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FINAL REPORT

"TOXICITY STUDY OF THE SANTA CLARA RIVER, SAN GABRIEL RIVER, AND CALLEGUAS CREEK"

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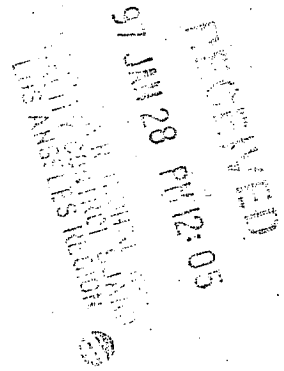

EXECUTIVE SUMMARY

Quarterly toxicity bioassays were conducted on water samples collected from the Santa Clara River, San Gabriel River, and Calleguas Creek drainages. Test species included fish (*Pimephales promelas*), invertebrate (*Ceriodaphnia dubia*) and algae (*Selenastrum capricornutum*). Both acute and chronic toxicity was demonstrated with all three organisms on a frequent basis and was confirmed with Toxicity Identification Evaluation (TIE) procedures. Suspected toxicants included metals and organic chemicals such as pesticides. Elevated ammonia concentrations may also have contributed to toxicity in some cases. In addition, fish collected from Calleguas Creek and the San Gabriel River exhibit increased incidence of lesions in various organ systems that are consistent with exposure to toxicants.

Collectively, these results underscore that aquatic life beneficial uses are impaired in certain reaches of these waterways. However, the severity and frequency of the responses suggest that conditions could be improved considerably by a program of toxicant identification and source reduction and/or treatment. Thus, more intensive sampling in conjunction with TIEs and increasingly sensitive analytical chemistry is likely to be productive in terms of identifying toxicants, their sources, and temporal variations in their concentrations in the receiving water. The effectiveness of source control and treatment would be determined on the basis of follow-up monitoring programs.

TABLE OF CONTENTS

INTRODUCTION

This study addressed water quality in three watersheds of the Los Angeles Region. Following United States Environmental Protection Agency (EPA) guidelines for toxicity tests in freshwater systems (EPA, 1989) chronic toxicity was evaluated in the Santa Clara and San Gabriel Rivers and Calleguas Creek.

The Santa Clara and San Gabriel Rivers (Figures A & B) drain large areas of the Los Angeles Region. During dry weather, flows in many reaches (upper Santa Clara and lower San Gabriel) of these rivers consist primarily of reclaimed water from municipal wastewater treatment facilities (POTWs). Municipal waste water treatment facilities, their locations and average daily discharge are shown for Santa Clara River and Calleguas Creek (Fig. C) and for San Gabriel River (Fig. D). Additional flows are derived from industrial point sources and nonpoint sources, including agricultural and urban runoff. Parts of the Santa Clara River receive flow from rising ground water. Calleguas Creek (Fig. A) drains the Oxnard Plain; the watershed is largely agricultural but there are also four POTWs located within the drainage (Fig. C). Land uses associated with the different watersheds are shown in Figs. E and F. As Figure E shows, much of the areas immediately adjacent to the mid to lower reaches of the Santa Clara River and most areas of Calleguas Creek are in agriculture. There are scattered urban and industrial areas, but agriculture predominates. In addition, scattered areas associated with sand and gravel mining and oil extraction are also present in the Santa Clara River drainage. While the upper reaches of the San Gabriel River remain in a fairly natural state, the middle and lower reaches are dominated by urban and industrial uses.

A wide variety of beneficial uses are designated for the various reaches of these three rivers in the Regional Board's Basin Plan (California Regional Water Quality Control Board, Los Angeles

Region 1994). These include several beneficial uses (reach-specific) which pertain to the protection of instream aquatic resources: warm freshwater habitat, cold freshwater habitat, wildlife habitat, migration of aquatic organisms, spawning, reproduction, and/or early development, rare, threatened, or endangered species, and preservation of biological habitats.

The 1990 State Water Quality Assessment Report classified most of the Santa Clara and San Gabriel Rivers as "intermediate." This classification was based primarily on impairment or threat to beneficial uses of these waters. In addition, the tidal prism of the San Gabriel River was classified as "impaired" due to bio-accumulation of metals in fish tissues. The 1992 Water Quality Assessment revised the classification of the lower San Gabriel River to impaired due to high ammonia levels and acute toxicity found in an EPA study (Norberg-King and Englehorn, 1990). Calleguas Creek is also listed as "impaired" due to high levels of pesticides in sediment and biota.

The major objectives of this study were to:

1. Determine whether aquatic life beneficial uses are impaired in the Santa Clara and San Gabriel Rivers and/or in Calleguas Creek.
2. Test the usefulness of chronic toxicity studies, coupled with traditional water quality data, for assessment of water quality.
3. Identify specific pollutants and sources responsible for ambient toxicity in these waters.
4. Provide recommendations for practices or regulatory controls which can be used in toxicity reduction.

To achieve these objectives, the study comprised the following tasks:

Task 1: Collection and review of existing data, map preparation

This task reviewed existing reports and data on the watersheds to provide background for interpreting the analytical and bioassay results. Information on land use and point and non-point discharges were mapped to provide further understanding of potential sources of toxic inputs to the watersheds.

Task 2: Toxicity testing

In this task, bioassays were conducted quarterly using (EPA, 1989) procedures for conducting short-term tests to evaluate chronic toxicity. Test organisms were fathead minnows (*Pimephales promelas*), cladocerans (*Ceriodaphnia dubia*), and green algae (*Selenastrum capricornutum*). Data from these tests were used to identify the frequency and severity of toxicity. In addition, based on the sampling location, candidate sources of toxicity could be derived.

Task 3: Toxicity identification evaluations (TIEs)

Phases 1 and 2 TIE procedures were applied to selected toxic samples to provide additional information on the characteristics of the toxic constituent(s). Where possible, these data were coupled with those from land use and discharge location to further identify possible sources of contaminants.

Task 4: Histopathological investigation of field-caught fish

Histological investigations were conducted on fish sampled from the San Gabriel and Calleguas Creek watersheds. The presence of histologic alterations was used to assess the health of fish living in the watersheds from which the water samples were collected for toxicity testing.

This final report describes the results of the data review, toxicity tests, TIEs, and histopathological studies conducted on samples from Calleguas Creek and the Santa Clara and San Gabriel Rivers. It also includes maps for each of the watersheds and suggests possible sources of toxicity as well as recommendations for further studies to pinpoint specific causes and sources of toxicity. Detailed methods and results for the toxicity and TIE portions of this study have been submitted previously in a series of quarterly reports (Appendix A), which include the raw data associated with the individual testing events. However, the work related to the histopathological studies is treated in greater detail because the methods and findings have not been described previously. The overall findings and recommendations associated with the study are presented on the basis of individual watersheds.

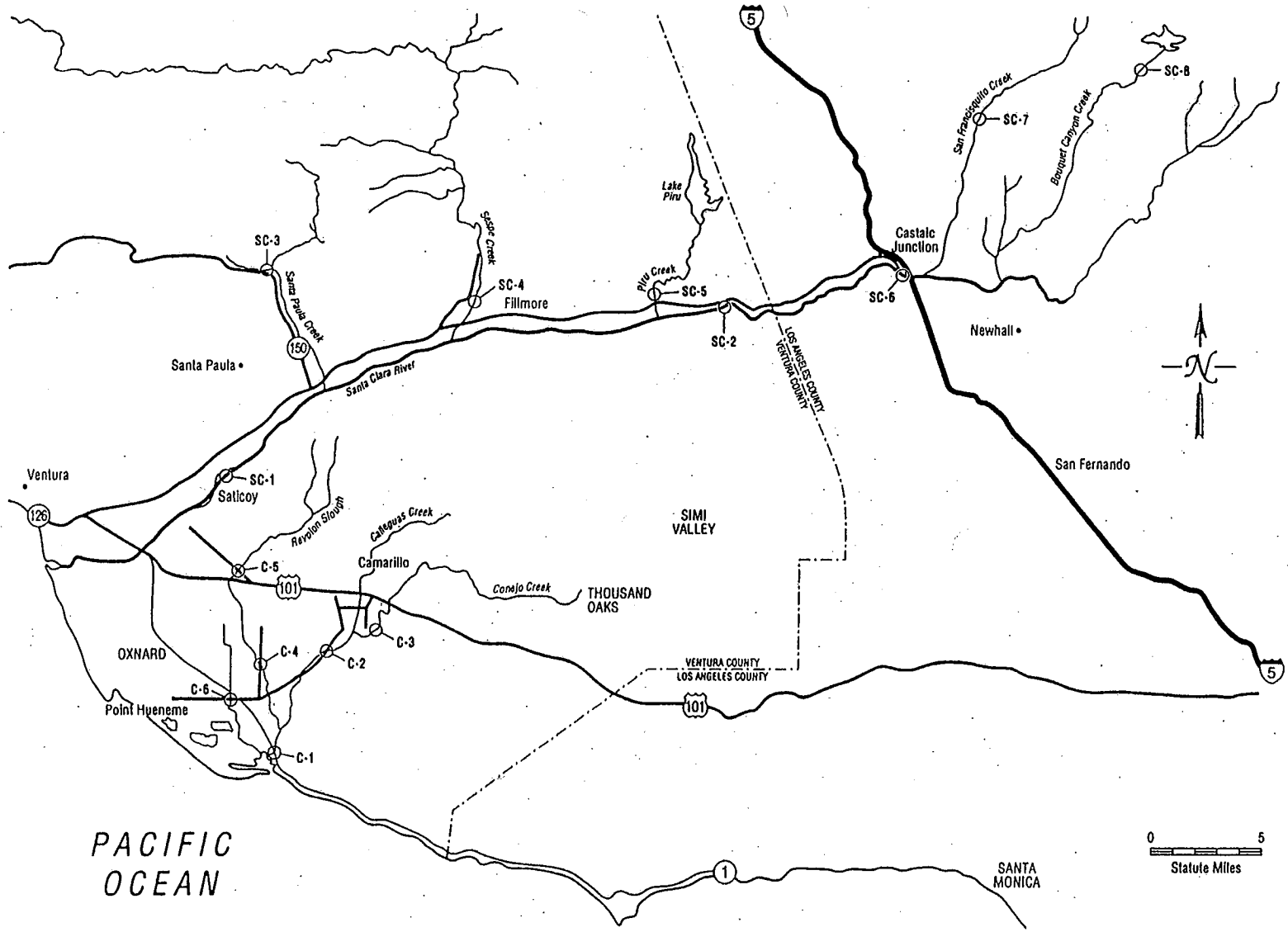

Figure A. Santa Clara River and Calleguas Creek Watershed and Sampling Locations

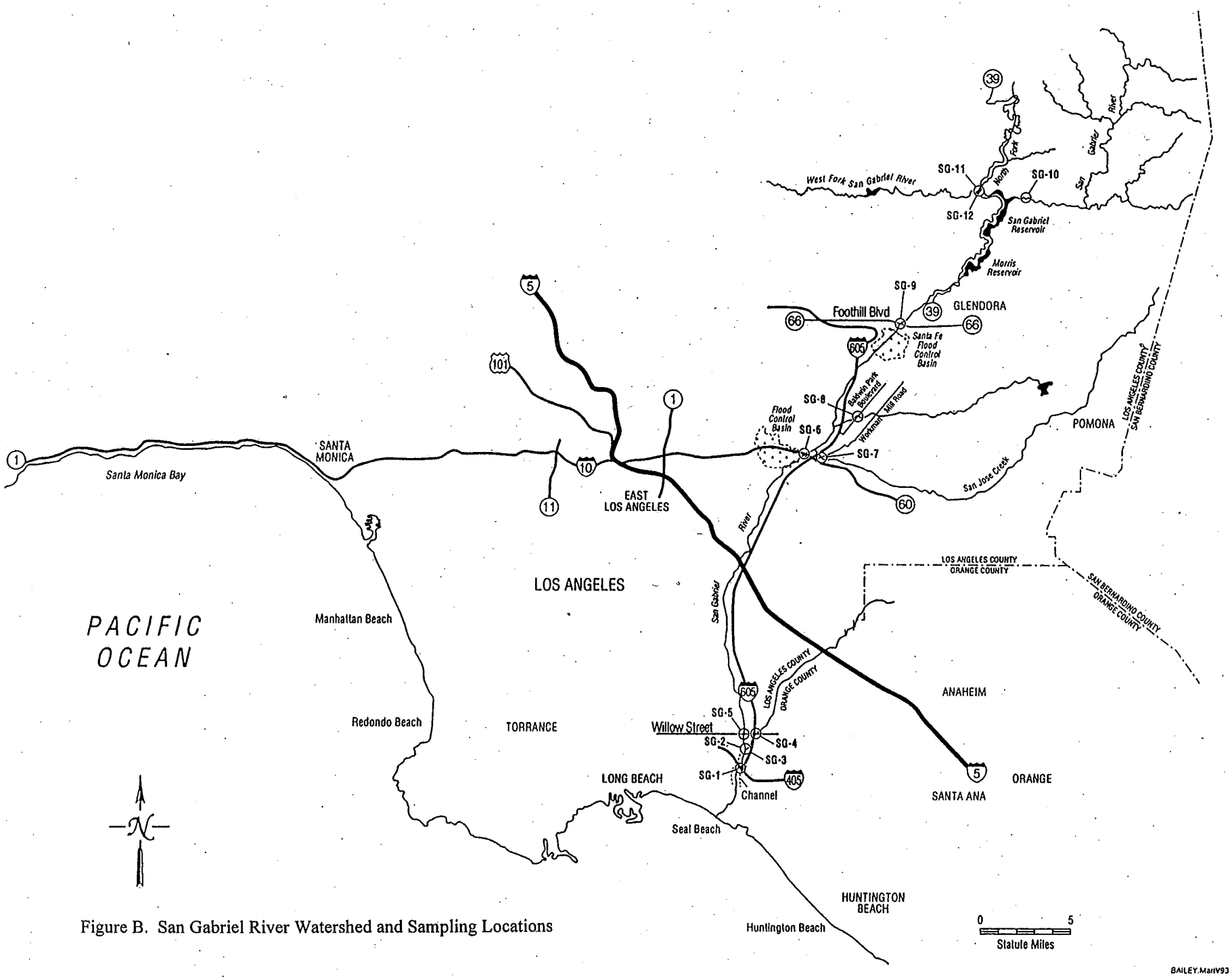

Figure B. San Gabriel River Watershed and Sampling Locations

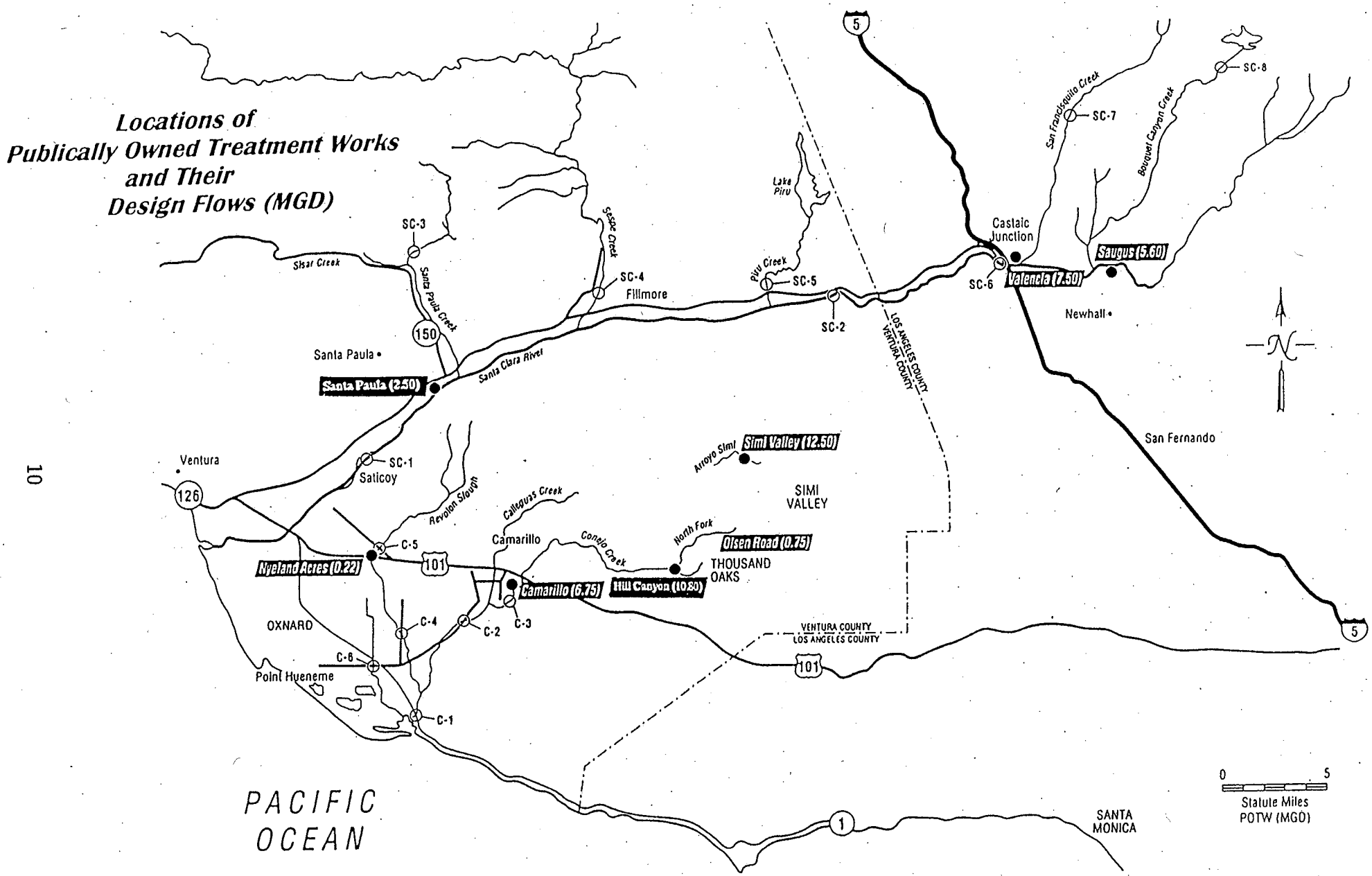

Figure C. Santa Clara River and Calleguas Creek

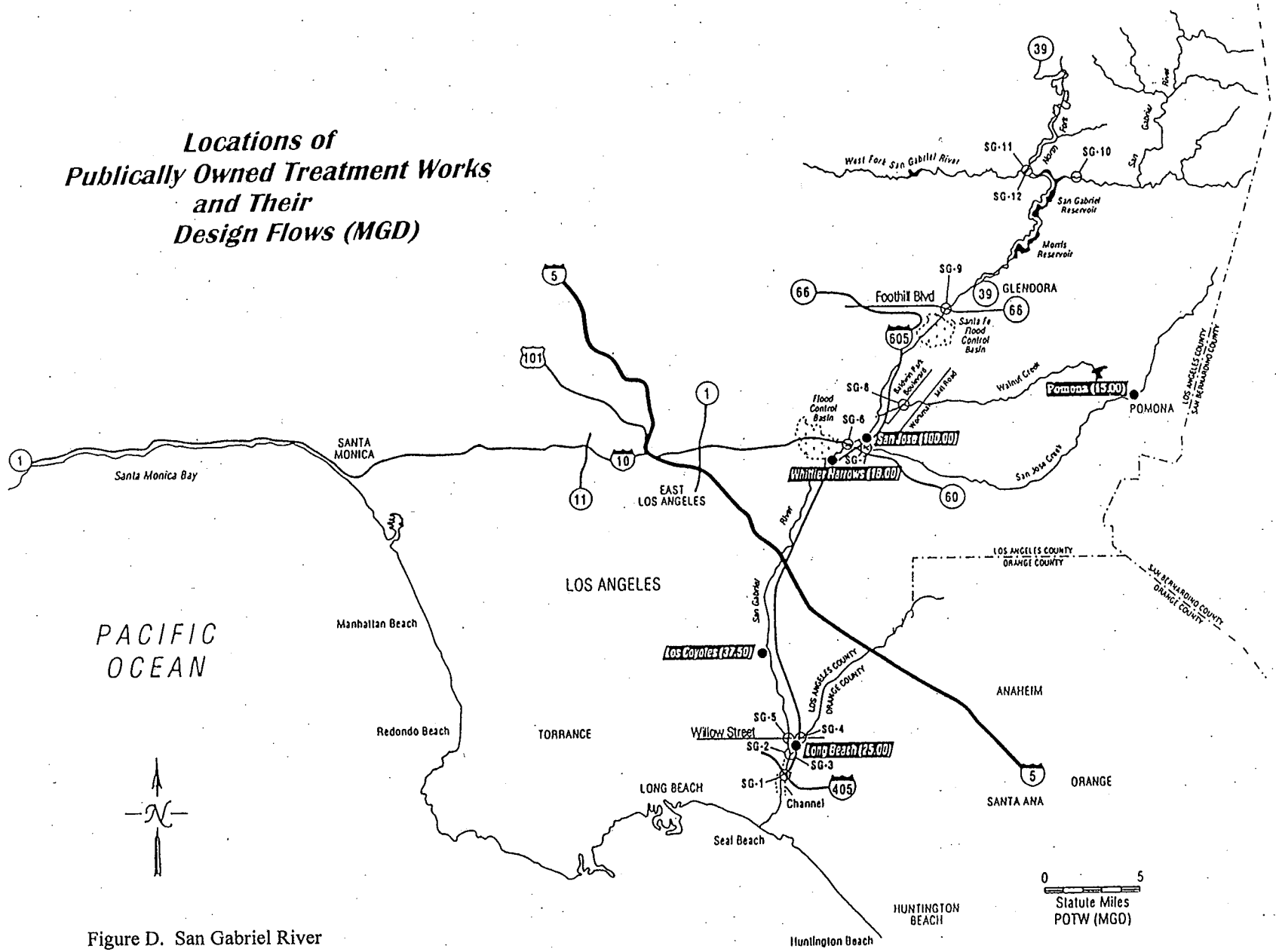

Figure D. San Gabriel River

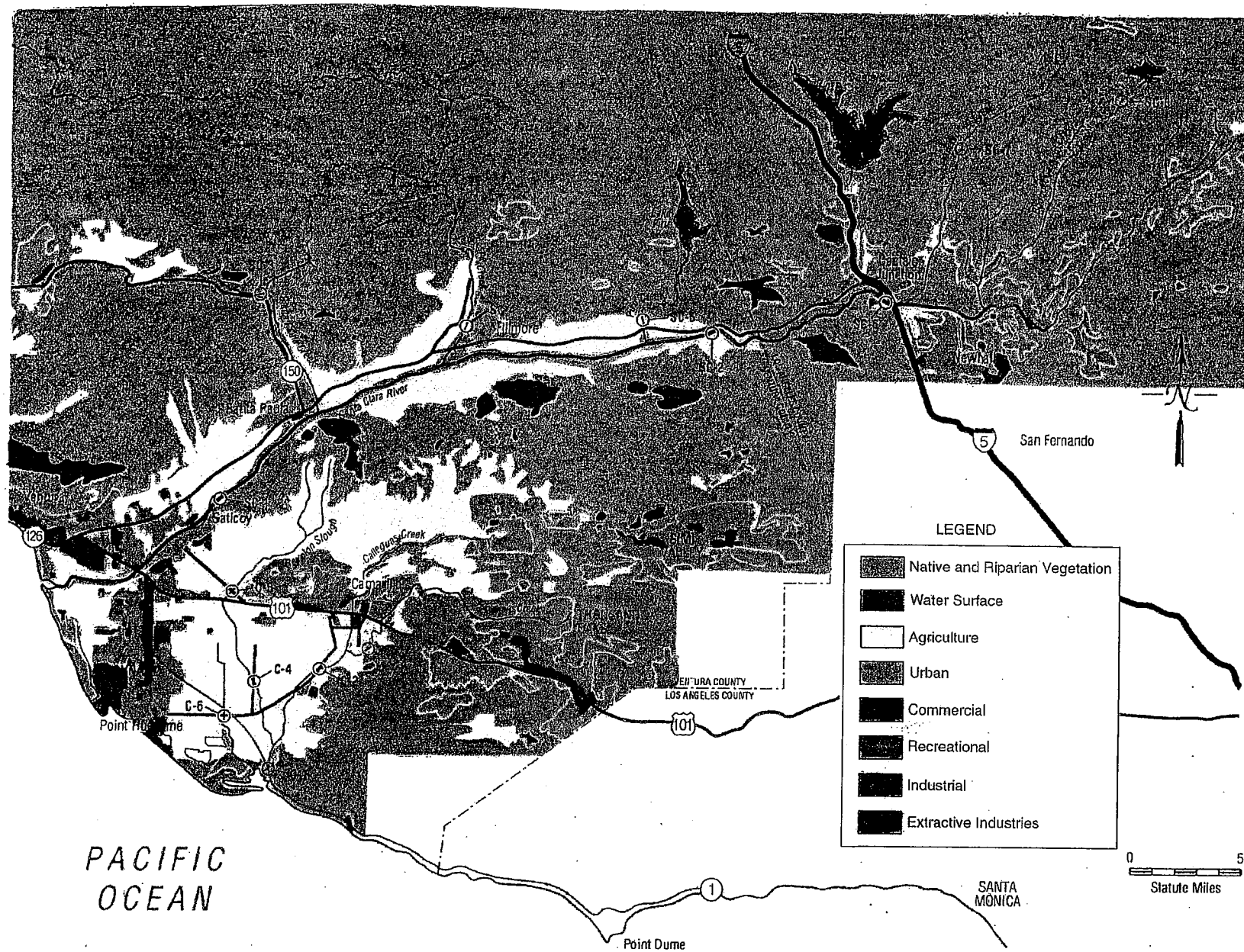

Figure E. Land Use in the Santa Clara River and Calleguas Creek Watersheds

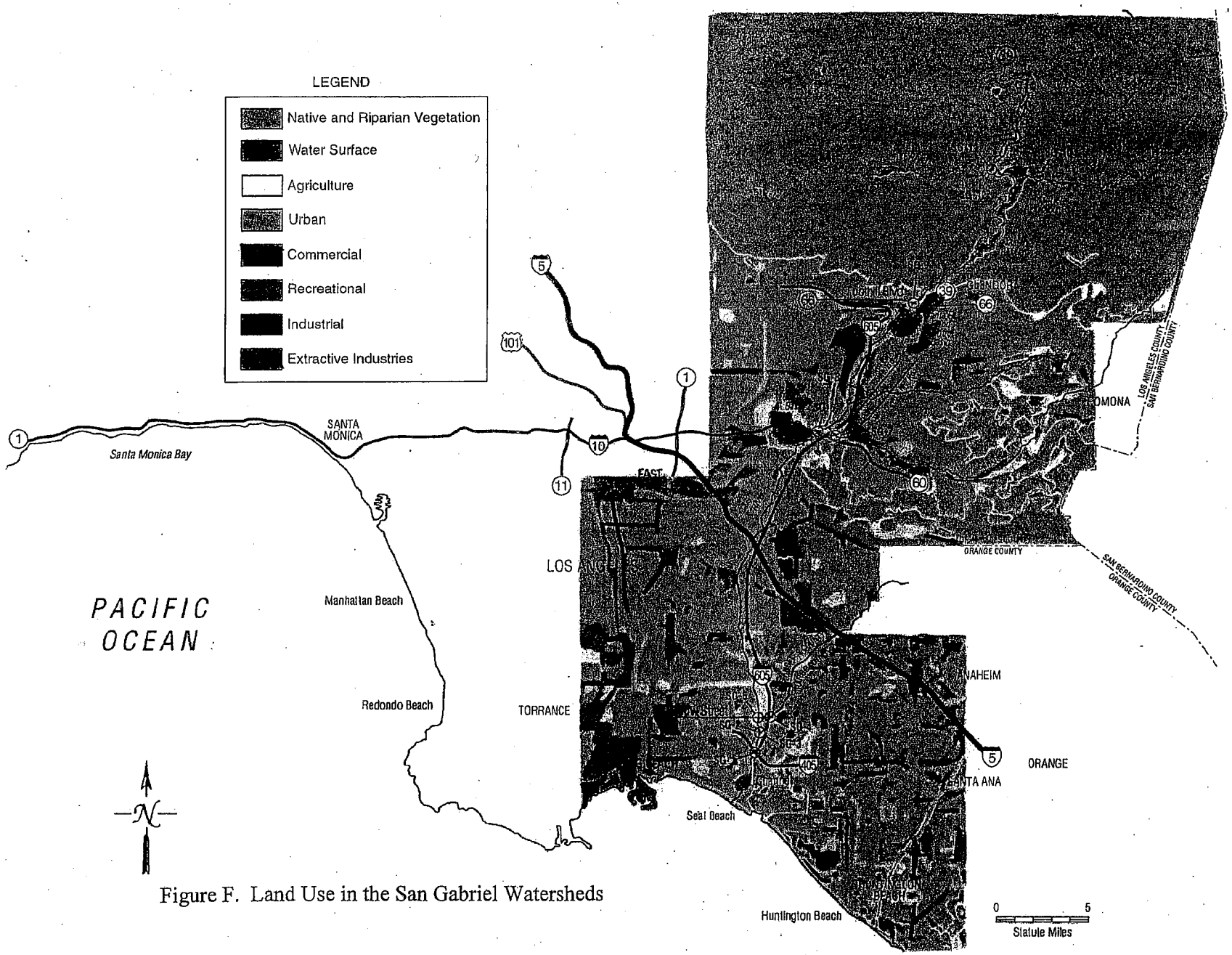

Figure F. Land Use in the San Gabriel Watersheds

MATERIALS AND METHODS

Historical Data Review

Copies of reports describing the San Gabriel River and previous findings of toxicity were submitted to the Aquatic Toxicology Laboratory, U.C. Davis by the Regional Board Contract Manager. These were reviewed and summarized to provide a historical perspective on toxicity studies in this watershed. Maps detailing land use information were obtained from the Department of Water Resources. The information on the maps was reduced in scale and incorporated into maps of each watershed.

Toxicity Studies

Samples were collected quarterly from each watershed. There were a total of twelve sampling sites in the San Gabriel River watershed. These were sampled in June, September and December 1992, and also in March 1993. There were six sampling sites in the Calleguas Creek drainage. These were sampled in July and October 1992 and in January and April 1993. There were eight sites sampled in the Santa Clara River watershed. These were sampled and tested in conjunction with samples from Calleguas Creek.

Grab samples, 100% river water, were used for all tests. Samples were collected by Regional Board Staff in 2- and 4-liter glass bottles and shipped overnight on ice to the Aquatic Toxicology Laboratory at U.C. Davis. At Davis, they were stored in the dark at 4°C. Testing was initiated on all samples within 48 hr of collection. The bioassay methods followed published United States Environmental Protection Agency guidelines (EPA, 1989) for conducting short-term chronic toxicity tests with freshwater organisms. The test species were fathead minnows (*Pimephales promelas*),

a cladoceran (*Ceriodaphnia dubia*) and a green algae (*Selenastrum capricornutum*). Brief descriptions of the procedures follow.

Fathead Minnows - ten < 24-hr post-hatch fathead minnow larvae were exposed in 250 ml sample water in each of four replicate 600-ml beakers. Test solutions were renewed on a daily basis by draining approximately 80% of the solutions from each beaker and refilling the beaker with fresh sample. Mortalities were monitored daily. The fish were fed *Artemia nauplii* 3X daily and the test was concluded after seven-days of exposure. At termination, the surviving fish were anesthetized with MS-222, rinsed and dried to constant weight. Dry weights for each replicate were determined with a Mettler Model AE100 balance. Test endpoints were survival and growth.

Ceriodaphnia dubia - ten < 24 hr neonates were exposed individually to each sample in 20 ml glass scintillation vials. Test solutions were renewed on a daily basis by capturing each neonate in a pipet, discarding the old solution, adding new solution, and replacing the neonate. Any young present were counted and discarded prior to adding fresh solution. An algal:trout chow mix was added to the fresh solutions on a daily basis to provide nutrition for the test organism. The exposure lasted seven days. Test endpoints were survival and production of young.

Selenastrum capricornutum - four replicate 250-ml Erlenmeyer flasks were used for each sample. Samples were filtered through 0.45 μ prior to testing. Each flask contained 100 mls of sample and an initial inoculum of 10,000 cells/ml. Each sample was spiked with nutrients prior to exposure. During exposure, the flasks were maintained on orbital shakers (100 rpm) under constant illumination (400 ft candles). The position of the flasks on the shakers was changed 2X daily to minimize position effects. The exposure was terminated after four days and cell numbers were measured with an electronic particle counter (Coulter Electronics). The test endpoint was cell

numbers.

Controls - control procedures for the fathead minnows and *C. dubia* were similar to the procedures described above for the test solutions except that the test organisms were maintained in laboratory reference water (SSEPAMH) and diluted Institute of Ecology well water (Dilute EI). SSEPAMH was prepared according to EPA moderately hard standards by adding the specified four salts to Sierra Spring water. Dilute EI was made by diluting laboratory well water to EPA moderately hard standards with glass-distilled water. Algal control water was prepared by adding EPA nutrient salts (w/o EDTA) to glass distilled water. The algal medium was passed through a 0.45 μm filter prior to use.

Water Quality Measurements

Water quality was monitored on freshly prepared samples used for renewal and on the 24-hr-old solutions. Measurements included dissolved oxygen (0- and 24-hr samples), pH (0- and 24-hr samples), conductivity (0-hr samples), and hardness and alkalinity (0-hr samples). Total ammonia (as mg/L NH_3-N) was measured if test organism mortality was $\geq$ 50%.

Reference Toxicity Tests

Monthly reference toxicant tests, using dilutions of NaCl, were conducted with all three test species as part of our overall Laboratory Quality Assurance Program initiated in 1992. Tests were conducted in the laboratory reference waters as described above. Appendix I provides information on those tests run concurrently with assays described herein.

Statistics

In cases where the parametric assumptions were met, mortality, growth, reproduction, and cell numbers in the sample waters were compared to performance in the control using Dunnett's Test

in the analysis of variance. In cases where parametric assumptions were not met, Kruskal-Wallis or the Wilcoxon Two-sample Test was used to differentiate between control and treatment effects.

Toxicity Identification Evaluations

Selected Phase 1 and 2 TIE procedures (EPA, 1985a; EPA, 1985b) were applied to toxic samples to characterize the cause of toxicity. The treated solutions were then exposed to the test organisms to see which of the treatments removed the toxicity.

Manipulations of sample pH may enhance, reduce or eliminate effluent toxicity. Compounds whose toxicity can be pH dependent include metals and ammonia. Effluent pHs were either lowered to pH 3 with 0.01 - 1.0 N HCl or elevated to pH 11 with 0.01 - 1.0 N NaOH. The pHs of all pH adjusted samples were returned to their initial measured pH prior to introduction of the animals.

The presence of chelatable materials in the effluent, including divalent metals, was evaluated by adding different volumes of 0.02 M EDTA to 10 mL portions of the sample (EPA 1991). If materials, such as copper, were present at toxic concentrations, the EDTA should selectively bind them and reduce their biological availability. Under these conditions, treated samples that contained metals should have exhibited less toxicity.

Different volumes of 0.16 M $Na_2S_2O_3$ were added to the samples to determine if oxidative compounds (such as chlorine) were present. Some metals will also be chelated by this reagent. Samples were also aerated to determine if the toxicant was volatile or oxidizable.

To evaluate the contribution of non-polar organic chemicals, including pesticides, to toxicity, 200-mL portions of each sample were passed through 3-mL SPE (C-18) columns (Baker) at a flow rate of 10 mL/min with a positive flow pump. The columns were subsequently eluted with MeOH

and an aliquot also tested for toxicity to determine if the sorbed material could be removed from the column. Because C-18 columns selectively remove non-polar organic materials from solution, reduced toxicity in the filtrate suggests that the original toxicity was due to organics that partitioned onto the column. For Phase 2, the SPE columns were eluted with 25, 50, 70, 75, 80, 85, 90, 95 and 100% methanol:water solutions. Toxic fractions were submitted to Eureka Laboratories, Sacramento, CA, for chemical analysis to identify candidate toxicants.

Chemical Analyses

Selected toxic samples were sent for chemical analysis to Eureka Laboratories, California. Not all analyses were conducted with every sample submitted. Methods used to analyze organic toxicants were:

Organochlorines and PCBs	EPA method 8080
Organophosphates	EPA method 614
Carbamates and ureas	EPA method 632
Thiocarbamates	EPA method 630

Methods used to analyze inorganic toxicants were:

Arsenic	EPA method 7060
Mercury	EPA method 7470
Metals	EPA method 6010
Selenium	EPA method 7740

Histopathology

Background and Scope

Environmental studies using fish as sentinel organisms are not new with investigations reported of varying magnitude (Brown, et al., 1979; Christensen, 1980; Hodgins, et al., 1977; Murchelano, 1982; Sindermann, 1979; Sparks, 1972; Wellings, 1969). In each of these, analysis of histologic alterations played a major role.

McCarthy and Shugart (1990a) reviewed difficulties in estimating the extent of exposure to toxic chemicals in the environmental and attributing adverse health or ecological effects to such exposure. Due to the above difficulties, interest has been growing in an approach that evaluates exposure and effects of environmental chemicals and other stressors by the use of "biological markers" in resident fishes. These biomarkers have been defined (McCarthy and Shugart (eds), 1990a; McCarthy and Shugart, 1990b) as "measurement of body fluids, cells or tissues that indicate in biochemical or cellular terms the presence of contaminants or the magnitude of the host response." Histopathology has a major role in this approach. Recently, (Hinton, et al., 1992) evaluated various histopathologic biomarkers and categorized them as "present" or "future." Present markers were those produced in laboratory exposures of fishes to specific chemical toxicants and those that were later verified in field studies where the same toxicants were found to be elevated in sediment or biota. Certain of the biomarkers are included in the EPA's Ecological Monitoring and Assessment Program (EMAP).

General Approach

With a thorough prior knowledge of normal fish anatomy, the investigator uses histological analysis to detect alterations in tissues and organs (Hinton and Couch, 1984) caused by exposure

to toxicants. When the concentration of a toxicant is sufficient to result only in cellular injury, but not in death of the cells, sublethal (adaptive) changes may be observed in affected cells. On the other hand, death of cells without death of the organism is followed by a series of cellular reactions and host responses. When tissues are properly fixed immediately after the animal is euthanized, toxicant-induced, antemortem necrosis can be differentiated from postmortem changes in the overall organ. In a similar fashion, Goede and Barton (1990) have developed a necropsy-based approach to assess fish health and condition. In that examination, grossly observable alterations on surfaces of fish and on their mesenteries and walls of body chambers and on principal organs heart, gills, thymus, kidney, gonads, gut and pancreas are examined.

A combination of the necropsy-based approach and the histological condition index was used in this study. Alterations from the expected normal gross anatomy and microscopic anatomy of resident fishes, fathead minnow (*Pimephales promelas*), goldfish (*Cyprinus carpio*), white croaker (*Genyonemus lineatus*) mosquito fish (*Gambusia affinis*), and tilapia (*Tilapia sp.*) were included in the investigation. All collection sites except Coyote Creek at Willow Street were below POTWS. An immediate question is the ability of fish to move about and be positioned above or below the site of effluent. Such movement could render interpretation as to site-associated causality difficult.

