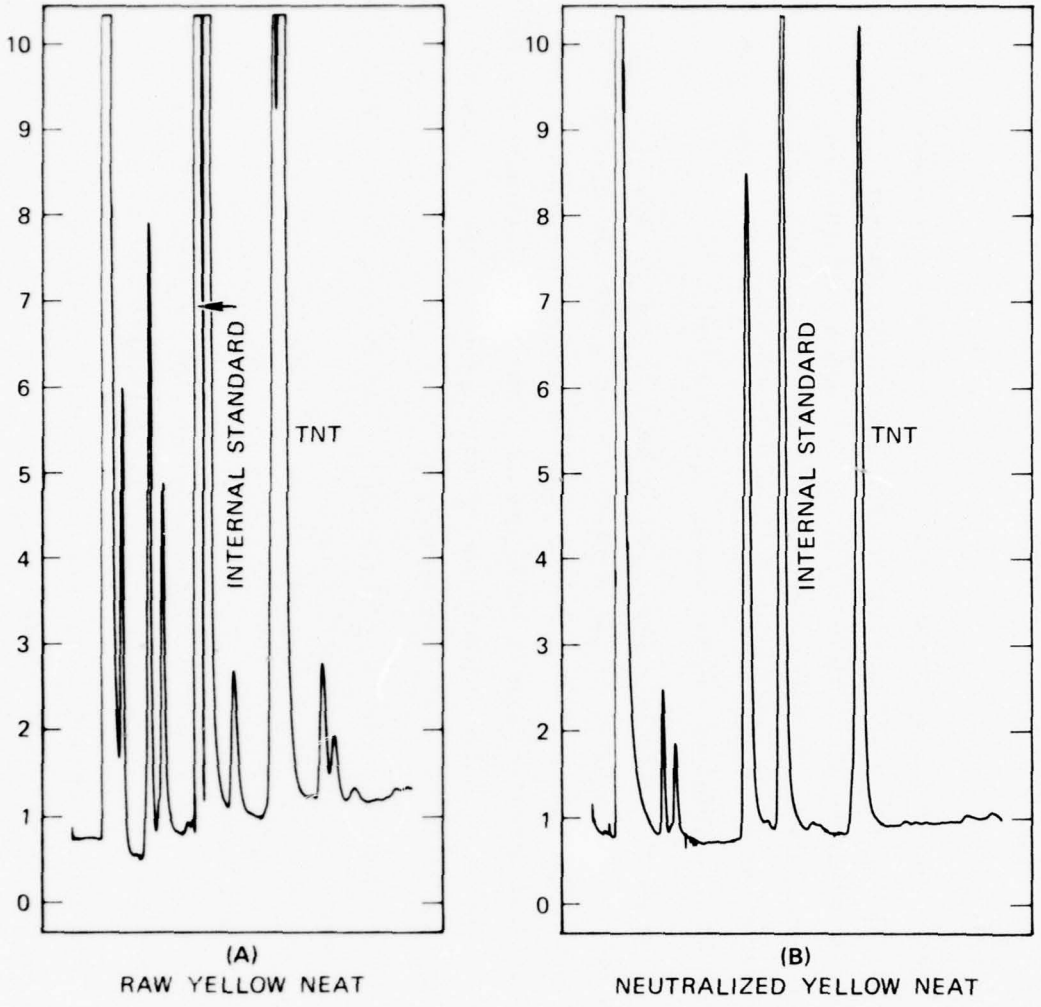


FIGURE 11 GAS CHROMATOGRAMS OF YELLOW WATER PRIOR TO (A) AND AFTER NEUTRALIZATION (B)

The following components were identified by gc/ms in raw yellow water (before neutralization):

- Ortho- and para-nitrotoluene
- 2,4- and 2,6-dinitrotoluene
- 2,4,6-TNT
- Trinitrobenzene.

Red Water

Red water results from the Sellite process, which removes isomeric trinitrotoluenes in the production of 2,4,6-trinitrotoluene. This water is piped to the evaporation plant for concentration. Two red water samples were profiled. One (Red Water I) was obtained before the water was mixed with continuous process water, and the other (Red Water II) was obtained before evaporation occurred.

The pH of Red Water I was found to be 7.7, and the TNT concentration was 104 ppm. Red Water II had a pH of 9.4 and a TNT concentration of 2.4 ppm. The hplc profiles of each solution appear in Figure 12, and the gas chromatograms appear in Figure 13. We cannot determine whether the change in profiles reflects the pH adjustment or results from the mixing and/or dilution with other waters.

The components identified by gc/ms in Red Water I (before mixing with continuous process water) were:

- 2,4- and 2,6-Dinitrotoluene
- Trinitrobenzene
- Mol wt 198 (possibly dinitrobenzylalcohol or dinitrocresol)
- Mol wt 197 (five isomers--possibly aminodinitrotoluenes)

Red Water II components (after mixing with continuous process wastewater) were identified as:

- 2,4- and 2,6-Dinitrotoluene
- Mol wt 197 (two isomers--possibly aminodinitrotoluenes)

Condensate Wastewater

Condensate wastewater results from the evaporation of Sellite process waters and yellow water from TNT continuous-process manufacturing.

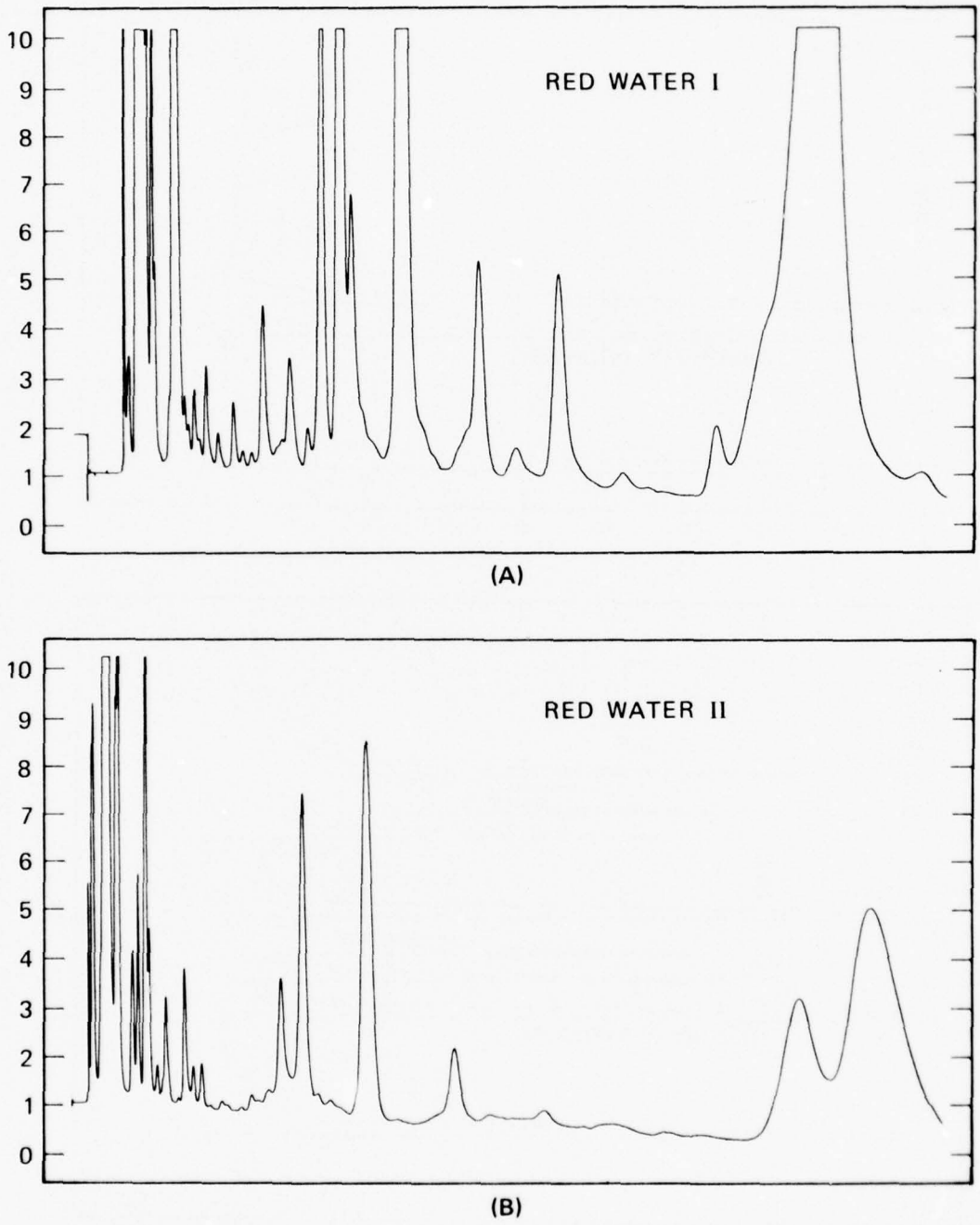


FIGURE 12 HPLC PROFILES OF RED WATER I (A) AND RED WATER II (B)

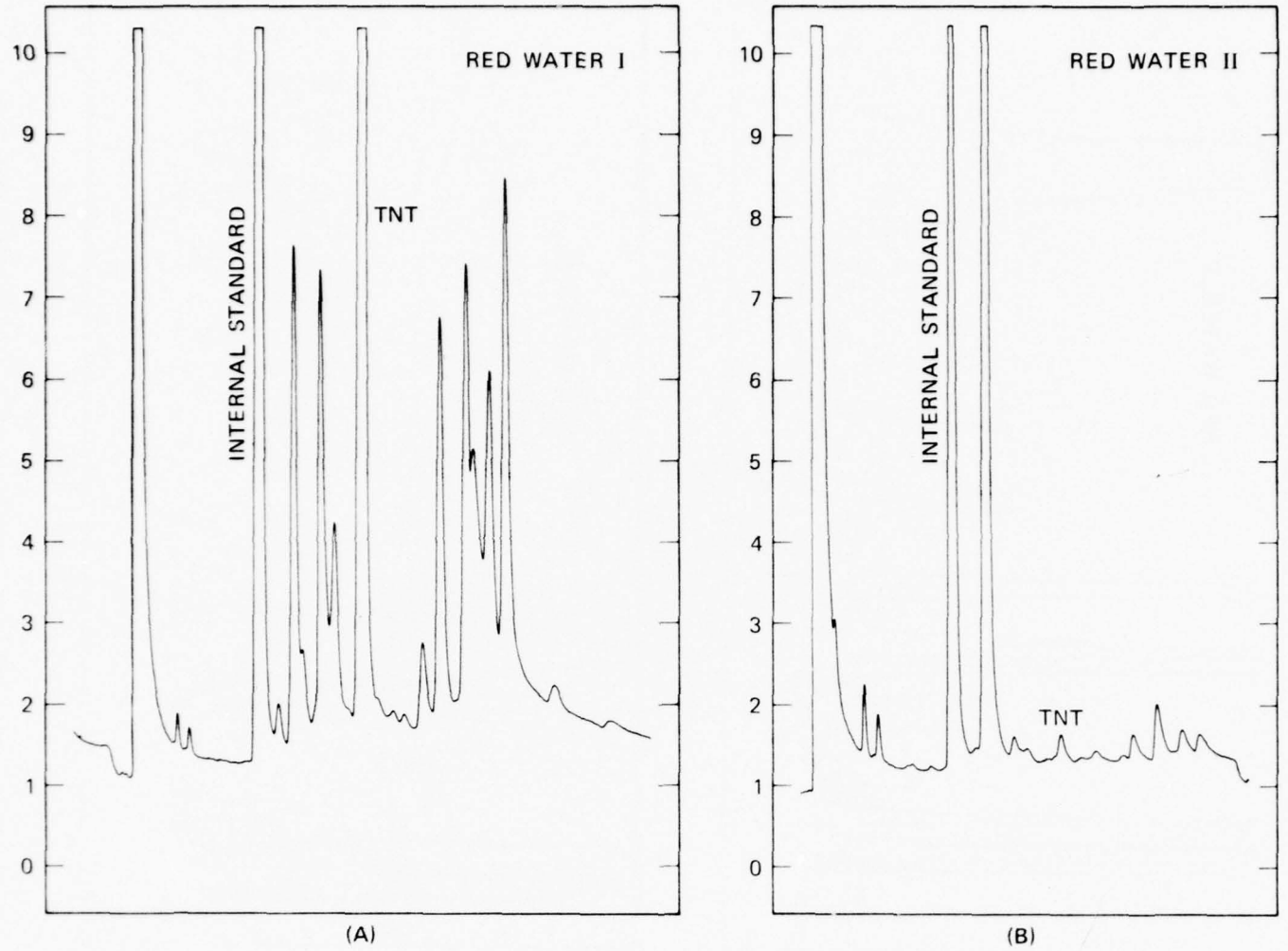


FIGURE 13 GAS CHROMATOGRAMS OF RED WATER I (A) AND RED WATER II (B)

The organic components in condensate water are steam distillates that condense when the water is cooled for discharge.