In general, the purpose of this portion of the investigation into chronic toxicity within San Gabriel and Santa Clara Rivers and Calleguas Creek was twofold. First, to determine whether histopathologic biomarkers indicative of exposure to anthropogenic chemicals would be found; and second, to determine the overall microscopic anatomical condition (i.e., health) of these resident fishes. We asked the question "Were alterations present? If so, did prevalence differ with respect to site?" In addition, we were able to differentiate between alterations that were likely more directly

related to infectious disease and/or its sequel, inflammation, and those that were more likely related to toxicant exposure. In some instances, the two (infectious disease and alterations due to toxicant) may be related. Parasitic disease secondary to debilitation and immunosuppression of the host may have made itself manifest under the conditions of this study. Fish culturists are aware that a variety of environmental stressors affect the immune system and result in disease outbreaks and mortality. Anderson (1990) reviewed immunosuppression and disease and cited various challenge tests (metals and corticosteroid drugs) that demonstrated suppression of disease resistance in exposed fish. Field investigations with redbreast sunfish (*Lepomis auritus*) in a Tennessee stream receiving a single point source industrial discharge including PCBs and metals, revealed greatest alteration in a battery of bioindicators at the discharge site and a downstream gradient at three subsequent sites. Volume of tissue occupied by parasites was greatest and functional tissue volume lowest at the discharge site suggesting depression in host resistance (Adams, et al., 1989).

The ability to compare and contrast results in different sites was somewhat facilitated by the finding of sufficient numbers of fishes of the same species. The bulk of the fish included in the study were fathead minnows followed by tilapia and, then, the other species.

General Materials and Methods

Fish were collected by electroshocking or netting and rapidly transported to the bank where necropsy was performed. During necropsy, fish were rapidly opened with scalpel and scissors and placed in fixative solution (Bouin's fluid). This facilitated, by direct contact with various internal organs, penetration of fixative. Upon identification of individuals as to species and site, fish were placed in ten volumes of fluid as above and stored until transportation to UC Davis. At the UC Davis laboratory, fish were removed from Bouin's fixative solution, rinsed in 50% ethanol, and

stored in 70% ethanol until time of further processing. Each fish was cut, with a clean fresh razor blade, into right and left halves by a midline incision from tip of snout through the caudal end of celomic cavity. A transverse section was made immediately caudal to the celomic cavity and this portion of the fish was discarded after examination for gross anatomical lesions. In this way, parasagittal sections through head contained: epidermis and dermis; subdermal connective tissue; lateral line canals; snout; eye; occasional olfactory lamellae; brain including telencephalon, mesencephalon, and rhombencephalon; spinal cord; branchial cavity with gill arches and thymus; pharyngeal, esophageal, and stomach or cranial intestinal bulb portions of digestive tract; head kidney; and inner ear. Larger specimens included a minimum of two sections through the head and two sections through trunk. In smaller specimens, trunk and head remained attached. Parasagittal sections of trunk segments included the following important visceral organs: stomach or intestinal bulb (fathead minnows and goldfish), intestine; liver; gallbladder; exocrine pancreas; mesentery; celomic cavity wall; heart; pericardial cavity; occasional head kidney; trunk kidney; gonad; epaxial and hypaxial skeletal musculature, notochord, and spinal column. In addition, many of the sections contained portions of swimbladder. By orienting the sections in a midsagittal plain, we were able to gain information from the majority of the major organs of individual fish.

All tissues for histopathologic analysis were dehydrated by passage through a graded series of ethanol solutions, cleared in xylene, and embedded in paraffin. Upon embedment, paraffin blocks were sectioned at 6-8 μ thickness and resultant tissue sections were stained by hematoxylin and eosin (H&E) for routine survey. Without knowledge of the site, each of the histopathologic slides was read by a skilled examiner and changes from the normal microscopic anatomy were described for each individual fish. Later, the percentage incidence of alterations was established for each of the

collection sites and representative lesions were photographed for demonstration of alterations in the final report.

Due to the fact that large numbers of fish were made available at one time, some of the individual fish in the study underwent autolysis (necrosis following the extinction of life) and therefore certain cellular alterations in intestinal loops, gill, and ventral stomach, of some fishes were not detectable due to autolysis. However, major lesions affecting architecture of these organs were possible to detect despite autolysis.

RESULTS

SAN GABRIEL RIVER

Background

The headwaters of the San Gabriel River arise in the San Gabriel mountains. Its drainage basin includes the eastern one-half of the Los Angeles basin and the river enters the Pacific ocean near Seal Beach. Since the 1930s, the river has been heavily managed; both dams and concrete and rip-rap modifications have extensively altered the streambed and flow characteristics of the river. In fact, flows in the lower 60 percent of the river are comprised primarily of effluents from sewage treatment plants, industrial discharges, and urban non-point source run-off (SDLAC 1990).

In 1990, EPA conducted a series of toxicity bioassays on samples from the San Gabriel River (Norberg-King and Englehorn, 1990). The test organisms included fathead minnows, *Ceriodaphnia dubia*, and duckweed (*Lemna sp.*). Two of the three samples collected in March 1990 produced acute mortality with fathead minnow larvae and *C. dubia*. Interestingly, neither of these two samples affected duckweed, but the remaining sample, which was collected upstream, reduced the number of fronds on duckweed plants by about 40% when compared with the control (Norberg-King and Englehorn, 1990). The etiologic agent(s) responsible for alterations in the test species were not defined.

For samples collected in June, waters from the same two sites again significantly reduced the survival of exposed fathead minnow larvae and *C. dubia*. In addition, reproductive output of *C. dubia* exposed to the third sample was reduced approximately 25% when compared with the control. No effects on duckweed were observed with any of the three samples (Norberg-King and Engelhorn 1990).

San Gabriel River

Samples were also collected and tested in August, 1990. The two downstream sites again produced acute effects on survival of both fathead minnow larvae and *C. dubia*. The upstream sample also reduced the reproductive output in *C. dubia* over 25% when compared with the controls. Similar to results obtained with the March, 1990 sample, duckweed frond production in the upstream sample was also reduced when compared with the control (Norberg-King and Englehorn, 1990). Since these workers used no TIE follow-up, the agent(s) responsible are not known.

In a communication to the Regional Board, the Sanitation Districts of Los Angeles County commented on the results of the Norberg-King and Englehorn (1990) tests described above (SDLAC 1990). The major issues raised were pH in the bioassays and precise location of the sampling points for the two downstream sites located below two of the District's discharge points for treated municipal effluent. The District suggested pH may have been high enough in bioassay media to result in toxicity from unionized ammonia. The District also pointed out that their permits allowed a chlorine residual in the river from some distance downstream of the discharge points. Thus, if samples were collected in the designated reach, and not dechlorinated prior to testing, the chlorine residual may have been sufficient to cause toxicity.

With respect to the sites sampled in this study, the following descriptions apply. The lower river is concrete-lined and is dominated by tertiary-treated effluent from three POTWs. SG-2 and SG-5 measure this water. SG-1 is slightly downstream of these sites and is located in a semi-natural area that has some tidal influence. SG-4 is located on a concrete-lined tributary (Coyote Creek) composed primarily of urban runoff. SG-3 is further downstream on Coyote Creek and also receives discharge from a major POTW.

Moving upstream, SG-6 is in the middle reach of the river where the channel is unlined. This flow is primarily from one to two (depending on diversions) upstream POTWs. SG-7 is on a tributary (San Jose Creek) and is several miles downstream of a major POTW. This site also contains urban runoff, and may have some other sources. SG-8 is located on another tributary (Walnut Creek) and primarily measures urban runoff from a highly commercialized/industrialized area. This site occasionally receives upstream releases of "clean" drinking water by the Metropolitan Water District. Further upstream, SG-9 receives releases from upstream reservoirs and some urban runoff. SG 10-12 are fairly pristine mountain streams which are heavily used recreationally on weekends and in the summer. A diagram (Fig. G) of the sites and photographs portraying these sites are located at the end of the section on the San Gabriel River.

Toxicity Studies

The results of toxicity tests are summarized in Table 1 presented at the end of this section. Beginning at the upstream portion of the watershed at site SG-12, located on the West Fork of the San Gabriel River, survival of fathead minnow larvae was reduced only once, a sample collected in December, 1992. Growth was reduced in tests with the sample collected in September, 1992. There were no adverse effects on ceriodaphnid reproduction or survival in any of the samples tested from this location. Algae also did not exhibit adverse effects in samples from SG-12.

Samples from site SG-11, North Fork San Gabriel River, did not affect the survival of fathead minnow larvae or *C. dubia*. However, minnow growth was reduced in the September, 1992 sample and *C. dubia* reproduction was inhibited in the sample collected in March, 1993. Samples

from site SG-11 produced no effects on algae.

Samples collected from site SG-10, East Fork San Gabriel River, did not affect fathead minnow survival, *C. dubia* survival and reproduction, or algal cell numbers. However, the growth of fathead minnow larvae was reduced in the September, 1992 sample from this site.

Samples from site SG-9, San Gabriel River at Foothill Blvd., were not tested with all three species in one of the testing events (12/3/92). The fathead minnow test uses the most sample volume per test requiring tests to be restricted to algae and *ceriodaphnia*. Insufficient test water at the time of assay arose due to a broken container during shipment. However, none of the samples tested produced evidence of adverse effects.

The survival of fathead minnow larvae exposed to samples from site SG-8, located on Walnut Creek, was reduced in the March, 1993 sample. Growth was reduced in samples collected in September and December, 1992. No *C. dubia* survived exposure to samples collected from SG-8 in June and December, 1992. Algae exhibited no adverse effects in any of the samples collected from this site.

Fathead minnow survival was reduced at site SG-7, located on San Jose Creek, in the sample collected in December 1992. Growth was reduced in the September 1992 sample. There were no effects on *C. dubia* or algae when exposed to any of the samples from site SG-7.

Samples from site SG-6, San Gabriel River at Peck Road, were only collected in December, 1992 and March, 1993. Growth of fathead minnow larvae and reproduction of *C. dubia* were both reduced when exposed to water collected in December, 1992 at this site.

All of the species were affected by all of the samples collected at site SG-5, the San Gabriel

River at Willow Road. The effects were appreciable, with zero survival in *C. dubia* in all of the testing events, and in fathead minnow larvae, zero survival in three of the four events. Algal cell numbers were reduced to levels less than 30% of the control values at site SG-5.

The survival of fathead minnow larvae exposed to water from site SG-4, located on Coyote Creek, was reduced in samples collected in June, 1992 and March, 1993. There were no adverse effects observed in *C. dubia*, but algal cell numbers were reduced in the March, 1993 sample from site SG-4. This reduction exceeded 90% compared with the control.

The survival of fathead minnow larvae exposed to waters from site SG-3, located downstream of SG-4 on Coyote Creek, was reduced in samples collected in June and December, 1992. Growth was also reduced in the samples collected in September, 1992. Total mortality occurred in *C. dubia* exposed to all of the samples collected from this site. Algal cell numbers were reduced when exposed to samples collected in September and December, 1992 and in March, 1993 from site SG-3.

Samples from site SG-2, located on San Gabriel River near the end of the low flow channel, reduced the survival of fathead minnow larvae in each of the testing events and mortality exceeded 95% in three of the four events. None of the *C. dubia* exposed to samples from this site survived the 7-day exposure period. Algal cell numbers were reduced markedly in each sample tested from site SG-2.

Samples from the tidal prism of the San Gabriel River (SG-1) reduced fathead minnow survival in all of the testing events. The survival of *C. dubia* was also reduced in the June, 1992 and March, 1993 testing events. Algal cell numbers were reduced in all of the testing events with water samples from this site.

Toxicity Identification Evaluations

The results of TIEs conducted on samples from the San Gabriel River are summarized in Table 2. A sample collected from site SG-2 in June, 1992 exhibited total mortality within 24 hours to *C. dubia*. This sample was extracted with an SPE column and the column eluted with different methanol:water fractions. All toxicity was removed following SPE treatment and the toxicity was present in the 80 and 85% methanol fractions. This sample was retested to determine the effects of other treatments. When adjusted to pH 3 toxicity was removed but it remained at pH 11. Aeration also removed toxicity, but treatment with EDTA and sodium thiosulfate did not.

A sample collected at site SG-2 in September, 1992 produced 100% mortality in *C. dubia* within 48 hrs. This sample was only treated with EDTA which appeared to partially delay the mortality. However, the significance of this result is difficult to judge since no other treatments were run on this sample.

A sample collected in December, 1992 at site SG-2 produced 100% mortality in *C. dubia* within 48 hrs. Toxicity appeared to be lower at the time the TIE was conducted, so it was difficult to evaluate the results of all of the TIE treatments against background mortality. Nonetheless, 100% mortality occurred in the 85% methanol:water fraction, and 80% mortality occurred in the 90% methanol:water fraction. Thus, an organic constituent was again implicated as the cause of toxicity. These methanol fractions were subsequently treated with EDTA, which had no effect, further suggesting an organic material as the cause of toxicity.

A sample from site SG-2 collected in March, 1993 demonstrated toxicity to both fathead minnows and *C. dubia*. pH treatment had no effect on the toxicity of the samples to *C. dubia*, nor

did treatment with EDTA, sodium thiosulfate, or aeration. However, the SPE column removed the toxicity which was recovered in a methanol eluate from the column. Thus, an organic constituent was again implicated as the source of toxicity to *C. dubia*. Unfortunately, by the time the TIE was performed with fathead minnows (13 days after collection), insufficient toxicity remained in the sample.

A sample collected from site SG-3 in June, 1992 produced 100% mortality in *C. dubia* within 24 hrs. Aeration eliminated the toxicity as did filtration and treatment with SPE column. EDTA and sodium thiosulfate had no effect on toxicity. Treatment with pH 3 and 11 postponed the onset of death. No methanol elution of the column was made but the lack of effect of EDTA and sodium thiosulfate suggest that the toxic constituent was not a divalent cation or an oxidizing agent.

Another sample from site SG-3, collected in September, 1992 produced 90% mortality in *C. dubia* within 48 hrs. This sample was treated only with EDTA which may have reduced the onset of toxicity for 24 hrs.

A sample from site SG-3 collected in December, 1992 also exhibited toxicity to *C. dubia*. Treatment with an SPE column eliminated the toxicity, which was confirmed in a methanol elution of the column. Treatment with pH 3 and 11, aeration, and filtration appeared to postpone the onset of the effect, but EDTA and sodium thiosulfate had no effect. These data suggest that an organic constituent was responsible for toxicity.

A sample from site SG-5 (June 1992) produced acute mortality in *C. dubia*. Aeration at pH 3 reduced toxicity, as did filtration at pH 3, and treatment with pH 3 alone. pH 11 had no effect on toxicity, nor did sodium thiosulfate. Treatment with SPE columns also reduced the toxicity and

EDTA may have postponed the onset of effects by 24 hrs. No methanol elution from the column was made.

Another sample from site SG-5, collected in September, 1992 also exhibited toxicity to *C. dubia*. EDTA was the only treatment applied to this sample and may have postponed the effect for 24 hrs.

Another sample obtained from this site in December, 1992, also produced mortality in *C. dubia*. Filtration reduced toxicity as did treatment with SPE columns. Toxicity was confirmed in the 85% methanol fraction eluted from the column. These data suggest that an organic chemical was responsible for the observed effect.

A sample collected from site SG-5 in March, 1993 produced toxicity to *C. dubia* and fathead minnows. Treatment with pH 3 and 11 had no effect on the toxicity to *C. dubia*. Similarly, EDTA, aeration, and sodium thiosulfate also had no effect on toxicity. Treatment with SPE column reduced the toxicity and toxicity was confirmed in the methanol elution of the column. Unfortunately, toxicity was no longer manifest in this sample to fathead minnows when the TIE was initiated 13 days after collection.

A sample collected from site SG-8 in June, 1992 produced acute mortality in *C. dubia*. Treatment with an SPE column eliminated the toxicity but no methanol elution was run. Treatment with EDTA and sodium thiosulfate had no effect on toxicity. Treatment with pH 3 and 11 and aeration may have reduced the toxicity. These data suggest that an organic constituent was responsible for toxicity.

A sample collected from site SG-8 in December, 1992 initially exhibited toxicity to *C. dubia* but no toxicity remained when the TIE was initiated four days later.

Chemical Analyses

Chemical analyses results are shown in Table 3. Hardness values are given in Table 3 for all chemical analyses that detected metals in the sample. Chemical results by date are discussed below.

Ceriodaphnid mortality was 100% in waters collected from SG-8 on June 3, 1992. Of the pesticides detected, diazinon at a concentration of 3.6 μg/l was approximately 9X above the ceriodaphnid LC50 (Bailey et al., 1996). Passage of the sample through a C18 column removed toxicity to ceriodaphnids, indicating that the source of toxicity was an organic.

Both fathead minnows and ceriodaphnids were significantly affected by a sample collected from SG-2 on September 9, 1992. Based on EDTA TIE manipulations with ceriodaphnids, a metal scan of the samples was conducted. Metals detected were barium, molybdenum and zinc. Metal toxicity is dependent on a number of biotic and abiotic factors, therefore, it is difficult to determine if one or more of these metals were responsible for the observed toxicity, however, with respect to zinc, 4 day EC50s for *Chlorella vulgaris* and *Chlorella saccarophila* were 2400 and 7100 μg/l (Reyes et al. State of California Regional Water Quality Board Report, in prep.). No hardness values were reported for either of these zinc values. For ceriodaphnids, acute zinc toxicity was observed at 32 μg/l and a hardness of 45 mg/l as $CaCO_3$ (Reyes et al., State of California Regional Water Quality Board Report, in prep). A life-cycle test using fathead minnows conducted at a hardness of 46 mg/l resulted in a chronic value of 106.3 μg/l (Reyes et al, State of California Regional Water Quality Board Report, in prep). Hardness of SG-2 collected in September was 220 mg/l as $CaCO_3$.

Like SG-2 collected in September, only ceriodaphnid EDTA TIEs were conducted on the

sample collected at SG-3 on September 9, 1992. Analytical results were similar to SG-2. A metals scan detected barium, molybdenum and zinc. Metal toxicity is dependent on a number of biotic and abiotic factors, therefore, it is difficult to determine if one or more of these metals were responsible for the observed toxicity, however, with respect to zinc, 4 day EC50s for *Chlorella vulgaris* and *Chlorella saccarophila* were 2400 and 7100 μg/l (Reyes et al, State of California Regional Water Quality Board Report, in prep). No hardness values were reported for either of these zinc values. For ceriodaphnids, acute zinc toxicity was observed at 32 μg/l and a hardness of 45 mg/l as $CaCO_3$ (Reyes et al, State of California Regional Water Quality Board Report, in prep). A life-cycle test using fathead minnows conducted at a hardness of 46 mg/l resulted in a chronic value of 106.3 μg/l (Reyes et al, State of California Regional Water Quality Board Report, in prep). Hardness of SG-3 collected in September was 200 mg/l as $CaCO_3$.

Both ceriodaphnids and algae were significantly affected by the sample collected at SG-5 on September 9, 1992. Only ceriodaphnid EDTA TIEs were conducted. A chemical analysis of the sample detected three metals, barium, molybdenum and zinc. Metal toxicity is dependent on a number of biotic and abiotic factors, therefore, it is difficult to determine if one or more of these metals were responsible for the observed toxicity, however, with respect to zinc, 4 day EC50s for *Chlorella vulgaris* and *Chlorella saccarophila* were 2400 and 7100 μg/l (Reyes et al, State of California Regional Water Quality Board Report, in prep). No hardness values were reported for either of these zinc values. For ceriodaphnids, acute zinc toxicity was observed at 32 μg/l and a hardness of 45 mg/l as $CaCO_3$ (Reyes et al, State of California Regional Water Quality Board Report, in prep). A life-cycle test using fathead minnows conducted at a hardness of 46 mg/l

resulted in a chronic value of 106.3 μg/l (Reyes et al, State of California Regional Water Quality Board Report, in prep). Hardness of SG-5 collected in September was 200 mg/l as $CaCO_3$.

Both ceriodaphnids and fathead minnows were significantly affected by the sample collected on December 2, 1992 at SG-2. Due to the loss of sample toxicity, TIEs conducted with ceriodaphnids were inconclusive. The waters collected from this site were analyzed for metals and organophosphate pesticides. Three metals, barium, molybdenum and zinc were detected at this site. No organophosphate pesticides were detected. Results of phase II TIEs indicated that the cause of toxicity may have been an organic. If an organic was responsible for toxicity, then it may have decayed to a concentration below the detection limit and led to the negative TIE results.

All three species were significantly affected by the sample collected on December 2, 1992 at SG-3. Ceriodaphnid TIE results indicated an organic as the source of toxicity, however, no organophosphate pesticides were detected, however, three metals, barium, molybdenum and zinc were detected.

Histopathology

Fish were collected from four sites in the lower San Gabriel River watershed. The sites included Coyote Creek above and below the Long Beach wastewater treatment plant outfall, the San Gabriel River at the confluence of Coyote Creek, and from the tidal prism at College Park Drive. The sites and fish species collected are summarized below.

Collection Sites, Identification Codes, Fish Species, Number of Individuals for Histopathology Studies in the San Gabriel River and its Tributaries

Site	Fish Species	Number
Coyote Creek at Willow Street (above outfall)	*Tilapia sp.* *Gambusia affinis*	19 3
Coyote Creek below outfall	*Tilapia sp.*	15
San Gabriel River Tidal Prism at confluence with Coyote Creek just south of end of concrete channel	*Tilapia sp.*	21
San Gabriel River Tidal Prism at College Park Drive*	*Tilapia sp.* *Genyonemus lineatus*	30 1

*Fish from this site were collected using boat and suspended gill nets

Coyote Creek above Outfall at Willow Street

Fish collected at this site included 19 tilapia (*Tilapia sp.*) and 3 *Gambusia affinis*. Inflammatory foci of both the eye including choroid and optic nerve and the trigeminal nerve were observed at equal frequency (27%). These inflammatory foci involved either eosinophilic granular leukocytes or macrophages containing similarly staining granules. The inflammatory cells were clustered around the choroid rete, the optic nerve (Figure 1, Histopathology figures located at end of this section), the trigeminal ganglion, the trigeminal nerve and its branches, and associated areolar connective tissue of the adjacent head (Figures 2 and 3). Alterations in glial cells produced a swollen appearance around nerve axons. A 5% frequency of gill parasitism primarily involving lamellae and filaments was observed. No evidence of necrosis and of aneurysm formation was seen. Inflammation of the gill and adjacent branchial cavity wall (inner epithelial layer covering

operculum) was seen at 27% incidence. The buccal cavity roof in one fish showed extensive infiltration of eosinophilic granular leukocytes (Figure 4). One of the individuals studied showed adhesions and granuloma formation within the peritoneal cavity and involving adjacent mesentery. Inflammation of mesentery was present at a 9% frequency. A nematode parasite was in the gut lumen of one fish (Figure 5). Within livers, 3 of the 22 individuals showed perihepatic venous inflammation and necrosis (a 14% frequency) (Figure 6). More than half of the individuals showed glomerular and tubular regeneration. However, the extent of this condition was judged to be within normal limits. Fourteen percent of the individuals analyzed showed tubular epithelial hyaline granules. These granular formations occupied the majority of the cytoplasm of these epithelial cells. This apparently degenerative response was associated with further change in 18% of the individuals examined. This advanced change involved calcification of tubular epithelium (18% frequency). In addition, inflammatory foci within interstitium of kidney were observed at equal frequency to tubular calcification. Heart, specifically atrium, showed signs of alteration (Figure 7).

Coyote Creek Below the Outfall

Fifteen tilapia were collected from this site. When the head region of one of these fish was sectioned in a parasagittal plane, various organs could be identified and analyzed (Figure 8). Figure 9 shows those organs that are visible in a parasagittal plane through the trunk region of the fish. Eosinophilic granular leukocyte infiltration of eye was seen in one fish. However, the same type of inflammation much more frequently involved the trigeminal nerve (73% frequency). In the gill, no parasites were observed. However, necrosis of mitochondria-rich cells was seen with a 33% frequency. The gills were free of aneurysms. In addition to necrosis of mitochondria-rich cells, the

pavement respiratory epithelial cells of the secondary lamellae were also necrotic (frequency = 27%) (Figure 10). Inflammation of gill and branchial cavity was not observed. The livers of these fish were free of alterations. In addition, there were no adhesions, granuloma, or inflammatory foci within the peritoneal cavity and/or its mesenteries. Hyalin degeneration of kidney tubular epithelial cells (Figures 9 and 11) was seen at high frequency (60%). The skin was free of inflammatory foci. Sections through gut revealed no parasites and food particles were observed in all of the fish examined indicating their recent feeding status. The stomach was free of lesions. In one fish, an atretic ovarian follicle was observed. Inflammation of the buccal cavity epithelium was seen at a frequency of 13%. Hyperplasia of the gas gland epithelium and debris within the lumen of the gland was seen at a 20% frequency (Figure 9).

San Gabriel River Tidal Prism at Confluence with Coyote Creek just South of End of Concrete Channel

A total of 21 tilapia (*Tilapia sp.*) were collected at this site. The central nervous system was free of alteration. Extensive inflammation of the trigeminal ganglion was observed with cells that had characteristics of eosinophilic granular leukocytes. An alternative would be that the cells were macrophages having phagocytosed eosinophilic granules. Whatever the exact nature of the cell, the process was inflammatory in nature and involved the trigeminal nerve and its branches. The cells in question were associated with a swollen feature of the nerve indicating damage to the glial cells. The frequency of this abnormality was 33%. Gill necrosis was observed in 3 of the animals studied and this involved mitochondria-rich (chloride) cells and pavement respiratory epithelium. The frequency for this lesion was 14%. No aneurysms of the gill were found and no parasites were

observed. No lesions were seen within the peritoneal cavity and an absence of adhesions and granulomas within this space was observed. No liver alterations were encountered. Inflammation of gill arches and branchial cavity epithelium was observed in 2 of the individuals studied. The frequency of this alteration was 9%. Two of the individuals showed renal pathology. In one of these, extensive severe tubular epithelial hyalinization had occurred. This was associated with disruption of the nephron wall at that site. In another individual, interstitial inflammation was observed. Skin necrosis was found in 2 of the 21 animals observed. One gut parasite was found and appeared to be a tapeworm. No changes were seen within ovary or testis, and the buccal epithelium was free of change. No alterations were observed in exocrine pancreas, heart, and pericardial cavities.

San Gabriel River Tidal Prism at College Park Drive

A total of 30 tilapia (*Tilapia sp.*) and 1 white croaker (*Genyonemus lineatus*) were examined from this site. Histopathologic examination revealed severe inflammation in submucosa and circular muscularis of the stomach (Figures 12 and 13). The inflammatory cells were eosinophilic granular leukocytes or macrophages which contained eosinophilic granules. In addition to this change, the white croaker showed mild inflammation around bile structures in the liver and inflammatory response in the wall of the heart. In addition, macrophage aggregates were present in the liver at a frequency of 3 per 10 X field. The white croaker also showed mild inflammation of the gill and two flukes (parasitic trematodes) were attached to gill structures. In the 30 tilapia, fairly consistent involvement of the eosinophilic granular leukocytes in inflammatory foci around the trigeminal ganglion and branches of the trigeminal nerve were seen. The frequency of this lesion was 30%.

In addition to the changes within the 5th cranial nerve, alterations were seen in gills that indicated that 3 of the 30 individuals showed aneurysm formation in blood vessels of secondary lamellae. In addition, inflammation of gill arch and filaments and adjacent regions of the branchial cavity wall were seen. The frequency for this lesion was 17%. Inflammation of the liver in areas adjacent to arterial structures and large tributaries of the hepatic venous system were seen (Figures 14 and 15). The inflammatory cells were usually eosinophilic granular leukocytes (Figure 16). The frequency for this change was 13%. Two of the fish showed inclusion bodies within hepatocytes (Figure 16). These were quite frequently seen and were close in resemblance to the tubular epithelium hyaline granules of the kidney. In addition, 4 fish showed interstitial inflammation of the kidney and 5 showed extensive degeneration with tubular epithelium showing hyaline change (Figure 17). The frequency for the latter was 17%. Some of the tubular degenerative changes had advanced to the formation of tubular deposits of calcium and this characterized 2 of the 30 individuals (Figure 17). Heart ventricular mineralization was also seen (Figure 18) in 4 of the 30 individuals examined. Skin necrosis involved 2 of the 30 individuals and was a consistent change in the affected fish. A large skin lesion was observed on one tilapia (Figures 19, 20, 21). One fish showed a parasite within the gut lumen.

Summary of Findings

Because sampling and testing were conducted on a quarterly basis, the data set imposes limitations on the extent of conclusions that can be drawn. For example, only very limited conclusions can be drawn with respect to the frequency of toxicity at different sites, the causative

toxicants, as well as seasonal trends. Nonetheless, it is possible to compare upstream and downstream sites with respect to overall frequency of effects, the species involved, the association of the different sites with POTWs, and the characteristics of the toxic constituents found in the TIEs. Finally, the histopathologic conditions may be compared between the four sites that were sampled.

Although the TIE portion of testing emphasized samples that caused acute mortality to *C. dubia*, virtually all of the results implicated organic chemicals as causing toxicity. These results suggest an organic toxicant that is labile at low pHs. One candidate chemical, the organophosphate pesticide, diazinon, is labile at low pHs, is removed by SPE columns and elutes in the 80-85% range of methanol. Used primarily for urban and agricultural pest control, this pesticide is also found in effluent from POTWs.

Ammonia levels were also generally elevated at SG-5 and sites located further downstream. Earlier work suggested that ammonia may play a role in toxicity in this watershed (Norberg-King and Englehorn, 1990). However, no TIEs were conducted by EPA to confirm the contribution of ammonia to toxicity.

Collectively, these data suggest that water quality in this system is impaired and that there is an incremental downstream gradient to toxicity. Generally, sites above SG-5 were associated with intermittent toxicity that affected only one of the test species in a given testing event. The exception to this is site SG-8, which was located on Walnut Creek, a tributary of the San Gabriel River. This site exhibited more consistent toxicity and, in particular, was highly toxic to *C. dubia* in two of the four testing events. The land use map suggests urban and industrial sources may contribute to toxicity at this site.

Conversely, beginning at SG-5, multiple species were affected within the same testing event and the effects were generally large. The fact that all three species responded suggests that multiple toxicants were present in the samples. In a notable exception, samples from SG-4, a tributary to the main river, generally exhibited less toxicity than samples collected from the main river in this same reach.

Inflammatory foci of both eye and the fifth cranial or the trigeminal nerve were prominent findings in fish collected from Coyote Creek above the outfall at Willow Street. It would be impossible to directly attribute this infectious process to toxicity (Rogers and Gaines Jr, 1975; Wolke, 1975). However, evidence is accumulating which indicates that metals and some organics such as polychlorinated biphenyls interfere with the immune system of the host (Anderson, 1990; Zeeman and Brindley, 1981). With a compromise in the immune system, parasites and bacteria may establish infestation (Anderson, 1990). It is possible that the infectious lesions of eye and trigeminal nerve reflect prior immunoincompetence. Hawkes and Stehr (1982) reported lesions of brain and eye in embryo surf smelt exposed to crude oil. An additional finding was inflammation of the liver in perihepatic venous sites. This condition could have followed prior hepatocyte necrosis (Hinton, et al., 1992; Meyers and Hendricks, 1985). Fourteen percent of the individuals analyzed showed tubular epithelial hyaline granules. This condition is degenerative and is often associated with necrosis of tubular epithelial cells (Meyers and Hendricks, 1985). Further stages of the process were indicated by the blue, calcified foci in tubule walls. This tertiary repair followed cellular necrosis (Reimschuessel, et al., 1989; Reimschuessel, et al., 1990). Renal tubular cellular necrosis is a manifestation of exposure to various toxicants including salts of metals (Meyers and Hendricks,

1985) and may correlate with water column toxicity in the three species tests. Even if the inflammation was not associated with contaminants, the fact that a sizeable fraction (25%) of the fish examined showed disease, indicates that the fish are compromised and would likely be endangered further by deterioration of water quality (Sindermann, 1993).

In the fish from the downstream site of Coyote Creek below the outfall, a higher percentage (73) showed inflammation of the trigeminal nerve. Also, necrosis of mitochondria-rich (chloride) cells and pavement epithelium of secondary lamellae were seen. Gills of fish from contaminated sites (Hinton, 1993a; Hinton and Laurén, 1990; Sindermann, 1993) have been shown to contain various lesions and necrosis in the above cell types is a common finding. Also, kidney tubular epithelial cell degeneration was present at higher prevalence than at the upstream site. Taken together, it would appear that fish below the outfall show evidence of tissue alteration which is higher in prevalence and more severe than at other sites. Clearly, these fish are not normal and would likely be susceptible to additional stress from deteriorating water quality.

Additional studies of upstream and downstream sites in the San Gabriel River Tidal Prism revealed toxicity. Inflammatory lesions were prevalent at about 30% in fish from both sites. Gill toxicity reactions were seen at equal frequency. In the upper site, only two fish showed extensive tubular epithelial hyalinization of kidney while 5 of their counterparts from the lower site were positive for the same lesion. In addition, the lesions had advanced in the downstream affected fish to the point at which tubular deposits of calcium were prominent in two fish. Heart ventricle also showed mineralization, a likely sequel to systemic infection (Ferguson, 1989). Skin necrosis, likely a direct result of toxicity in the water column characterized two of the 30 fish at the lower site.

The analysis of fish collected from the San Gabriel River and its tributaries suggests that a sizeable portion of the individuals are victims of infectious disease and a smaller portion reveal signs of toxicity. These are not healthy fish and their tissue conditions do not resemble those of fishes from reference habitats previously investigated by this group (Adams, et al., 1989; Ashley, 1975; Baumann, et al., 1987; Brown, et al., 1979; Ellis, 1985; Ellis, et al., 1978; Hinton, et al., 1984b). Epithelial necrosis of gill and skin are adverse effects likely associated with toxicity within the water column (Hawkes, 1977; Meyers and Hendricks, 1985; Ribelin and Migaki (eds), 1975).

Recommendations

Although the data are limited to only four testing events, the results suggest impaired water quality, particularly in the lower portion of the watershed. Toxicity was primarily evidenced by effects on *C. dubia*, but the fish and algae were also affected. The relatively poor water quality demonstrated by the toxicity tests was corroborated by the histological findings which indicated that fish collected in these areas exhibited tissue damage consistent with compromised water quality.

In general, the TIEs uniformly implicated non-polar organic chemicals as the source of toxicity to *C. dubia*. In cases where methanol:water fractionation was performed, the toxic fractions were contained in the 80-90% methanol fractions. These fractions are consistent with organic compounds such as the organophosphate pesticides diazinon and chlorpyrifos. Although purely speculative with respect to causing toxicity in these particular samples, these two pesticides are widely used in urban applications and frequently reach acutely toxic concentrations in stormwater and POTW discharges. The presence or absence of these chemicals could be readily confirmed

using current research methodology.

The consistency of toxicity even in the limited sampling program described herein suggests that water quality in the San Gabriel River should be markedly improved by a program that identifies toxicants present in the river in conjunction with a follow-up program to reduce their concentrations. Consequently, a more intensive sampling program should be implemented. This should also provide greater clarification of the role ammonia plays with respect to adverse water quality. Based on toxicity, samples should be subjected to TIEs to determine which constituents, including ammonia and pesticides, are major contributors to toxicity. By implementing Phase II and III TIE procedures, using sensitive analytical chemistry methods, it should be possible to determine the identities of these toxicants. Once these constituents are characterized, programs aimed at reducing their concentrations should be implemented, in conjunction with follow up monitoring to evaluate the program's effectiveness.