We obtained one sample of JAAP condensate water in October 1974 and one from VAAP in November 1974. Both samples had identical gas chromatographic profiles, shown in Figure 14, and the components were identified by gc/ms as isomeric dinitrotoluenes and nitrated benzenes.

A later sampling of condensate wastewater from JAAP in September 1975 showed a different profile, presented in Figure 15. This sample contained 2,6- and 3,4-dinitrotoluenes, which were absent in the first sampling, and was devoid of m-dinitrobenzene and 3,5-dinitrotoluene. However, in both cases, 2,4-dinitrotoluene was the major component. Figure 16 shows the concentration of the components found in each sampling.

The difference in profiles of JAAP wastewater may reflect the manufacturing change from batch to the continuous process for TNT production.

On irradiation of condensate wastewater, the only components found to undergo photodegradations were the dinitrotoluenes containing an ortho-nitro group. m-Dinitrobenzene, trinitrobenzene, and 3,4- and 3,5-dinitrotoluenes remained unchanged. Thus, the generation of new photolytic degradation products from condensate wastewater will result from the photodecomposition of 2,4-, 2,6-, and 2,5-dinitrotoluenes.

Toxicology

Mice given a toxic oral dose of the pink water residues generally exhibited lassitude, cyanosis, occasional muscular twitching, convulsions, and red urine. Mortality usually occurred within the first 24 hr or not at all. Animals that survived the treatment appeared to have fully recovered within 2 to 3 days after treatment. Surviving animals autopsied at the end of the 14-day observation period did not have any gross pathological lesions attributable to the treatment.

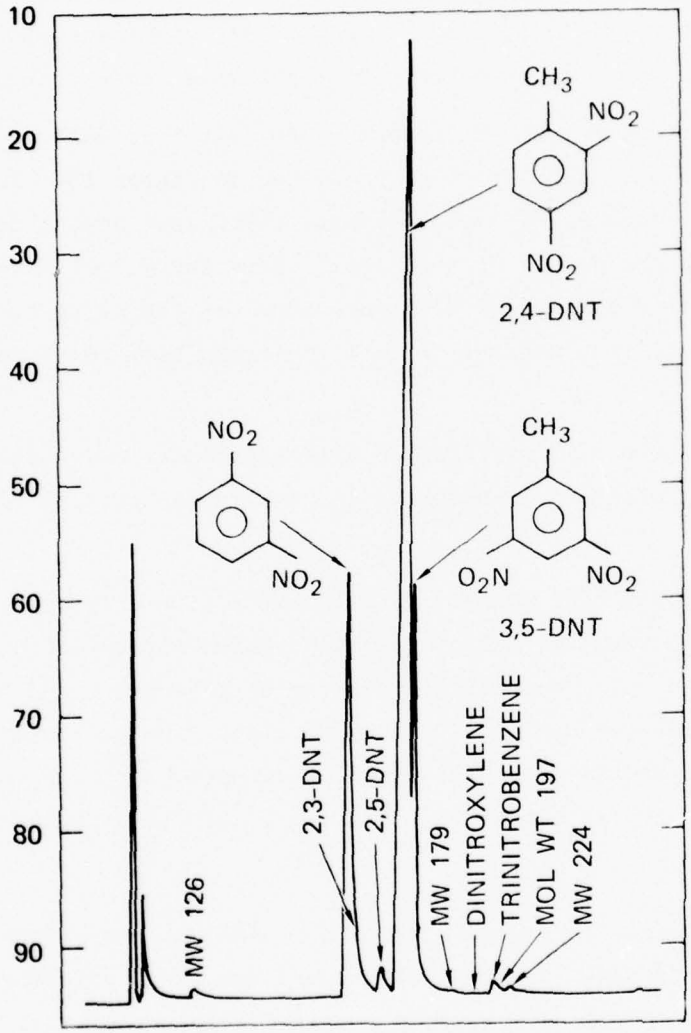


FIGURE 14 GAS CHROMATOGRAM OF BENZENE EXTRACT OF CONDENSATE WATER

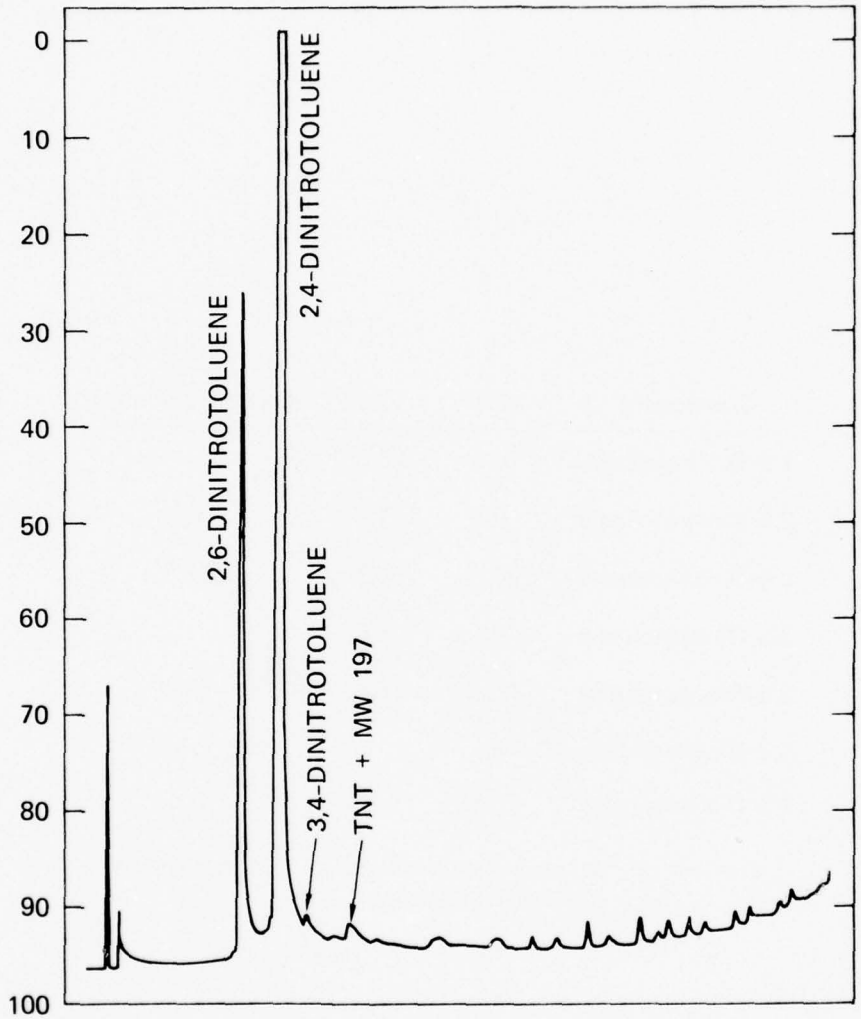


FIGURE 15 JAAP CONDENSATE WASTE WATER — SECOND SAMPLING

Component	JAAP	VAAP	JAAP (Second Sampling)
1,3-Dinitrobenzene	18.8	23.5	
2,5-Dinitrotoluene	0.8	1.3	Trace
2,4-Dinitrotoluene	26.3	50.5	62.0
3,5-Dinitrotoluene	4.1	8.6	
2,6-Dinitrotoluene	Trace	Trace	14.1
3,4-Dinitrotoluene			Trace
2,3-Dinitrotoluene	Trace	Trace	

FIGURE 16 NITROAROMATICS FOUND IN CONDENSATE WASTEWATERS (ppm)

Acute Oral Toxicity of Pink Water Fractions

Initial acute oral toxicity studies were determined with nonirradiated Joliet LAP water residues. These residues were further divided into aqueous and benzene fractions. Table 3 presents the results of these studies. The LD_{50} of the neat residue in male and female mice was 1.3 g/kg. However, the LD_{50} for the benzene fraction was only 0.5 g/kg, whereas that for the aqueous fraction was 2.5 g/kg. Therefore, the toxic components of the neat residue apparently are extracted principally into the benzene fraction.

Table 3

ACUTE ORAL LD_{50} OF JOLIET LAP WATER FRACTIONS IN MICE
95% Confidence Limits in Parentheses

Compound	pH	Percentage of uv Irradiation	LD50 (g/kg)
Neat residue	7.0	0	1.3 (1.1-1.5)
Benzene residue	7.0	0	0.5 (0.3-0.8)
Aqueous residue	7.0	0	2.5 (1.6-3.8)

Acute Oral Toxicity of Neat uv-Irradiated Residues

We determined the acute oral LD_{50} of neat residues of pink water that had been irradiated to 50 or 100% disappearance of the TNT content at pH 5.0, 7.0, or 9.4. Table 4 presents the results of these studies. The 50% irradiation at pH 5.0 or 9.4 did not substantially alter the toxicity of the neat residue from that of the unirradiated group. The toxicity of the residue irradiated 50% at pH 7.0 was slightly reduced, whereas the toxicity was markedly reduced in the 100% irradiated group at all three pHs.

Acute Oral Toxicity of Aqueous uv-Irradiated Fractions

The acute oral LD_{50} was determined on the aqueous fraction of pink water that had been irradiated to 50 and 100% disappearance of the TNT content at pH 5.0, 7.0, and 9.4. Table 5 presents the results.

Table 4

ACUTE ORAL LD_{50} OF JOLIET LAP WATER NEAT RESIDUE IN MICE

95% Confidence Limits in Parentheses

pH	Percentage of uv Irradiation	LD50 (g/kg)
7.0	0	1.3 (1.1-1.5)
5.0	50	1.5 (1.2-1.7)
5.0	100	4.9 (4.5-5.3)
7.0	50	2.6 (2.3-2.8)
7.0	100	~ 5
9.4	50	1.6 (1.3-2.0)
9.4	100	4.2 (4.0-4.9)

Table 5

ACUTE ORAL LD_{50} OF JOLIET LAP WATER AQUEOUS RESIDUE IN MICE

95% Confidence Limits in Parentheses

pH	Percentage of uv Irradiation	LD50 (g/kg)
7.0	0	2.5 (1.6-3.8)
5.0	50	4.2 (3.6-5.0)
5.0	100	4.7 (4.3-5.0)
7.0	50	4.7 (4.4-5.0)
7.0	100	> 5
9.4	50	3.9 (3.5-4.4)
9.4	100	4.4 (4.0-4.9)

The 100% irradiated fractions were less toxic to mice than the non-irradiated fraction. The 50% irradiated groups were not significantly differnt from the nonirradiated controls mainly because of the wide confidence intervals.

Confirmatory Acute Oral Toxicity of Irradiated LAP Water Residues

We administered the neat and aqueous residues from uv-irradiated and nonirradiated LAP water to male and female mice to confirm the acute toxicity studies. Table 6 shows the results of these confirmatory tests. These results confirm the previously determined LD_{50} values and also demonstrate that male and female mice displayed no difference in sensitivity to the compounds.

Acute Oral Toxicity of Condensate Water Residues

We determined the acute oral toxicity to mice of synthetic condensate water and of TNT simultaneously. The results, tabulated below, indicate that condensate water neat residues are approximately three times more toxic to mice than TNT. We confirmed these findings by repeating the LD_{50} determination of condensate water residue later. The miscellaneous oral LD_{50}s were as follows:

	LD50
TNT	830 (761-905) mg/kg)
Synthetic condensate water	250 (180-340) mg/kg
Synthetic condensate water (repeated)	280 (230-340) mg/kg

Comparison of Synthetic Pink Water and TNT Oral Toxicity

The acute oral toxicity of aqueous and benzene extracts of synthetic pink water to male mice was compared with the acute oral toxicity of TNT. The highest dose of the aqueous extract of synthetic pink water was 2.70 g/kg; this dose killed only one of five mice. However, the benzene extract killed three of five mice at a dose of 1.00 g/kg, but no deaths occurred at 0.67 g/kg. This compares favorably with the

Table 6

ORAL CONFIRMATORY LD_{50} TESTS ON JOLIET LAP WATER IN MICE

Residue	pH	Percentage of uv Irradiation	Dose g/kg	Mortality		
				Male	Female	Total
Neat	7.0	0	1.3	1/5	0/5	1/10
Benzene	7.0	0	0.5	2/5	1/5	3/10
Aqueous	7.0	0	2.5	3/5	5/5	8/10
Neat	5.0	50	1.5	1/5	0/5	1/10
	5.0	100	4.9	3/5	5/5	8/10
	7.0	50	2.6	4/5	4/5	8/10
	7.0	100	5.0	4/5	5/5	9/10
	9.4	50	1.6	2/5	2/5	4/10
	9.4	100	4.2	4/5	2/5	6/10
Aqueous	4.0	50	4.2	2/5	3/5	5/10
	4.0	100	4.7	3/5	2/5	5/10
	7.0	50	4.7	4/5	4/5	8/10
	7.0	100	5.0	3/5	5/5	8/10
	9.4	50	3.9	5/5	4/5	9/10
	9.4	100	4.4	2/5	3/5	5/10

LD_{50} of the JAAP LAP water residues in Table 2. Further, it suggests that the toxic component in LAP water is probably TNT.