Diagram and Photographs of Sampling Sites

San Gabriel River

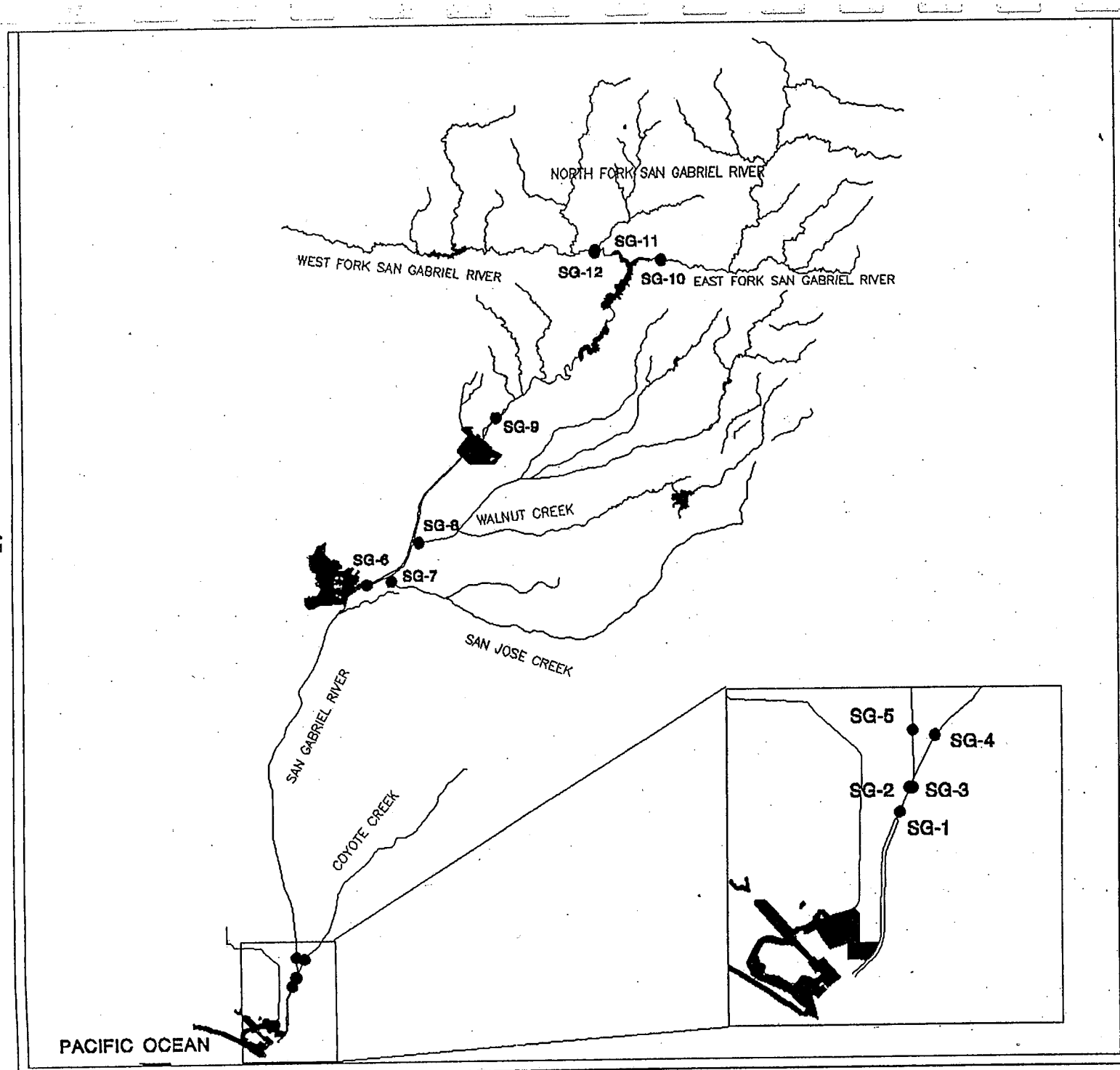

Figure G. San Gabriel River Toxicity Sampling Sites

SG-1: San Gabriel River at Tidal Prism
SG-2: San Gabriel River near end of concrete low flow channel
SG-3: Coyote Creek near end of concrete low flow channel
SG-4: Coyote Creek at Willow
SG-5: San Gabriel River at Willow
SG-6: San Gabriel River at Peck Rd.
SG-7: San Jose Creek at Workman Mill Road
SG-8: Walnut Creek at Baldwin Park Blvd.
SG-9: San Gabriel River at Foothill Blvd.
SG-10: San Gabriel River (East Fork) at Fire Camp
SG-11: North Fork San Gabriel River
SG-12: West Fork San Gabriel River west of Azusa County Road

CALIFORNIA
REGIONAL
WATER QUALITY
CONTROL BOARD
LOS ANGELES REGION
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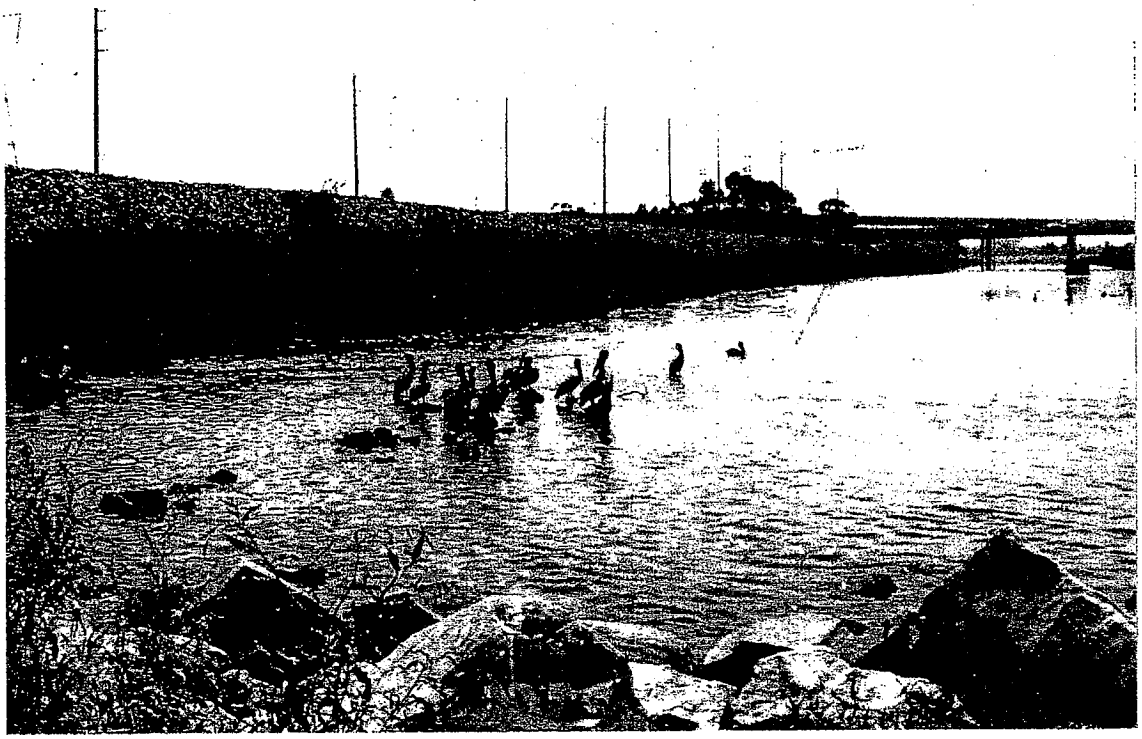
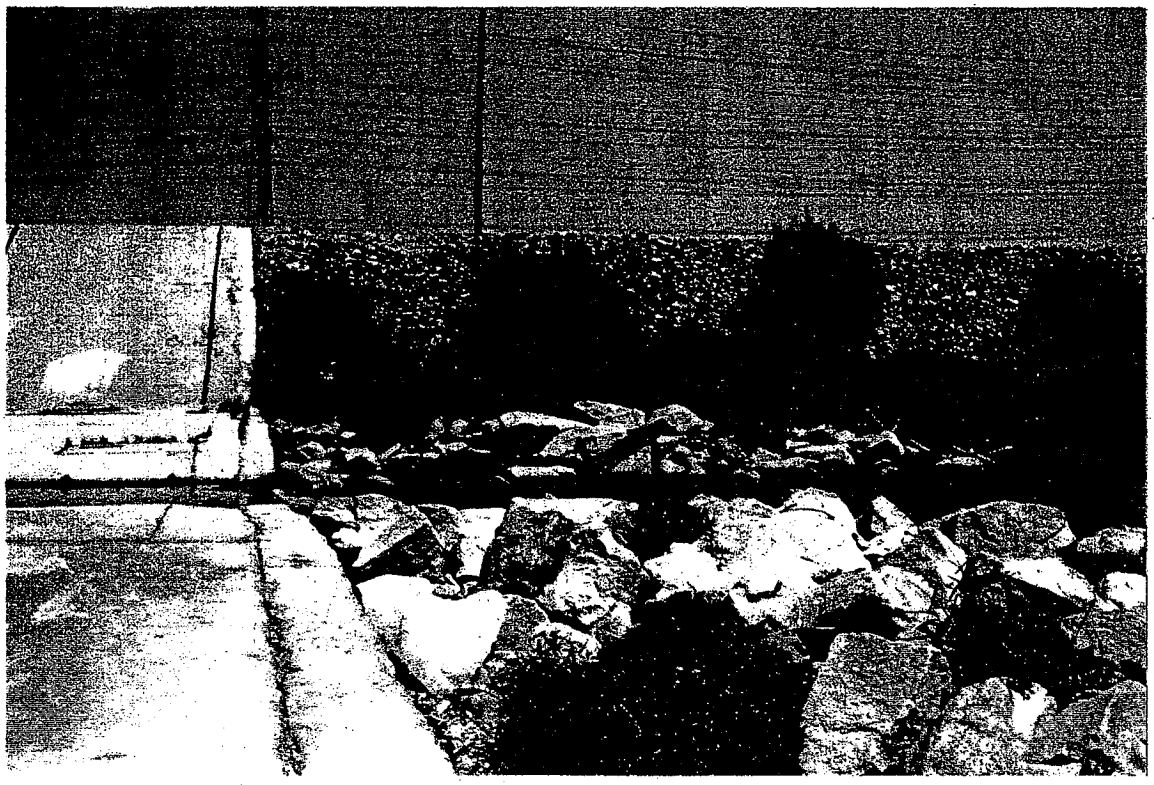
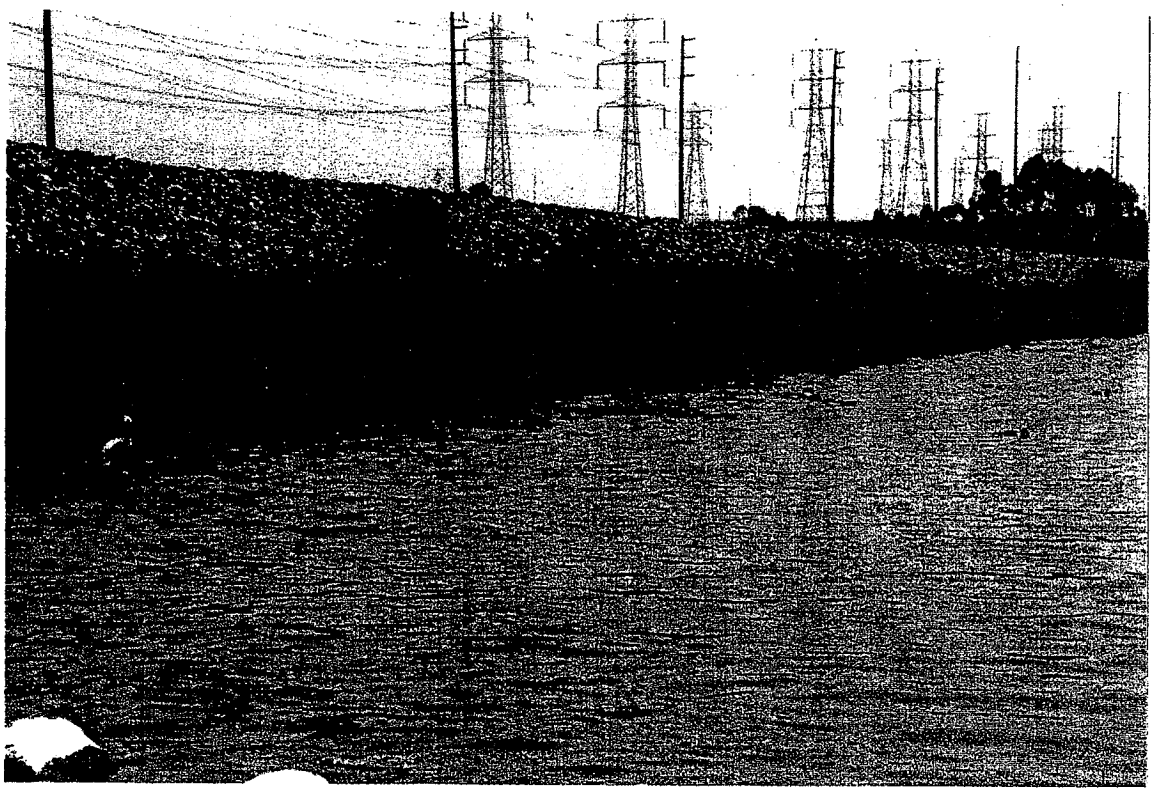
SG-1 SAN GABRIEL RIVER TIDAL PRISM AT 405 FREEWAY
6/3/92



PELICANS IN THE UPPER SAN GABRIEL TIDAL PRISM JUST SOUTH OF CONCRETE LINING
9/9/92

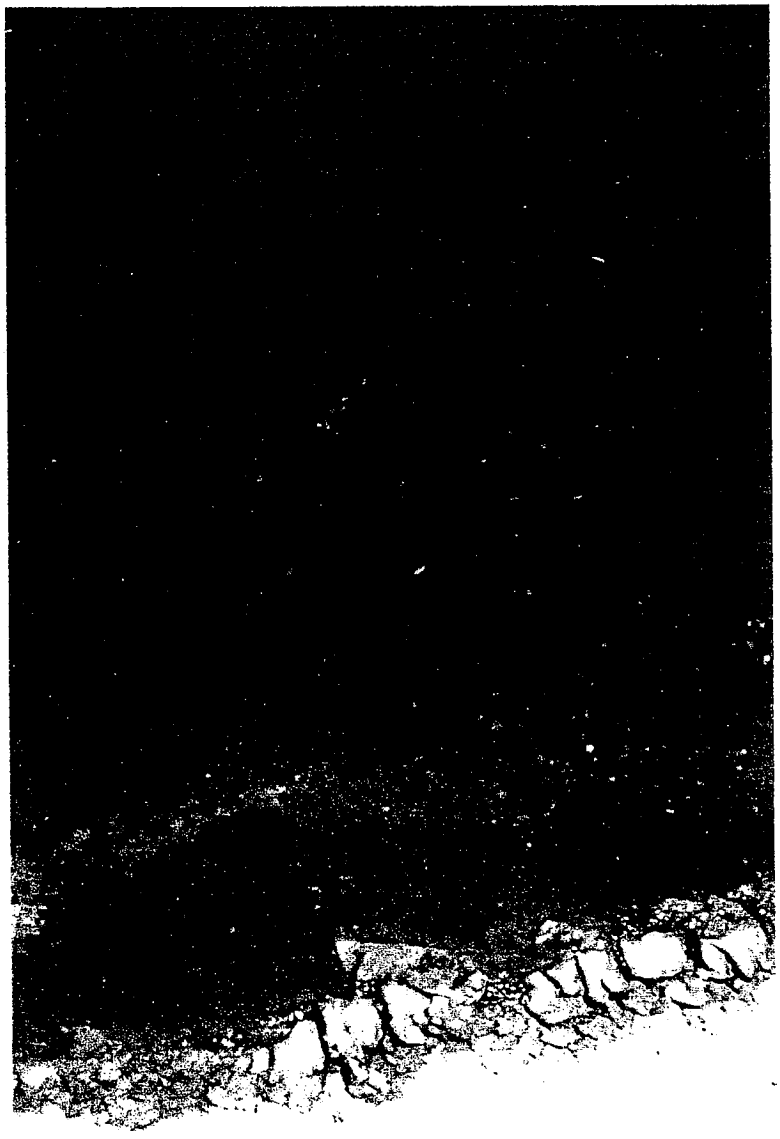


SG-3 SAN GABRIEL RIVER AT END OF CONCRETE CHANNEL
(COYOTE CREEK SIDE)
6/3/92

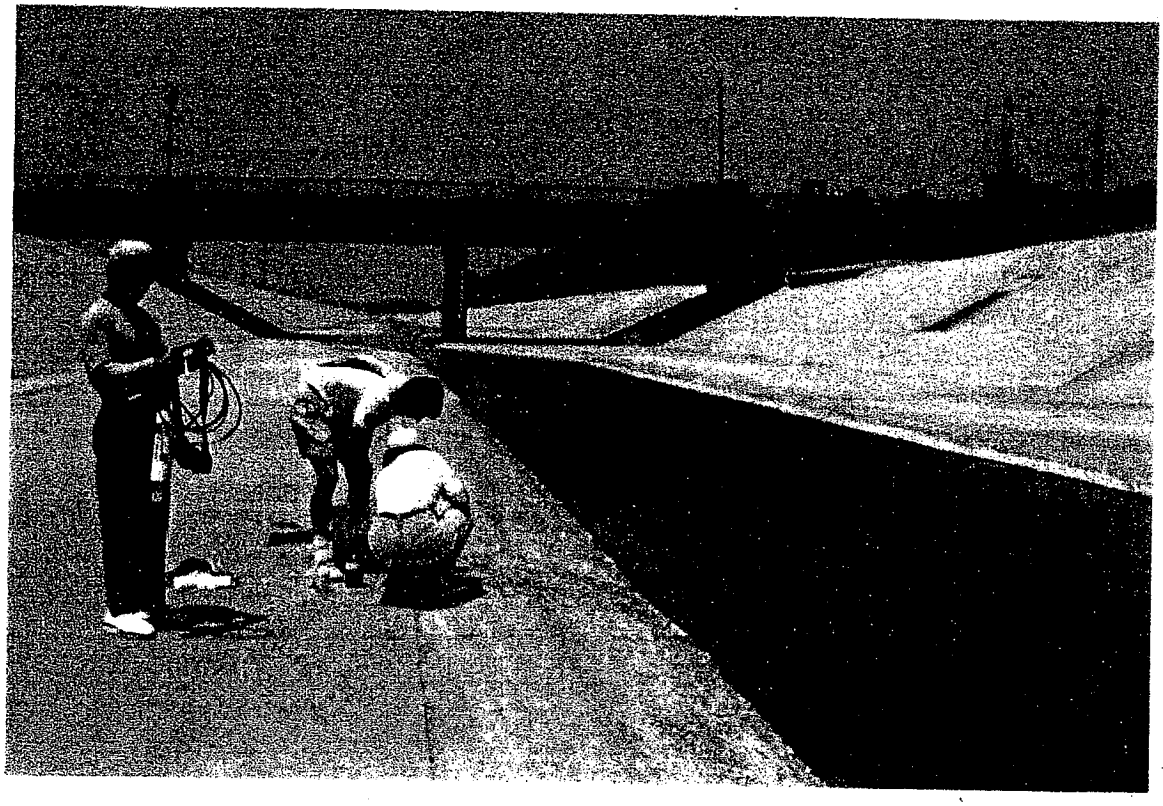


DOWNSTREAM VIEW TOWARD TIDAL PRISM

SG-4 COYOTE CREEK AT WILLOW 6/3/92

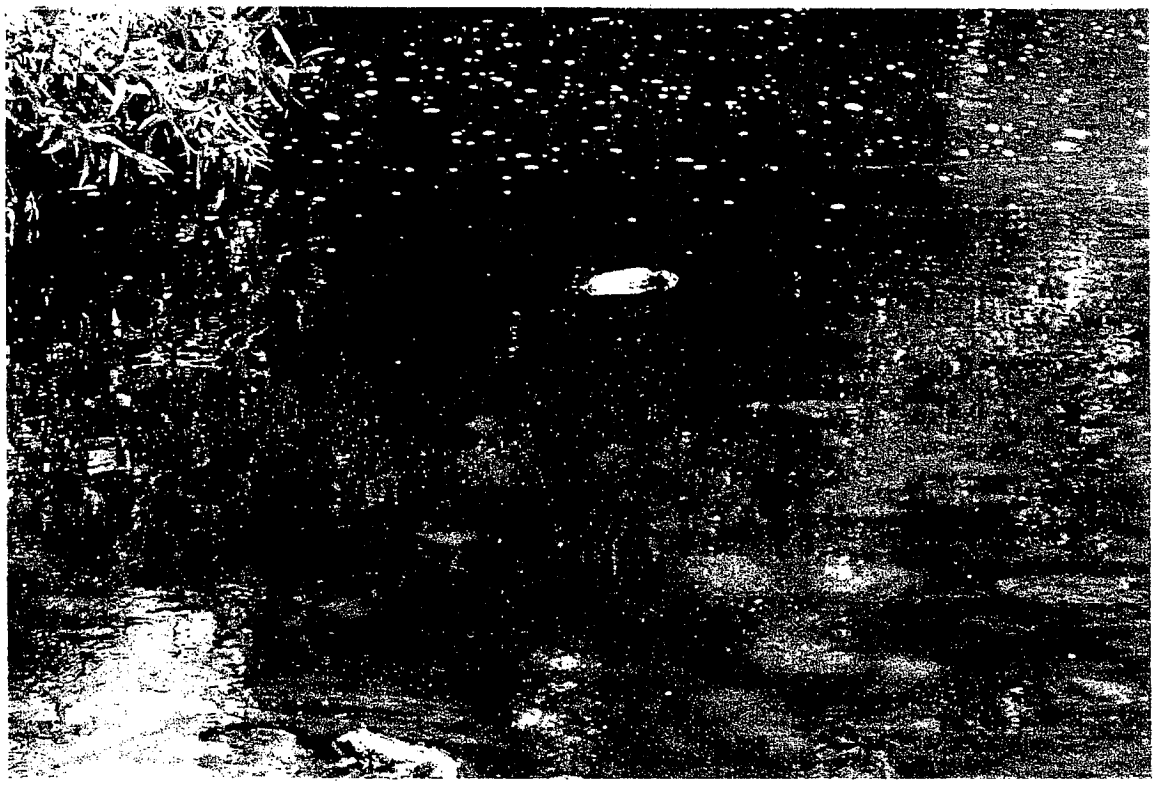


CLOSE UP VIEW
OF WATER AT
WESTERN EDGE OF
COYOTE CREEK

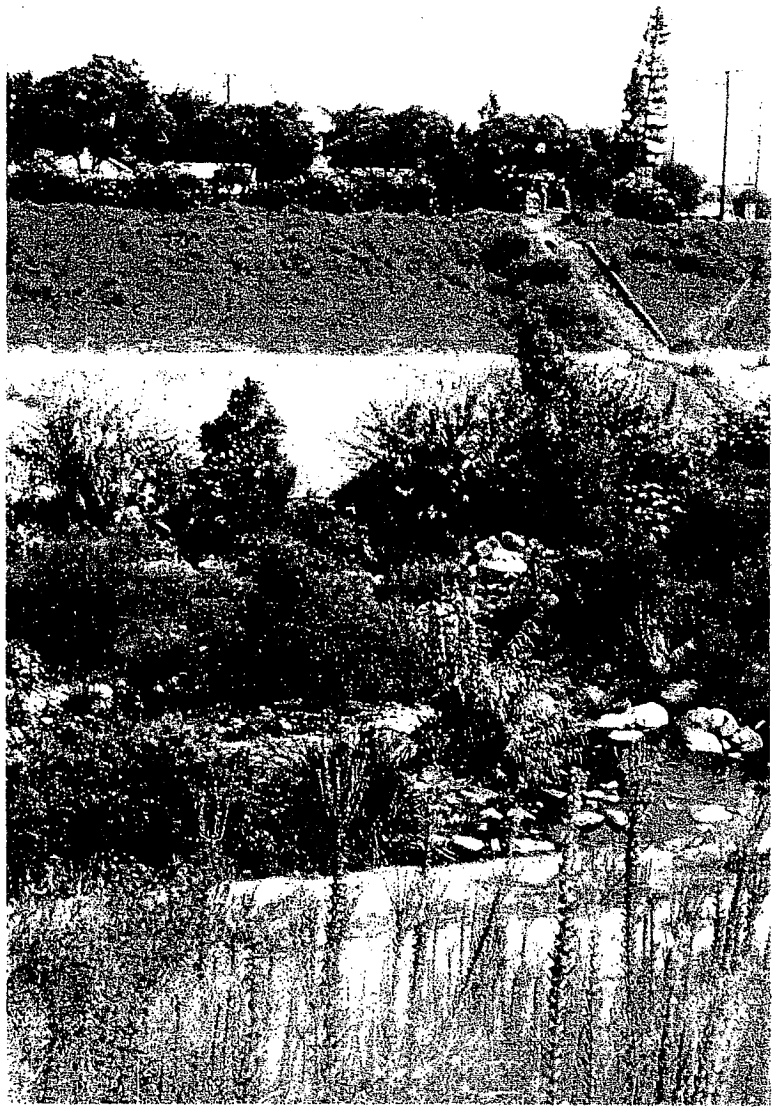


SG-5 SAN GABRIEL RIVER AT WILLOW
6/3/92

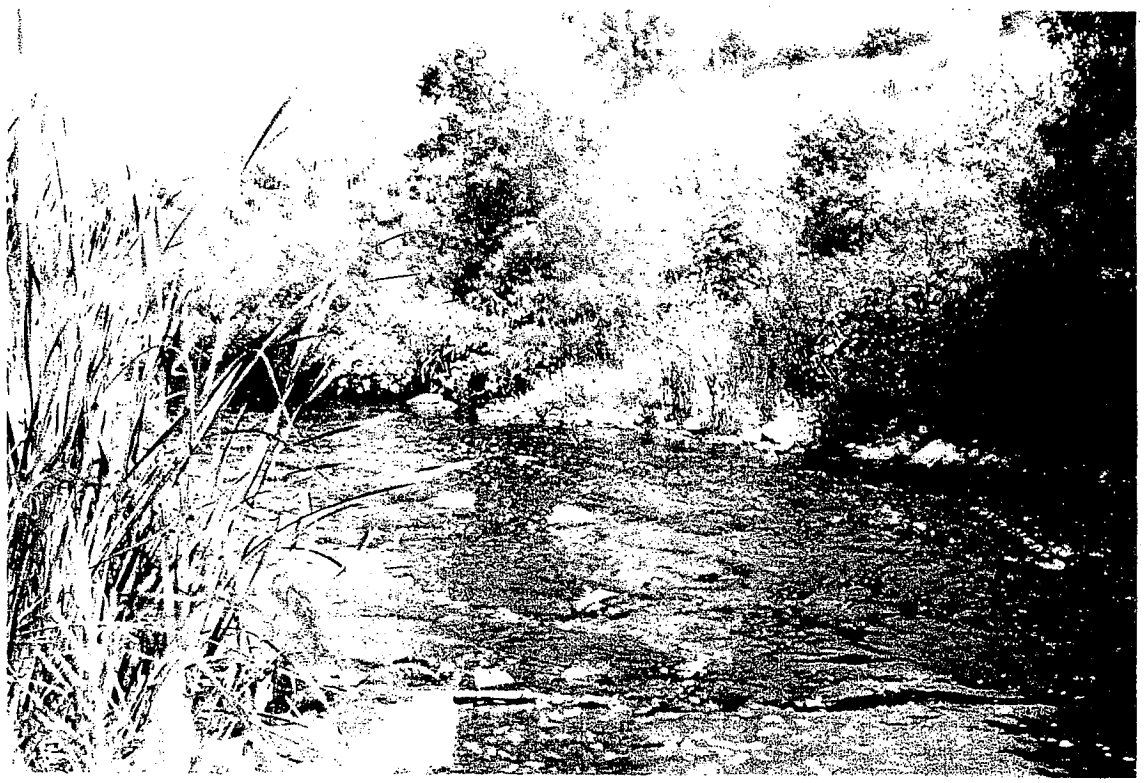
SG-7 SAN JOSE CREEK AT WORKMAN MILL ROAD
6/3/92

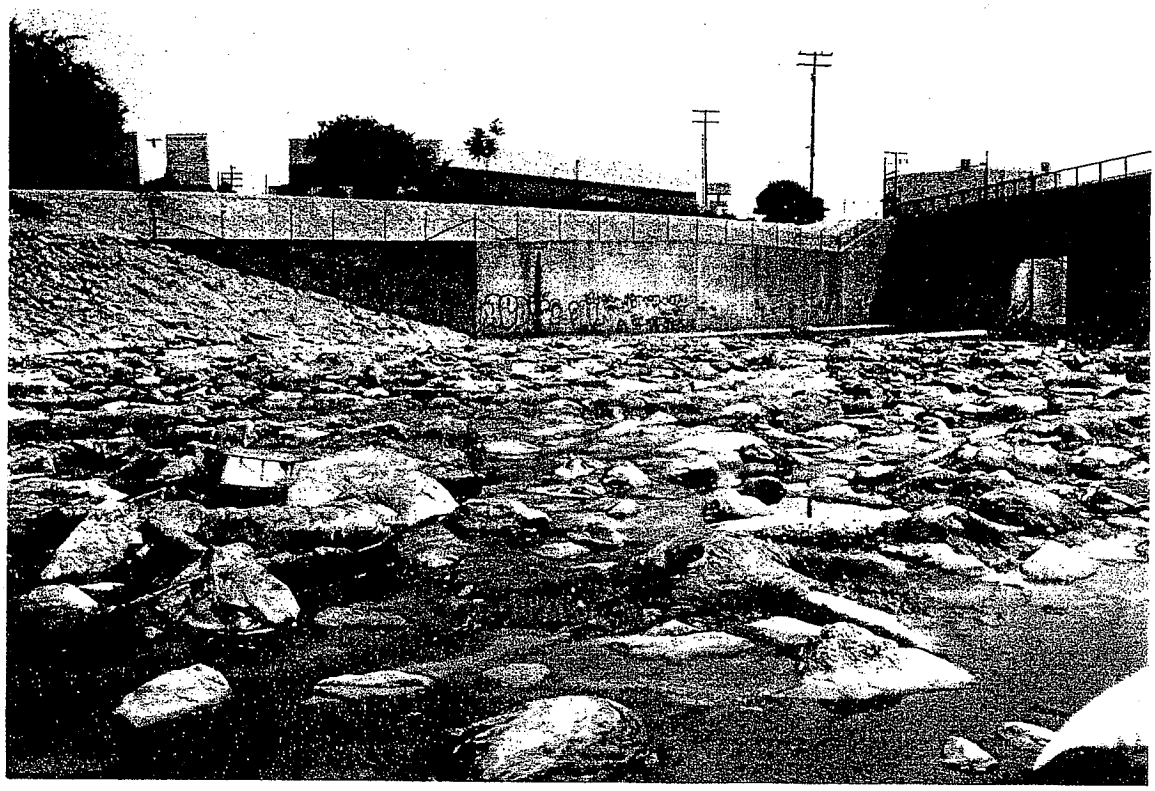


WATER APPEARS VERY GREEN DUE TO ABUNDANT ALGAE



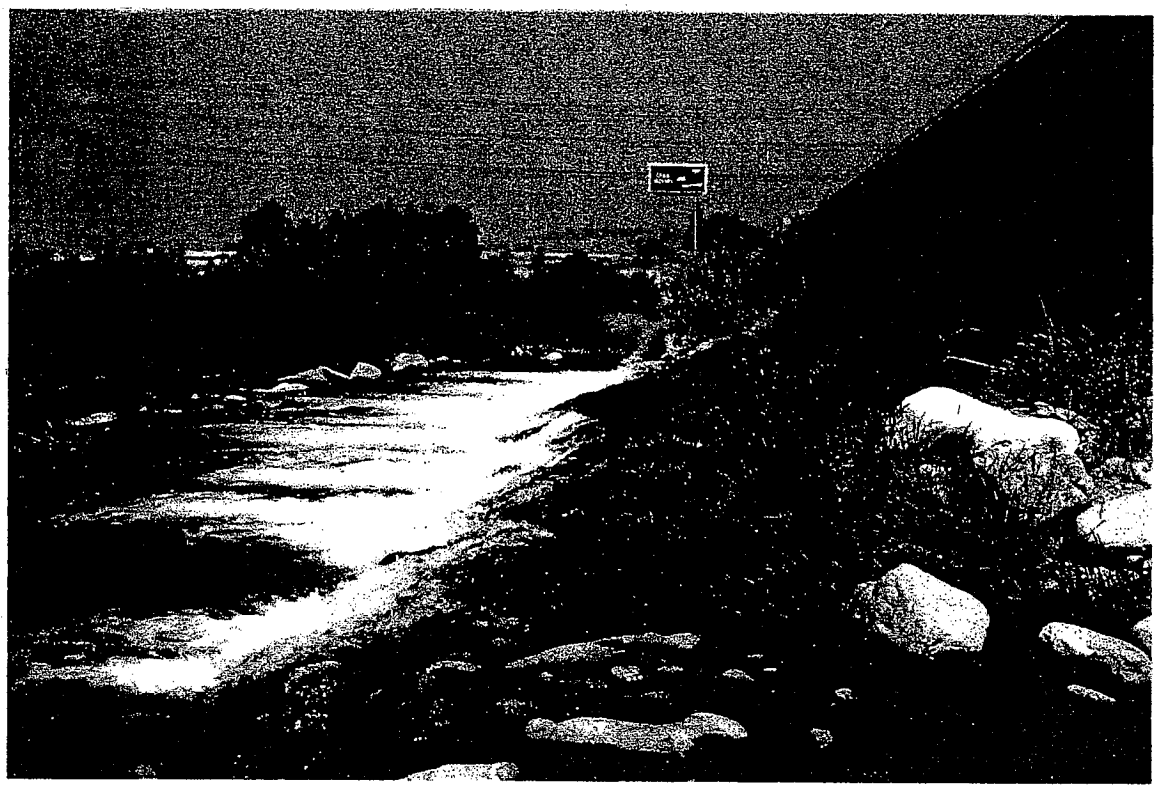
SG-7
SAN JOSE CREEK
AT WORKMAN MILL ROAD
9/9/92



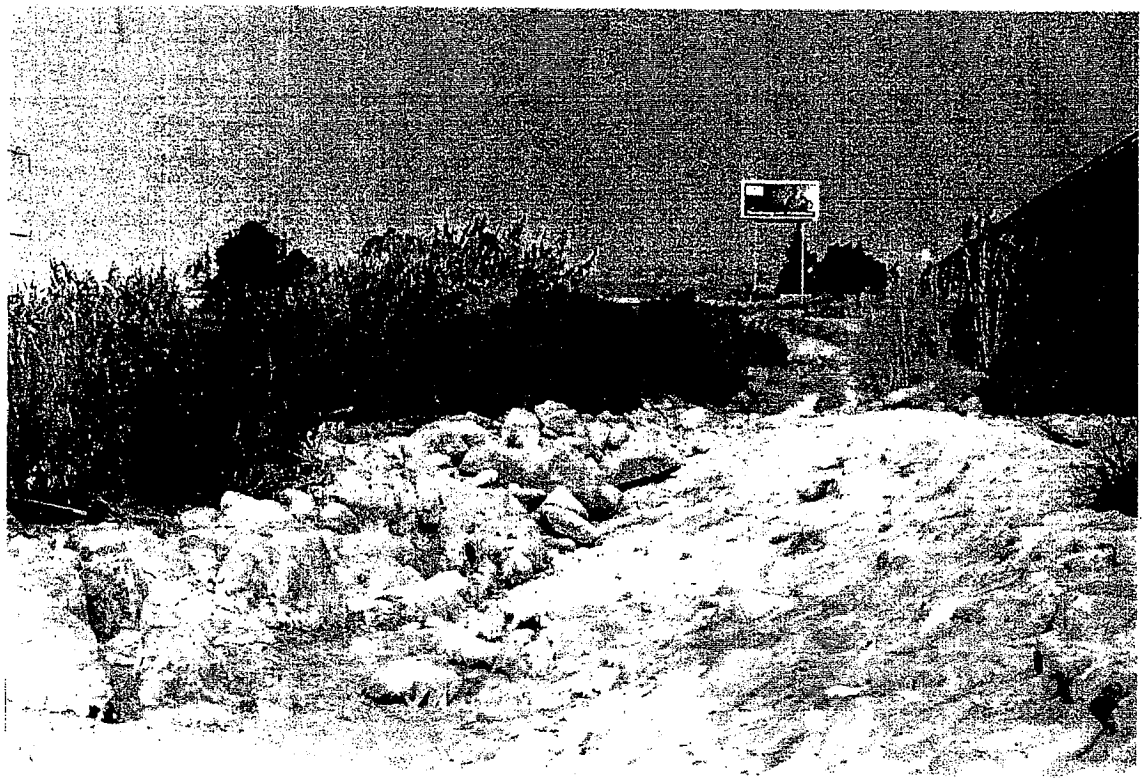


SG-8 WALNUT CREEK AT BALDWIN AVENUE
6/3/92

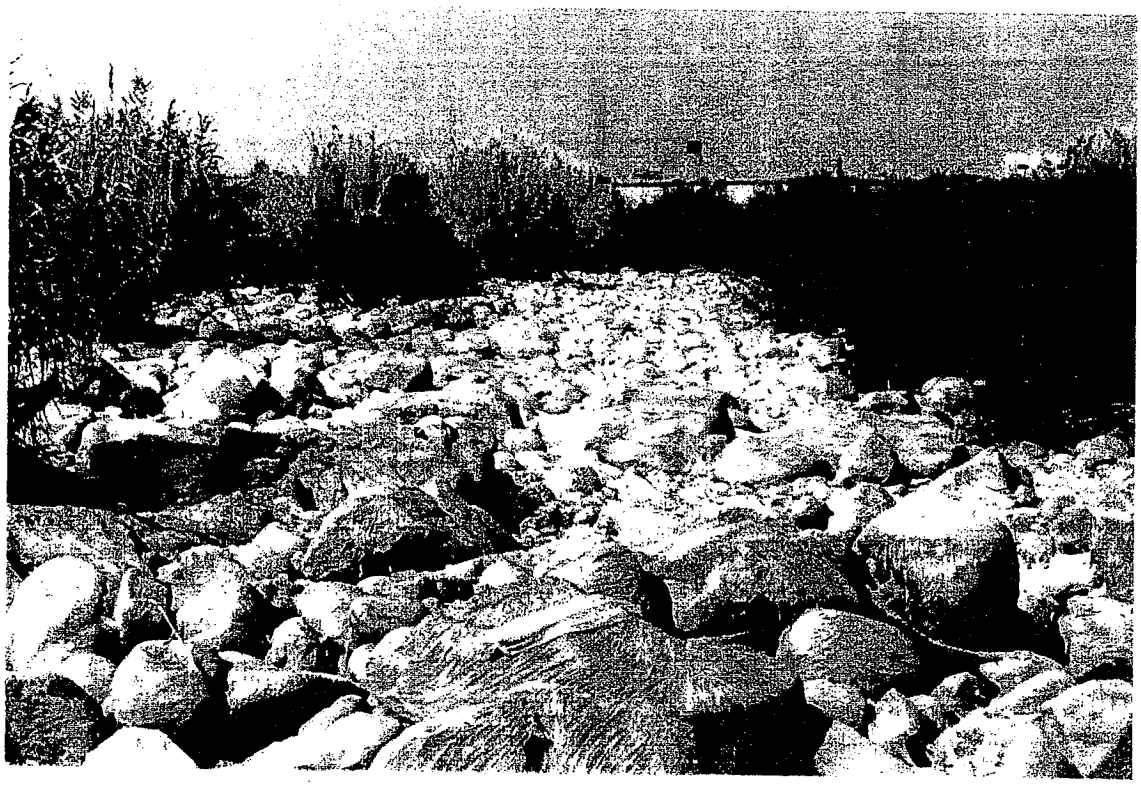
AT THIS TIME VISUAL ASSESSMENT SHOWED NUMEROUS MIDGE FLY LARVAE AND AN UNPLEASANT ODOR WAS DETECTED

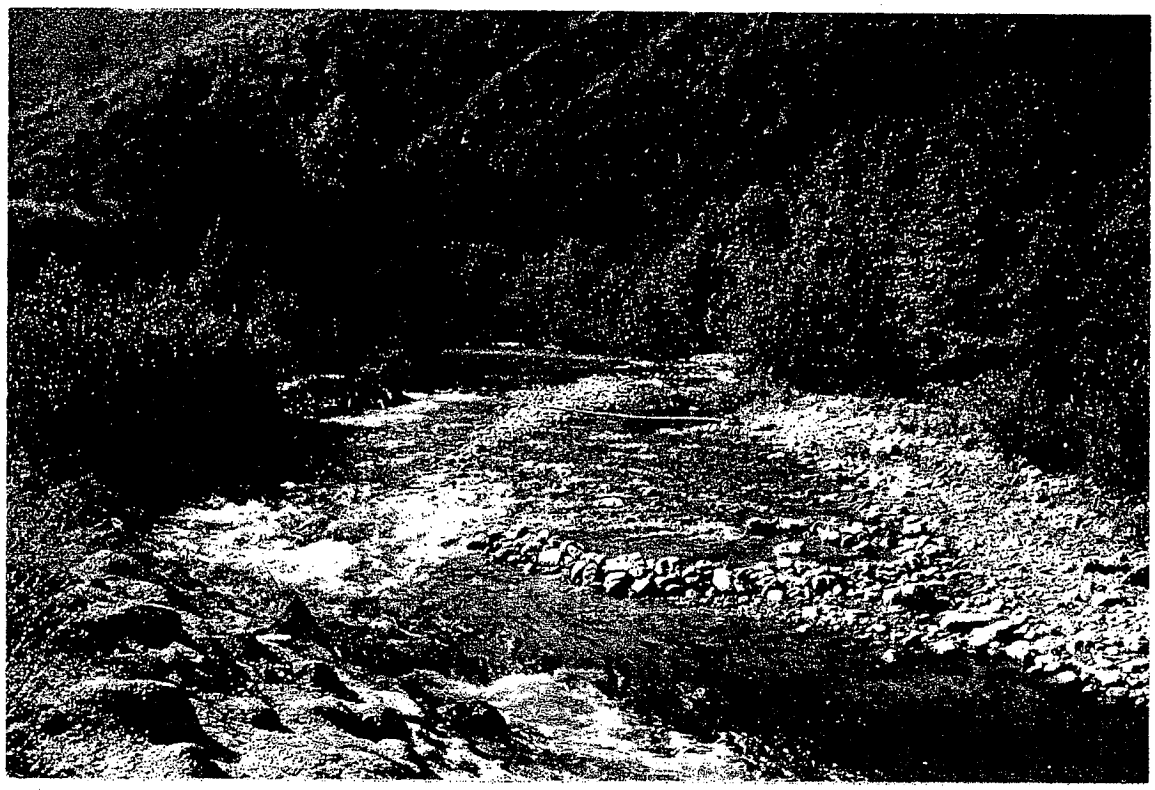


SG-9 SAMPLING SITE ON SAN GABRIEL RIVER AT FOOTHILL BOULEVARD
6/3/92

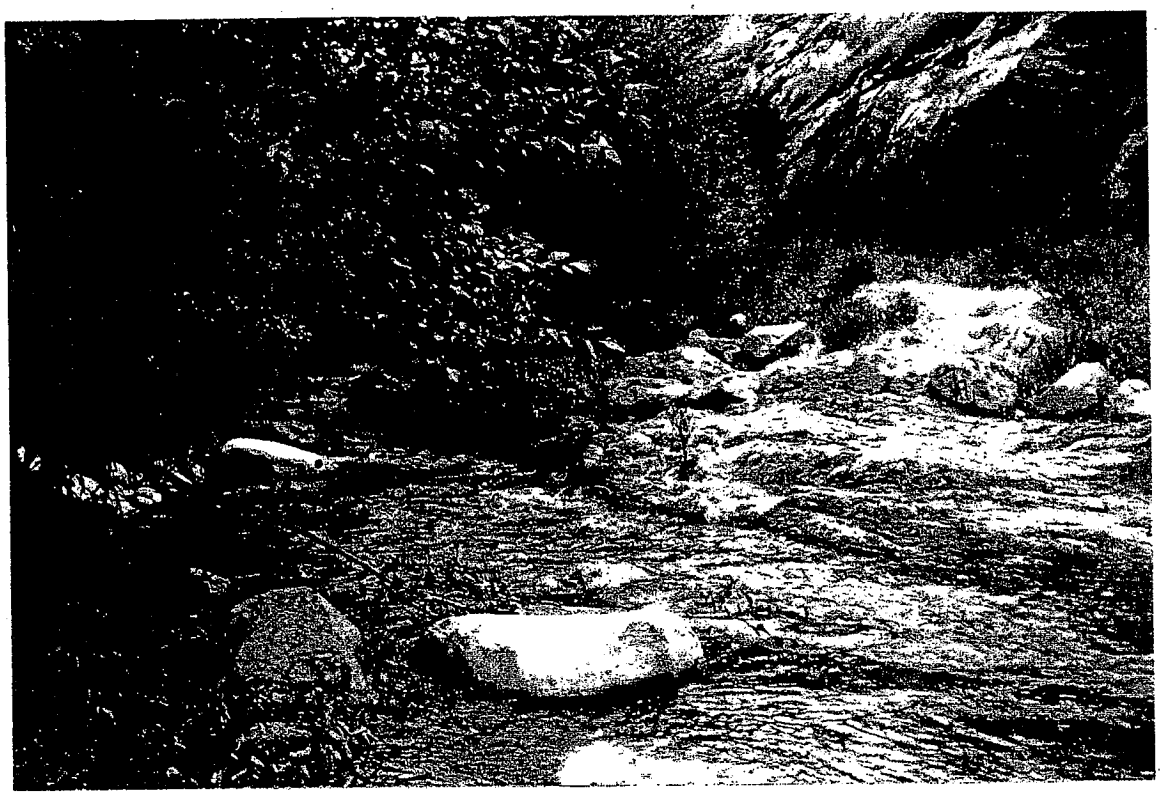


SG-9 SAMPLING SITE ON SAN GABRIEL RIVER AT FOOTHILL BLVD
9/9/92
DRY





SG-10 SAN GABRIEL RIVER - EAST FORK AT FIRE CAMP ROAD.
6/3/92



SG-11 NORTH FORK - SAN GABRIEL RIVER
6/3/92
(ACROSS FROM PARKING LOT)