Skin and Eye Irritation Studies

Skin irritation studies were done using the 0 and 100% irradiated neat residue. No skin irritation was observed, but a considerable amount of red skin staining occurred, especially with the 100% irradiated residue.

Eye irritation studies were done using the nonirradiated, neat LAP residue. Table 7 presents the results of these tests. Essentially no eye irritation was seen when the eyes were washed 30 sec or 5 min after instillation of the test material. When the material was left in the eye for 24 hr, we observed some irritation, including iritis and corneal opacity, for up to 3 days. However, the irritation was nearly absent by the fourth day, and the rabbits had completely recovered by the seventh day.

Conclusions

The acute oral toxicity of the neat and aqueous residues of LAP water suggests that the toxic component found in this wastewater is mostly extractable with benzene. However, uv irradiation of the wastewater also reduces the toxicity of the residue. Because approximately 90% of the organic material found in LAP water is TNT, and because irradiation as well as benzene extraction removes the TNT content of the wastewater, TNT may be the principal toxic component. We determined the LD_{50} of TNT in mice to be 803 mg/kg, whereas that of the neat nonirradiated residue of Lap water was 1300 mg/kg. If the weight of the salts and other solids are subtracted from the 1300 mg/kg figure, the toxicity of the LAP water neat residue and the TNT are probably not significantly different.

The acute toxicity of the condensate water is probably more predictable since the composition is known. The toxicity most likely is the result of the additive toxicities of the known individual components.

Table 7

RESULTS OF EYE IRRITATION STUDIES IN RABBITS
TREATED WITH NONIRRADIATED RESIDUE FROM JOLIET LAP WATER

Time Eyes Washed After Treatment	Time After Administration	Animal 1			Animal 2			Animal 3			Totals* for Animal:		
		Cornea	Iris	Conjunctiva	Cornea	Iris	Conjunctiva	Cornea	Iris	Conjunctiva	1	2	3
30 sec	24 hr	0	0	4	0	0	4	0	0	6	4	4	6
	48 hr	0	0	6	0	0	4	0	0	6	6	4	6
	72 hr	0	0	4	0	0	2	0	0	4	4	2	4
	4 days	0	0	2	0	0	0	0	0	4	2	0	4
	7 days	0	0	0	0	0	0	0	0	0	0	0	0
5 min	24 hr	0	0	6	0	0	14	0	0	8	6	14	8
	48 hr	0	0	4	0	0	8	0	0	2	4	8	2
	72 hr	0	0	2	0	0	6	0	0	4	2	6	4
	4 days	0	0	2	0	0	4	0	0	4	2	4	4
	7 days	0	0	0	0	0	4	0	0	0	0	4	0
24 hr	24 hr	15	5	12	15	5	16	10	5	14	32	36	29
	48 hr	10	0	6	15	5	12	5	0	10	16	32	15
	72 hr	0	0	4	5	5	10	0	0	6	4	20	6
	4 days	0	0	6	0	5	6	0	0	4	6	11	4
	7 days	0	0	2	0	0	4	0	0	2	2	4	2

* Total possible score: cornea, 80; iris, 10; conjunctiva, 20.

The skin and eye irritation studies suggested that none of the material is an irritant. The slight conjunctivitis seen with the material not washed from the eye for 24 hr is probably the result of physical irritation rather than chemical irritation.

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Appendix A

THIN-LAYER, GAS, AND LIQUID CHROMATOGRAPHIC TECHNIQUES

Appendix A

THIN-LAYER, GAS, AND LIQUID CHROMATOGRAPHIC TECHNIQUES

Thin-Layer Chromatography

Tlc was useful only for the benzene extracts of irradiated solutions. We identified nitroaromatics by comparing R_f values with those of reference compounds and color produced from a dimethyl sulfoxide/ethylenediamine (DMSO/EDA) spray forming characteristic Meisenheimer complexes. Tlc was performed under the following conditions:

- Support--Eastman silica gel sheets, F-254.
- Solvent--Benzene.
- Detection--Uv (quenching) DMSO/EDA (10/1) spray.

Gas Chromatography

Gc was useful for monitoring concentrations of the primary components--TNT and dinitrotoluenes. Only minor photolytic degradation products were observable and characterized by gas chromatography/mass spectrometry (gc/ms). The following procedures were used to determine TNT and 2,4-DNT.

Before and after irradiation, 20 ml of the wastewater solution was extracted with an equal volume of diethyl ether. The ether solution was dried over anhydrous magnesium sulfate and rotary evaporated to dryness. The residue was dissolved in acetone, and an internal standard was added (m-dinitrobenzene for TNT and for 2,4-DNT). The solutions were analyzed by gc under the following conditions:

- Instrument--Varian 2700 gas chromatograph equipped with an HP Model 3380A integrator recorder.
- Column--6 ft x 2 mm glass column packed with 10% DC-200 on 80/100 mesh Gas-Chrom Q.
- Temperature--150-250° programmed at 4°/min.

- Flow rate--20 ml/min N_2.
- Detector--Flame ionization.
- Sensitivity--1 x 10^{-10} amps/mv.

Liquid Chromatography

High-pressure liquid chromatography (hplc) was the most useful analytical technique for profiling wastewater solutions. The following hplc conditions were used to "fingerprint" solutions:

- Instrument--Spectra-Physics Model 3500B liquid chromatograph.
- Column and packing--1/4 in x 30 cm μ Porasil (Waters Associates).
- Detector--uv at 254 nm.
- Sensitivity--0.16 aufs.
- Solvent--(A) 35% hexane and 65% dichloromethane isocratic; (B) 75% hexane and 25% $CH_2Cl_2/CH_3OH/Et_2NH$ (100/200/0.5) isocratic.
- Flow rate--(A) 1.2 ml/min; (B) 2.8 ml/min.
- Pressure drop--(A) 240 psi; (B) 2200 psi.
- Temperature--Ambient.
- Chart speed--0.5 cm/min.
- Sample size--(A) 20 μl; (B) 10 μl.

Conditions (A) were used for benzene extracts, and conditions (B) were used for ion-pairs of aqueous-phase components extracted from water with dichloromethane after the addition of tetrabutylammonium hydroxide.