SG-12 WEST FORK - SAN GABRIEL RIVER
6/3/92

Summary Tabulation of Toxicity Tests and TIEs

San Gabriel River

Table 1. Summary of toxicity tests conducted on samples from the San Gabriel River Watershed

Site	Parameter	Test Date 6/4/92	Test Date 9/10/92	Test Date 12/3/92	Test Date 3/4/93
SG-12	Fathead Minnow				
	Survival (%)	98.3	100	69.3*	93.3
	Growth (mg)	0.448	0.302*	0.407	0.596
	Ceriodaphnia				
	Survival (%)	100	100	100	90
	Reproduction	30.7	24.7	26.78	24.9
	Selenastrum cap.				
	Cells/ml	3443360	2372060	1883180	2093053
SG-11	Fathead Minnow				
	Survival (%)	95.1	100	81.7	100
	Growth (mg)	0.493	0.165*	0.37	0.545
	Ceriodaphnia				
	Survival (%)	100	90	100	100
	Reproduction	24.3	25.9	23.1	18.3*
	Selenastrum cap.				
	Cells/ml	2927093	2271120	2001560	2175100

Table 1. Summary of toxicity tests conducted on samples from the San Gabriel River Watershed

Site	Parameter	Test Date 6/4/92	Test Date 9/10/92	Test Date 12/3/92	Test Date 3/4/93
SG-10	Fathead minnow				
	Survival (%)	100	100	N/A	96.8
	Growth (mg)	0.477	0.14*	N/A	0.539
	Ceriodaphnia				
	Survival (%)	100	100	90	100
	Reproduction	25.6	23	20.5	25.8
	Selenastrum cap.				
	Cells/ml	2872453	2214860	1798240	2259073
SG-9	Fathead minnow				
	Survival (%)	96.9	N/A	N/A	100
	Growth (mg)	0.4	N/A	N/A	0.552
	Ceriodaphnia				
	Survival (%)	100	N/A	100	100
	Reproduction	25.2	N/A	22.5	21.2
	Selenastrum cap.				
	Cells/ml	3671720	N/A	1298180	2261153

Table 1. Summary of toxicity tests conducted on samples from the San Gabriel River Watershed

Site	Parameter	Test Date 6/4/92	Test Date 9/10/92	Test Date 12/3/92	Test Date 3/4/93
SG-8	Fathead minnow				
	Survival (%)	98.3	95	98.1	85*
	Growth (mg)	0.483	0.343*	0.306*	0.495
	Ceriodaphnia				
	Survival (%)	0*	100	0*	100
	Reproduction	0*	34.1	0*	28.9
	Selenastrum cap				
	Cells/ml	2225973	1777600	1055800	2046573
SG-7	Fathead minnow				
	Survival (%)	90.3	87.9	70	93.3
	Growth (mg)	0.505	0.298*	0.369	0.508
	Ceriodaphnia				
	Survival (%)	90	88.9	100	
	Reproduction	27	23	27.9	33
	Selenastrum cap.				
	Cells/ml	3090940	1451380	2006780	755493

Table 1. Summary of toxicity tests conducted on samples from the San Gabriel River Watershed

Site	Parameter	Test Date 6/4/92	Test Date 9/10/92	Test Date 12/3/92	Test Date 3/4/93
SG-6	Fathead minnow				
	Survival (%)	N/A	N/A	85.1	91.6
	Growth (mg)	N/A	N/A	0.249	0.554
	Ceriodaphnia				
	Survival (%)	N/A	N/A	100	100
	Reproduction	N/A	N/A	0	25
	Selenastrum cap.				
	Cells/ml	N/A	N/A	1510080	2367420
SG-5	Fathead Minnow				
	Survival (%)	0*	79.2	1.5*	0*
	Growth (mg)	0*	0.242*	0.025*	0*
	Ceriodaphnia				
	Survival (%)	0*	0*	0*	0*
	Reproduction	0*	0*	0*	0*
	Selenastrum cap.				
	Cells/ml	45440	463940*	72500*	35980*

Table 1. Summary of toxicity tests conducted on samples from the San Gabriel River Watershed

Site	Parameter	Test Date 6/4/92	Test Date 9/10/92	Test Date 12/3/92	Test Date 3/4/93
SG-4	Fathead Minnow				
	Survival (%)	56.8*	88.3	91.8	80*
	Growth (mg)	0.487	0.349	0.336	0.484
	Ceriodaphnia				
	Survival (%)	N/A	100	80	100
	Reproduction	N/A	30.1	23.1	21.3
	Selenastrum cap.				
	Cells/ml	2434260	1604100	1192400	38080*
SG-3	Fathead Minnow				
	Survival (%)	70*	79.8	49*	96.7
	Growth (mg)	0.345	0.229*	0.247*	0.452
	Ceriodaphnia				
	Survival (%)	0*	0*	0*	0*
	Reproduction	0*	1*	0*	3.4*
	Selenastrum cap.				
	Cells/ml	2622560	797840*	974520*	40473*

Table 1. Summary of toxicity tests conducted on samples from the San Gabriel River Watershed

Site	Parameter	Test Date 6/4/92	Test Date 9/10/92	Test Date 12/3/92	Test Date 3/4/93
SG-2	Fathead Minnow				
	Survival (%)	0*	70*	1.6*	0*
	Growth (mg)	0*	0.242*	0.125*	0*
	Ceriodaphnia				
	Survival (%)	0*	0*	0*	0*
	Reproduction	0*	0.4*	0*	0*
	Selenastrum cap.				
	Cells/ml	46040	808480	68780	42306
SG-1	Fathead Minnow				
	Survival (%)	0*	33.9*	15*	6.7*
	Growth (mg)	0*	0.229*	0.446	0.183*
	Ceriodaphnia				
	Survival (%)	0*	90	90	0*
	Reproduction	8.7*	28.5	20.9	0.4*
	Selenastrum cap.				
	Cells/ml	465780	579520*	569960*	43120*

Table 1. Summary of toxicity tests conducted on samples from the San Gabriel River Watershed

Site	Parameter	Test Date 6/4/92	Test Date 9/10/92	Test Date 12/3/92	Test Date 3/4/93
Control	Fathead Minnow				
	Survival (%)	98.3	95	98.3	100
	Growth (mg)	0.435	0.422	0.433	0.502
	Ceriodaphnia				
	Survival (%)	80	100	100	100
	Reproduction	22.5	24.2	21.3	23
	Selenastrum cap.				
	Cells/ml	2875120	1576940	1268520	797153

*= Significance @ $P \leq 0.05$; see Methods for statistical tests.

Table 2. Summary of Results of Toxicity Identification Evaluations

Site	Date	Species	pH 3	pH 11	Grad. pH	EDTA	Na2S203	Air	Fil.	SPE Col.	Meth Elute
SG-2	6/3/92	Cerio	+	-	-	-	-	+	+/-	+	+
	9/9/92	Cerio	N/T	N/T	N/T	+a	N/T	N/T	N/T	N/T	N/T
	12/2/92	Cerio	*	*	*	*	*	*	*	*	+
	3/3/93	Cerio	-	-	N/T	-	-	-	N/T	+	+
	3/3/93	Fathead	*	*	N/T	*	*	*	N/T	*	*
SG-3	6/27/92	Cerio	+a	+a	+	-	-	+	+	+	N/T
	9/9/92	Cerio	N/T	N/T	N/T	+/-	N/T	N/T	N/T	N/T	N/T
	12/4/92	Cerio	+a	+a	+	-	-	+/-	+/-	+	+
SG-5	6/3/92	Cerio	+	+/-	+	+a	-	+/-	+/-	+	N/T
	9/9/92	Cerio	N/T	N/T	N/T	+a	N/T	N/T	N/T	N/T	N/T
	12/2/92	Cerio	N/T	N/T	N/T	N/T	N/T	N/T	+	+	+
	3/3/93	Cerio	-	-	N/T	-	-	-	N/T	+	+
	3/3/93	Fathead	*	*	N/T	*	*	*	N/T	*	*
SG-8	6/3/92	Cerio	+/-	+/-	-	-	-	+/-	N/T	+	N/T
	12/2/92	Cerio	*	*	*	*	*	*	*	*	*

Key: N/T = Not tested; + = Test passed; - = Test failed
+/- = Ambiguous results
* = Sample no longer toxic at time of TIE
a = 24 hour delay in mortality
++ = Test passed using helium and air

Table 3. Summary of Analytical Chemistry Results for San Gabriel River (6/92 to 4/93)

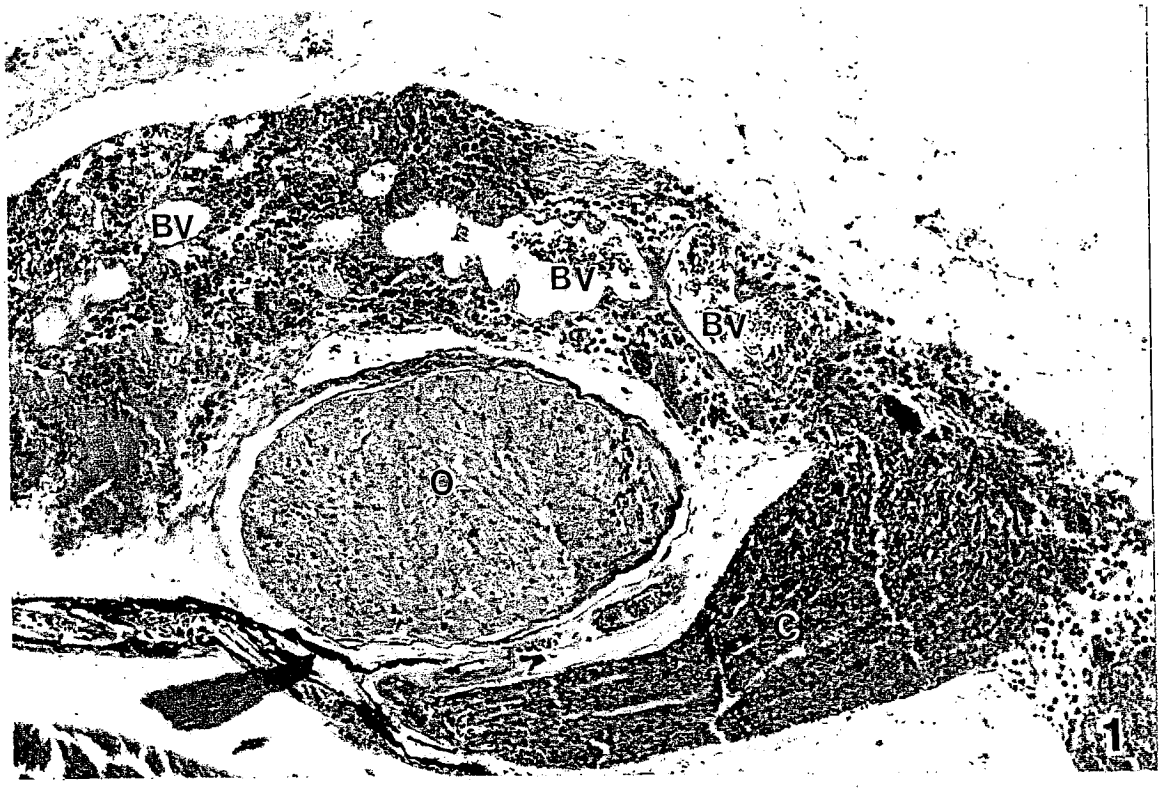
Date Sampled	Sample ID (Site #)	Eureka Laboratories	Hardness/ Alkalinity	µg/l (ppb)	Detection Limits (ppb)
6/3/92	SG-8	g-BHC (Lindane) Diazinon		0.011 3.600	0.009 0.600
9/9/92	SG-2	Barium Molybdenum Zinc	Hardness = 220 Alkalinity = 55	30.0 30.0 30.0	20.0 20.0 10.0
9/9/92	SG-3	Barium Molybdenum Zinc	Hardness = 200 Alkalinity = 55	30.0 20.0 40.0	20.0 20.0 10.0
9/9/92	SG-5	Barium Molybdenum Zinc	Hardness = 200 Alkalinity = 50	30.0 20.0 30.0	20.0 20.0 10.0
12/2/92	SG-2	Barium Molybdenum Zinc* No organophosphorus pesticides detected	Hardness = 300 Alkalinity = 260	60.0 30.0 100.0	20.0 20.0 10.0
12/2/92	SG-3	Barium Molybdenum Zinc* No organophosphorus pesticides detected	Hardness = 280 Alkalinity = 240	40.0 20.0 70.0	20.0 20.0 10.0

*Zn was found on the method blank, but not greater than three times the detection limit.

Illustrations of Histological Lesions

San Gabriel River

Figures 1 - 21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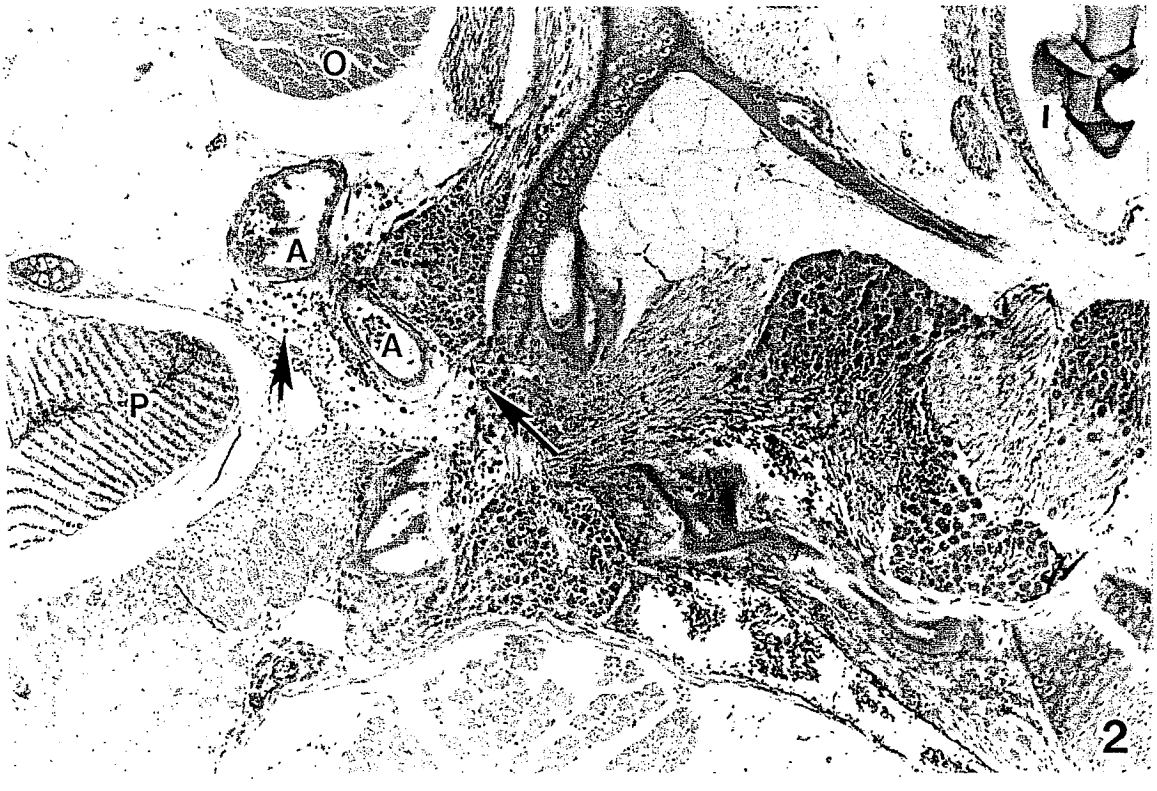
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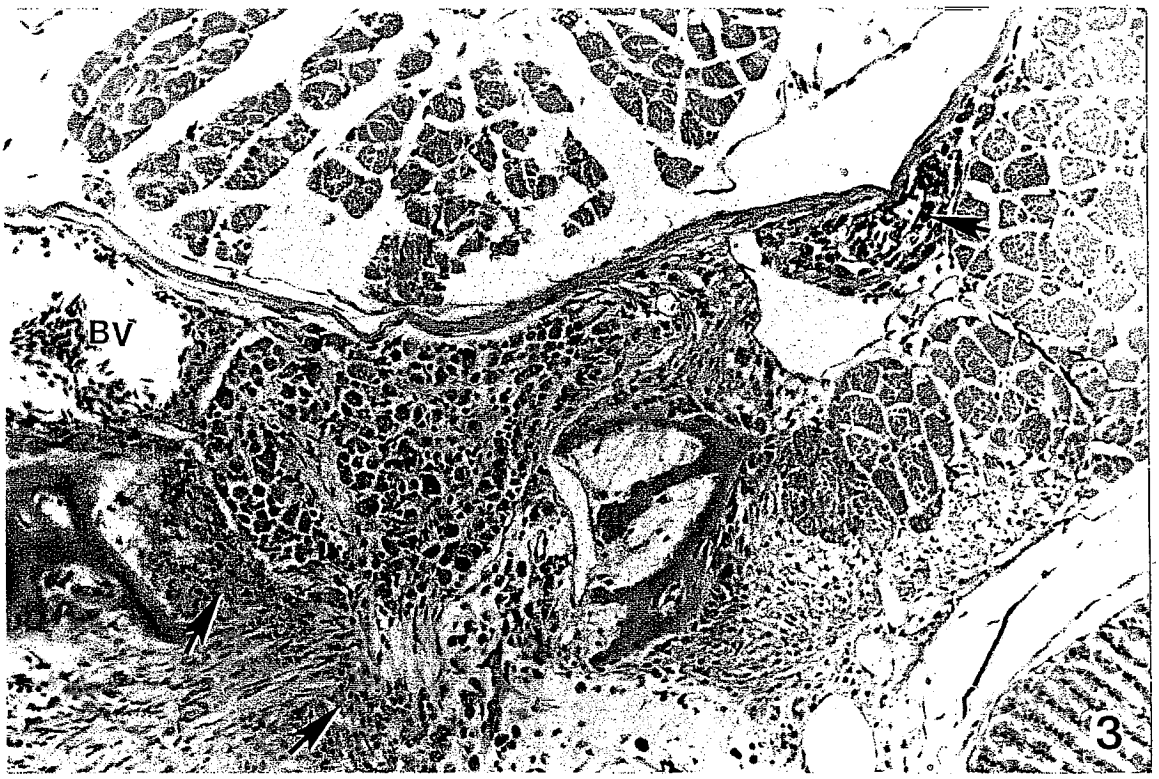
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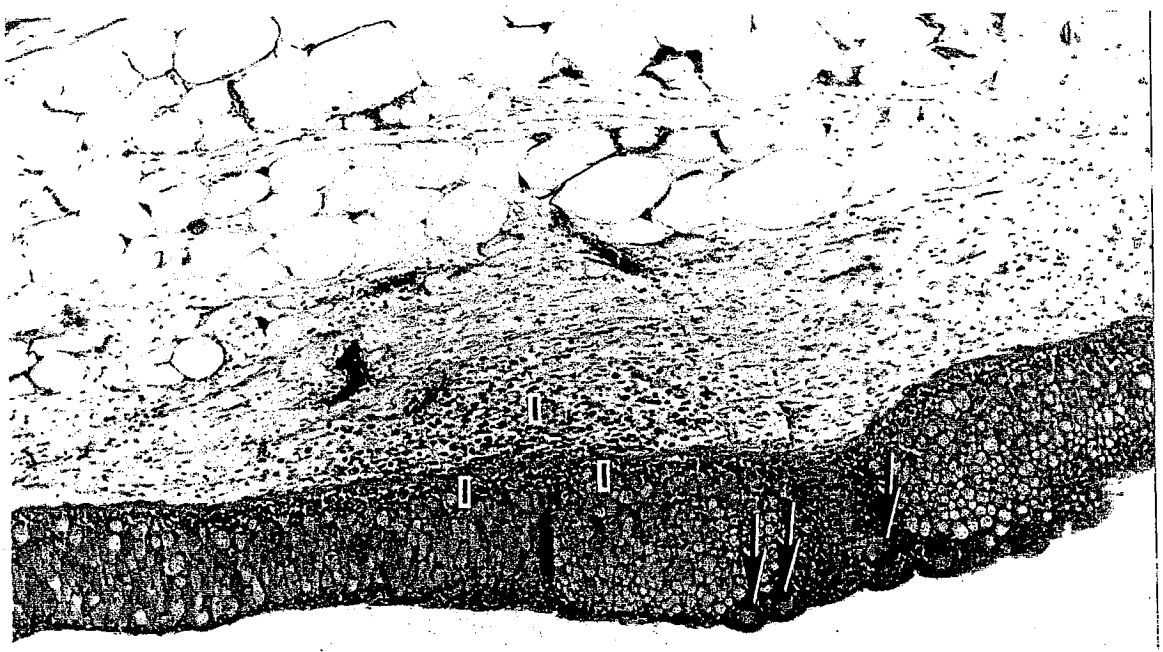
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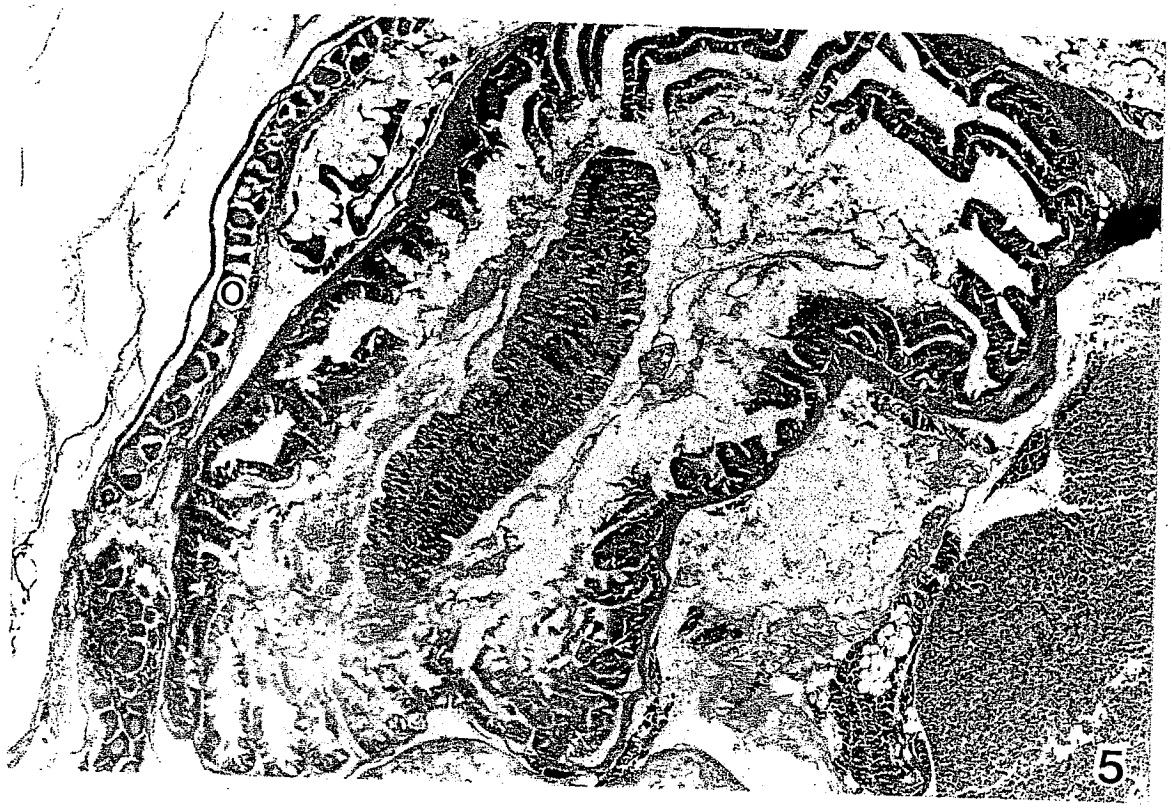

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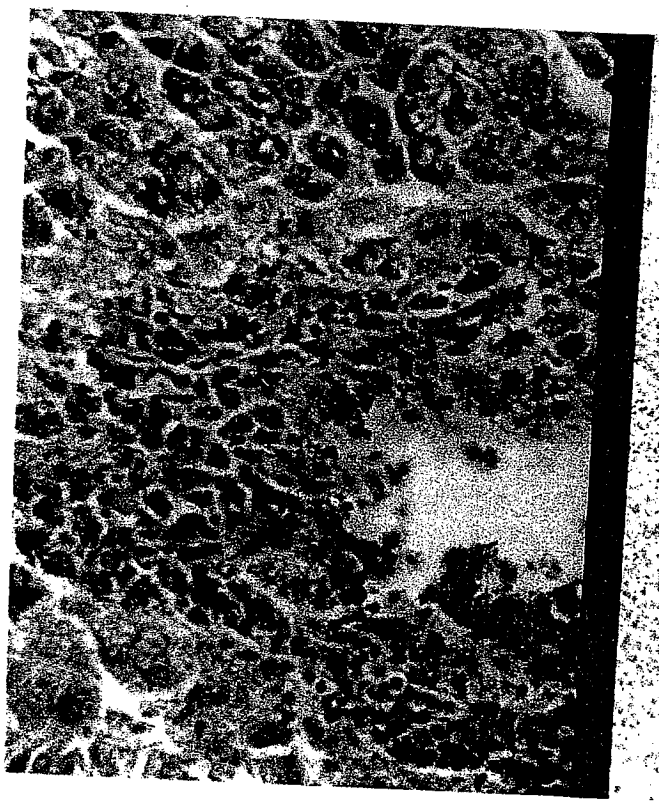

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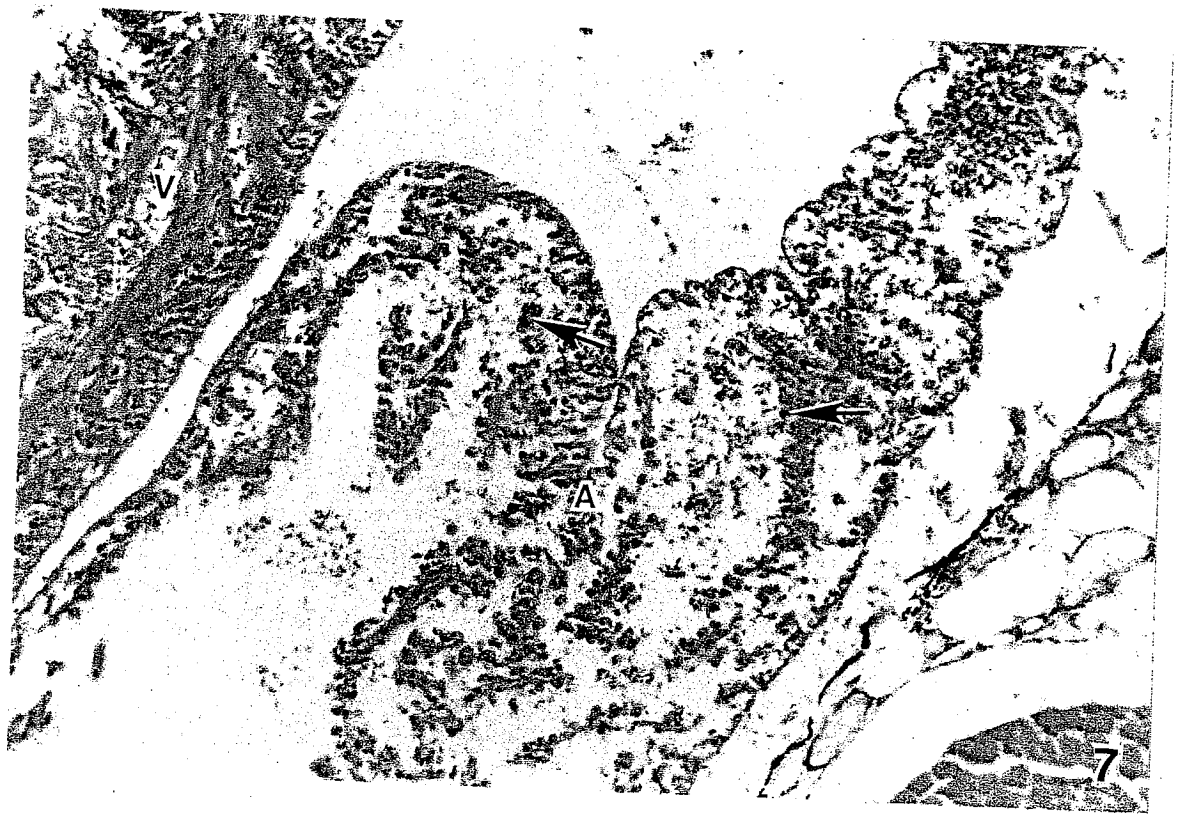
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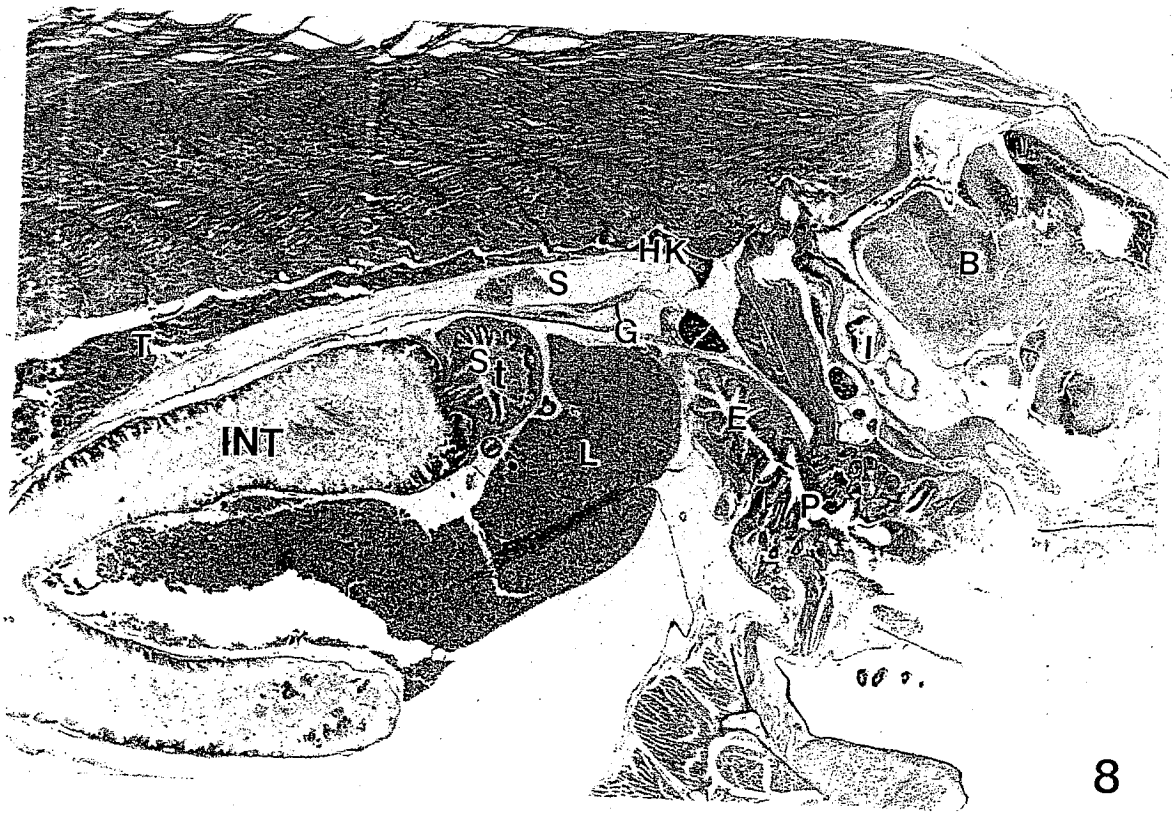
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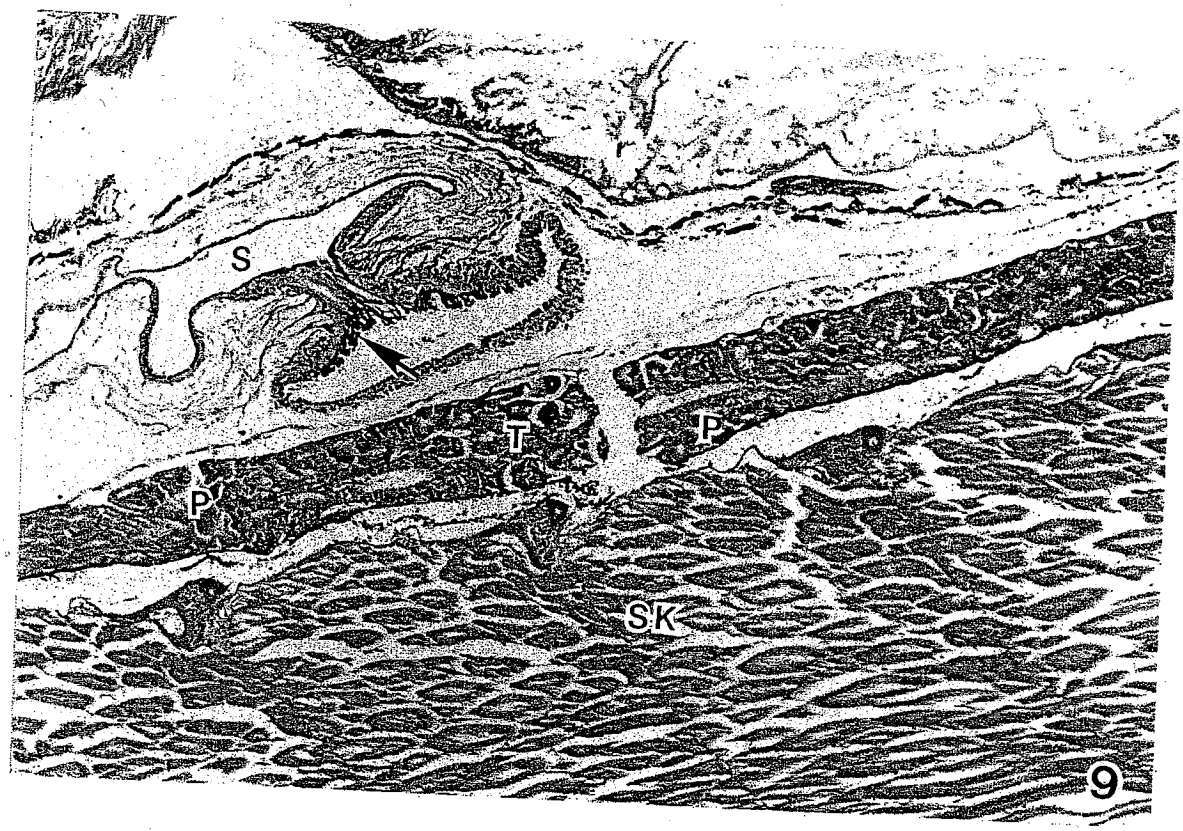

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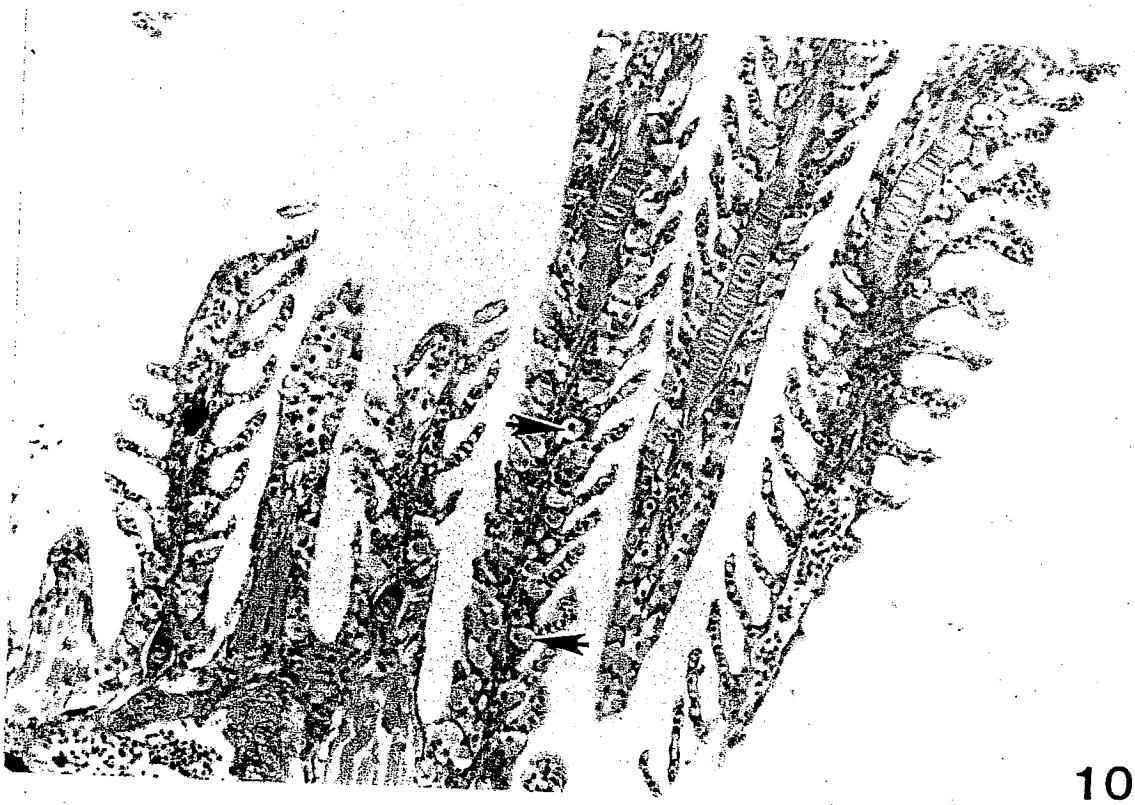