Appendix B

CHROMATOGRAPHIC PROFILES OF EXTRACTS

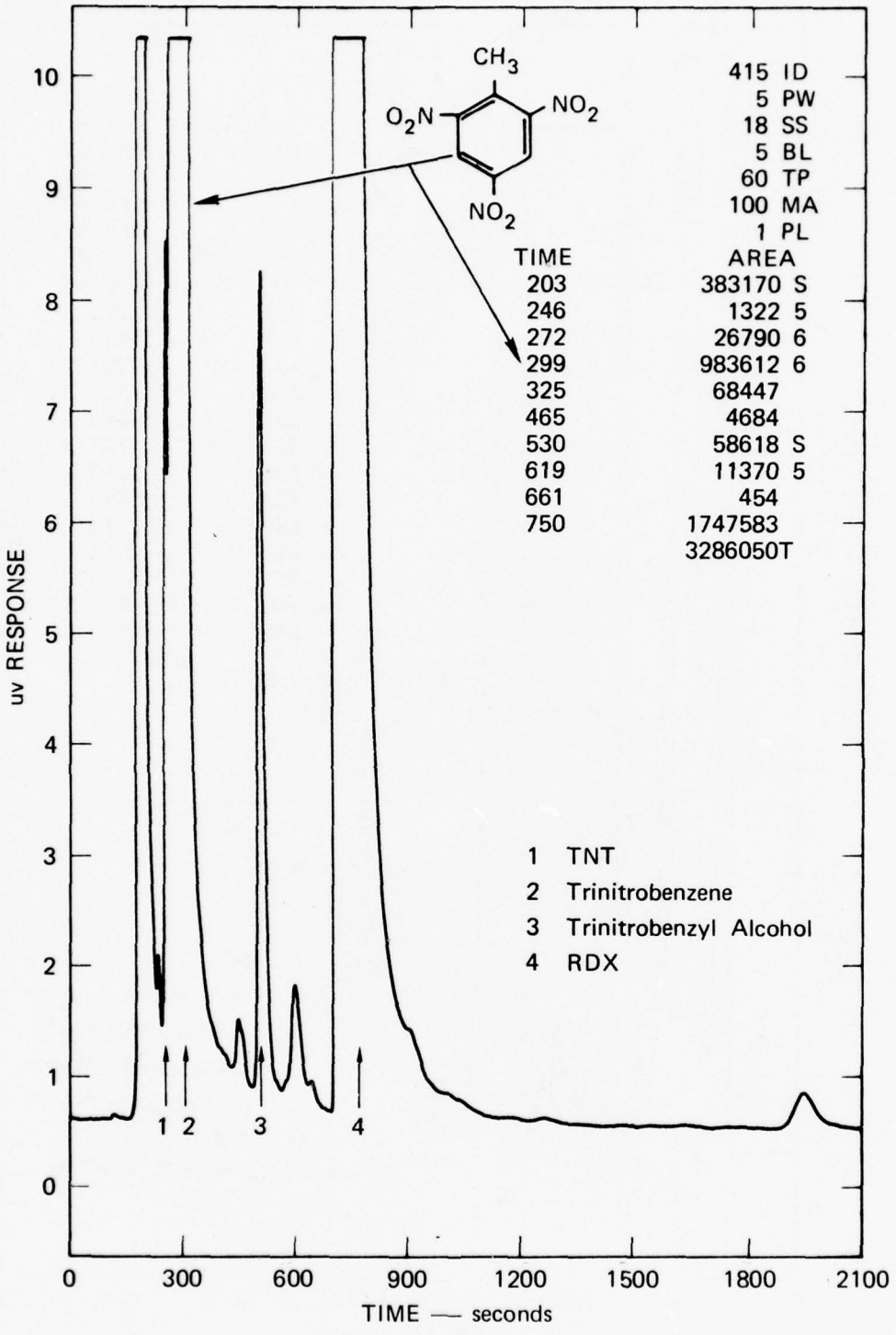

FIGURE B-1 LAP WASTEWATER AT pH 5, 0 HOUR IRRADIATION

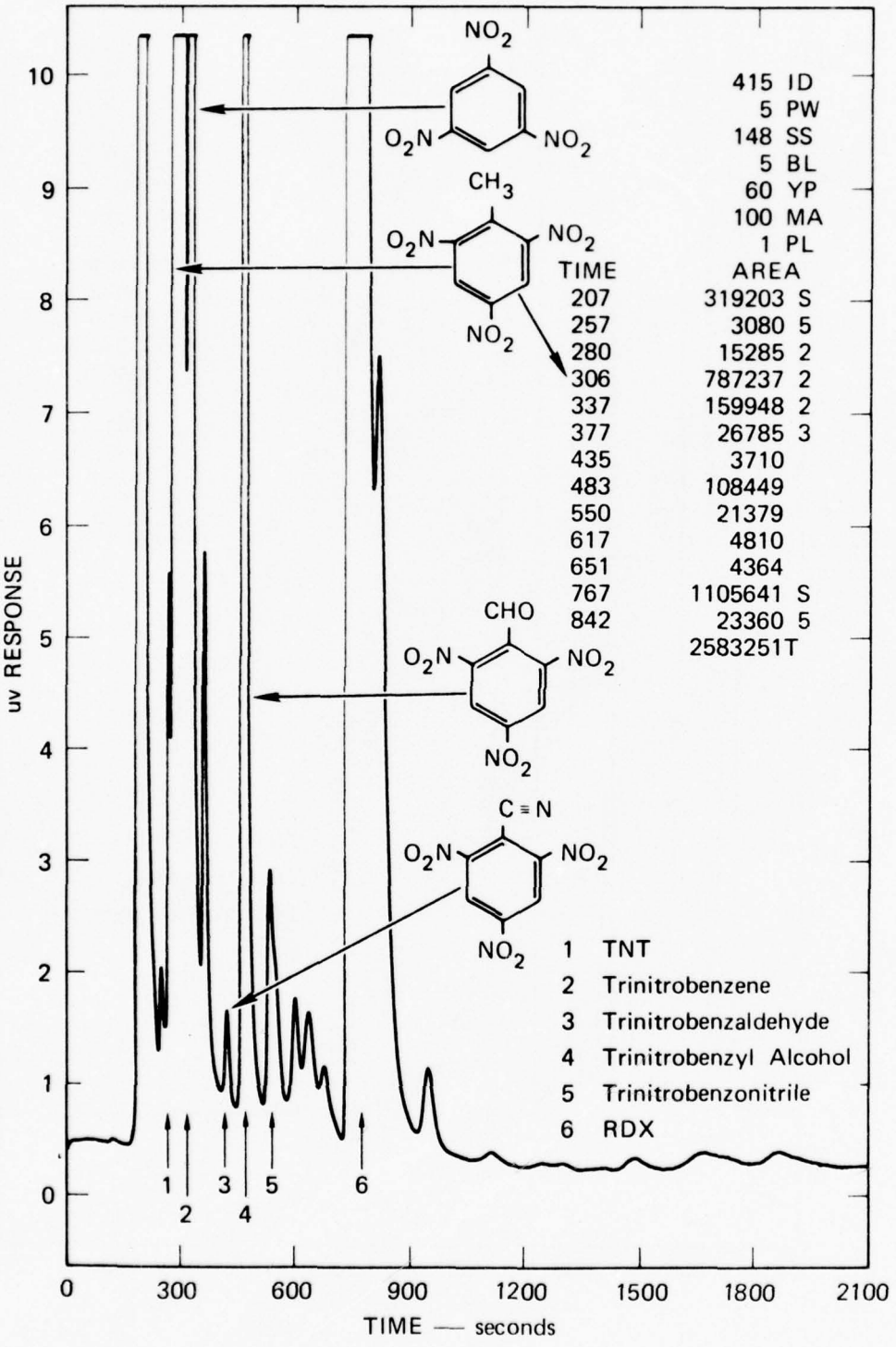

FIGURE B-2 LAP WASTEWATER AT pH 5, 2 HOURS IRRADIATION

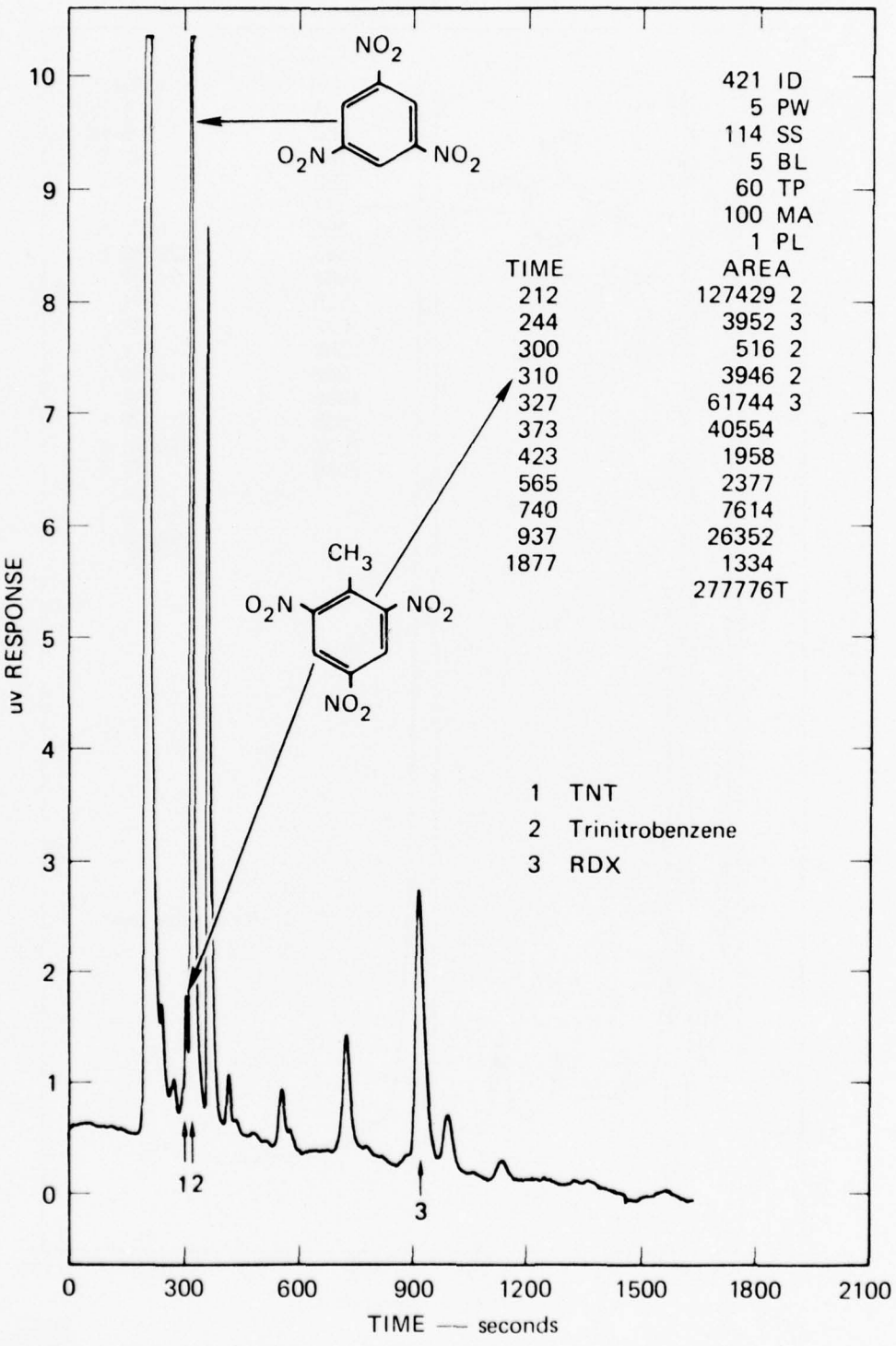

FIGURE B-3 LAP WASTEWATER AT pH 5, 8 HOURS IRRADIATION

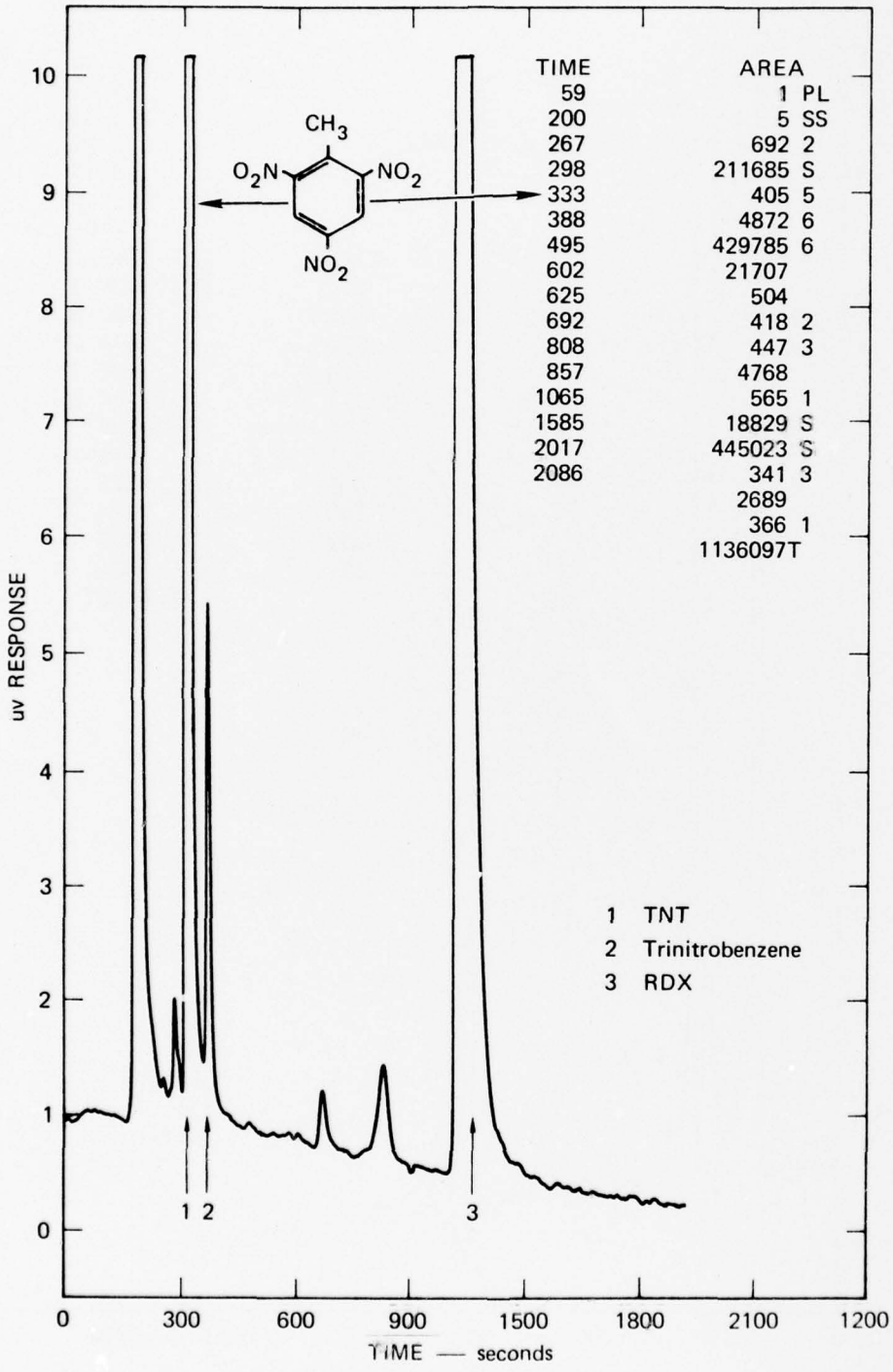

FIGURE B-4 LAP WASTEWATER AT pH 9.4, 0 HOUR IRRADIATION

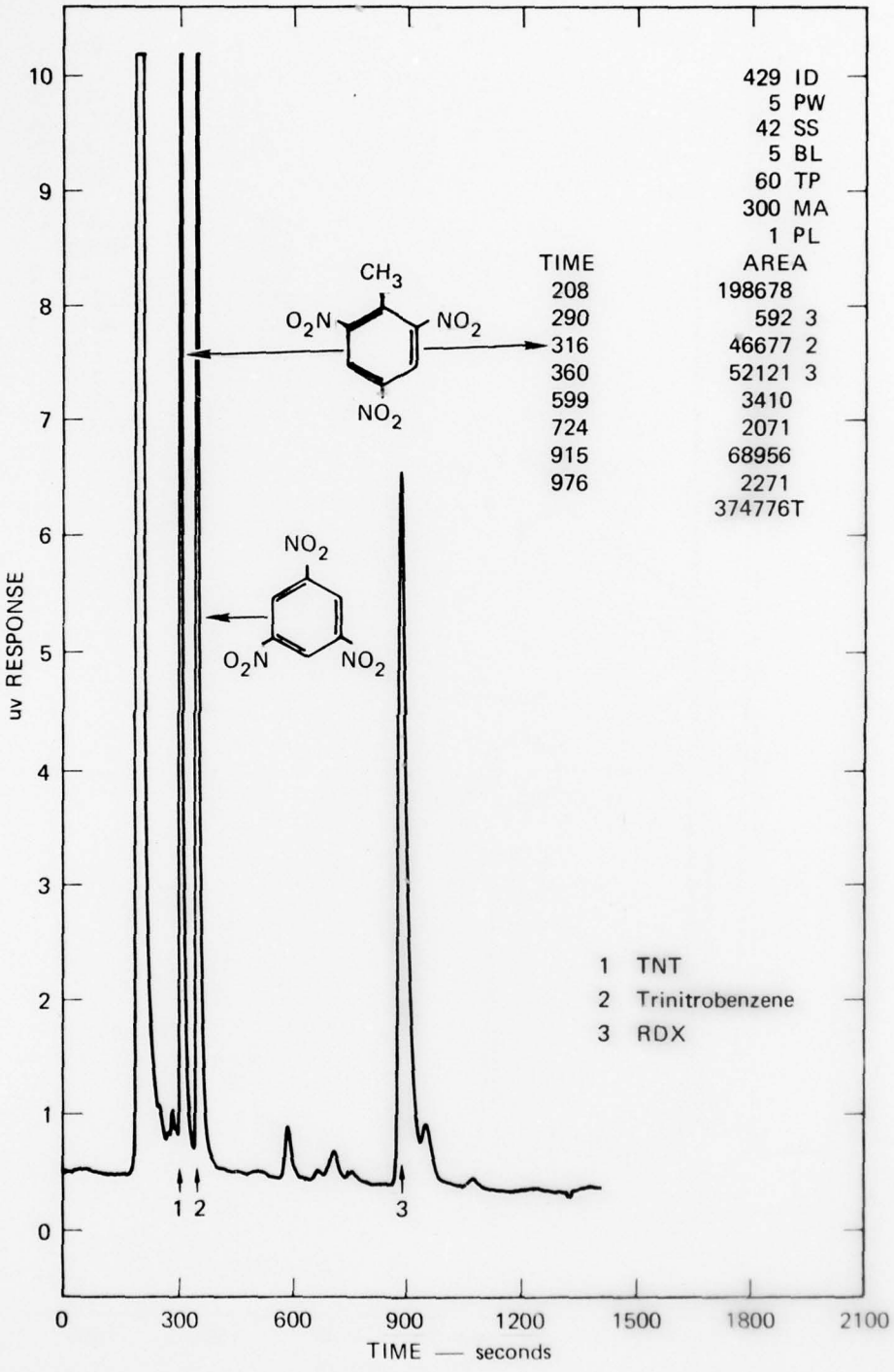

FIGURE B-5 LAP WASTEWATER AT pH 9.4, 2 HOURS IRRADIATION

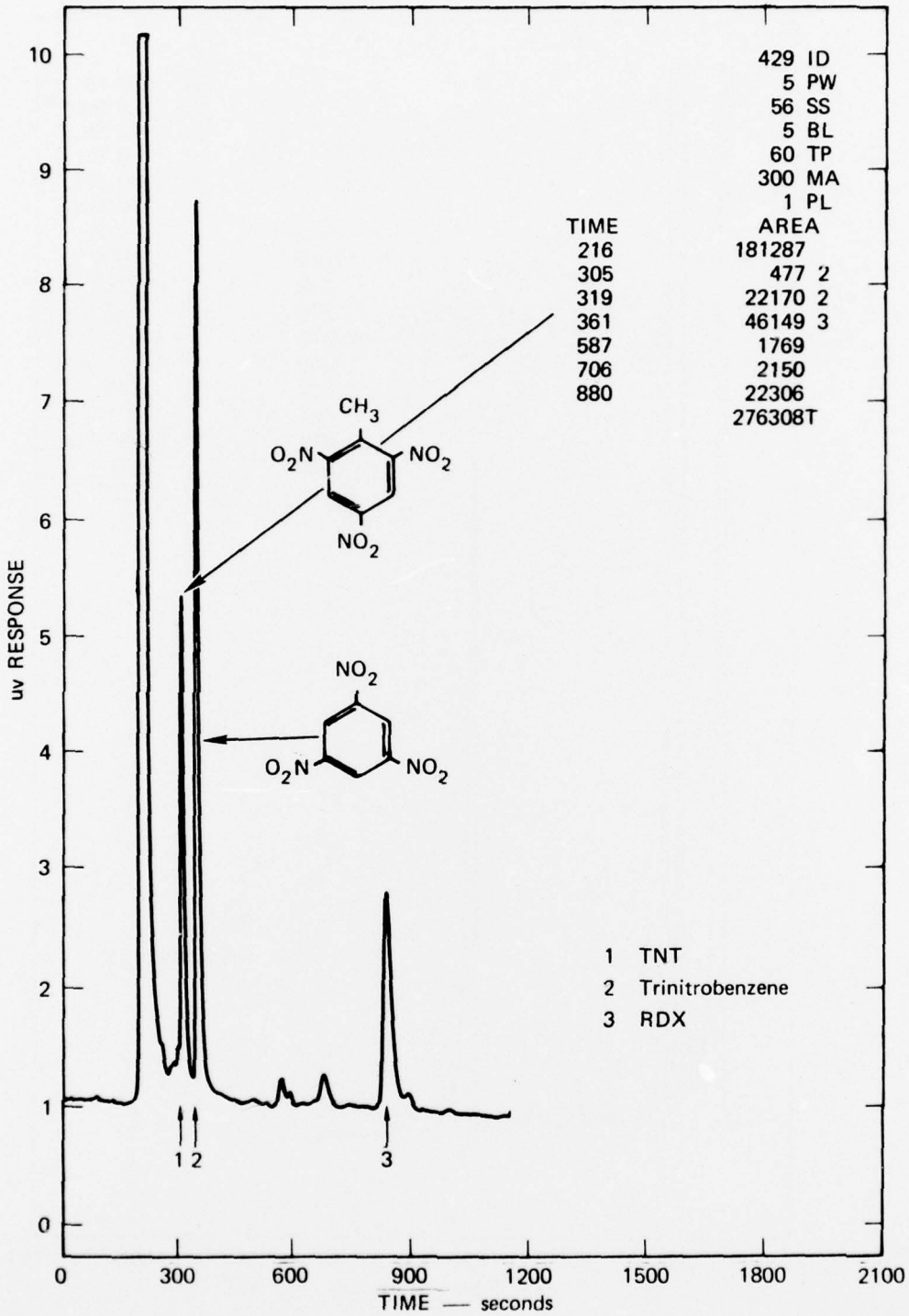

FIGURE B-6 LAP WASTEWATER AT pH 9.4, 7 HOURS IRRADIATION

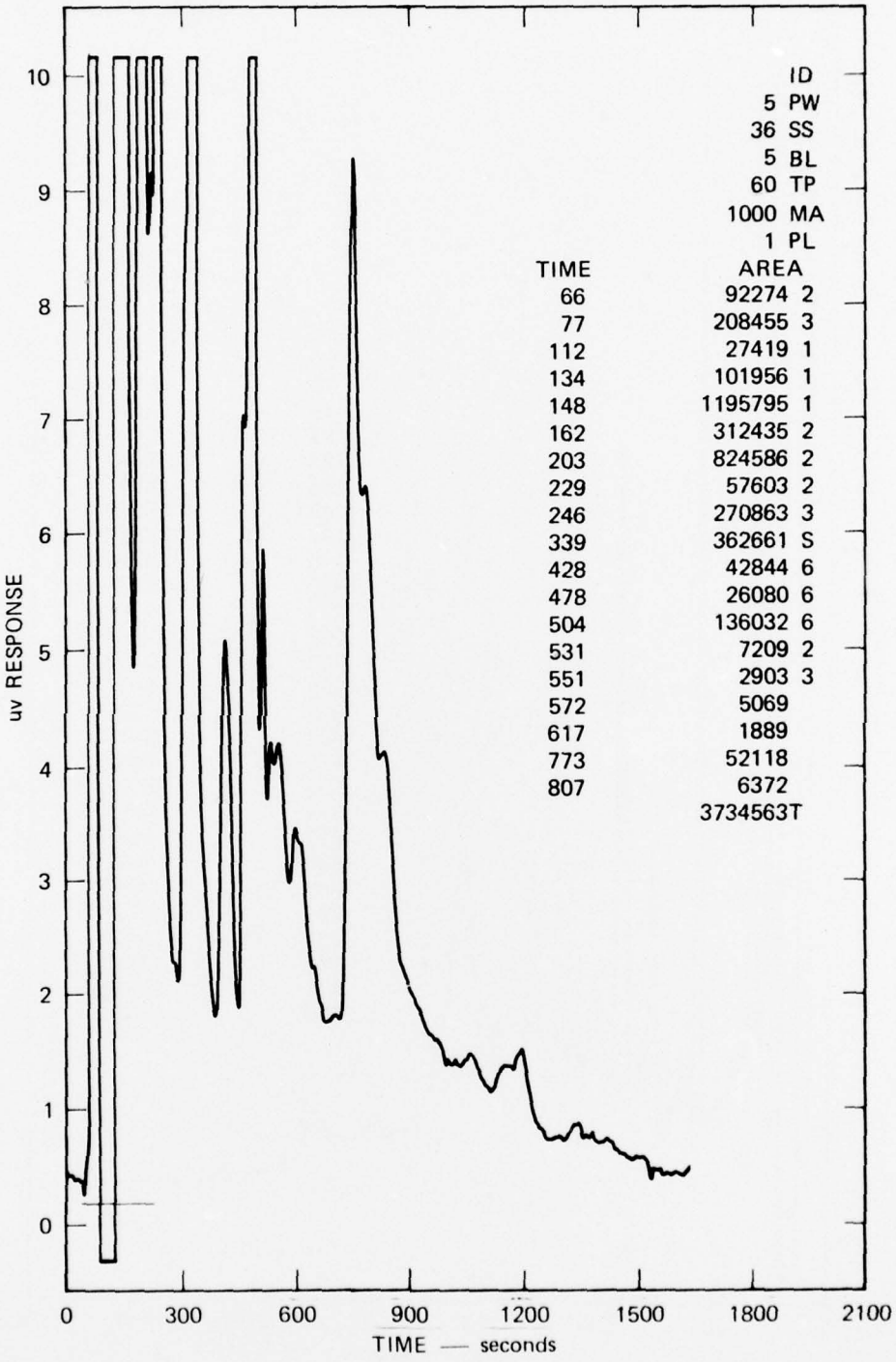

FIGURE B-7 LAP WASTEWATER, pH 5, 50% DEGRADATION, NEAT RESIDUE

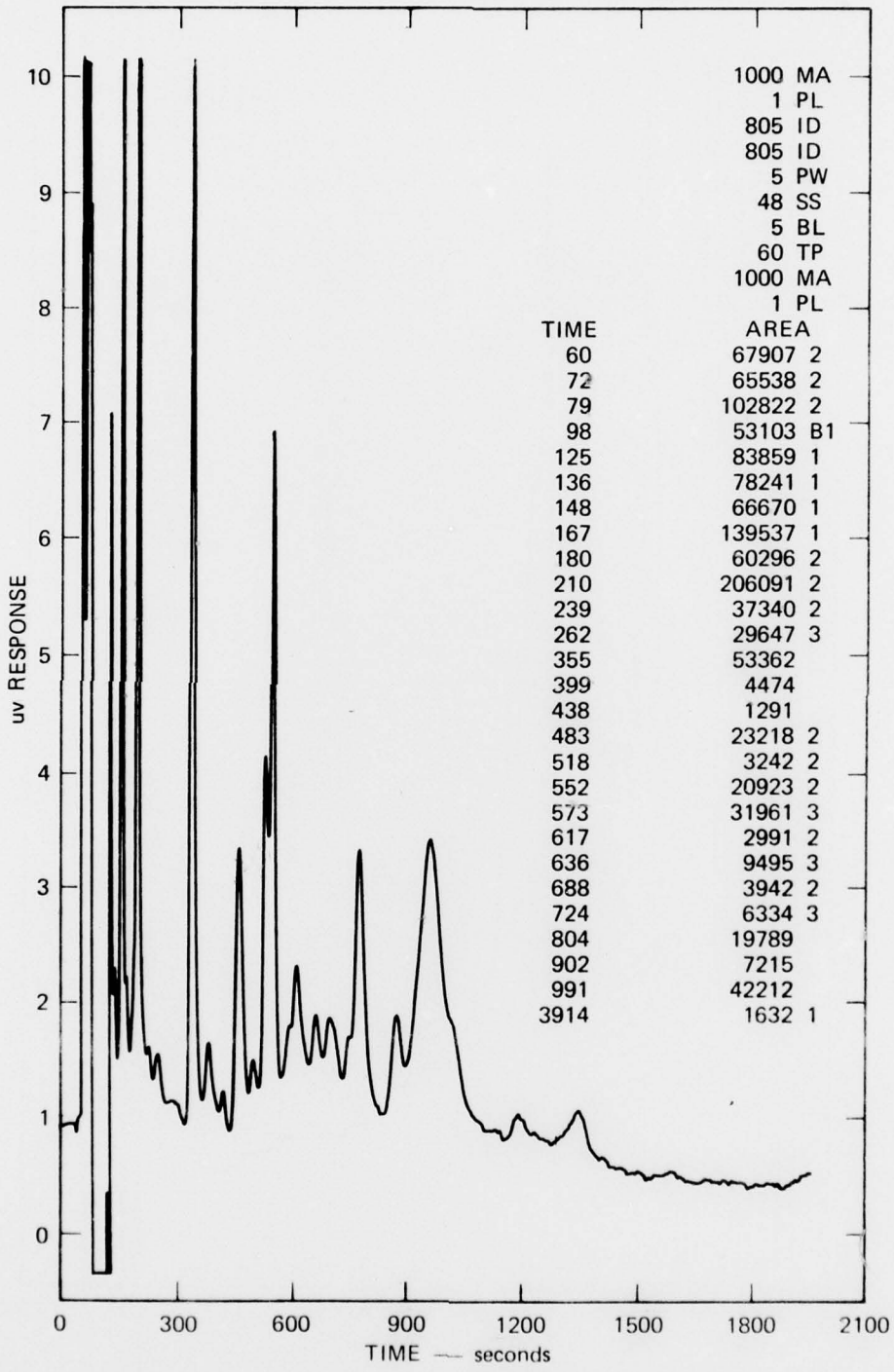