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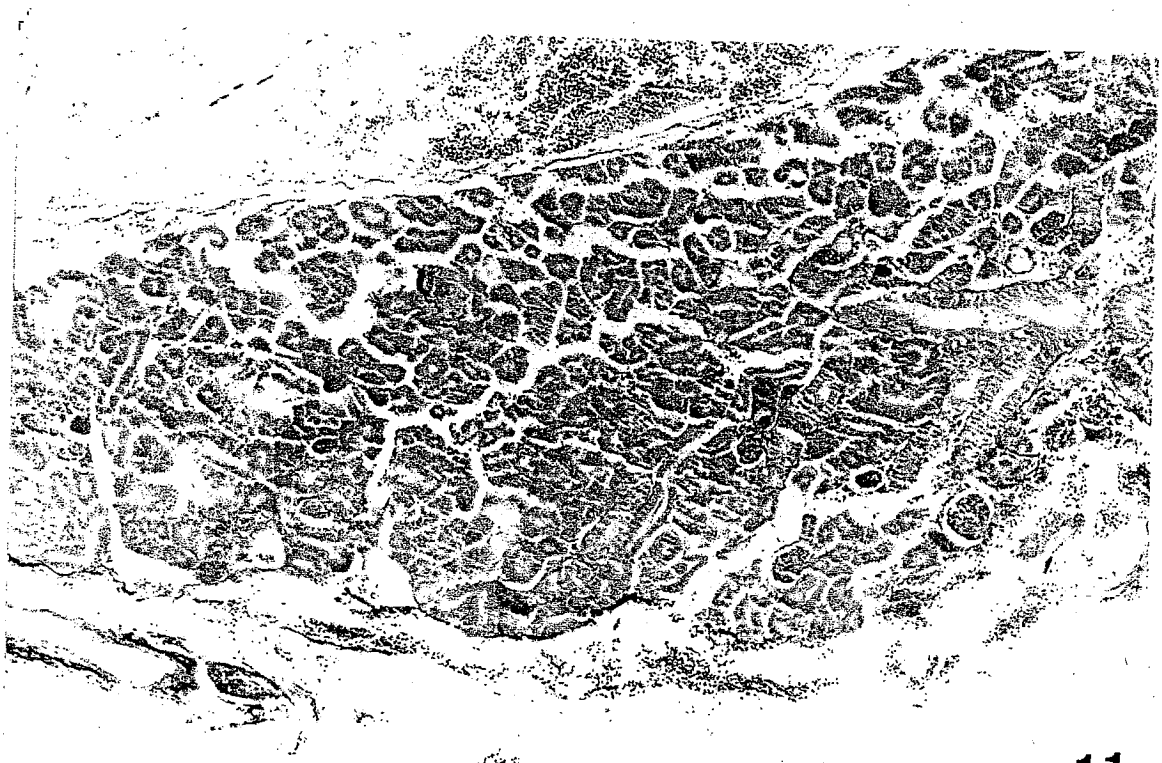

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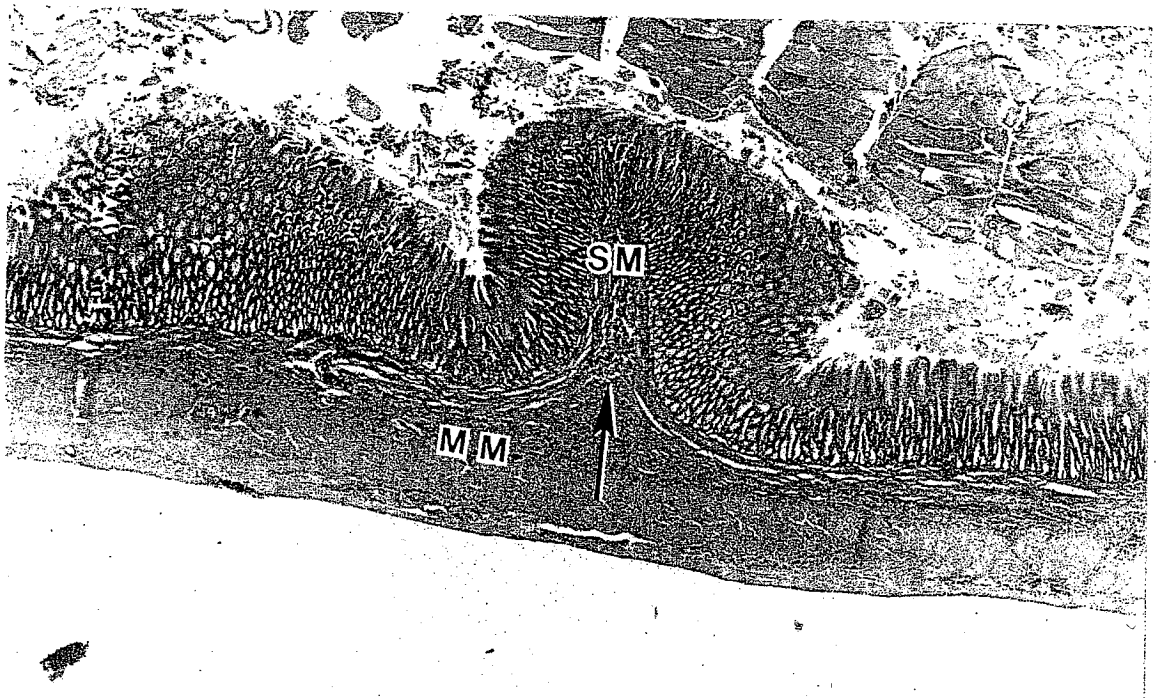

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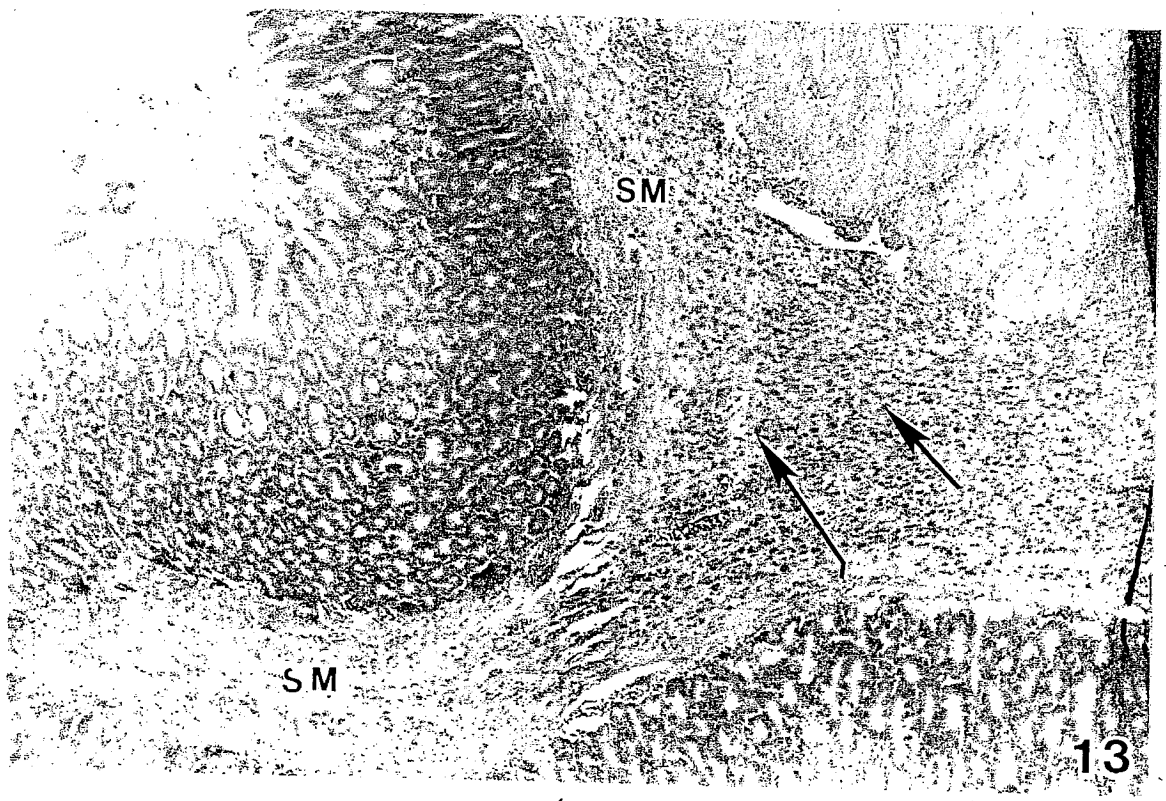

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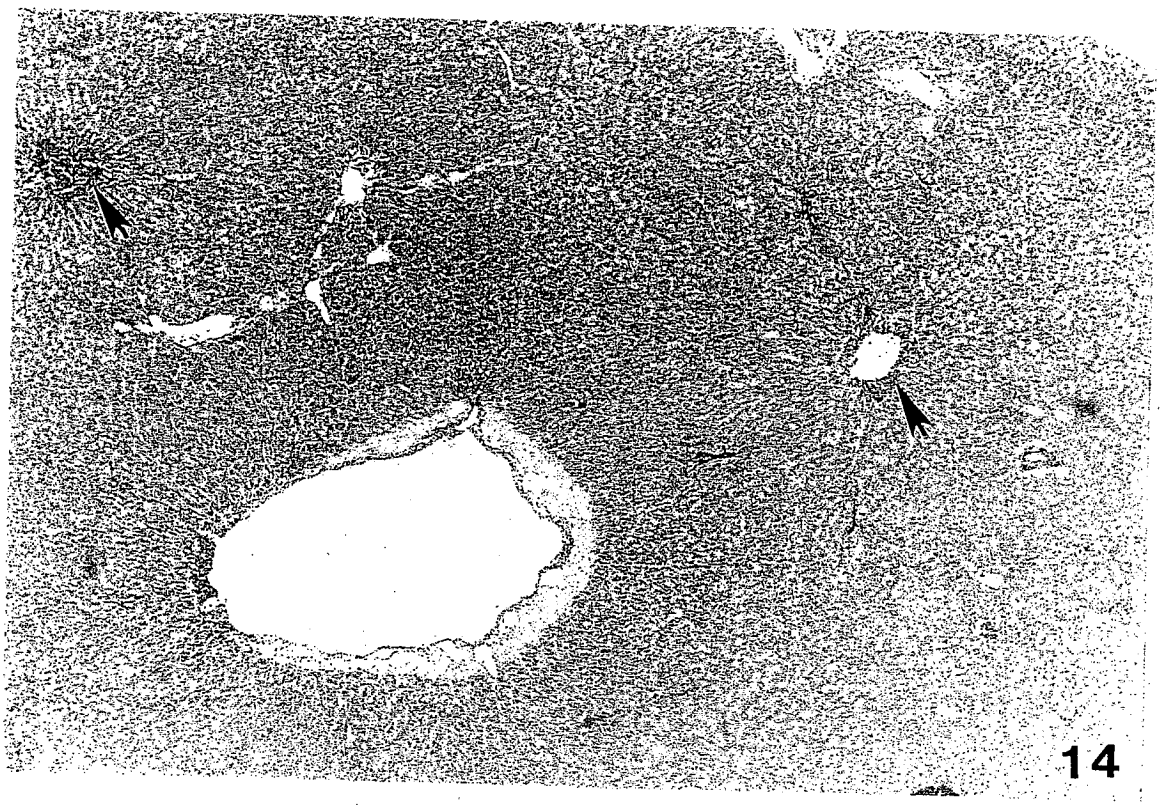

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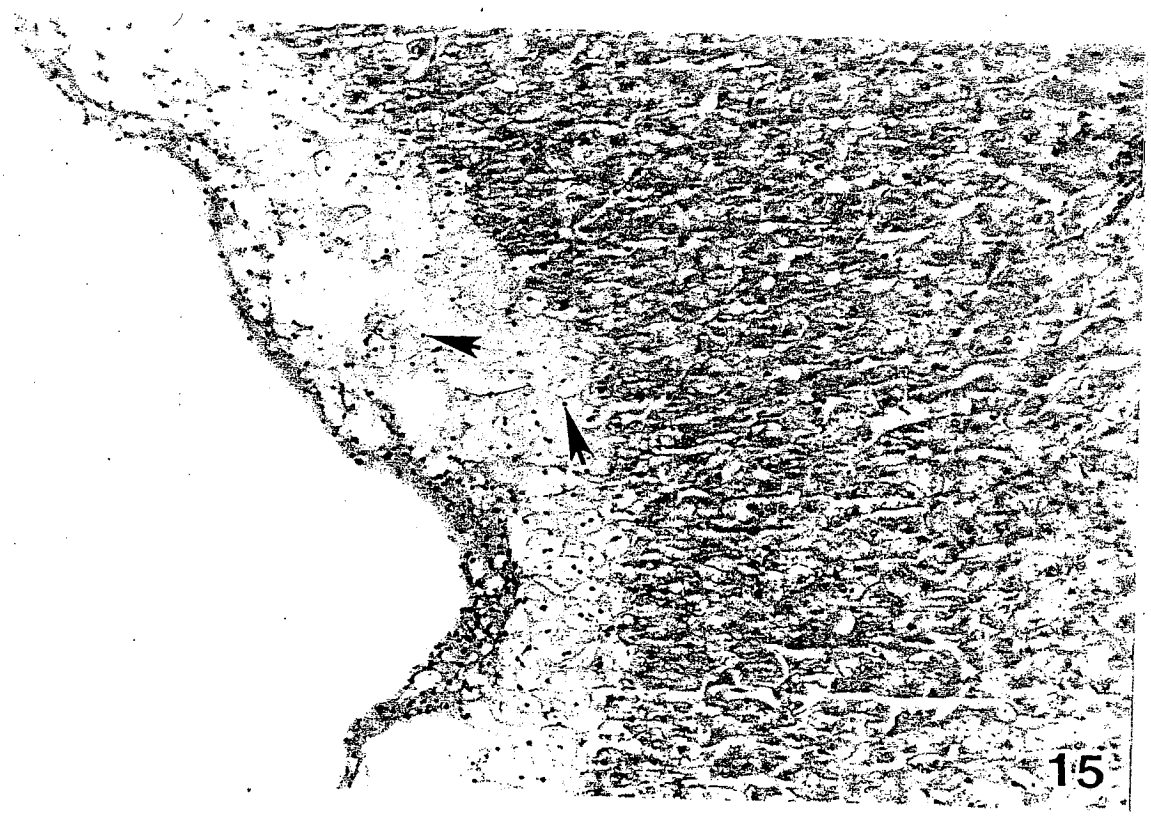

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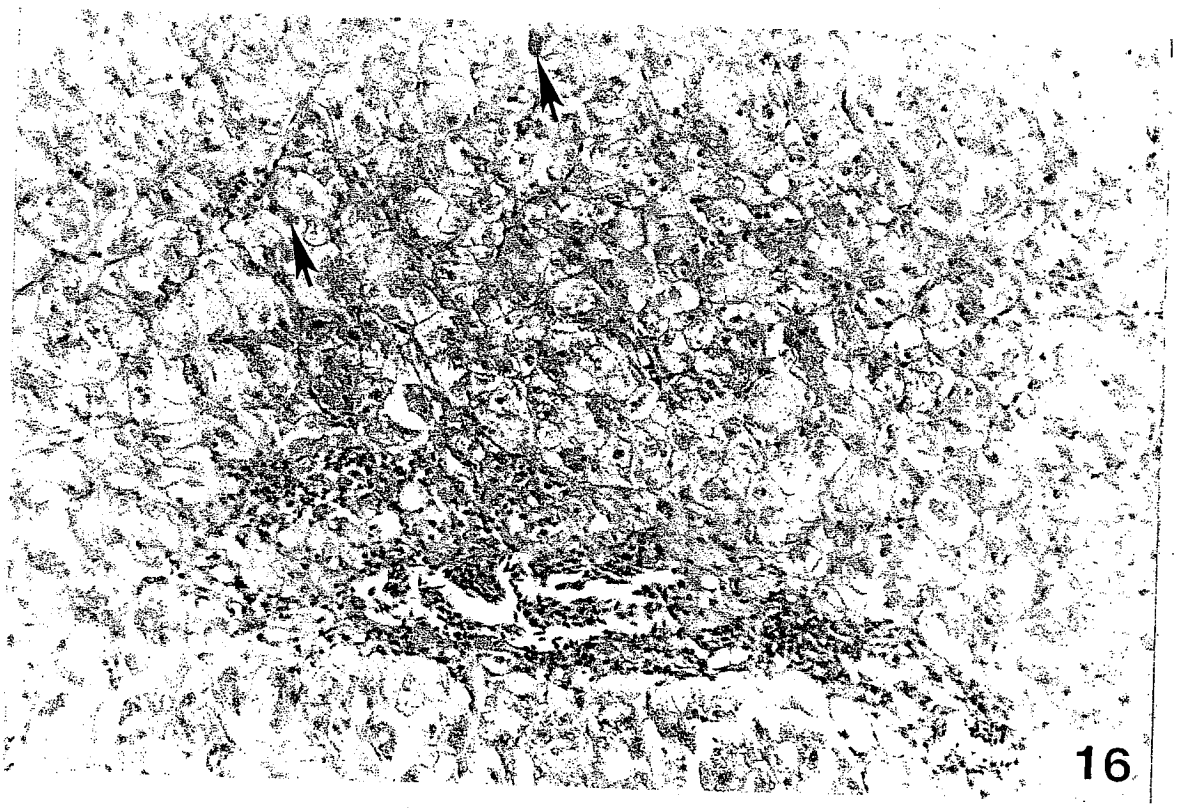
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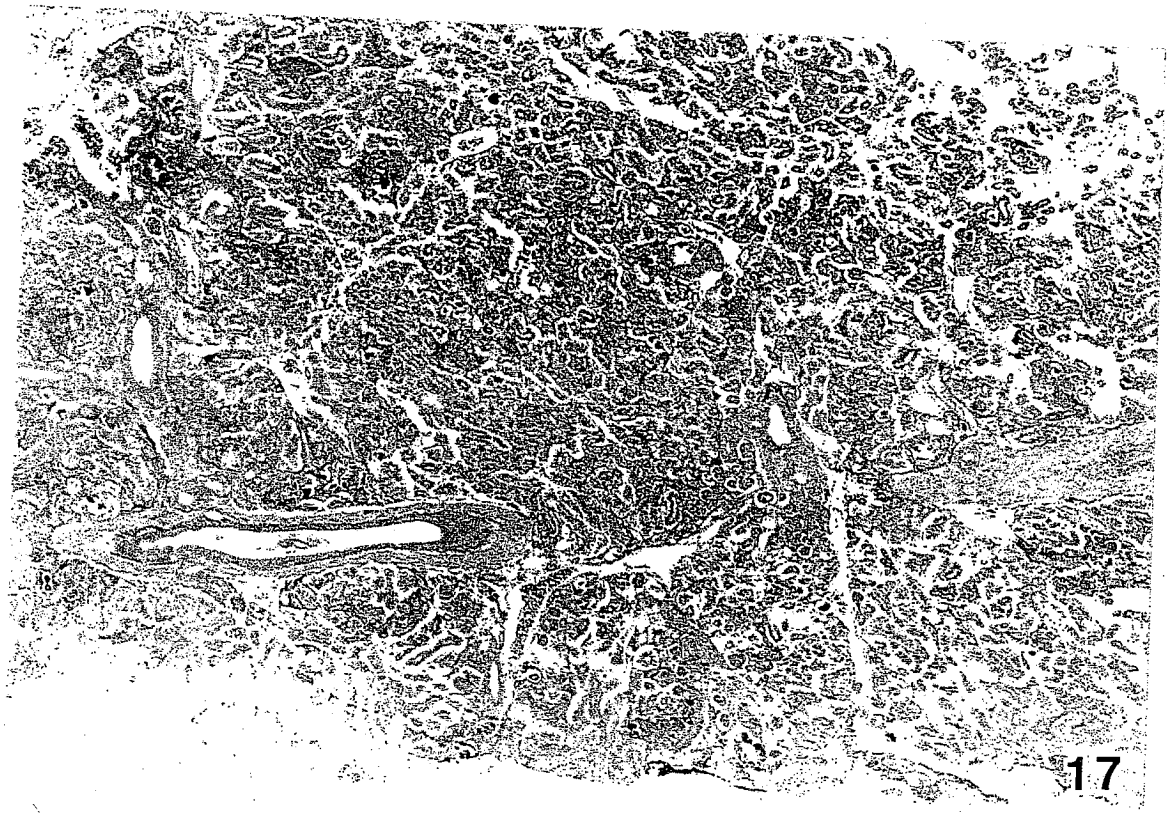

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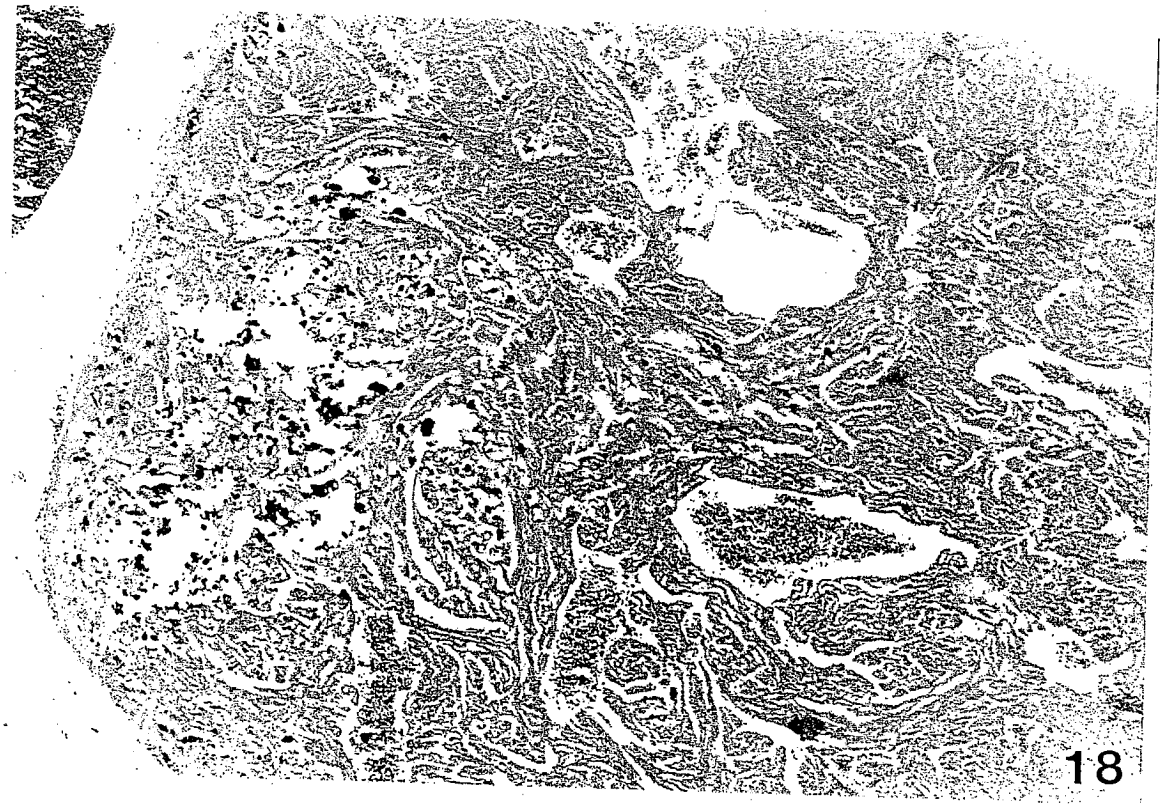

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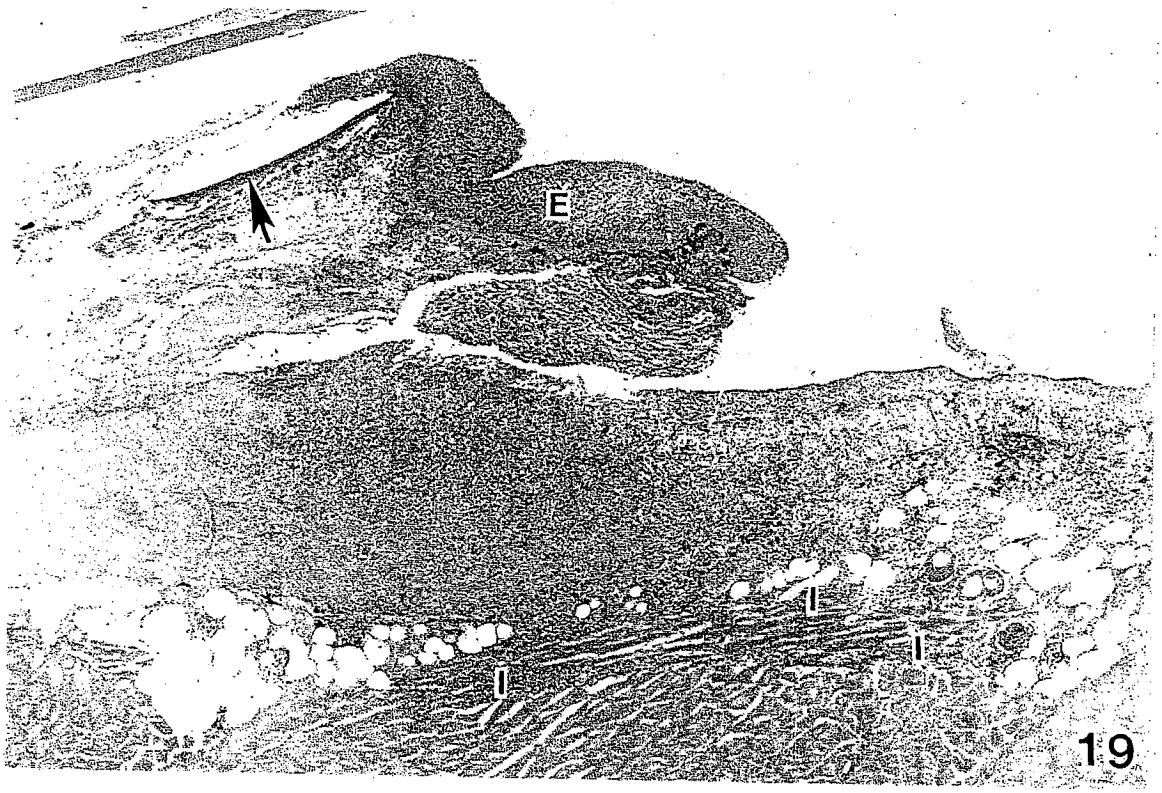
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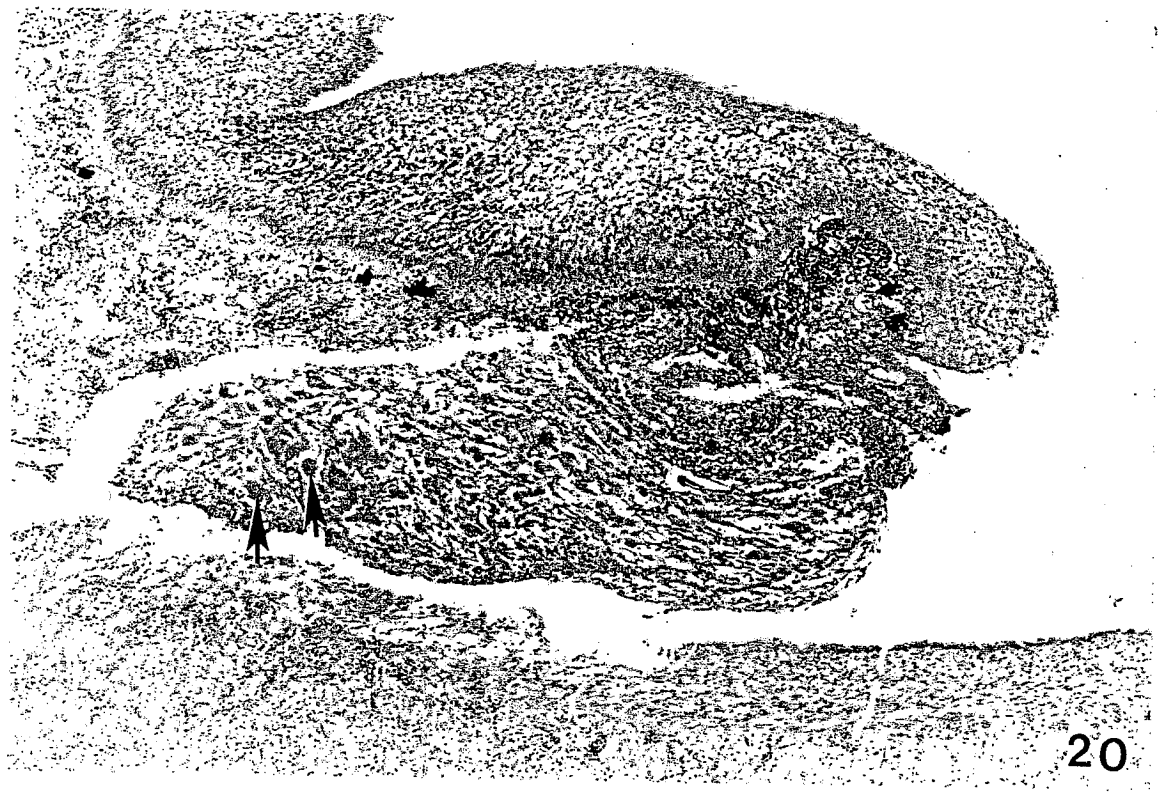

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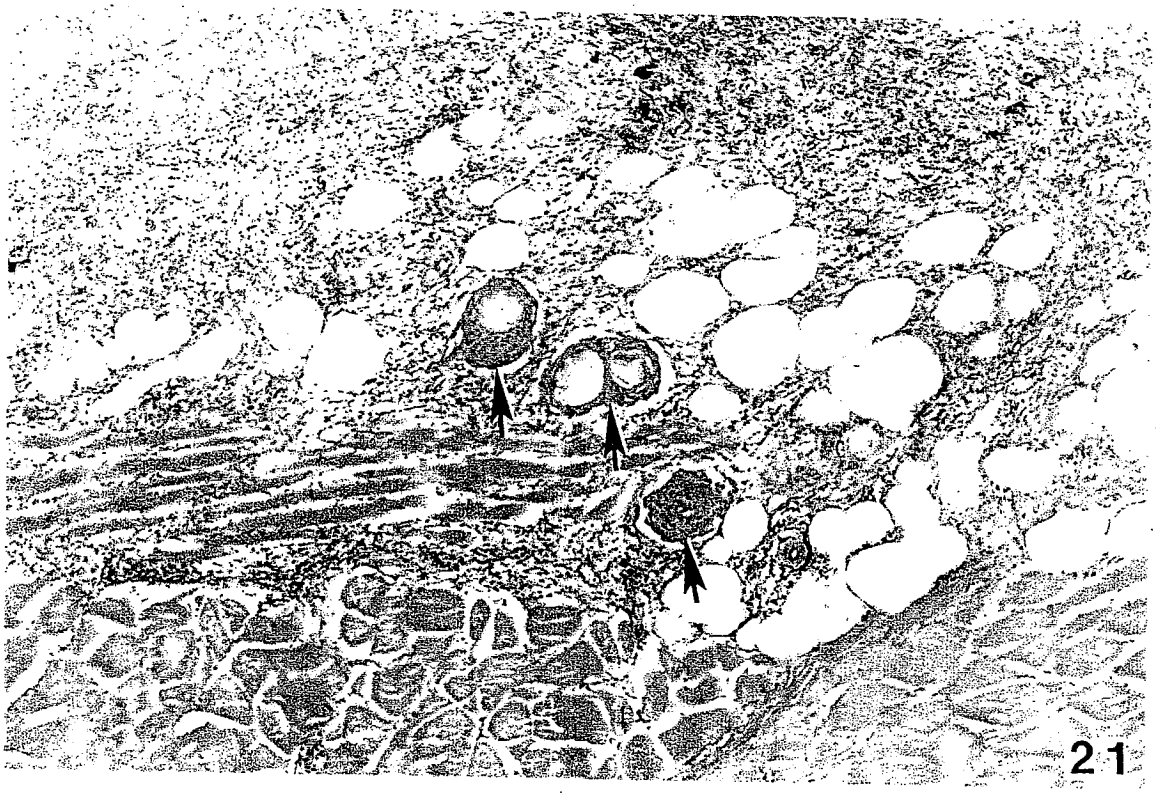


21

RESULTS

CALLEGUAS CREEK

Background

Calleguas Creek drains the Oxnard Plain, which is largely agricultural. This watershed has a history of pesticide contamination, in the water column, in the sediments, and in fish tissues. Body burdens of chlordane, DDT and toxaphene in fish are the highest found in the state. External lesions have been regularly reported on resident fish (Personal Communication, Regional Board staff). The most upstream site, C-3, is located on a tributary, Conejo Creek, downstream of two POTWs. C-2 is located on Calleguas Creek downstream of the confluence with Conejo Creek. C-4 and C-5 are located on another tributary, Revolon Slough, which is primarily comprised of agricultural runoff. C-6 is located on an agricultural return drain that enters Calleguas Creek and C-1 is located on Calleguas Creek just upstream from where it enters the Pacific Ocean.

A diagram of the sampling sites (Fig. H) and photographs of individual sites are presented at the end of the section on Calleguas Creek.

Toxicity Studies

The results of the toxicity tests are summarized in Table 4, presented at the end of this section. All of the sites produced measurable adverse effects with all three of the test species, with the exception of site C-2 in which no adverse effects were demonstrated with fathead minnow larvae. Samples from Site C-5, Beardsley Wash, reduced the survival of fathead minnow larvae in July 1992 and January 1993. One hundred percent mortality occurred in *C. dubia* exposed to samples collected in October, 1992 and in January and April, 1993. Finally, algal cell numbers were reduced in the July, 1992 and January and April, 1993 testing events.

Calleguas Creek

At site C-3, located on Conejo Creek, fathead minnow survival was reduced in the sample collected in October, 1992. One hundred percent mortality occurred in *C. dubia* exposed to samples collected in July and October, 1992, but no mortality occurred in either of the samples tested in 1993. Algal cell numbers were reduced in samples collected in July 1992 and in January and April, 1993.

As indicated previously, there were no adverse effects observed in fathead minnow larvae exposed to samples from site C-2, Calleguas Creek at Heuneme-Lewis Road. However, *C. dubia* exhibited 70 and 100% mortality, respectively, in samples collected in July and October, 1992. Furthermore, young production was reduced in samples collected in January, 1993. Algal cell numbers were reduced in the July 1992 and in the January and April, 1993 testing events.

Fathead minnow larvae exposed to samples from site C-4, Revolon Slough, exhibited reduced survival in the January and April, 1993 testing events. All of the exposed ceriodaphnids died in the sample collected in October 1992, and the average young production was reduced approximately 25%, compared with the control in the April 1993 testing event. Cell numbers were reduced in the algae in the July 1992 and January and April 1993 testing events with water from site C-4.

Samples collected from C-6, the "Duck Pond Agricultural Drain," reduced the survival of fathead minnow larvae in July and October, 1992 and in April, 1993. Growth of the larvae exposed to the January 1993 sample was almost 25% less than the controls. All of the *C. dubia* exposed to the sample collected in January, 1993 died. Reproduction was also reduced after exposure to the sample collected in April, 1993, when compared with its control. Algal cell numbers were decreased

in each of the four samples collected at site C-6.

Samples collected from site C-1, Calleguas Creek at the Pacific Coast Highway, reduced survival of larval fathead minnows in the July, 1992 and January, 1993 testing events. Ceriodaphnid survival was not affected in any of the four samples tested, but reproduction was reduced in tests with samples collected in October, 1992 and in January and April, 1993. *S. capricornutum* exhibited reduced cell numbers in samples collected in July, 1992 and April, 1993.

Collectively, these results confirm impairment of water quality in the Calleguas Creek watershed. Waters from virtually all sites proved toxic to all of the test organisms on multiple occasions. However, the waters from sampling sites were generally not toxic in all of the quarterly events, and the species affected (i.e., showing toxicity) also often varied with the event. This pattern suggests intermittent inputs into the system, and no single substance responsible for the observed effects. These toxicities could be the result of agricultural inputs or inputs from POTWs or both. Additional effort (more toxicant identification evaluations from samples collected more often) at this site would yield a more definitive cause-effect relationship.

Toxicity Identification Evaluations

The results of tests conducted on samples from the Calleguas Creek watershed are summarized in Table 5.

The sample collected at site C-2 in October, 1992 exhibited toxicity to *C. dubia* (total mortality within 48 hr.). Treatment of the sample with pH 3, aeration and solid phase extraction (SPE) column eliminated the toxicity. Moreover, the methanol extract from the SPE column also

exhibited toxicity while EDTA and sodium thiosulfate were not effective in reducing toxicity. These results suggest an organic constituent with labile properties at low pHs and possessing volatile characteristics.

Two samples from site C-3 were subjected to TIEs with *C. dubia* as the test organism. The first sample, collected in July, 1992, produced complete mortality within 48 hr. Filtration did not remove toxicity, but treatment with an SPE column did so. Elution of the column with a series of methanol:water fractions further showed that the toxicity was not restricted to the 90% methanol fraction but that some overlap into the 85 and 95% fractions occurred. These results suggested that an organic constituent caused the observed toxicity.

The second sample from site C-3 was collected in October, 1992, and killed 100% of the test organisms within 48 hours. Neither EDTA nor sodium thiosulfate affected toxicity, but toxicity was eliminated by treatment with SPE column, and, thereafter, was present in the methanol elution from that column. Treatment with pH 3 and pH 11 and aeration also reduced toxicity. These results suggest an organic constituent was responsible for the observed toxic effects.

One sample collected at site C-4 in October, 1992 produced 100% mortality in *C. dubia* within 48 hrs. Treatment with pH 3, EDTA and sodium thiosulfate had no effect on toxicity. However, passing the sample through an SPE column eliminated toxicity. Toxicity was present in the methanol elution of the SPE column. Treatment with pH 11, aeration and filtration also reduced toxicity. These results also implicate an organic constituent as the cause of toxicity.

Another sample from site C-4 (January, 1993) also produced elevated mortalities in fathead minnows. In this case, EDTA eliminated the toxicity but treatment with sodium thiosulfate did not.

Treatment with SPE column also eliminated toxicity but no toxicity was present in the methanol elution of the column. These results suggest that a divalent cation caused the toxicity. However, it probably was not Cd, Cu, Hg, or Ag since treatment with sodium thiosulfate also reduces the toxicity associated with these ions. (EPA, 1993).

Two samples collected at site C-5 were investigated for their effects on *C. dubia*. The first, collected in January 1993, produced 100% mortality within 48 hrs. With the exception of aeration and passage over an SPE column, none of the treatments reduced toxicity. To differentiate between volatility and oxidation, the sample was aerated with the inert gas helium, as well as with air. Both of these treatments eliminated toxicity, suggesting that a volatile organic was responsible for toxicity.

A second sample from site C-5 was collected in April, 1993 and killed all of the exposed *C. dubia* within 24 hrs. Treatment with pH 3 and pH 11 did not affect toxicity. Treatment with EDTA and with sodium thiosulfate also had no effect. Aeration eliminated toxicity as did treatment with an SPE column. Toxicity was confirmed in methanol eluate from the column. These results suggest an organic constituent as the toxic component.

A sample collected from site C-6 on October 22, 1992 significantly affected fathead minnows and algae. TIEs conducted with fatheads suggested that a divalent cation and/or organic toxicant might be responsible for toxicity.

A sample collected on April 1, 1993 at C-6 significantly affected all three test species. TIEs conducted with fathead minnows suggested that a divalent cation and/or organic toxicant might be responsible.

Chemical Analyses Results

Chemical results for Calleguas Creek samples sent for analysis are shown in Table 6. Chemical results are discussed by date below.

Both ceriodaphnids and fathead minnows were significantly affected by the sample collected on July 20, 1992 at site C-3. Ceriodaphnid TIE results indicated an organic as the source of the toxicity, however, no organochlorines, PCBs or organophosphates were detected in this sample.

Both fathead minnows and algae were significantly affected by the sample collected on July 20, 1992 at site C-6. No TIEs were conducted on this sample, therefore, it is difficult to determine the cause of toxicity. No organochlorines, PCBs or organophosphates were detected in this sample, however, three metals, barium, molybdenum and zinc, were detected in this sample. Metal toxicity is dependent on a number of biotic and abiotic factors, therefore, it is difficult to determine if one or more of these metals were responsible for the observed toxicity, however, with respect to zinc, 4 day EC50s for *Chlorella vulgaris* and *Chlorella saccarophila* were 2400 and 7100 μg/l (Reyes et al, State of California, Regional Water Quality Board Report, in prep). A life-cycle test using fathead minnows conducted at a hardness of 46 mg/l resulted in a chronic value of 106.3 μg/l (Reyes et al, State of California, Regional Water Quality Board Report, in prep). Hardness of C-6 collected in July was 1640 mg/l as $CaCO_3$.

Only ceriodaphnids were significantly affected by the sample collected on October 22, 1992 at site C-2. TIE results indicated that the source of ceriodaphnid toxicity was an organic, however, no organophosphate pesticides were detected.

Calleguas Creek

Both ceriodaphnids and fathead minnows were significantly affected by the sample collected at C-3 on October 22, 1992. TIE results indicated that the source of ceriodaphnid toxicity was an organic, however, no pesticides were detected.

Only ceriodaphnids were significantly affected by the sample collected on October 22, 1992 at site C-4. TIE results indicated that the source of ceriodaphnid toxicity was an organic, however, no pesticides were detected.

Both fathead minnows and algae were significantly affected by the sample collected on October 22, 1994 at C-6. TIEs suggested a divalent cation and/or organic toxicant might be responsible for fathead toxicity. Arsenic, barium, molybdenum, selenium, thallium and zinc were all detected. No thiocarbamates were detected. Metal toxicity is dependent on a number of biotic and abiotic factors, therefore, it is difficult to determine if one or more of these metals were responsible for the observed toxicity, however, with respect to zinc, 4 day EC50s for *Chlorella vulgaris* and *Chlorella saccarophila* were 2400 and 7100 μg/l (Reyes et al, State of California, Regional Water Quality Board Report, in prep). No hardness values were reported for either of these zinc values. For ceriodaphnids, acute zinc toxicity was observed at 32 μg/l and a hardness of 45 mg/l as $CaCO_3$ (Reyes et al, State of California, Regional Water Quality Board Report, in prep). A life-cycle test using fathead minnows conducted at a hardness of 46 mg/l resulted in a chronic value of 106.3 μg/l (Reyes et al, State of California, Regional Water Quality Board Report, in prep). Hardness of C-6 collected in October was 1840 mg/l as $CaCO_3$).

Both ceriodaphnids and algae were significantly affected by the sample collected at C-2 on January 20, 1993. No TIEs were conducted, therefore, it is difficult to estimate potential cause(s)

of toxicity. No organophosphate pesticides were detected.

Only algal toxicity was observed in the sample collected at C-3 on January 29, 1993. No TIEs were conducted on this sample and it is difficult to predict cause(s) of algal toxicity. No organophosphates were detected by chemical analytical methods.

Both fathead minnows and algae were significantly affected by the sample collected at C-4 on January 20, 1993. TIEs conducted with fathead minnows suggested that a divalent cation was responsible for fathead mortality, however, only organophosphate analyses were conducted on this sample, therefore, it is difficult to determine if metals were responsible. No organophosphates were detected.

Only ceriodaphnids were significantly affected by the sample collected at C-5 on January 20, 1993. TIEs with ceriodaphnids suggested that a volatile organic was responsible for toxicity. No organophosphate pesticides were detected, however, prior to analysis, the toxicant may have been lost through volatilization.

Both ceriodaphnids and algae were significantly affected by the sample collected at C-6 on January 20, 1993. However, as no TIEs were conducted on this sample, it is difficult to determine the nature of toxicity. No organophosphates were detected in the chemical analysis.

All three species were significantly affected by the sample collected on April 1, 1993 at site C-6. TIEs conducted with fathead suggested that a divalent cation and/or organic toxicant might be responsible for fathead toxicity. Only a metals analysis was conducted. Metals detected were barium, molybdenum, thallium and zinc. Metal toxicity is dependent on a number of biotic and abiotic factors, therefore, it is difficult to determine if one or more of these metals were responsible

for the observed toxicity, however, with respect to zinc, 4 day EC50s for *Chlorella vulgaris* and *Chlorella saccarophila* were 2400 and 7100 μg/l (Reyes et al, State of California, Regional Water Quality Board Report, in prep). No hardness values were reported for either of these zinc values. For ceriodaphnids, acute zinc toxicity was observed at 32 μg/l and a hardness of 45 mg/l as $CaCO_3$ (Reyes et al, State of California, Regional Water Quality Board Report, in prep). A life-cycle test using fathead minnows conducted at a hardness of 46 mg/l resulted in a chronic value of 106.3 μg/l (Reyes et al, State of California, Regional Water Quality Board Report, in prep). Hardness of C-6 collected in April was 1800 mg/l as $CaCO_3$).

Histopathology

The species of fish collected from the three sampling sites in the Calleguas Creek watershed are shown in the table below. Descriptions of alterations found at each site follow.

Collection sites, identification codes, fish species and number of individuals for Histopathology Studies on Calleguas Creek

Site	Fish Species	Number
Conejo Creek 1/2 river mile downstream of water collection site for toxicity studies†	*Pimephales promelas*	30
Revolon Slough at Wood Road at site where water was collected for toxicity studies	*Cyprinus carpio*	1
Calleguas Creek at Lewis Road at site where water was collected for toxicity studies	*Gambusia affinis* *Pimephales promelas* *Cyprinus carpio*	1 12 2

†This site was necessary since it provided shade on water surface where fish congregated

Histopathologic Studies of Fish from Conejo Creek

Thirty individual *Pimephales promelas* collected from this site were examined. The central nervous system including brain and spinal cord and the special sense organs, eye and internal ear, were free of alterations (Figures 22 and 23; Histopathology figures are presented at the end of this section). Spinal ganglia were also free of alterations. The gills did not reveal presence of any parasites and no necrosis of mitochondrial-rich (chloride) cells or of pavement respiratory epithelium was noted. A single aneurysm was found in the secondary lamellae of one fish. Given the fact that these fish were collected by gill net, it is entirely likely that the aneurysm may have resulted from that treatment. In one of the fish examined, hyperplasia of gill arch epithelial cells was detected. No abnormalities of the peritoneal cavity and mesenteries were observed. Similarly, the skin and buccal cavity epithelium were free of alterations. A single inflammatory focus in the body wall musculature was observed. The majority of the kidney sections revealed active regeneration of

glomeruli and tubules. However, the number and extent of this change was not considered to be outside of normal control ranges. Tubular epithelium of kidneys was free of hyaline droplet formation and revealed no other alterations. The interstitium of kidney was free of alteration. In one of the fish, perihepatic venus inflammation and adjacent hepatocellular necrosis was seen. No changes were observed with respect to the intrahepatic biliary system and one of the 30 individuals showed the presence of an encysted parasite within the liver. Hepatocytes of all fish were free of inclusions and there was no evidence of megalocytosis. Hepatic necrosis was seen in 2 of the 30 individuals examined (prevalence = 6.7%). Three of the 30 fish examined showed parasites within gut lumen (prevalence = 10%). All of the fish showed evidence of active feeding prior to their fixation. No changes were encountered in the exocrine pancreas and the heart and pericardial cavity were also free of alterations.

Revolon Slough at Wood Road

Histopathologic analysis was conducted on a single individual fish, a common carp (*Cyprinus carpio*). No other fish were collected although the equivalent amount of effort was made for collections as at other sites. The control type morphology of the liver is illustrated (Figure 24). The only alterations found within this single goldfish involved the kidney, skin, and heart. In the kidney, the tubular epithelium showed hyaline droplet change, particularly prominent in the proximal tubule. Histologic evidence in the form of basophilic clumps of cells with small lumens and capillary tufts signified regeneration of glomeruli and renal tubules (Hinton, et al., 1984b; Reimschuessel, et al., 1990). Normal histologic features of the intestine are shown (Figure 25). A single focus of inflammation was found in skin and subjacent dermis and upper portion of flank

muscle. This appeared to be an open sore or wound that was largely filled with white blood cells and debris (Figures 26 and 27). These may be due to opportunistic bacterial infection secondary to trauma (Sindermann, 1990; Sindermann, 1993). The atrial phagocytes of heart showed cytoplasmic enlargement characteristic of activation (Ferguson, 1989). These cells are often involved when a systemic inflammatory process is present (Ferguson, 1989). In addition, the myocardium of the heart ventricular wall was inflamed. This was particularly prominent at the epicardium. The presence of food particles within esophagus and intestine signified active feeding of this individual prior to its collection.

All goldfish (*Cyprinus carpio*) collected had external lesions of the skin (Fig. 35). Regional Board staff (personal communication) have stated that fish collected from this area routinely have external lesions.

Calleguas Creek at Lewis Road

The fish from Calleguas Creek at Lewis Road were collected from the same site where water was collected for the toxicity studies. One *Gambusia affinis*, 12 *Pimephales promelas* and 2 *Cyprinus carpio* were caught. The central nervous system including brain and spinal cord was free of alterations. Special sense organs such as eye and olfactory organ were also normal. Parasites were present in the peritoneal cavities of 2 of the 15 fish examined (prevalence = 13%) (Rogers and Gaines Jr, 1975; Wolke, 1975). However, upon close examination of the mesentery, there were no associated inflammatory lesions and no evidence for adhesions or for granuloma formation was found. Examination of the trigeminal nerve and its ganglion also revealed a lack of alteration. The gills were free of parasites in all of the fish examined (Figure 28). This does not necessarily mean

that certain parasites were not attached to the gill originally. It is known that attachment can be lost during storage of material in fixative and subsequent processing steps (Rogers and Gaines Jr, 1975). One of the fish examined showed selective necrosis of the mitochondria-rich (chloride) cells and this same individual showed necrosis of the surface respiratory epithelium of the secondary lamellae in gill. These lesions resembled those seen after metal exposure (Mallatt, 1985). Filament epithelial hyperplasia (Smith, 1984) is shown (Figure 29). No aneurysms were present. Inflammation of the gill and branchial cavity were present in 20% of the individuals. One of the fish, a goldfish (*Cyprinus carpio*) showed defective alignment and structure of the filament cartilage in the gill. After examination of gill, transporting epithelial cells and interstitium of kidney were carefully examined. The kidneys revealed a normal amount of glomerular and tubular regeneration. These, extremely basophilic, cell clusters apparently occur even during young adult and adult life of various fish species (Hinton, et al., 1984b). The extent and relative number of regenerating tubules and glomeruli were regarded as within the range of normal. Two of the 15 fish (13% prevalence) showed tubular epithelial change (Figure 30). This included appearance of hyaline granules within epithelial cells and accentuated intercellular space. In addition, 4 of the 15 fish examined showed cellular casts within the lumens of the distal nephron and adjacent collecting ducts. The prevalence for this condition was 27%. In some, but not all, of the fish showing cellular casts and tubular epithelial change, interstitial inflammation of the kidney was found. The prevalence for this condition was 33%. Four of the 15 fish examined (a prevalence of 27%) showed fatty vacuolation of hepatocytes in livers. In addition, the single *Gambusia affinis* studied showed single cell hepatocyte necrosis and foci of hepatocellular necrosis (Hinton, 1993a; Hinton, 1993b). A single *Pimephales promelas*

showed the presence of a basophilic adenoma of the liver (Figures 31 and 32). This tumor had a discrete margin and was stained differently than the surrounding liver (Hinton, et al., 1992). One of the fish from this site revealed the presence of parasites within the liver. In addition, one of the fish showed necrosis of hepatocytes surrounding large tributaries of the hepatic veins. No evidence for bile duct proliferation and associated inflammation was seen. There were no hepatocyte inclusions and no evidence for megalocytosis was seen. Examination of the digestive system including esophagus, stomach or intestinal bulb (cyprinid fishes) (Hinton, et al., 1984b) and intestine indicated that all of the fish had been actively feeding immediately prior to their capture. A single individual showed a large nematode within the gut lumen. The exocrine pancreas was free of alteration. Two of the individuals showed inflammation of the myocardium and epicardium of the heart. Inflammatory cells were also found in the pericardium cavity of these individuals. In one fish, a large inflammatory focus was seen within the flank skeletal muscle. A normal change associated with breeding activity was present in male *Pimephales promelas*. This is the presence of keratinized epithelium with hyperpigmentation of the dermis (Hinton, et al., 1984b). Taken together, these are breeding tubercles that are prominent on the heads of actively breeding males of this species. There was no indication of skin inflammation and buccal cavity epithelium was also free of change.

Figure 35 illustrates the examples of external lesions found in fish inhabiting this drainage. All goldfish (*Cyprinus carpio*) collected had external lesions of the skin. Regional Board staff (personal communication) have stated that fish collected from the area routinely have external lesions.

Summary of Findings

Multiple historical lines of evidences including fish body burdens of environmental contaminants, sediment toxicant chemistry, repeated findings of external lesions; and, results reported herein including toxicity tests, toxicant identification evaluations, and histopathology confirm that water quality is impaired in this watershed. All of the sites were toxic to all of the test organisms on multiple occasions and follow-up TIEs generally associated toxicity with non-polar organic chemicals. This category would include pesticides. However, the sites were generally not toxic in all of the events, and the species affected also often varied with the event. This pattern suggests intermittent, but severe, inputs into the system, and no one substance responsible for the observed effects. This pattern is consistent with the surrounding land use being primarily agricultural and may be associated with applications of different pesticides to different crops. For these short-term toxicity tests, the responses are probably due to inputs into the water column, rather than mobilization from the sediments, which would be expected to maintain relatively uniform concentrations in the water column. Histopathologic analysis of Conejo Creek fish revealed a near control appearance. Despite a thorough analysis of each organ system, alterations from normal were rare and did not fit alterations directly attributable to exposure to toxicants within the water column.

Only one fish was collected from Revolon Slough, but analysis of all internal organ systems revealed near normal morphology. Evidence of skin inflammation was present and the lesion showed characteristics which would be expected after invasion of a traumatic wound to the body surface by opportunistic bacteria (Ferguson, 1989). Similar changes in underlying dermis and adjacent skeletal muscle of body wall were consistent with bacterial spread. In all likelihood, the

skin lesion led to a generalized systemic infectious process which was evident by the extensive rounded nature of atrial (heart) phagocytes (Ferguson, 1989). The kidney alterations might have resulted from toxicant exposure (Reimschuessel, et al., 1989). Metals and certain organic compounds have been shown to produce similar lesions in fish exposed in laboratory studies (Meyers and Hendricks, 1985; Reimschuessel, et al., 1990). The hyaline droplets in these cells and the presence of cellular casts in distal portions of the nephron, suggest degeneration and necrosis of cells in proximal tubules (see review Meyers & Hendricks 1985).

The fish of the Calleguas Creek collections apparently resided in lower reaches of the watershed and, when compared to their counterparts in Conejo Creek and Revolon Slough, appeared to pay a greater cost for residence at their particular habitat. Evidence of necrosis of mitochondria-rich (chloride) cells and pavement, respiratory epithelium was seen in gill of one fish. This change is closely associated with metal exposure and may follow ammonia exposure (Mallatt, 1985; Smith and Piper, 1975). The filament epithelial hyperplasia could have been in response to prior toxicity. Inflammation of the gill and branchial cavity could have followed earlier necrosis of cells in this epithelial tissue. However, this change could be due to bacterial infection (Ferguson, 1989). Kidneys in two of the 15 fish showed tubular epithelial hyaline granule change. In addition, cellular casts (in higher prevalence) could be indicative of prior toxicant induced cellular degeneration and necrosis (Meyers and Hendricks, 1985). Fatty vacuolation of hepatocytes is a hallmark of toxicity and may be produced by exposure to organochlorines, polychlorinated biphenyls, pesticides, and other compounds (Couch, 1985; Hinton and Laurén, 1990a). Similarly the hepatic necrosis in the single mosquito fish (*Gambusia affinis*) was likely due to exposure to hepatotoxic substances

(Hinton, 1993a). The liver tumor, basophilic adenoma, seen in one fathead minnow (*Pimephales promelas*) is a biomarker lesion closely associated with exposure to carcinogenic toxicants (Baumann, 1992; Hinton, et al., 1992; Myers, et al., 1987).

Whereas the quarterly water samples and toxicity tests thereof argue for impairment of water quality, they represent grab samples that are not indicative of the variations with time at a given site. However, histopathology is an integration over time and when coupled with the toxicity tests provides additional evidence of multiple stressor effects in the lower reaches of the watershed (Hinton et al., 1992).

Recommendations

Toxicity tests indicated that water quality is frequently impaired in this watershed. Although only four testing events were completed, water samples from all of the sites were toxic at least half of the time. Furthermore, significant responses were seen with all three species, suggesting that a variety of toxicants were present at different times. This should not be surprising, given the mosaic of urban and agricultural land uses that occur in this drainage. The situation is further complicated by the contributions of POTWs that discharge into the watershed. Fish collected in Calleguas Creek also exhibited histological lesions consistent with poor water quality.

TIEs were generally conducted with *C. dubia*. In these instances, toxicity was uniformly associated with the non-polar organic fraction. Only one TIE was conducted with fathead minnows and suggested that metal ions were responsible for the toxicity of this sample. These results could be attributed to discharges from the POTWs, and/or from agricultural and urban areas since all three

could act as sources of organic and inorganic toxicants. Thus, further characterization of the toxic constituent(s) is necessary before the possible sources can be identified. Point or non-point source controls should be implemented as required to reduce inputs.

Because most of the water samples tested exhibited toxicity to one, or more, of the test species, the implication is that water quality could be markedly improved if the causes of toxicity could be identified and reduced to concentrations not associated with harmful effects. To achieve this goal, a sampling program should be initiated with more frequent sampling intervals. This would be of particular importance in identifying seasonal trends associated with different crop practices and pesticide applications in the watershed.

By conducting TIEs on the toxic samples, including Phase II and III identification and more sensitive characterization (analytical chemistry) procedures, the identities of major contributors to toxicity should be determined. Once this task has been completed, source control, or in the case of agricultural substances, modified application practices, should be implemented to reduce the concentrations of harmful substances to acceptable levels. Follow-up monitoring programs should then be implemented to evaluate the effectiveness of any reduction program.

Diagram and Photographs of Sampling Sites

Calleguas Creek

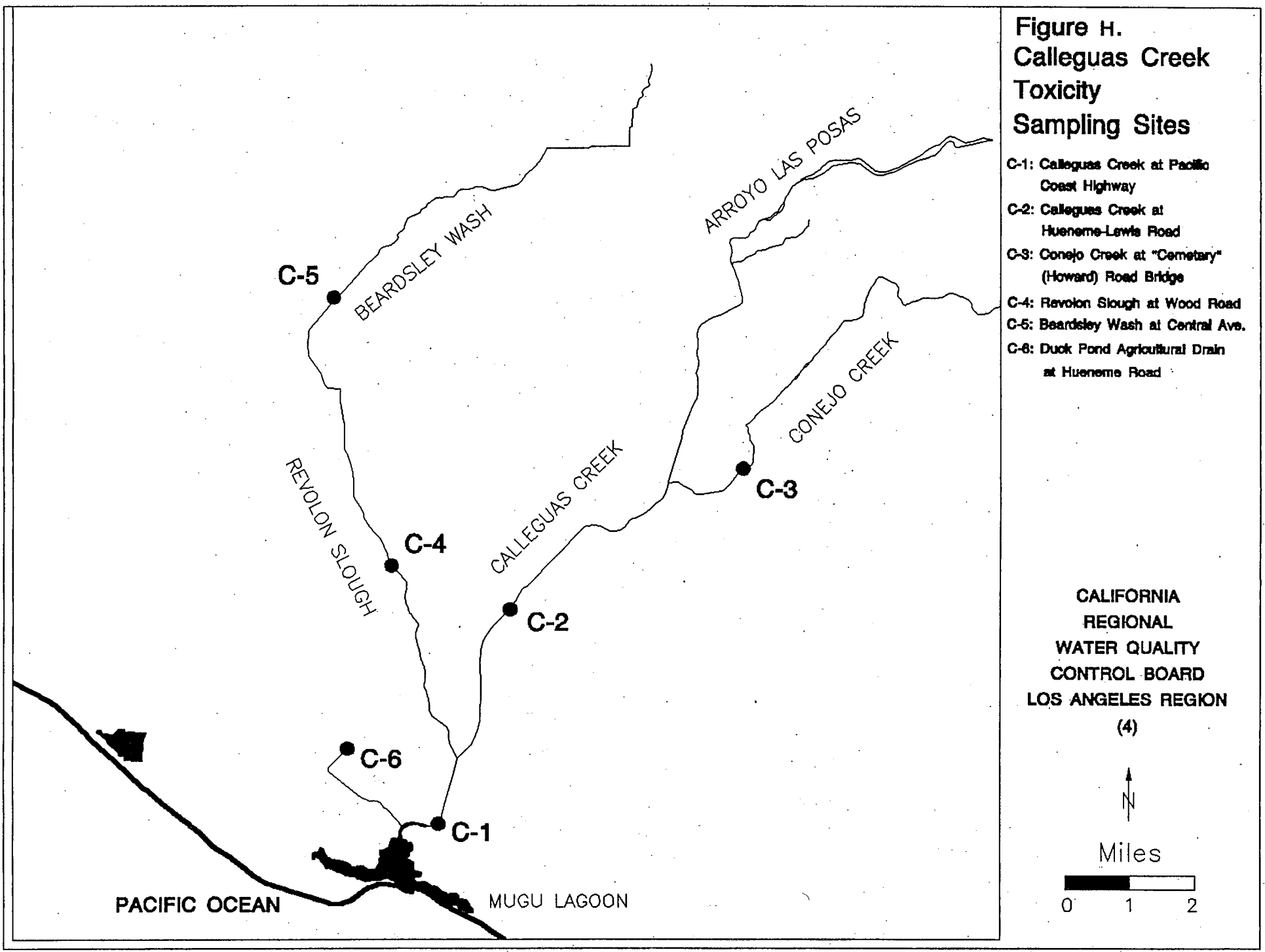


Figure H.
Calleguas Creek Toxicity Sampling Sites

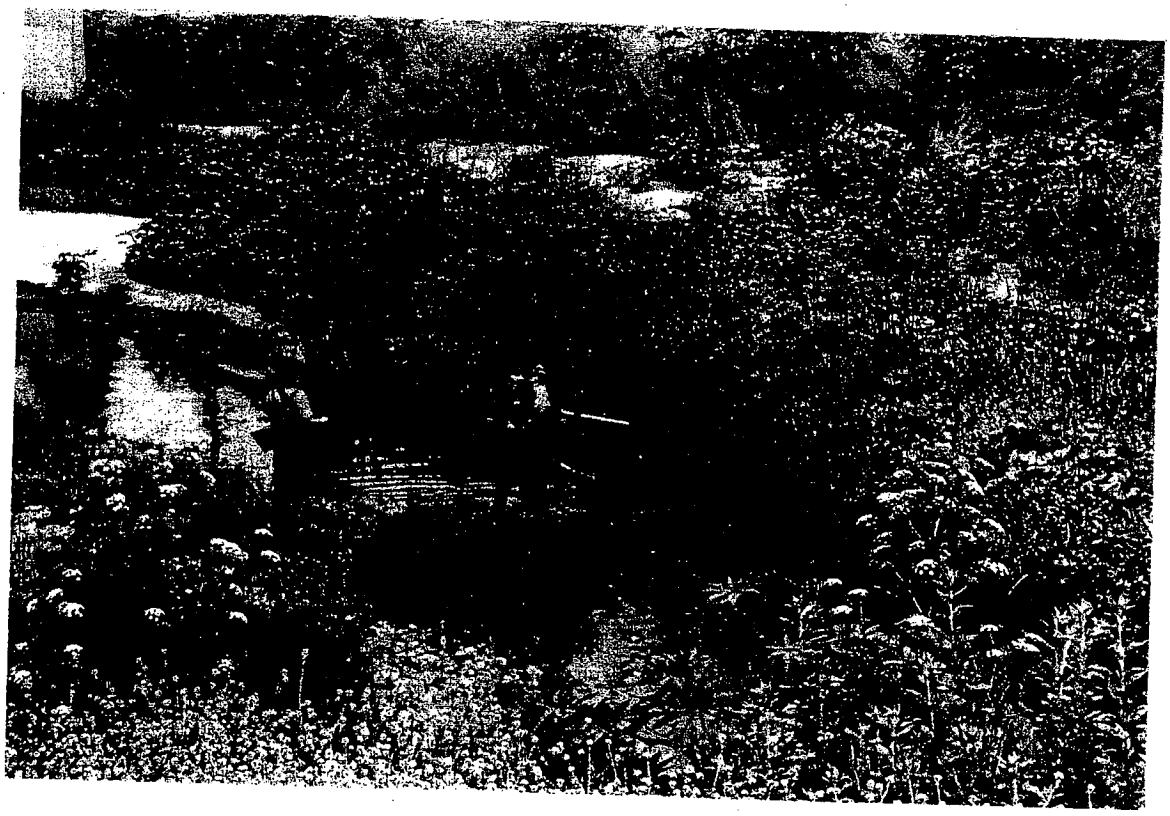
C-1: Calleguas Creek at Pacific Coast Highway
C-2: Calleguas Creek at Hueneme-Lewis Road
C-3: Conejo Creek at "Cemetary" (Howard) Road Bridge
C-4: Revolon Slough at Wood Road
C-5: Beardsley Wash at Central Ave.
C-6: Duck Pond Agricultural Drain at Hueneme Road

CALIFORNIA
REGIONAL
WATER QUALITY
CONTROL BOARD
LOS ANGELES REGION
(4)

N

Miles
0 1 2

C-4 REVOLON SLOUGH AT WOOD ROAD
6/2/92

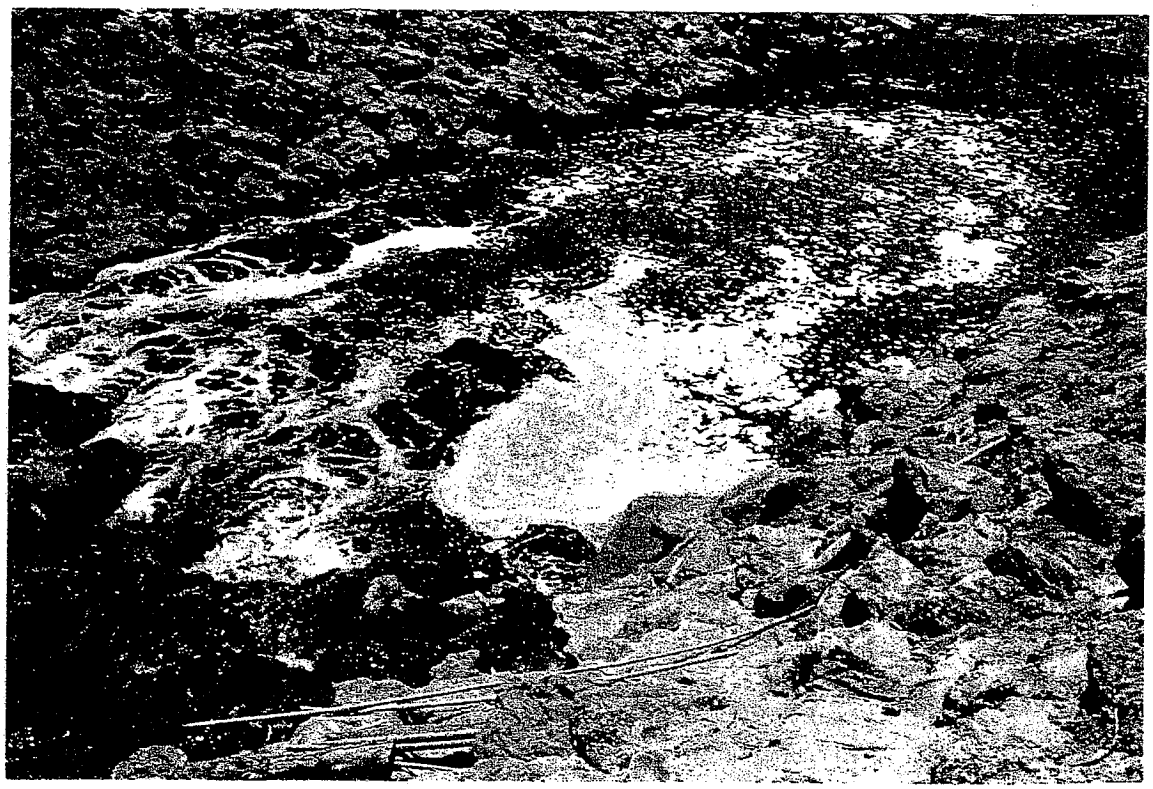


SHOCKING FOR FISH.

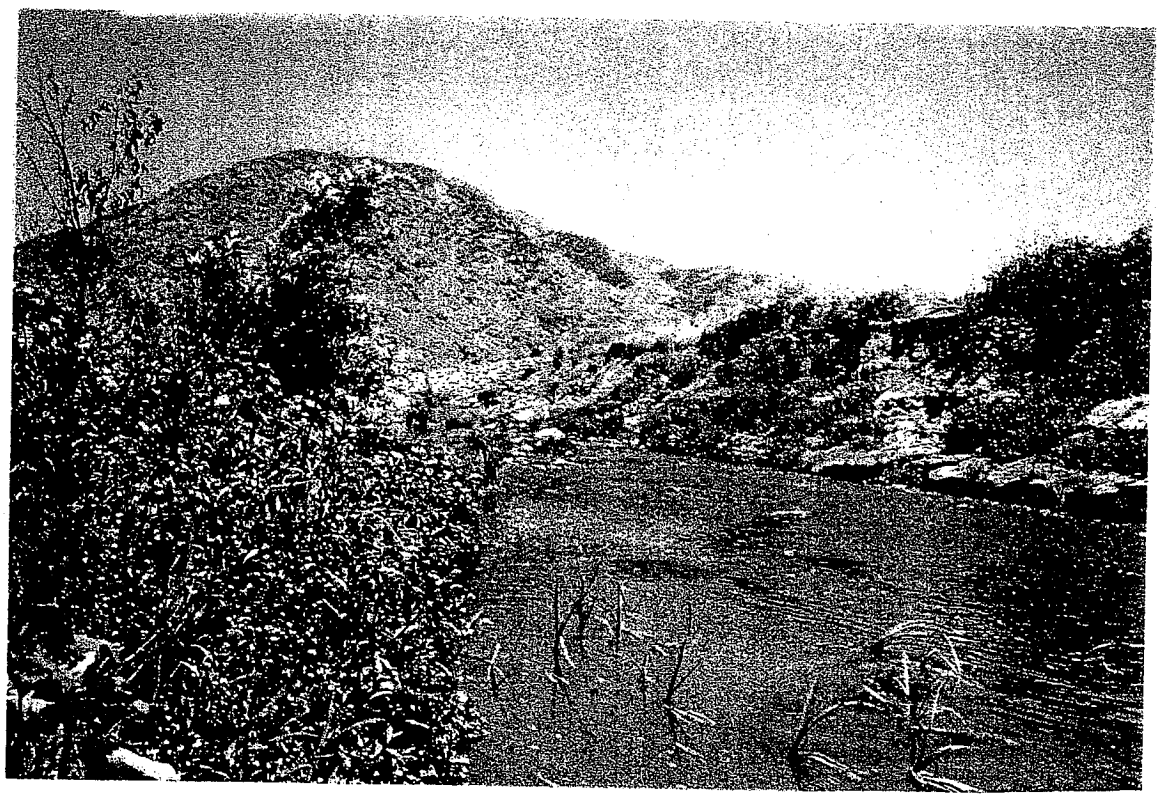
C-4 REVOLON SLOUGH AT WOOD ROAD
6/2/92

CLOSE - UP

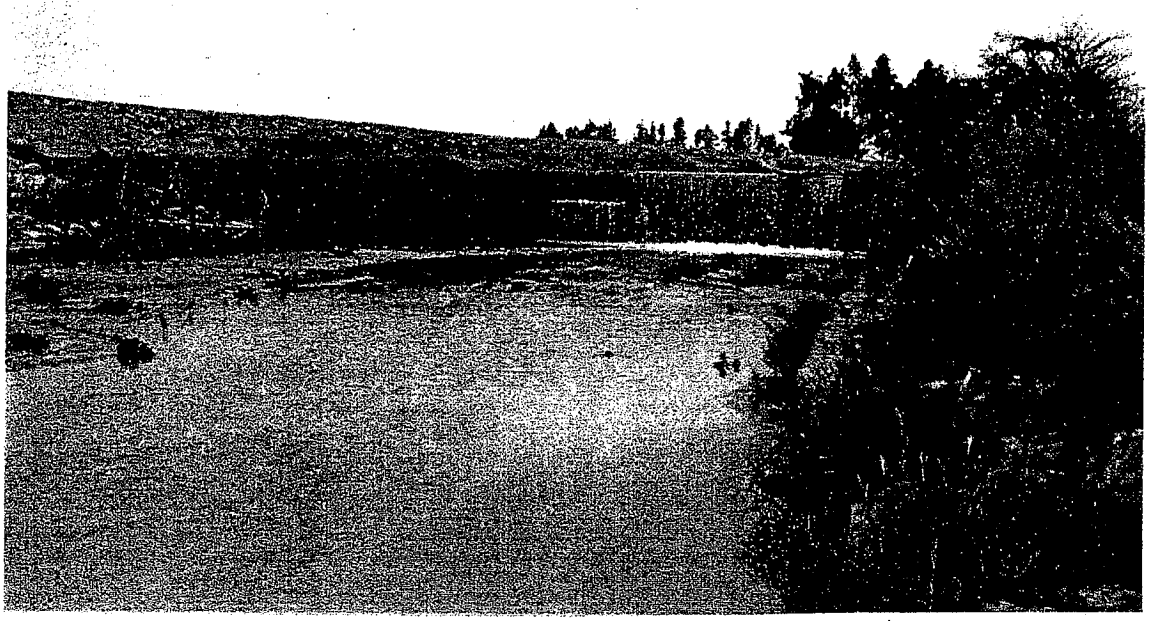
C-3 CONEJO CREEK AT HOWARD ROAD BRIDGE.
NOTE AGRICULTURAL DISCHARGE AT LEFT CENTER AND IN FOREGROUND. THE CAMARILLO POTW IS IMMEDIATELY UPSTREAM OF THIS SITE.



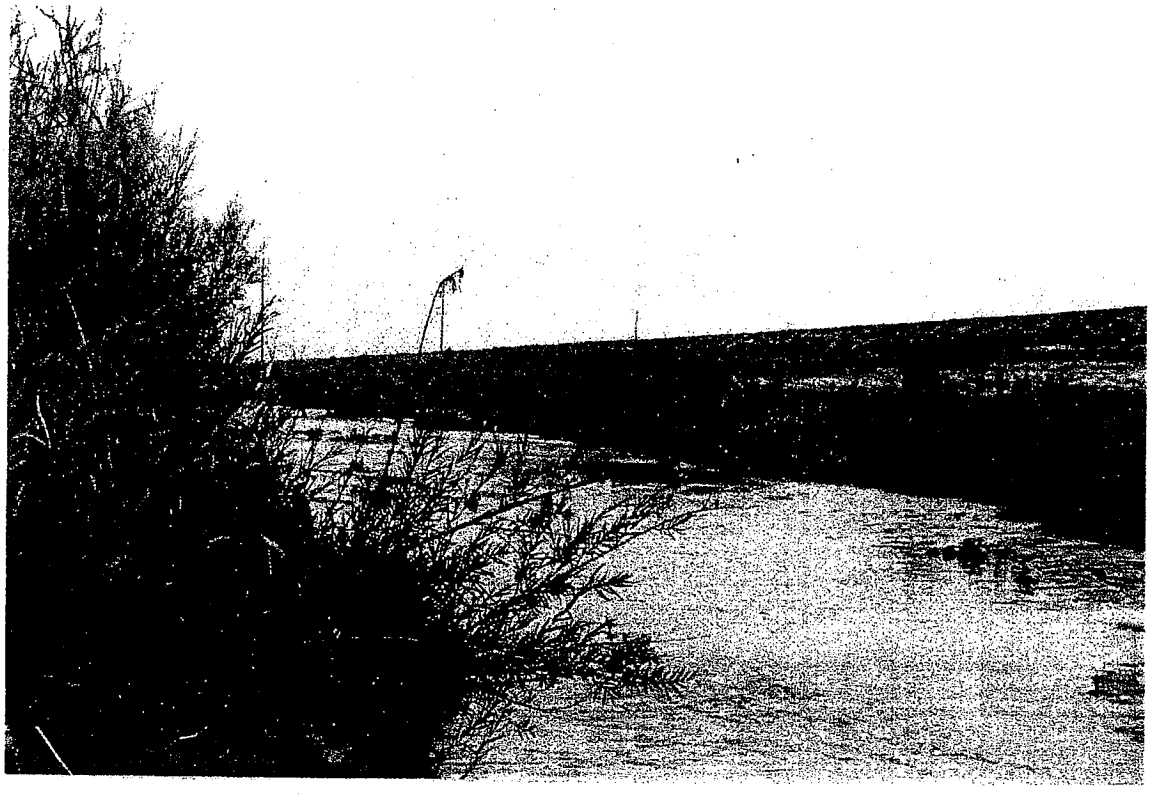
CONEJO CREEK AT SMITH RANCH, DOWNSTREAM OF SITE C-3
OUR FIRST ATTEMPTED FISH COLLECTION SITE
6/2/92



SITE FURTHER DOWNSTREAM BELOW RIP RAP WHERE SUCCESSFUL COLLECTION
OF FISH FOR HISTOPATHOLOGICAL ANALYSES WAS DONE



C-2 CALLEGUAS CREEK AT HUENEME - LEWIS ROAD
6/2/92
(NOTE: FISH COLLECTED FOR HISTOPATHOLOGY AT THIS SITE)



Summary Tabulation of Toxicity Tests and TIEs

Calleguas Creek

Table 4. Summary of toxicity tests conducted on samples from the Calleguas Creek Watershed

Site	Parameter	Test Date 7/23/92	Test Date 10/23/92	Test Date 1/21/93	Test Date 4/2/93
C-6	Fathead Minnow				
	Survival (%)	27.3*	22.3*	91.7	5.3*
	Growth (mg)	0.169*	0.229*	0.375*	0.1*
	Ceriodaphnia				
	Survival (%)	100	100	0*	100
	Reproduction	28.5	16.1	1.8*	18.1*
	Selenastrum cap.				
	Cells/ml	315100*	223000*	1043280*	221253*
C-5	Fathead Minnow				
	Survival (%)	68.2*	93.5	76.7	96.7
	Growth (mg)	0.251*	0.35	0.445	0.419
	Ceriodaphnia				
	Survival (%)	100	0*	0*	0*
	Reproduction	36.9	011*	0*	0*
	Selenastrum cap.				
	Cells/ml	148940	1587500	386613	565419

Table 4. Summary of toxicity tests conducted on samples from the Calleguas Creek Watershed					
Site	Parameter	Test Date 7/23/92	Test Date 10/23/92	Test Date 1/21/93	Test Date 4/2/93
C-4	Fathead minnow				
	Survival (%)	95.1	92.2	21.7*	27*
	Growth (mg)	0.367	0.291	0.07*	0.141*
	Ceriodaphnia				
	Survival (%)	100*	0*	100	90
	Reproduction	31	0*	27.8	21.5
	Selenastrum cap.				
	Cells/ml	870100*	1399660	419573*	235253*
C-3	Fathead minnow				
	Survival (%)	95	76.6*	96.7	91.7
	Growth (mg)	0.337*	0.226*	0.426	0.313
	Ceriodaphnia				
	Survival (%)	*0	0*	100	100
	Reproduction	0.4*	0*	25.7	25.8
	Selenastrum cap.				
	Cells/ml	60940*	1019600	1469340*	113160

Table 4. Summary of toxicity tests conducted on samples from the Calleguas Creek Watershed

Site	Parameter	Test Date 7/23/92	Test Date 10/23/92	Test Date 1/21/93	Test Date 4/2/93
C-2	Fathead minnow				
	Survival (%)	30*	0*	100	100
	Reproduction	27.4	0*	19*	25.4
	Selenastrum cap.				
	Cells/ml	36500*	1271573	1551480*	217866*
C-1	Fathead minnow				
	Survival (%)	74.9	89.3	78.3	96.7
	Growth (mg)	0.423	0.318	0.426	0.359
	Ceriodaphnia				
	Survival (%)	100	90	100	90
	Reproduction	28.4	15.4	17*	17*
	Selenastrum cap.				
	Cells/ml	49520*	754760	1567313*	381366*
Control	Fathead minnow				
	Survival (%)	95	93.9	95	96.7
	Growth (mg)	0.398	0.31	0.457	0.366

Table 4. Summary of toxicity tests conducted on samples from the Calleguas Creek Watershed					
Site	Parameter	Test Date 7/23/92	Test Date 10/23/92	Test Date 1/21/93	Test Date 4/2/93
	Ceriodaphnia				
	Survival (%)	100	100	100	100
	Reproduction	24.9	19.6	26.8	26
	Selenastrum cap.				
	Cells/ml	1176060	752440	1818940	2317313

*= significance @ $P \leq 0.05$; see Methods for statistical tests.