FIGURE B-8 LAP WASTEWATER, pH 5, 50% DEGRADATION, AQUEOUS EXTRACT

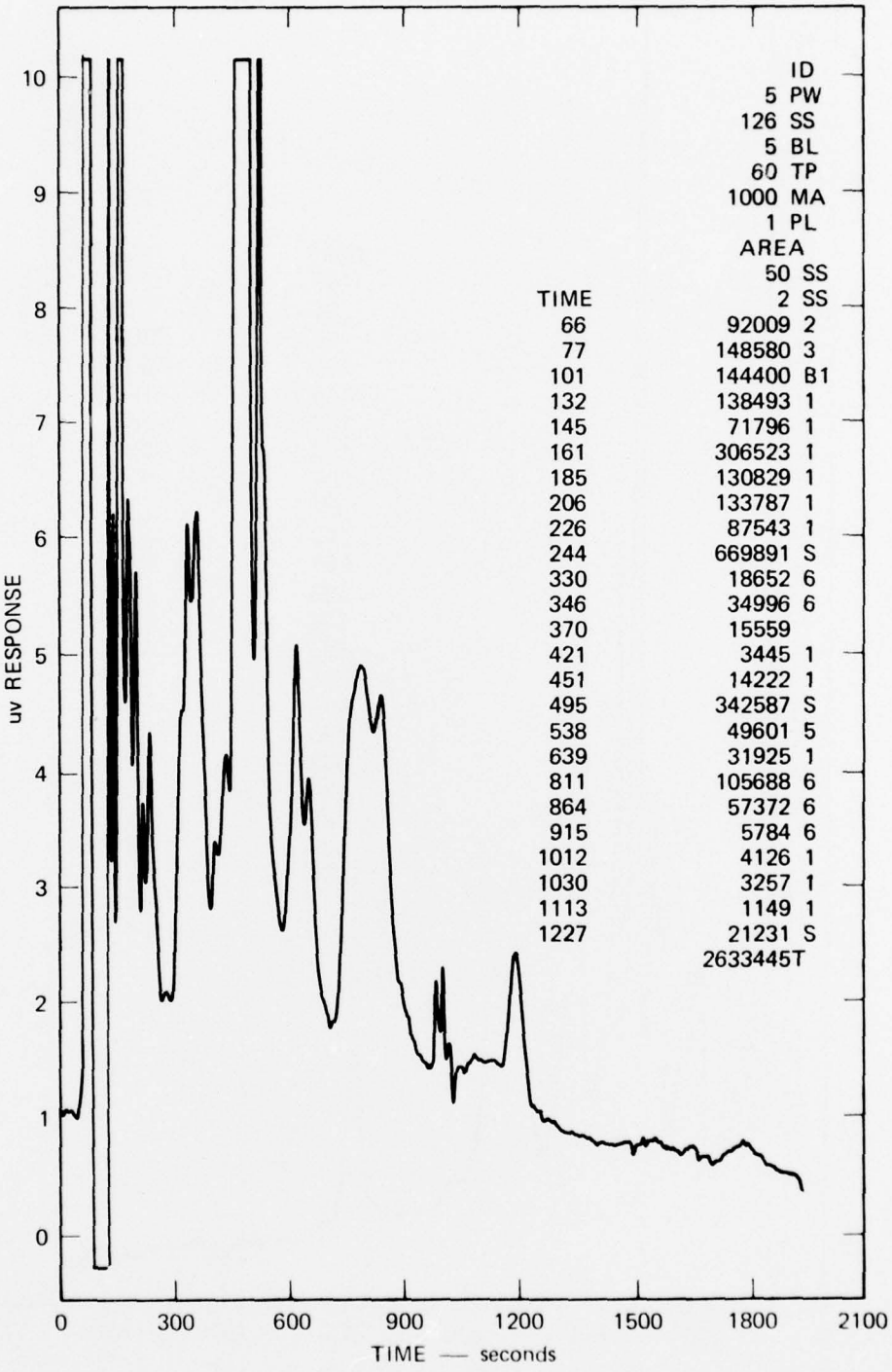

FIGURE B-9 LAP WASTEWATER, pH 6, 100% DEGRADATION, NEAT RESIDUE

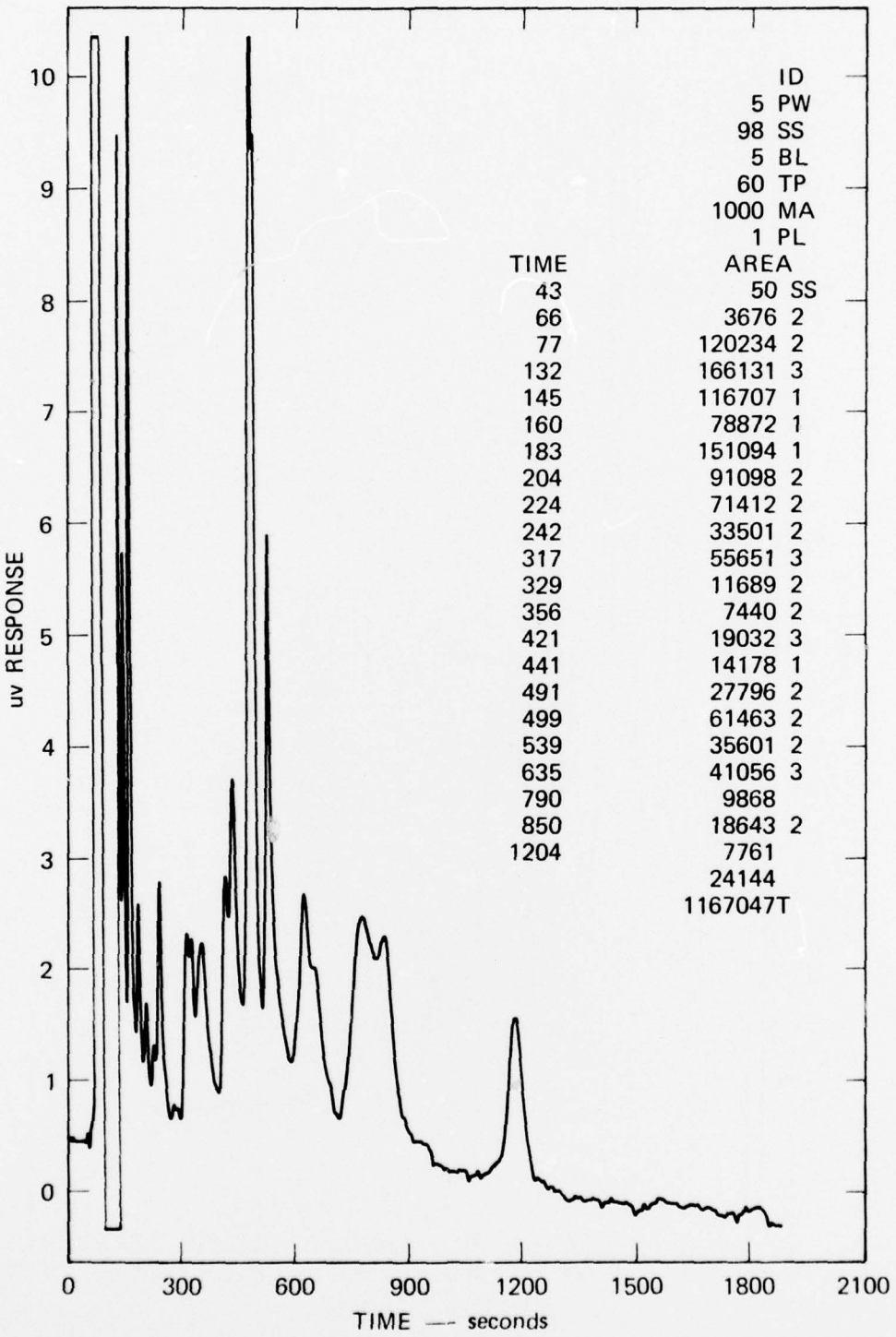

FIGURE B-10 LAP WASTEWATER, pH 5, 100% DEGRADATION, AQUEOUS EXTRACT

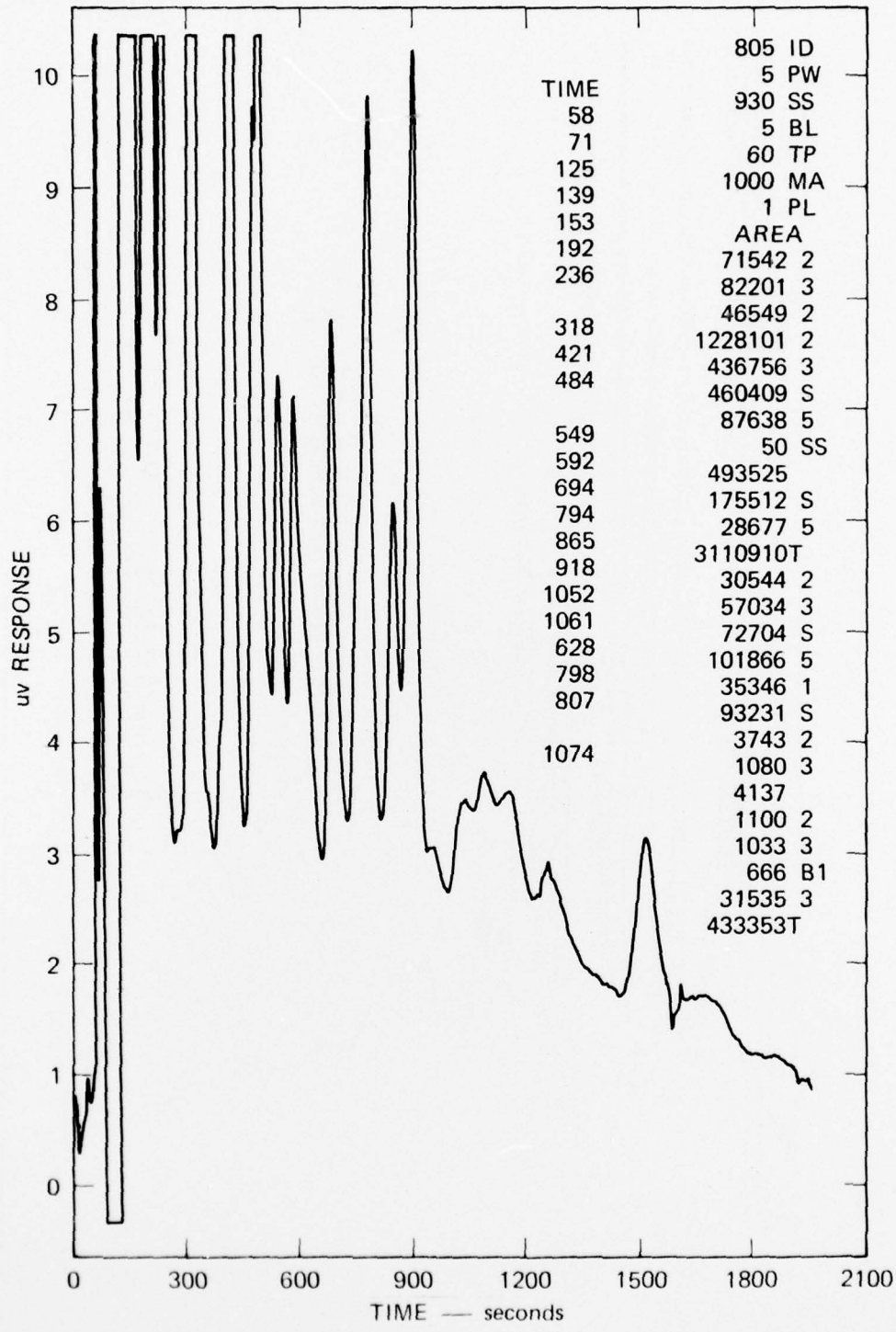

FIGURE B-11 LAP WASTEWATER, pH 7, 50% DEGRADATION, NEAT RESIDUE