Table 5. Summary of Results of Toxicity Identification Evaluations

Site	Date	Species	pH 3	pH 11	Grad. pH	EDTA	Na2S203	Air	Fil.	SPE Col.	Meth Elute
C-2	10/22/92	Cerio	+	+/-	-	-	-	+	+/-	+	+
C-3	7/22/92	Cerio	N/T	N/T	N/T	N/T	N/T	N/T	(-)	(+)	+
	10/22/92	Cerio	+a	+a	-	-	-	+a	+a	+	+
C-4	10/22/92	Cerio	-	+a	-	-	-	+a	+a	+	+
	1/20/93	Fathead	N/T	N/T	N/T	+	(-)	N/T	N/T	+	_
C-5	1/20/93	Cerio	-	-	N/T	-	-	++	N/T	+	-
	4/1/93	Cerio	-	-	N/T	-	-	+	N/T	+	+
C-6	10/22/92	Fathead	-	+	-	+	-	+	+/-	+	N/T
	2/1/93	Cerio	+	+	N/T	-	+	+	N/T	+	-
	4/1/93	Fathead	+	+	N/T	+	+/-	-	N/T	+	-

Key: N/T = Not tested; + = Test passed; - = Test failed
+/- = Ambiguous results
* = Sample no longer toxic at time of TIE
a = 24 hour delay in mortality
++ = Test passed using helium and air

Table 6. Summary of Analytical Chemistry Results for Calleguas Creek (6/92 to 4/93)

Date Sampled	Sample ID (Site #)	Eureka Laboratories	Hardness/ Alkalinity	µg/l (ppb)	Detection Limits (ppb)
7/20/92	C-3	No organochlorine pesticides or PCBs detected. No organophosphorus pesticides detected			
7/20/92	C-6	Barium Molybdenum Zinc No organochlorine pesticides or PCBs detected No organophosphorus pesticides detected	Hardness = 1640 Alkalinity = 260	40.0 90.0 30.0	20.0 20.0 10.0
10/22/92	C-2	No organophosphorus pesticides detected			
10/22/92	C-3	No organophosphorus pesticides detected No thiocarbamates detected No carbamates or urea pesticides detected			
10/22/92	C-4	No organophosphorus pesticides detected No thiocarbamates detected No carbamates or urea pesticides detected			
10/22/92	C-6	Arsenic Selenium Barium Molybdenum Thallium Zinc No thiocarbamates detected	Hardness = 1840 Alkalinity = 320	4.00 10.0 40.0 100.0 200.0 20.0	4.00 3.00 20.0 20.0 100.0 10.0 *6.70
1/20/93	C-2	No organophosphorus pesticides detected			
1/20/93	C-3	No organophosphorus pesticides detected			
1/20/93	C-4	No organophosphorus pesticides detected			
1/20/93	C-5	No organophosphorus pesticides detected			
1/20/93	C-6	No organophosphorus pesticides detected			
4/1/93	C-6	Barium Molybdenum Thallium Zinc	Hardness = 1800 Alkalinity = 360	40.0 100.0 200.0 20.0	20.0 20.0 100.0 10.0

*Higher detection limit due to insufficient sample provided

Illustrations of Histological Lesions

Calleguas Creek

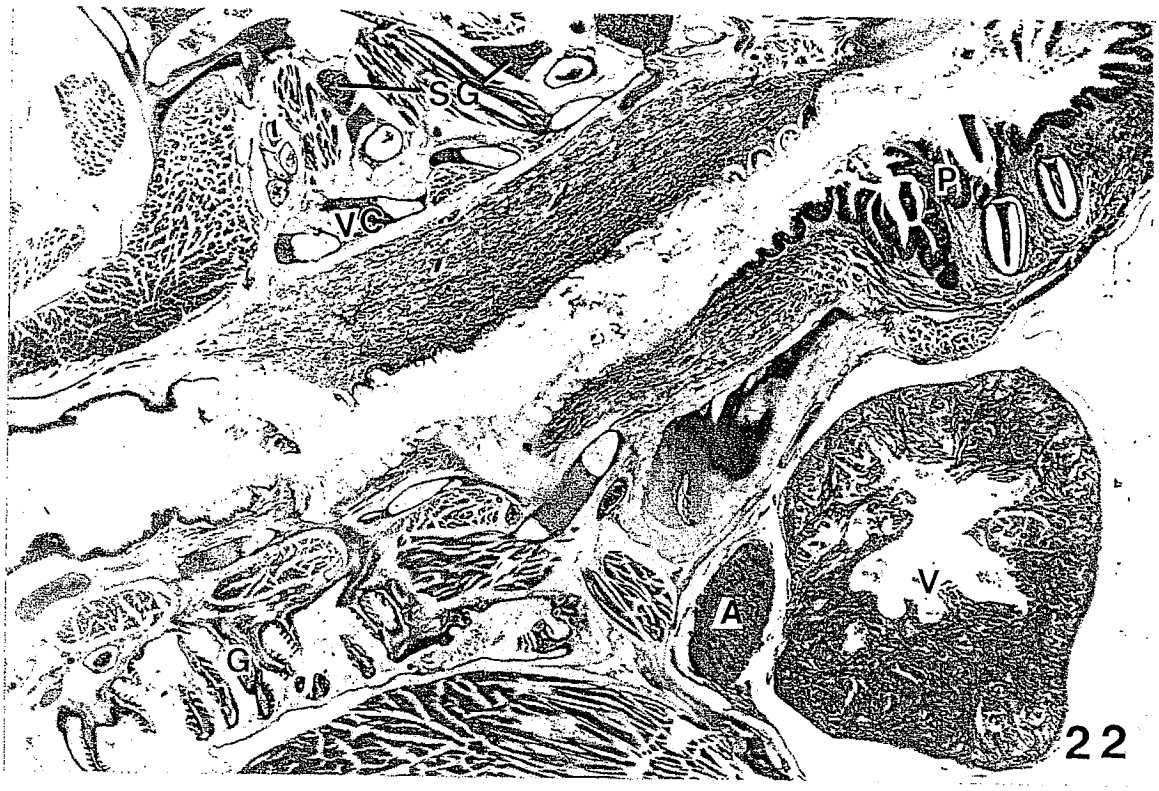
(Figures 22 - 35)

HISTOPATHOLOGY INVESTIGATIONS AT CALLEGUAS CREEK AND ITS TRIBUTARIES

FIGURE LEGENDS

Figure 22. This photomicrograph is from the head region of a *Pimephales promelas* collected from Conjeo Creek one-half river mile downstream from the collection site for toxicity studies. Although this figure does not illustrate pathologic alteration, it illustrates the types of organs that may be studied in a parasagittal section. The following tissues are represented: ventricle of heart (V), aortic bulb (A), gill (G), pharynx (P), vertical column (VC) and spinal ganglia (SG). Hematoxylin and eosin X 20.

Figure 23. This parasagittal section from the head region of the same fish as described in Figure 22 illustrates part of olfactory organ (O), buccal cavity (BC), and eye (E). Optic nerve (ON), ocular muscles (OM), trigeminal ganglion (T), are shown. A portion of the telencephalon of the brain (B) is also shown. Hematoxylin and eosin stain X 20.



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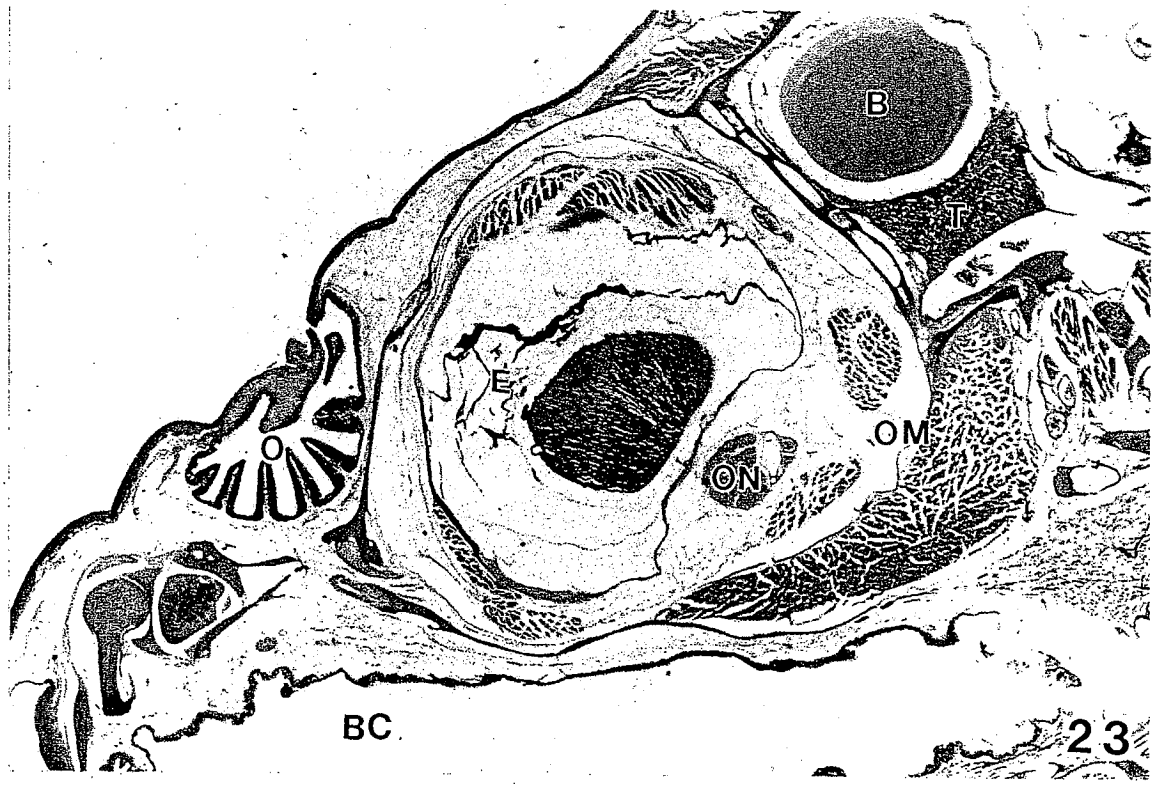
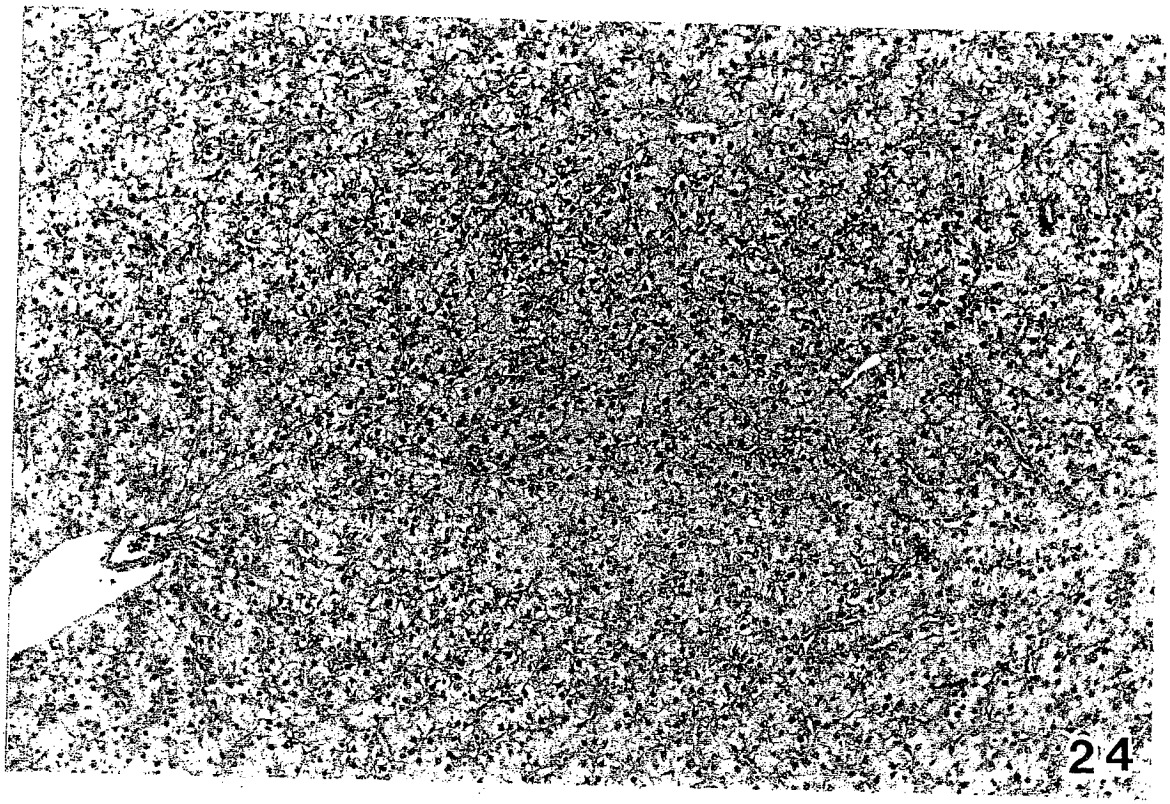


23

Figure 24. This photomicrograph illustrates liver morphology from a goldfish collected at Revolon Slough. The essential normal morphology of the liver is well illustrated. The light staining grey regions within hepatocytes, the major cells of the liver, indicate abundant glycogen. The blood vessel profiles are free of associated inflammatory cells. Hematoxylin and eosin stain X 100.

Figure 25. This photomicrograph from the goldfish collected at Revolon Slough shows features that are consistent with normal morphology of the intestine. The surface epithelial cells are light grey in staining because they contain abundant mucous. A small degree of inflammatory cells may be present in the mucosa and submucosa of this fish. Normal appearing pancreas is shown at the left of the field. Hematoxylin and eosin stain X40.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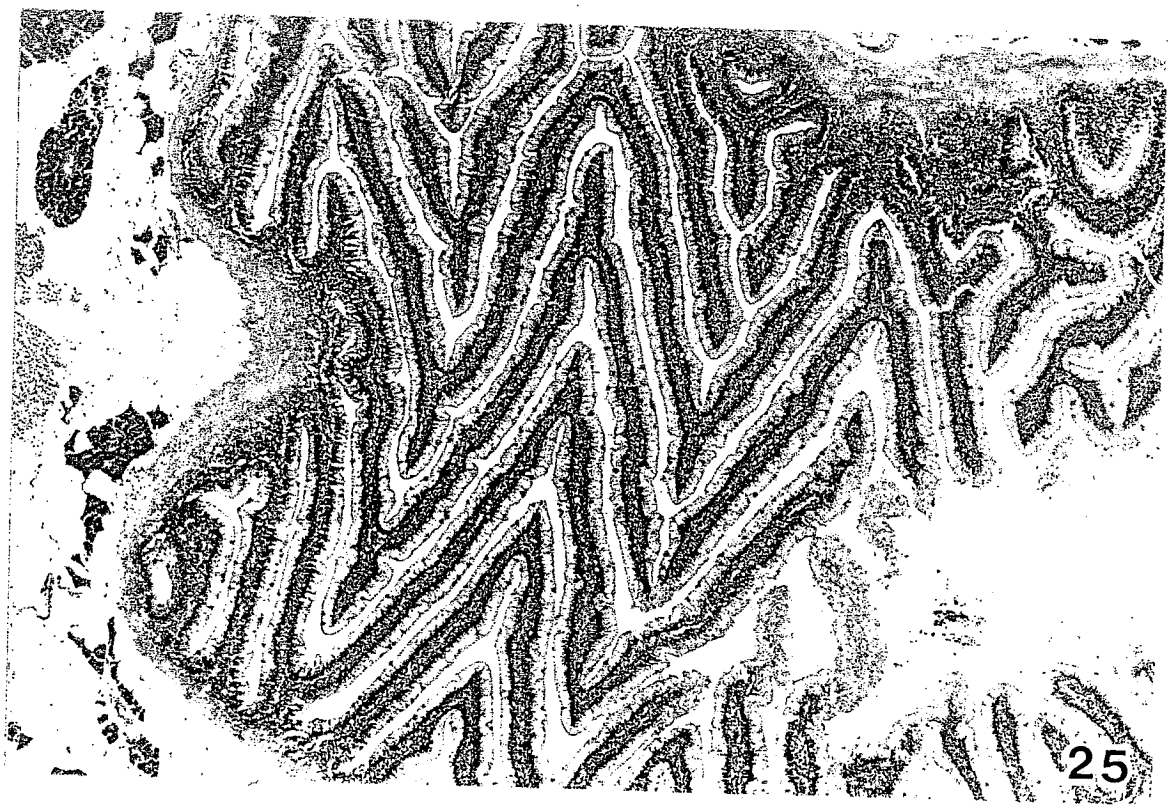

25

Figure 26. Although the intestine and liver of this goldfish collected from Revolon Slough were normal, a rather large surface lesion was located on the trunk of the animal. This surface lesion is illustrated in the split field as shown. In the left hand side of the field extensive inflammation and edema characterize the epidermis and dermis. Note the pink staining scale that is present at the middle portion of the field. Below the scale the region of the dermis shows inflammation and edema and this is contrasted with the thickened connective tissue at the bottom most portion of the dermis and the subjacent skeletal musculature of the trunk. Higher magnification view of a portion of this lesion is shown in the field at the right. Here inflammatory cells and wispy, edematous connective tissue are shown. Hematoxylin and eosin stain X 200 (right hand side of field) X 40 (left hand side of field).

Figure 27. A lower magnification view of the large surface lesion illustrated in Figure 26 above is shown. Note the extensive nature of this surface wound. It is likely that this type of lesion was associated with direct trauma to the body of the fish and was associated probably with secondary bacterial invasion following the traumatic injury. Hematoxylin and eosin stain X 20.

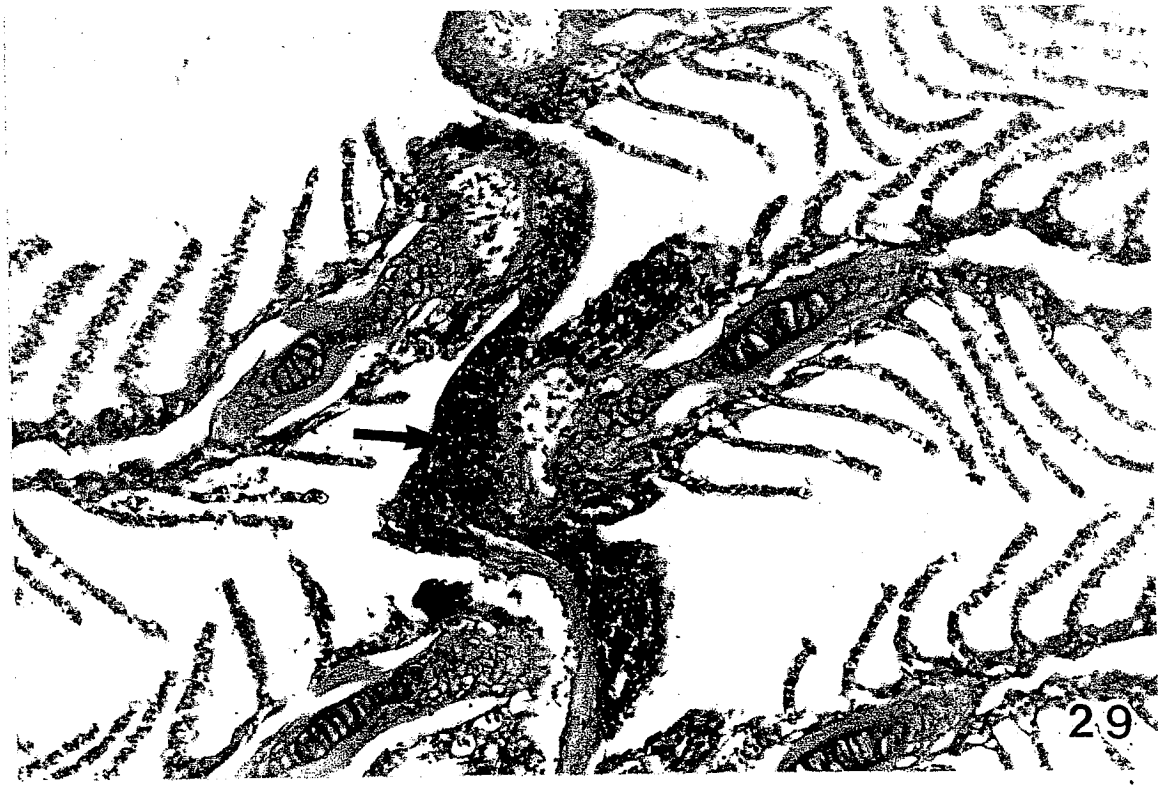
26

27

Figure 28. This photomicrograph is of the gill and illustrates secondary lamellae (2), primary lamellae (1), and, at the far right hand side of the field, attachment of filaments to the arch epithelium. Cartilage (C) is shown at the central most region of the gill filament. Hematoxylin and eosin stain X 200.

Figure 29. This photomicrograph illustrates features of the secondary lamellae, filaments and arch of a fathead minnow collected from Calleguas Creek at Lewis Road. The secondary lamellae appear normal while the filament epithelium shows hyperplasia (large arrow). Hematoxylin and eosin stain X 200.


2
1
C
28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Calleguas Creek

Figure 30. This photomicrograph shows a section through the trunk kidney of a male fathead minnow (*Pimephales promelas*) collected from the Calleguas Creek at Lewis Road at the same site where water was collected for toxicity studies. The major change shown in this organ involves the proximal tubular epithelial cells. These are visible in the center of the field and they show marked rearrangement. Large intercellular spaces and pale staining cytoplasm with pignotic nuclei indicate degeneration of this portion of the nephron. This change could be related to toxicants. Hematoxylin and eosin stain X 100.

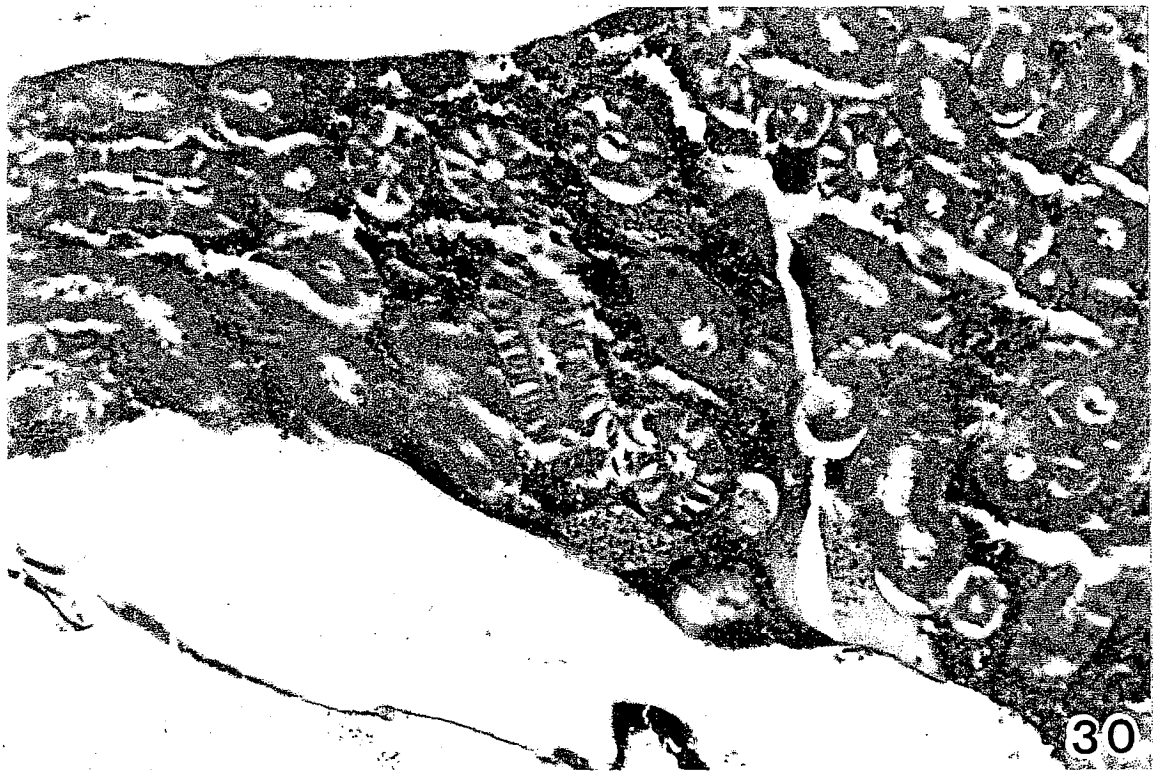
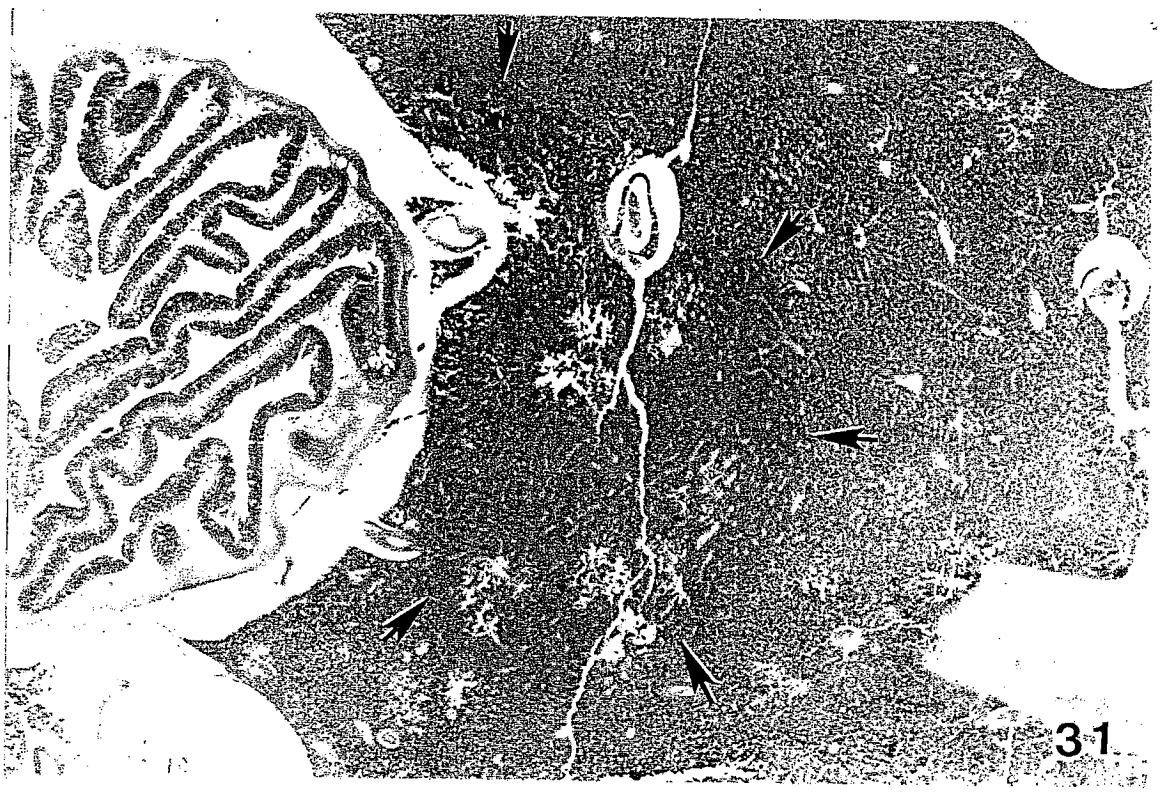

30

Figure 31. When contrasted with Figure 29 an obvious change in staining and architecture of a region of the liver is indicated in this photomicrograph taken from a fathead minnow (*Pimephales promelas*) collected from Calleguas Creek at Lewis Road. Arrows indicate the margin of this lesion which is a basophilic adenoma of the liver. This tumor is one of the present biomarker lesions indicative of exposure to anthropogenic toxicants. Hematoxylin and eosin stain X 40.

Figure 32. This higher magnification view of the basophilic adenoma described in Figure 30 above shows the difference in staining between cells of the adenoma and the adjacent liver. The normal portion of the liver is at the right hand side of the field while the adenoma is at the left hand side of the field. Note the increased basophilic staining over the adenoma and the thickened feature of hepatic tubules within this lesion. Hematoxylin and eosin stain X 200.



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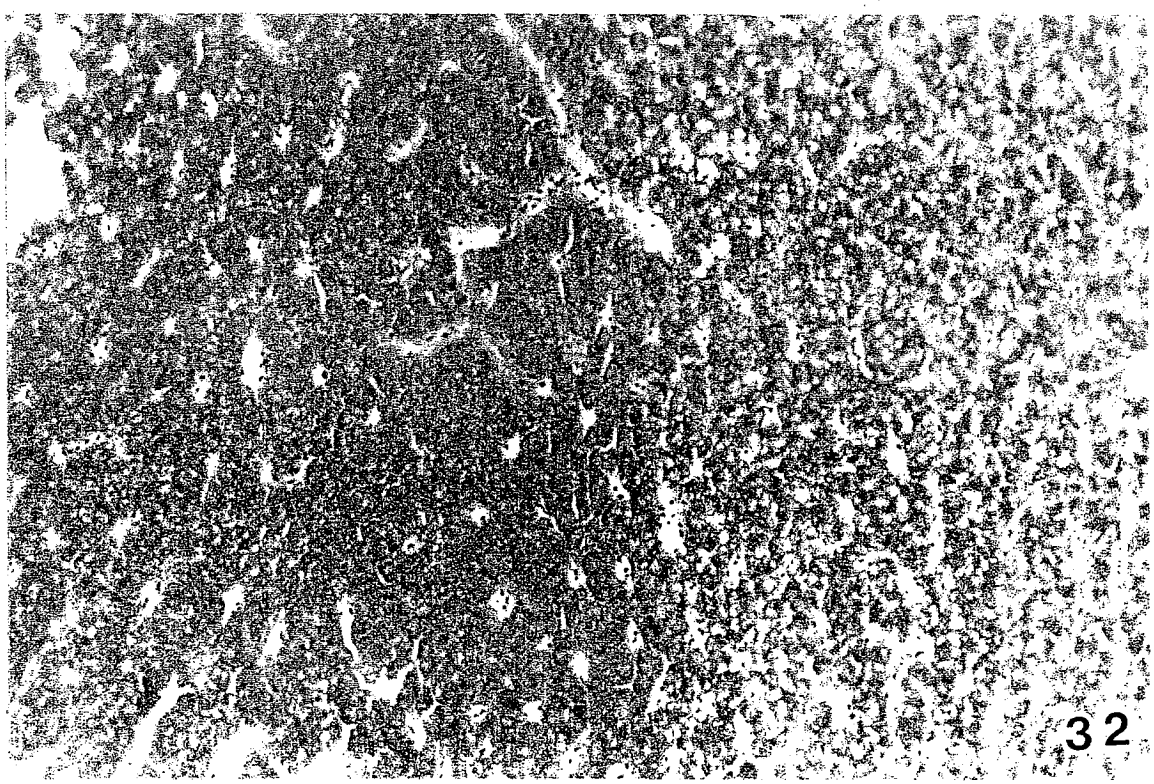
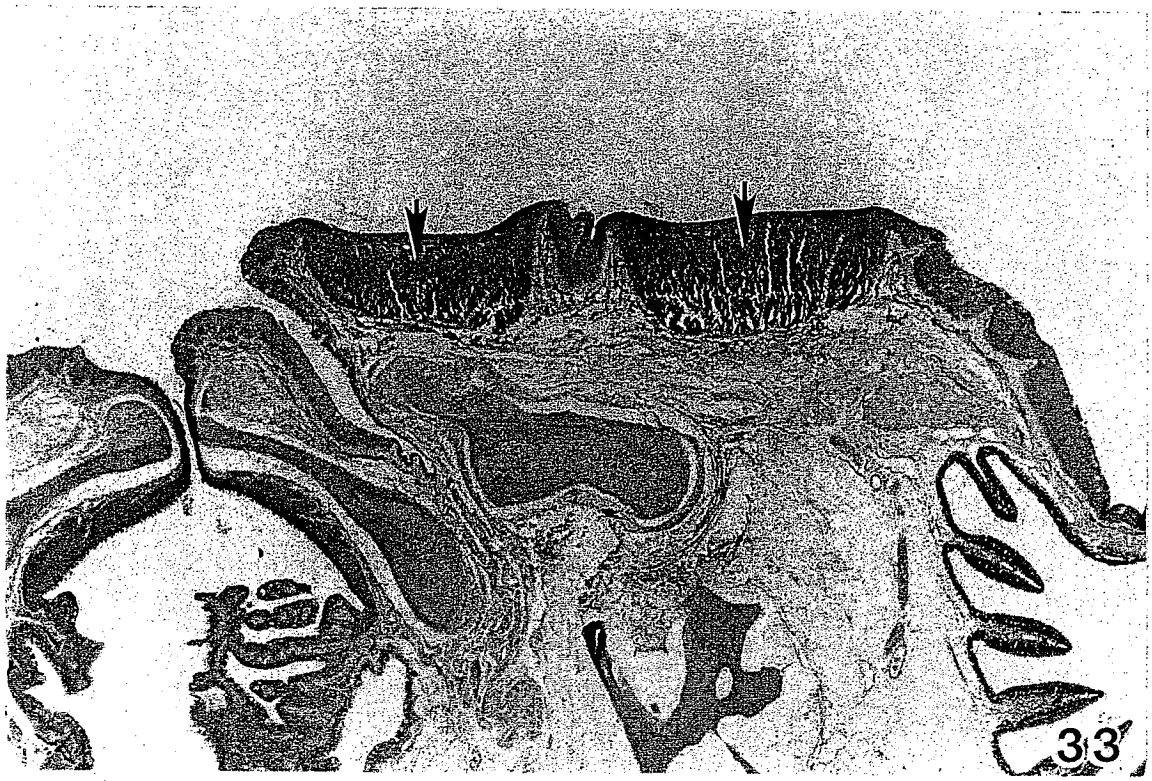


32

Calleguas Creek

Figure 33. This photomicrograph from the head region of a male fathead minnow (*Pimephales promelas*) collected from Calleguas creek at Lewis Road shows changes within the outer epithelium consistent with breeding. Note the positions indicated by the arrows where the epidermis is markedly thickened and stains with a pink coloration. Hematoxylin and eosin stain X 40.

Figure 34. This higher magnification view of a region shown in Figure 25 above indicates additional features of the so-called breeding tubercles on the heads of male fathead minnows. The arrow points to sloughed or desquamated cells at the outer surface of the head. These are keratinized epithelial cells. Keratinization of surface epithelial cells is a rare phenomenon in most teleost fish but is a normal constituent of breeding tubules within male fathead minnows. Note the extensive thickening of the epidermis when compared to regions at the right and left hand margins of the field. Hematoxylin and eosin stain X 200.