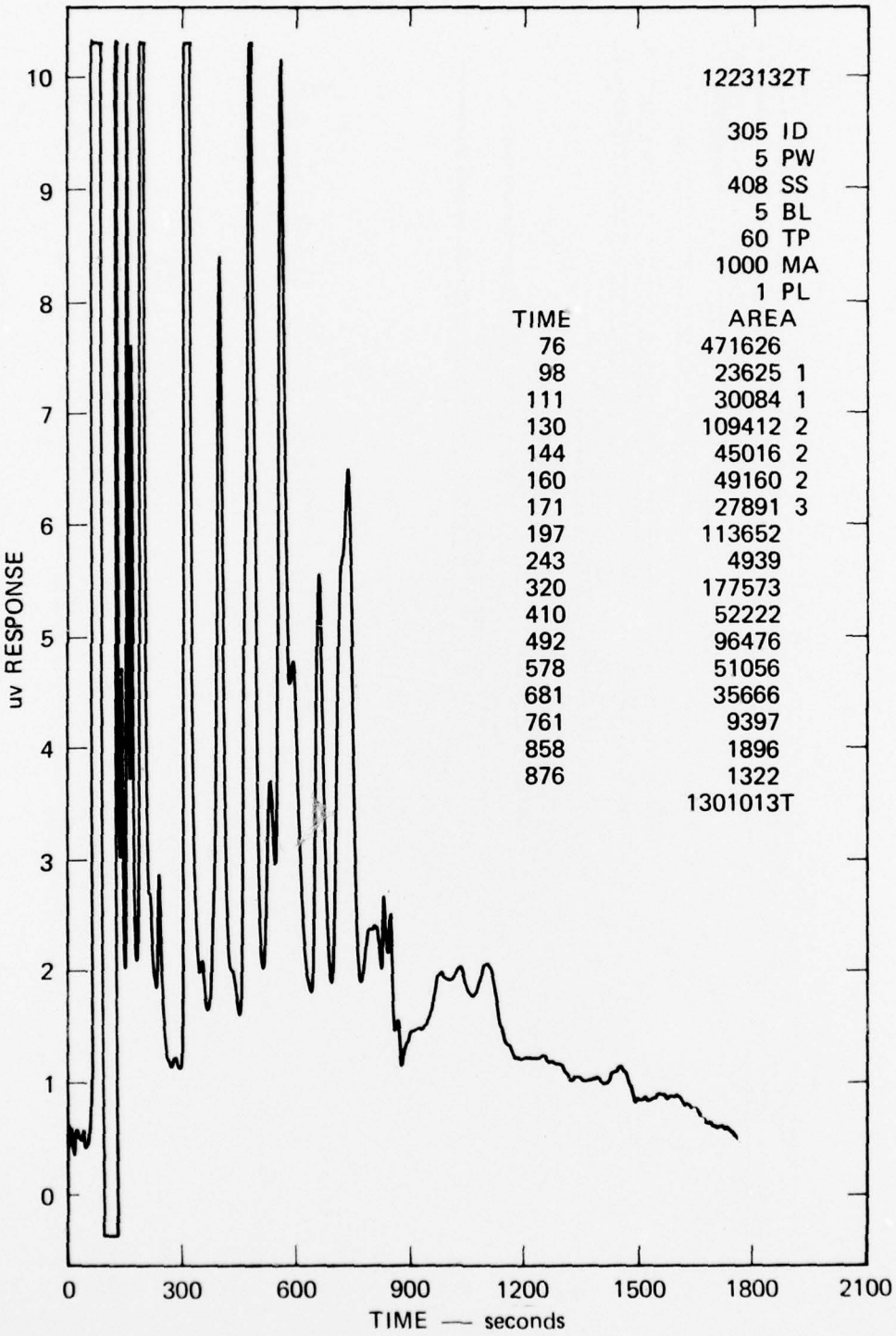

FIGURE B-12 LAP WASTEWATER, pH 7, 50% DEGRADATION, AQUEOUS EXTRACT

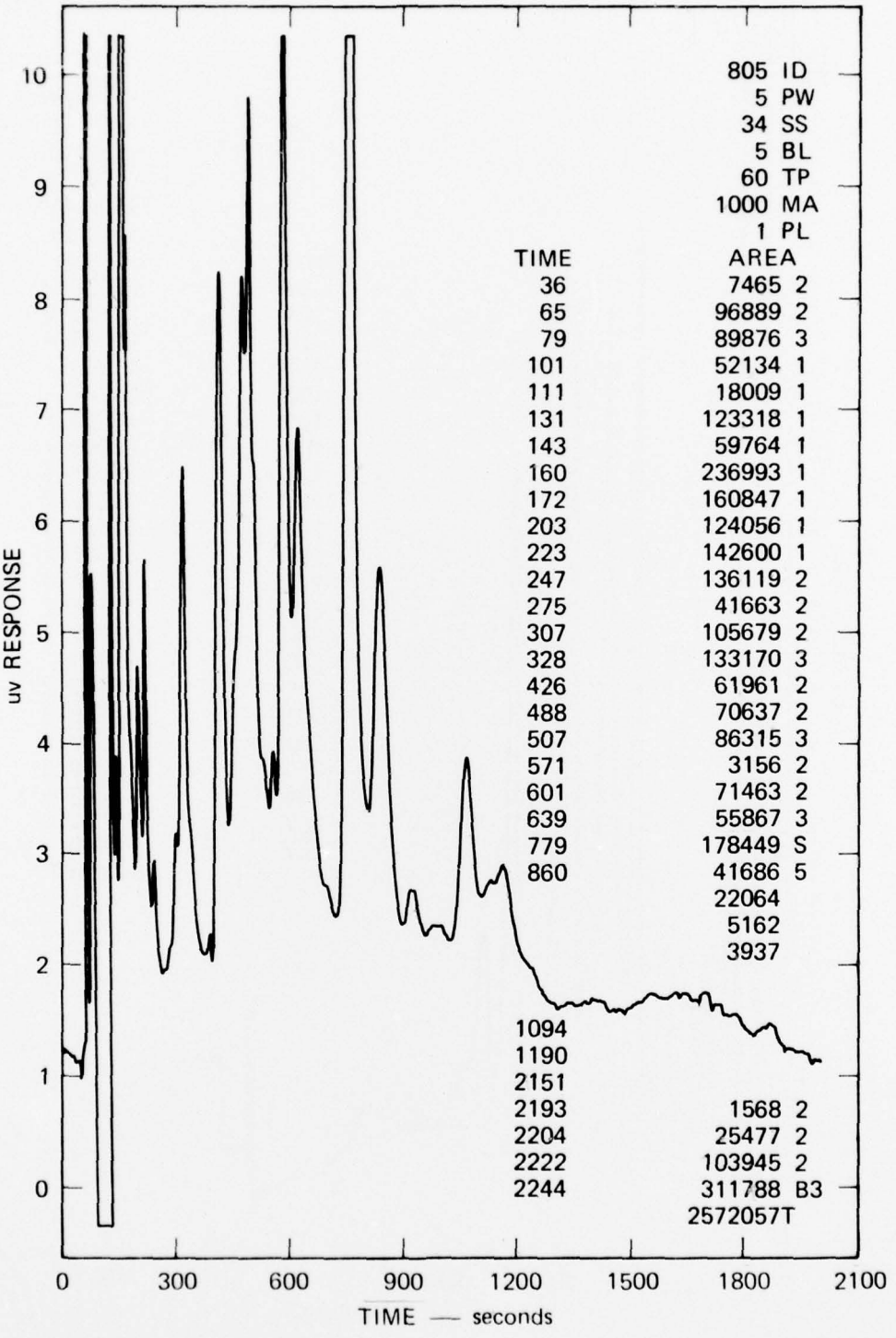

FIGURE B-13 LAP WASTEWATER, pH 7, 100% DEGRADATION, NEAT RESIDUE

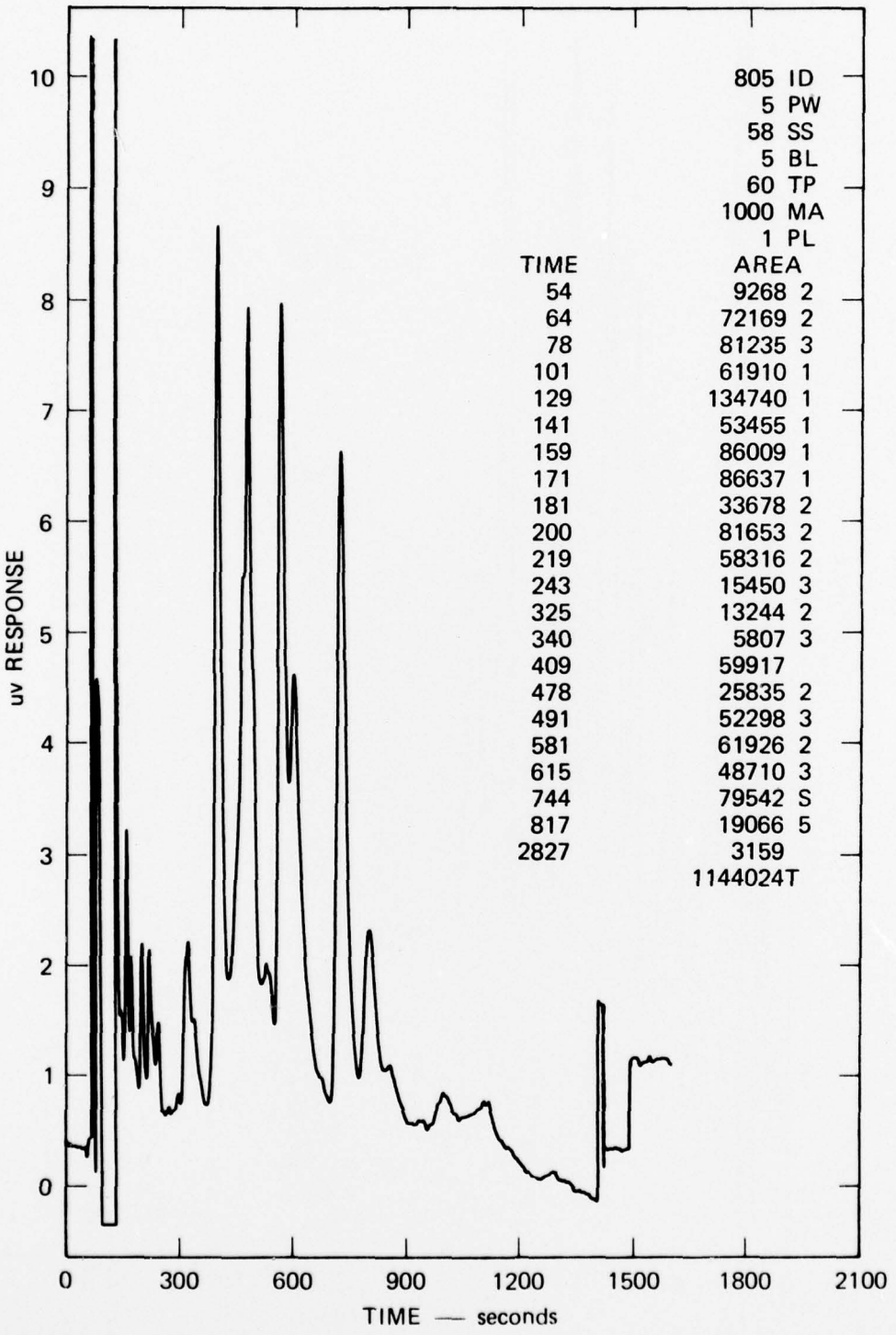

FIGURE B-14 LAP WASTEWATER, pH 7, 100% DEGRADATION, AQUEOUS EXTRACT

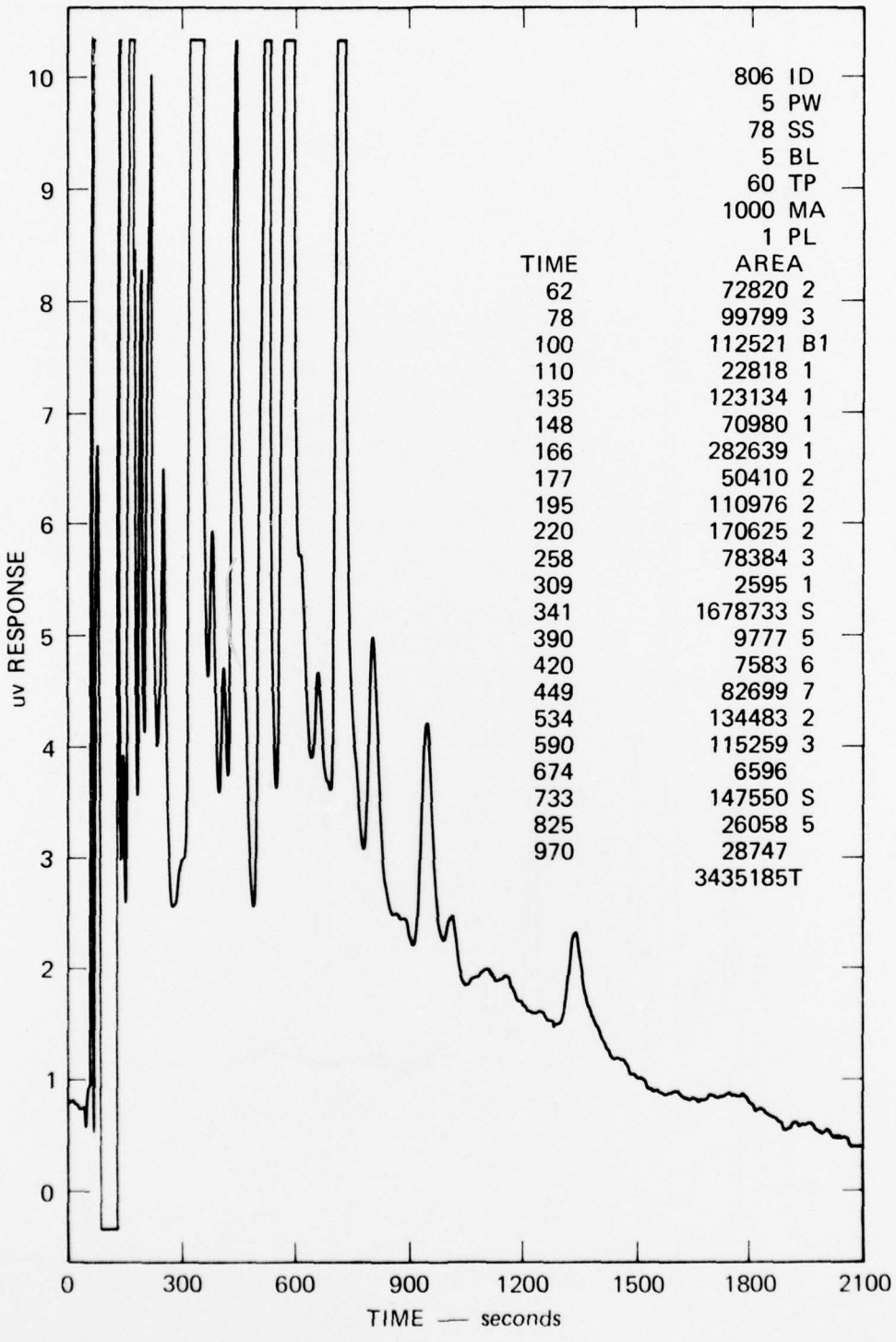

FIGURE B-15 LAP WASTEWATER, pH 9.4, 50% DEGRADATION, NEAT RESIDUE

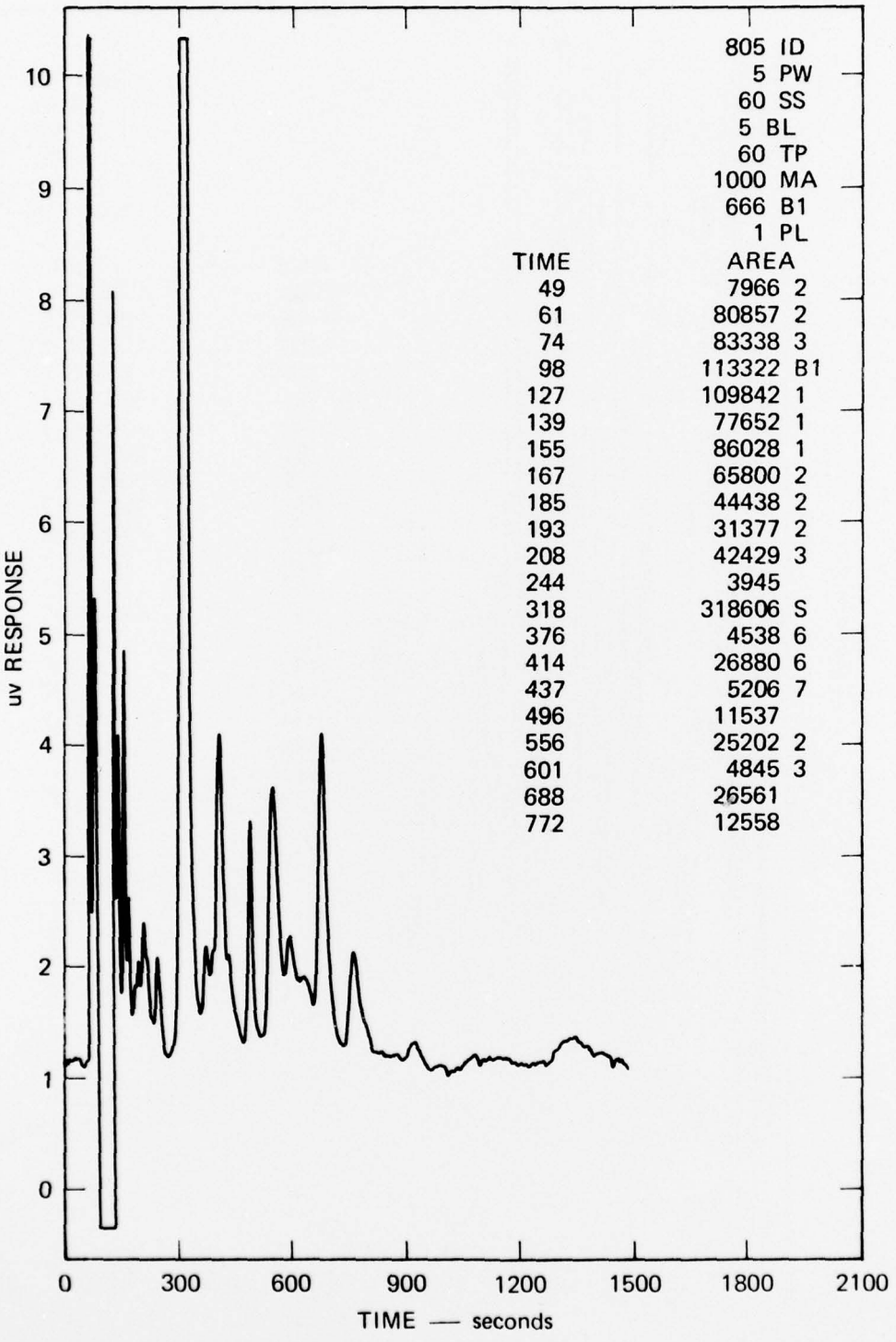

FIGURE B-16 LAP WASTEWATER, pH 9.4, 50% DEGRADATION, AQUEOUS EXTRACT

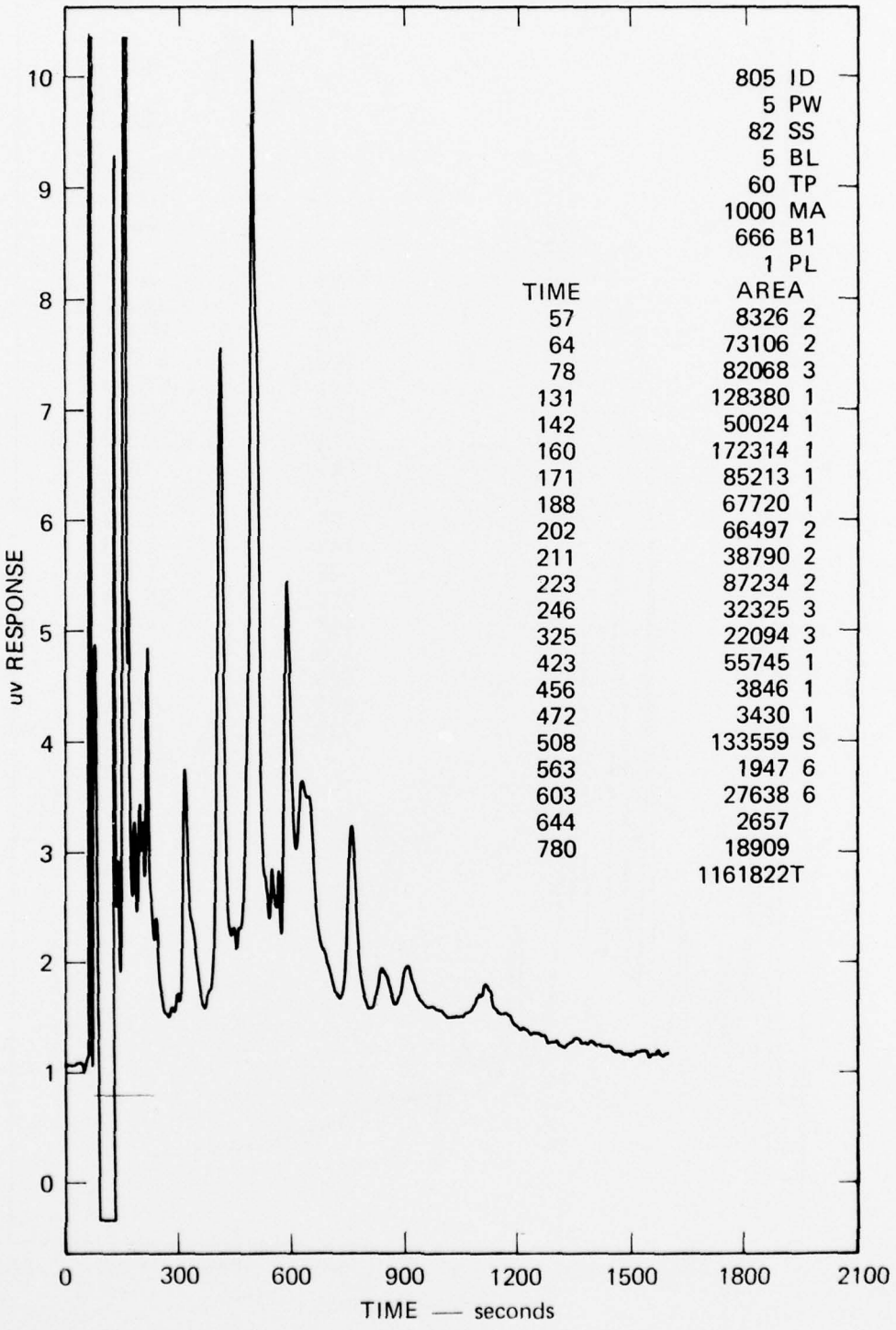

FIGURE B-17 LAP WASTEWATER, pH 9.4, 100% DEGRADATION, NEAT RESIDUE

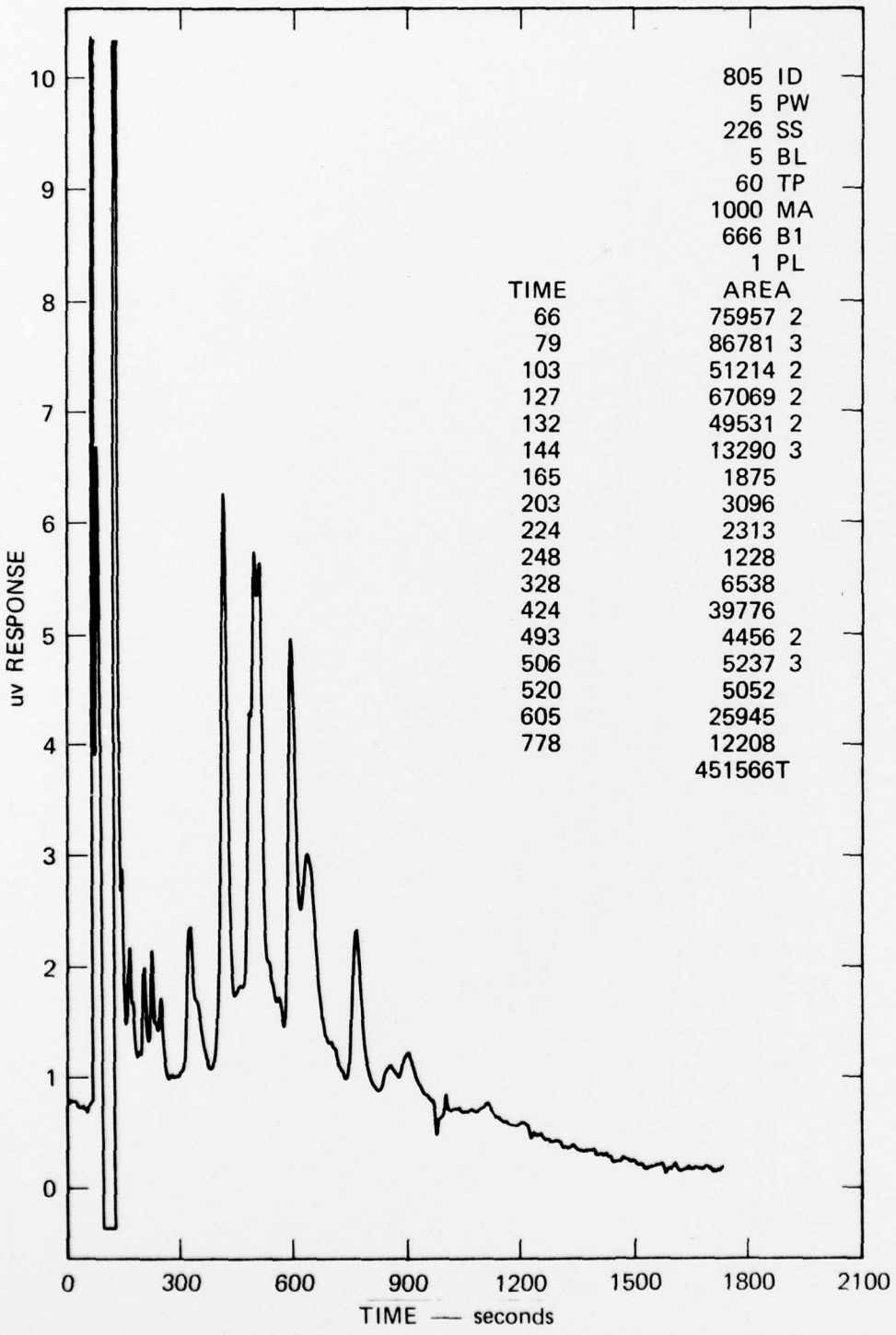

FIGURE B-18 LAP WASTEWATER, pH 9.4, 100% DEGRADATION, AQUEOUS EXTRACT