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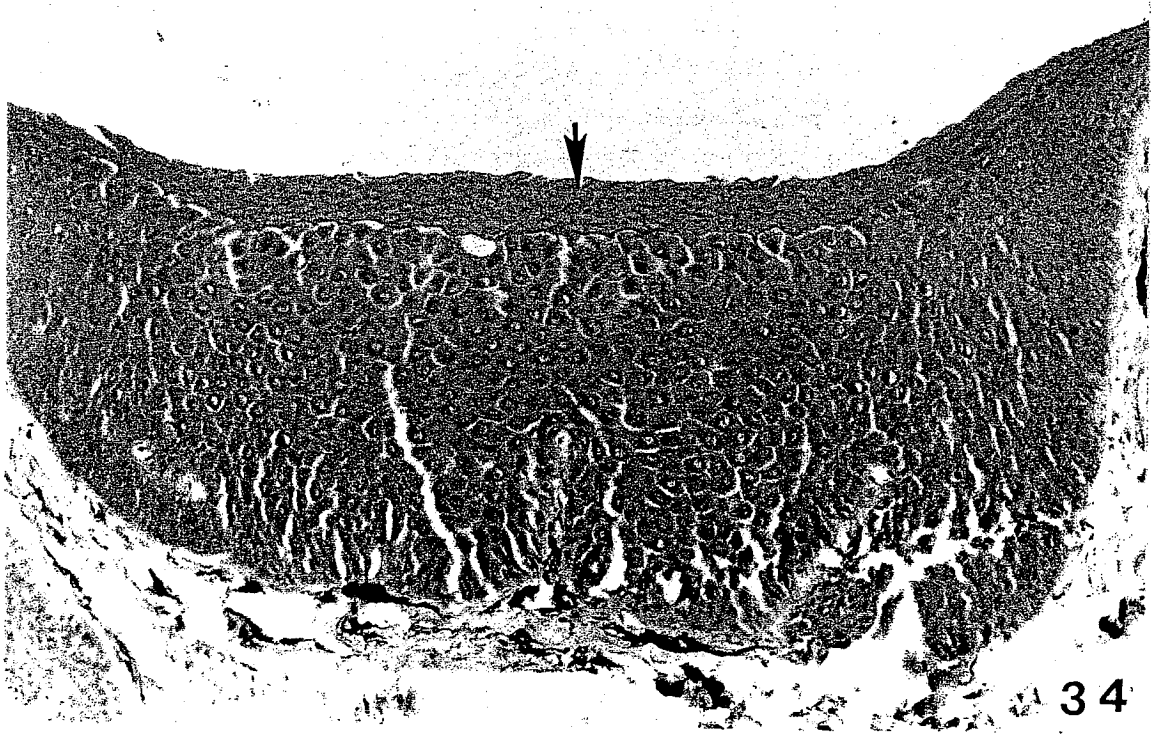
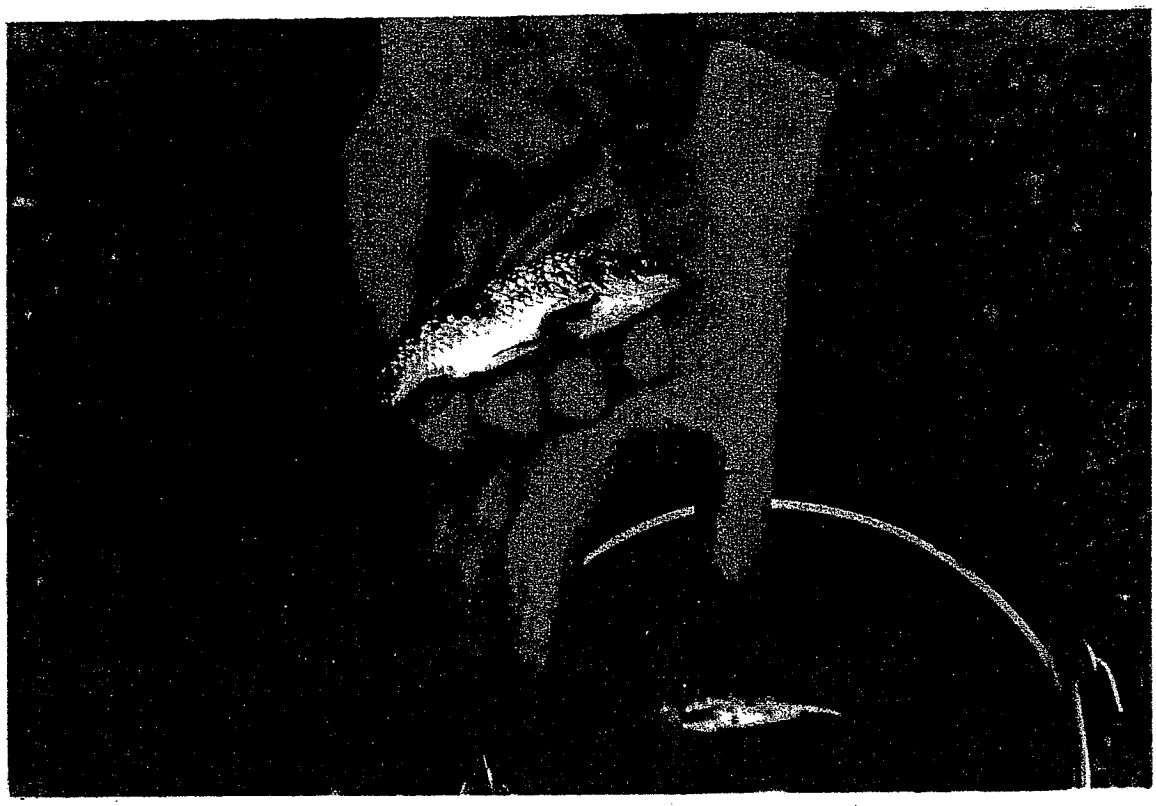


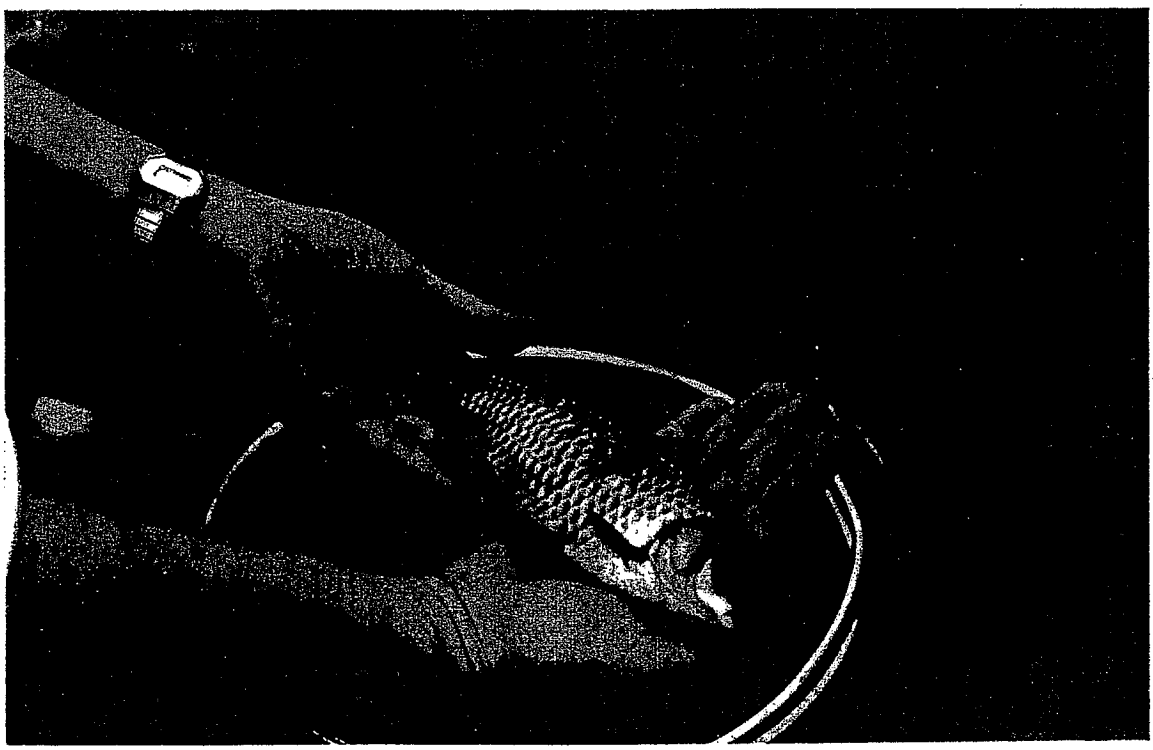
34

Calleguas Creek

Figure 35. Gross lesions on fish collected from Calleguas Creek and Revolon Slough



Calleguas Creek @ Lewis Road
6/13/90



Revolon Slough @ Wood Road
6/13/90

RESULTS

SANTA CLARA RIVER

Background

The Santa Clara River is one of the largest river basins in Southern California (4200 km^2) and also one of the few that remain in a relatively natural state. High quality riparian habitat is present along much of the river and is associated with a number of rare and endangered species. Currently, development is proceeding rapidly in parts of the watershed and channelization is occurring on tributaries in the upper areas. Two major POTWs discharge into the upper river in Los Angeles County.

Beginning at the upstream portion of the watershed, SC-7 is located on a natural intermittent tributary, San Francisquito Canyon Creek, which, because of its shallow nature, is often very eutrophic. SC-8 also samples water from a tributary, Bouquet Canyon Creek, located downstream of Bouquet Reservoir. SC-6, located on the Santa Clara River at Highway 99, is a natural channel with flows composed primarily of effluent from two POTWs. SC-5, located on a tributary, Piru Creek, is mainly imported water from Piru Reservoir just before it is diverted for groundwater recharge. SC-4 is on the lower part of Sespe Creek, a tributary of Santa Clara River which is pristine in its upper reaches. SC-3 is located on another tributary, Santa Paula Creek, a rural stream that cuts through an area with extensive natural oil seeps. SC-2 is located within a major corridor of citrus orchards along the river. Finally, SC-1 is located on the river at the Freeman Diversion site. This was the most downstream site sampled.

A diagram of these sites (Fig. I) and photographs of selected sites are presented at the conclusion of this section.

Toxicity Testing

The results of the toxicity tests are summarized in Tables 7 and 7a, presented at the end of this section and described here.

The survival of fathead minnow larvae was adversely affected when they were exposed to samples collected in July and October 1992, and January, 1993 at SC-8, located on Bouquet Canyon Creek. There were no effects on *C. dubia* survival or reproduction in any of the testing events using water from site SC-8. Cell numbers of algae were also unaffected in samples collected from this site.

Fathead minnow survival and growth were generally not adversely affected after exposure to water samples collected at SC-7 (San Francisquito Canyon Creek). In the one exception, April 1993, growth was reduced over 25%, compared with the control value. There were no effects on survival of *C. dubia*, but reproductive output was reduced after exposure to samples collected in January and April 1993. Algal cell numbers were reduced after exposure to the sample collected in July 1992 at this site. Since no sample was collected from this dry site on July 22, 1992, there are no toxicity results to report.

Samples collected from SC-6 (Santa Clara River at Highway 99) did not affect fathead minnow survival or growth. None of these samples affected survival in *C. dubia*, but reproduction was reduced in samples collected in January and April 1993. There were no adverse effects on algal cell number in any of the testing events using waters collected at site this site.

Fathead minnow survival was reduced in samples collected in July, 1992 and January, 1993 at site SC-5, located on Piru Creek. Ceriodaphnid survival was not affected when exposed to any of the samples collected from this site, but reproduction was reduced in the January, 1993 sample.

This sample from SC-5, also inhibited algal cell growth.

Survival of fathead minnow larvae exposed to samples from SC-4 (Sespe Creek) was reduced in the January, 1993 testing event and growth was reduced in the sample collected in April, 1993. Total mortality occurred in *C. dubia* tests with the sample collected in April, 1993 and reproduction in *C. dubia* was reduced in tests with the January, 1993 sample. There were no effects on algal cell numbers in any of the samples collected from this site.

There were no effects on fathead minnow survival or growth in tests with any of the samples collected at SC-3 (Santa Paula Creek). These samples did not affect the survival of *C. dubia*, but reproduction in these organisms was reduced in tests with samples collected in January and April, 1993. No effects on algae in any of the samples collected from site SC-3 were observed.

There were no effects on the survival of fathead minnow larvae exposed to any of the samples obtained at site SC-2 (Santa Clara River at Newhall Road). However, growth was reduced in the sample collected in July 1992. The results of the tests with *C. dubia* were similar to those obtained for SC-6 and 7. There were no effects on survival, but reproduction was reduced in the January, and April, 1993 testing events. Algal cell numbers were reduced only in the July 1992 testing event with sample water from this site.

A one time sampling of an agricultural drain (discharging into the river near SC-2) was made on July 21, 1992 (see Table 7-A). This sample inhibited algal cell growth as the only significant effect.

The growth of fathead minnow larvae was reduced upon exposure to water from SC-1 (Santa Clara River at the Saticoy Diversion) in the July, 1992 testing event. No other adverse effects on

fathead minnow survival or growth were encountered in tests with samples collected from this site. There were no effects on the survival of *C. dubia* in any of the samples collected at this site, however, reproduction was reduced in tests with the January and April, 1993 water samples. There were no effects on algae in any of the samples tested from this site.

Toxicity Identification Evaluations

A sample collected on January 19, 1993 at SC-2 produced 100% mortality in ceriodaphnids, however, toxicity was no longer present when a TIE was conducted. Without TIE data, it is difficult to determine the cause of toxicity, however, no organophosphates were detected in the sample.

A sample collected from SC-4 in March, 1993 produced 100% mortality in exposed *C. dubia*. However, toxicity was no longer present in the sample when the TIE was initiated two weeks later.

Chemical Analyses

Chemical results for the Santa Clara River samples sent for chemical analysis are shown in Table 8. Only one sample was analyzed chemically.

Histopathology

No specimens were collected for histopathologic examination from this watershed.

Summary of Findings

Compared with the results from the San Gabriel River and Calleguas Creek, water quality from this drainage appeared comparatively good. This conclusion is based on the overall lack of high mortalities in organisms exposed to the test samples. However, caution should be used in applying this conclusion because samples were only collected on a quarterly basis. These waters could be threatened or intermittently impaired. Nonetheless, all three of the test species responded to samples collected from this watershed. This suggests intermittent inputs of toxic materials into the system. This was especially true for the fathead minnows and algae which only responded in < 20 and in 33% of the samples, respectively. Conversely, the reproduction of *C. dubia* was inhibited in samples collected from virtually all of the sites, except SC-8, in January and April 1993. Although most of the effects observed were sublethal, no survival occurred in *C. dubia* exposed to the SC-4 sample collected in April 1993. In addition, only 44% survival occurred with fathead minnow larvae at SC-8 in October 1992. This may have been related to the use of copper sulfate in the upstream reservoir. It was confirmed that the reservoir was treated with copper sulfate for algae control on September 25, 1992. Forty-five and 55% survival of fathead minnow larvae was also observed in samples collected at SC-5 in July, 1992 and January, 1993, respectively. This site is also located downstream of a large reservoir. Algal cell numbers were reduced by approximately 50% or more in samples from SC-2 (July 1992) and SC-5 (January 1993).

Recommendations

Compared with results from the San Gabriel River and Calleguas Creek watersheds, samples from the Santa Clara River drainage exhibited reduced evidence of adverse effects. However, this may have been an artifact of the relatively low number of sampling events (four). Nonetheless, even though the incidence of acute mortality was low, all of the species exhibited signs of adverse effects at different times when exposed to samples collected from this watershed.

Because of the relatively low incidence of acute toxicity, no TIEs were performed on samples from this site. In most cases, chronic TIEs would have been necessary to identify the causes of toxicity.

Interestingly, the fact that this watershed is still in a comparatively natural state and, consequently, doesn't consistently exhibit high toxicity, may provide support for an increased monitoring program designed to eliminate toxic inputs and prevent further degradation of water quality. Thus, continued monitoring is recommended to evaluate trends in toxicity over time. TIEs, including Phase II and III identification and confirmation components, i.e., more sensitive analytical chemistry methods, should be conducted on toxic samples. Although chronic TIEs are more problematic and expensive than acute TIEs, the associated cost and effort would be justified in order to prevent further deterioration of the watershed. Analytical support for the TIEs should provide detection limits commensurate with determining effects at chronic toxicity levels. Once toxic constituents have been identified, it should be comparatively easy to determine the sources since the land use mosaic associated with the watershed is relatively simple and only three POTWs discharge into the basin. A follow-up monitoring program should also be maintained to monitor the effectiveness of any control programs and to identify new contaminants as they arise.

Diagram and Photographs of Sampling Sites

Santa Clara River

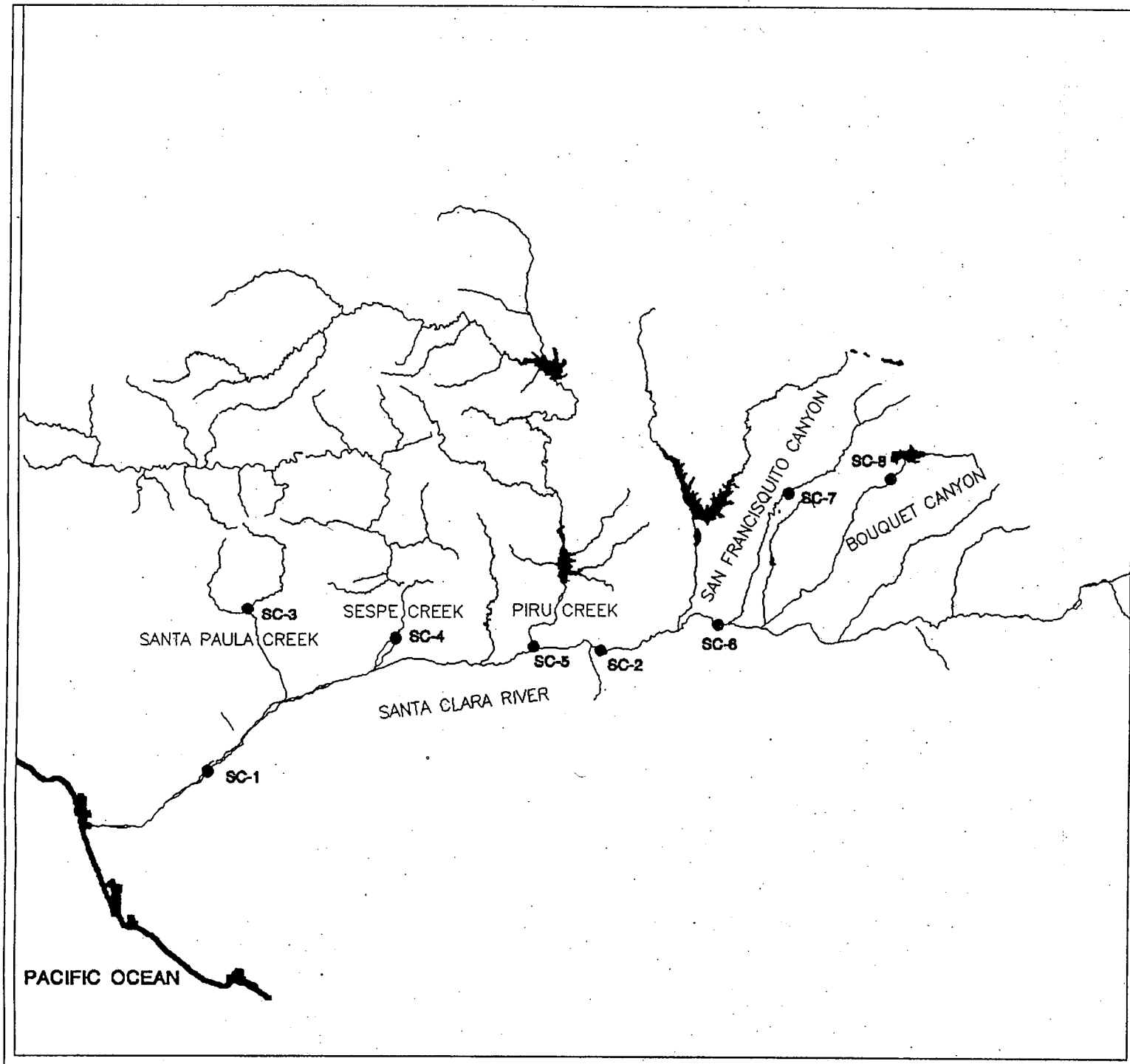


Figure 1. Santa Clara River Toxicity Sampling Sites

SC-1: Santa Clara River at Saticoy Diversion

SC-2: Santa Clara River at Newhall Ranch

SC-3: Santa Paula Creek at Hwy 150

SC-4: Sespe Creek at Old Telegraph Road

SC-5: Piru Creek at Diversion below Center St.

SC-6: Santa Clara River at old Hwy 99

SC-7: San Francisquito Canyon Creek (north of Powerhouse)

SC-8: Bouquet Canyon Creek at "Falls" Campground

CALIFORNIA
REGIONAL
WATER QUALITY
CONTROL BOARD
LOS ANGELES REGION
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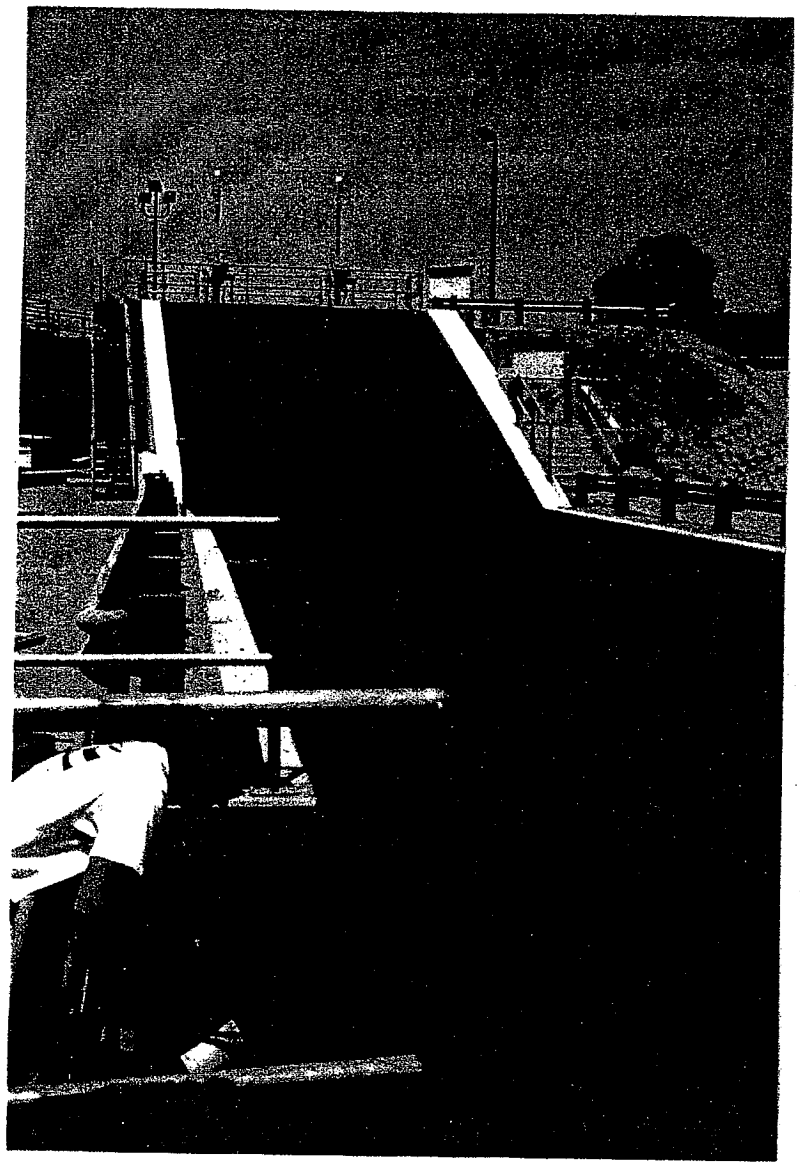
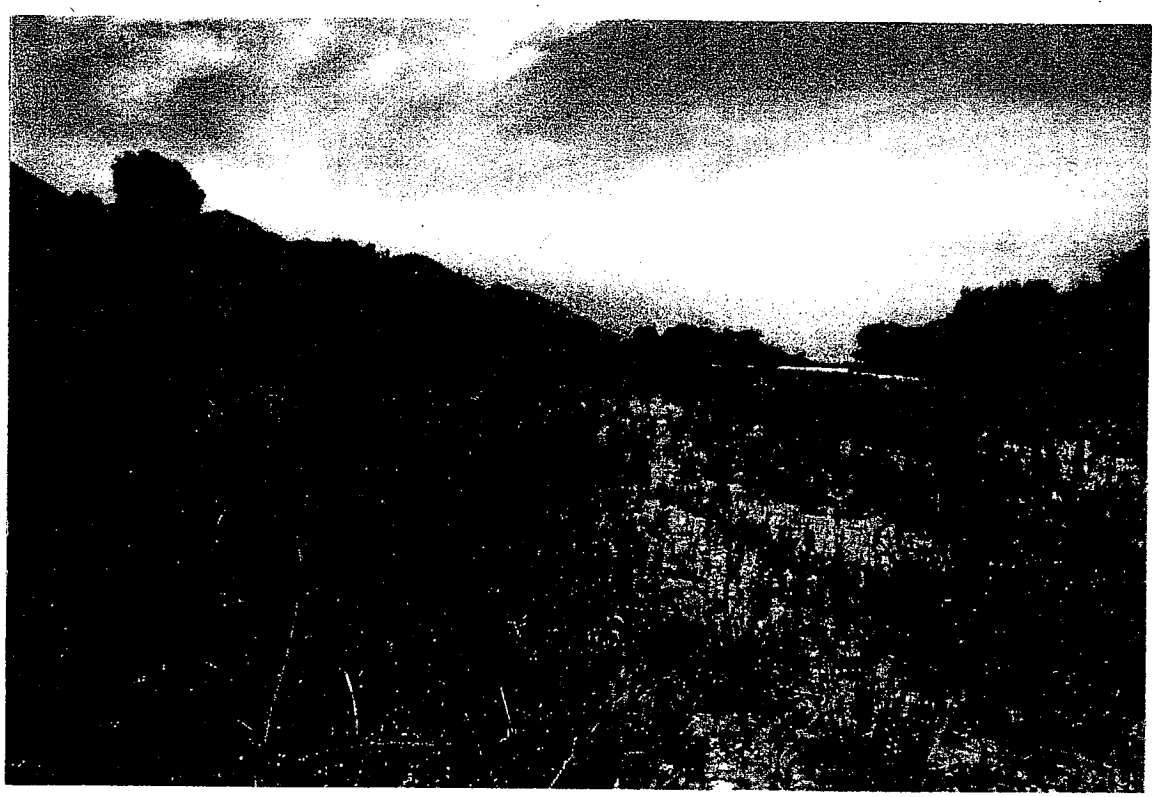
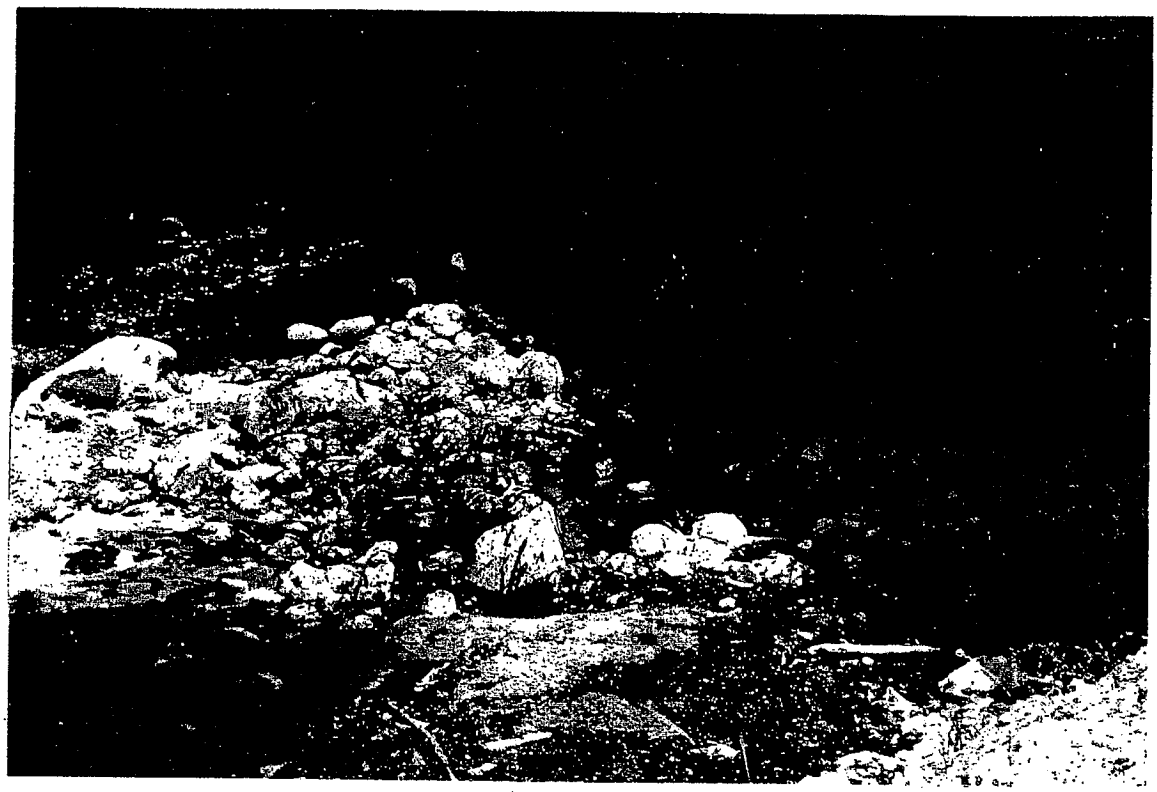


SC-1 SANTA CLARA RIVER AT FREEMAN DIVERSION
7/21/92

SC-2 SANTA CLARA RIVER AT NEWHALL RANCH
7/21/92

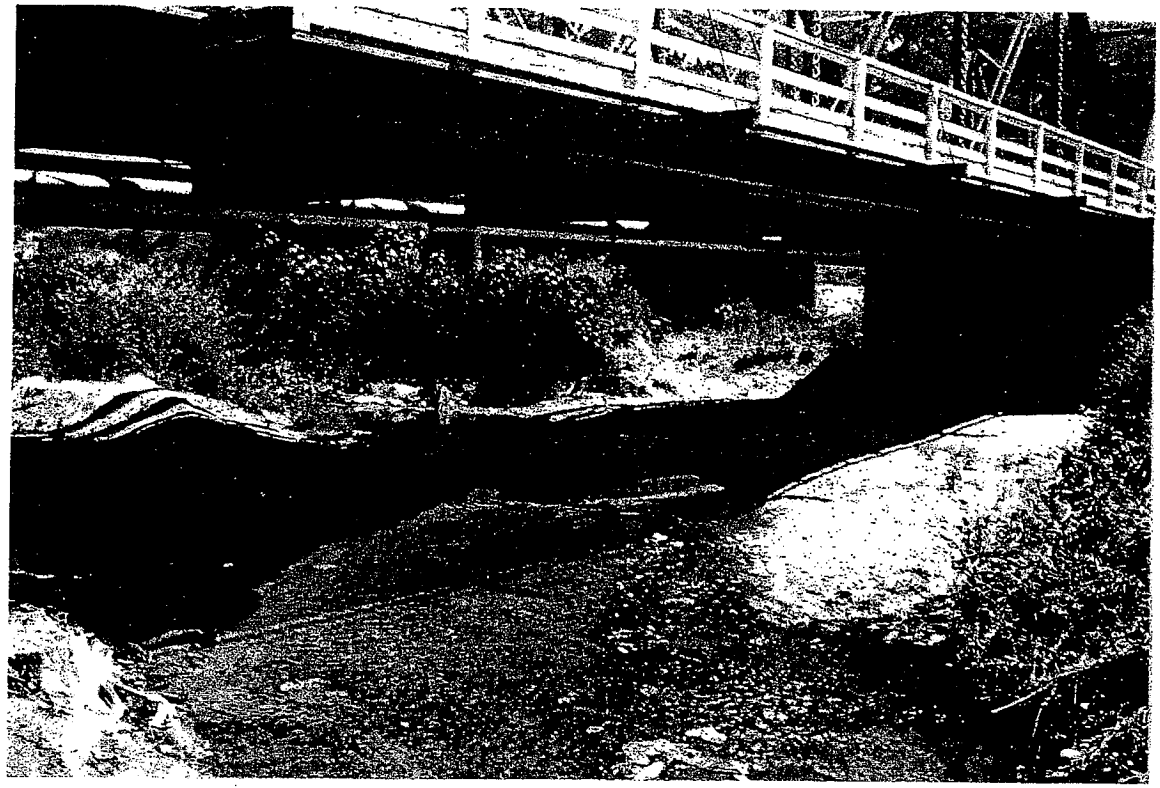


VIEW OF CROSS SECTION OF ENTIRE RIVERBED



SC-3 SANTA PAULA CREEK AT HWY. 150.
ACTIVE OIL SEEPS ARE PRESENT AT THIS SITE.
7/21/92

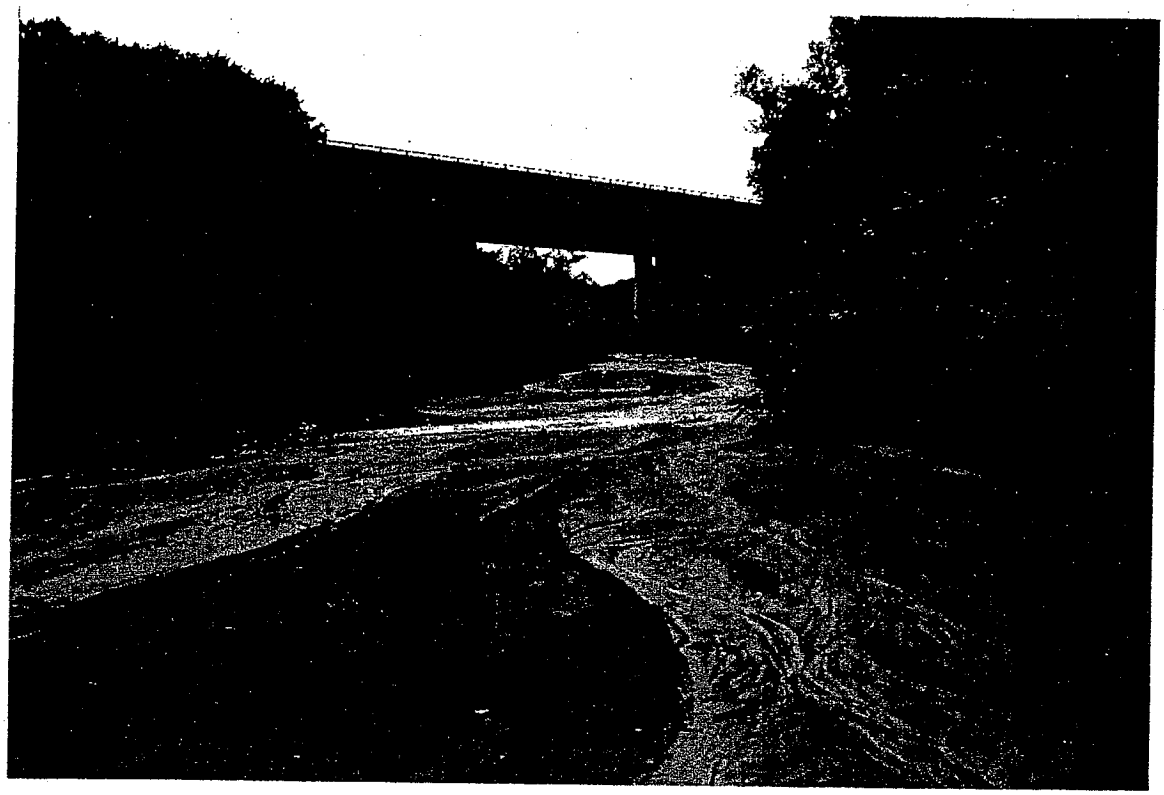
SC-4 SESPE CREEK AT OLD TELEGRAPH ROAD
7/21/92



(ABOVE DIVERSION)

SC-5 PIRU CREEK AT CENTER STREET
7/21/92

(BELOW DIVERSION)



SC-6 SANTA CLARA RIVER AT OLD ROAD (HIGHWAY 99)

ADDITIONAL COLLECTION SITE (AN AGRICULTURAL DRAIN) NEAR SC-2. WORK HERE WAS SELECTED WHEN AN SC-7 SITE, ORIGINALLY SCHEDULED FOR COLLECTION, PROVED DRY. THIS ALTERNATE SITE WAS SAMPLED ONE TIME ONLY (7/21/92).

SC-8 BOUQUET CANYON CREEK AT "THE FALLS" CAMPGROUND
5/7/92

Summary Tables of Toxicity Tests and TIEs

Santa Clara River

Table 7. Summary of toxicity tests conducted on samples from the Santa Clara River Watershed

Site	Parameter	Test Date 7/22/92	Test Date 10/22/92	Test Date 1/20/93	Test Date 4/1/93
SC-8	Fathead Minnow				
	Survival (%)	80.4	43.9*	76.7	90
	Growth (mg)	0.456	0.522	0.671	0.545
	Ceriodaphnia				
	Survival (%)	90	100	90	100
	Reproduction	40.6	18.44	25.7	22.4
	Selenastrum cap.				
	Cells/ml	2321160	2597720	1554920	1926946
SC-7	Fathead Minnow†				
	Survival (%)	N/A	98.3	91.7	75
	Growth (mg)	N/A	0.43	0.646	0.329*
	Ceriodaphnia				
	Survival (%)	N/A	100	100	100
	Reproduction	N/A	16.8	17.9*	15.7*
	Selenastrum cap.				
	Cells/ml	N/A	1708100	1958353	1616513

Table 7. Summary of toxicity tests conducted on samples from the Santa Clara River Watershed					
Site	Parameter	Test Date 7/22/92	Test Date 10/22/92	Test Date 1/20/93	Test Date 4/1/93
SC-6	Fathead minnow				
	Survival (%)	95	87.1	83.3	81.7
	Growth (mg)	0.417	0.396	0.515	0.468
	Ceriodaphnia				
	Survival (%)	100	100	100	90
	Reproduction	39.5	21.1	18*	11*
	Selenastrum cap.				
	Cells/ml	2078580	3047240	2024873	1464806
SC-5	Fathead minnow				
	Survival (%)	44.7	86.1	55	91.7
	Growth (mg)	0.341*	0.409	0.255*	0.578
	Ceriodaphnia				
	Survival (%)	90	100	100	100
	Reproduction	28.5	18.8	12.4*	25.5
	Selenastrum cap				
	Cells/ml	2111900	2636540	185293*	1639593

Table 7. Summary of toxicity tests conducted on samples from the Santa Clara River Watershed

Site	Parameter	Test Date 7/22/92	Test Date 10/22/92	Test Date 1/20/93	Test Date 4/1/93
SC-4	Fathead minnow				
	Survival (%)	93.3	90.6	73.3	75
	Growth (mg)	0.448	0.381	0.659	0.36*
	Ceriodaphnia				
	Survival (%)	100	100	90	0*
	Reproduction	38.3	19.3	12.1*	0*
	Selenastrum cap.				
	Cells/ml	2028660	2406420	1966633	1684160
SC-3	Fathead minnow				
	Survival (%)	100	98.5	88.4	80
	Growth (mg)	0.434	0.343	0.545	0.457
	Ceriodaphnia				
	Survival (%)	100	100	100	100
	Reproduction	33.6	21.6	18.7*	9*
	Selenastrum cap.				
	Cells/ml	2340460	2594620	1612133	1981380

Table 7. Summary of toxicity tests conducted on samples from the Santa Clara River Watershed					
Site	Parameter	Test Date 7/22/92	Test Date 10/22/92	Test Date 1/20/93	Test Date 4/1/93
SC-2	Fathead minnow				
	Survival (%)	88.3	98.5	81.6	91.7
	Growth (mg)	0.384*	0.408	0.662	0.476
	Ceriodaphnia				
	Survival (%)	100	100	100	80
	Reproduction	33.3	22.4	21.2*	12.5*
	Selenastrum cap.				
	Cells/ml	430900*	2288000	2037173	1911540
SC-1	Fathead minnow				
	Survival (%)	96.6	100	86.7	81.6
	Growth (mg)	0.382*	0.385	0.628	0.548
	Ceriodaphnia				
	Survival (%)	100	100	100	100
	Reproduction	28.33	20.7	17.7*	14.1*
	Selenastrum cap.				
	cells/ml	1007580	1766780	1826199	1374960

Table 7. Summary of toxicity tests conducted on samples from the Santa Clara River Watershed					
Site	Parameter	Test Date 7/22/92	Test Date 10/22/92	Test Date 1/20/93	Test Date 4/1/93
Control	Fathead Minnow				
	Survival (%)	100	100	96.7	83.4
	Growth (mg)	0.444	0.385	0.648	0.463
	Ceriodaphnia				
	Survival (%)	88.89	100	100	100
	Reproduction	33	16.9	25.7	25.4
Control	Selenastrum cap.				
	Cells/ml	805220	1068840	1747586	1639686

*= Significance @ $P < 0.05$; see Methods for statistical tests.

† SC-7 site was dry on 7/22/92. No sample taken.

Table 7a. Summary of toxicity tests conducted on sample from alternate site (Agricultural Drain near SC-2)		
Site	**Parameter**	**7/22/92**
Alternate	Fathead Minnow	
	Survival (%)	95.2
	Growth (mg)	0.44
	Ceriodaphnia	
	Survival (%)	100
	Reproduction	42.7
	Selenastrum cap.	
	Cells/ml	661460*

Table 8. Summary of Analytical Results for Santa Clara River (6/92 to 4/93)

Date Sampled	Sample ID (Site #)	Eureka Laboratories	µg/l (ppb)	Detection Limits (ppb)
1/19/93	SC-2	No organophosphorus pesticides detected		

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APPENDIX I

REFERENCE TOXICANT DATA

SUMMARY OF REFERENCE TOXICANT DATA FOR LA BOARD STUDY

No-Effect Concentrations of NaCl (µmhos/cm)

Test Species	5/13/92	6/4/92*	7/22/92*	9/9/92*	10/21/92 *	12/2/92 *	1/11/92	3/4/92*	4/2/93*
Fathead Minnow									
Survival	9100	9450	9000	9250	4685	8900	4250	1350	3850
Growth	9100	5000	4600	9250	4685	4625	4250	4525	7000
Ceriodaphnia dubia									
Survival	2500	1230	2500	2610	2550	2600	2550	2500	2500
Reproduction	2500	1230	1225	655	2550	2600	2550	625	230
Selenastrum capricornutum									
Cell numbers	<1000	<1000	1060	4100	2020	1200	<1000	2300	1000

*Tests run concurrently with LA Board bioassays