

**APPENDIX A**

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**TOXICITY IDENTIFICATION EVALUATION RESULTS**

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Application of Toxicity Identification Evaluation  
Procedures to the Ambient Waters of  
the Colusa Basin Drain, California

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**ABSTRACT--**Pesticides are applied to the rice fields in the Sacramento Valley to prevent the growth of plants, algae, and insects which reduce rice yields. Following the pesticide application, field water is released into agricultural drains which in turn discharge into the Sacramento River and Delta. Rice irrigation comprises the largest single use of irrigation water in the Sacramento Valley and since rice return flows are the primary source of drain effluent during the spring and summer (up to 33% of the total flow), these discharges can significantly affect drain water quality and resident aquatic organisms.

Toxicity to freshwater organisms was observed in the drain water during the period that coincides with the initial draining of the fields in 1986, 1987, and 1988. The Colusa Basin drain water was both acutely and chronically toxic to *Ceriodaphnia dubia*, but not the fathead minnow. In 1988, a toxicity identification evaluation (TIE) was conducted using *Ceriodaphnia* in an effort to identify the cause of toxicity. Through the non-polar concentration, methyl parathion and carbofuran were identified at levels of concern for the freshwater organisms used in the TIE. Mixture tests and chronic toxicity tests indicate that the levels of methyl parathion and carbofuran account for the toxicity observed in the drain water.

**Keywords--**Carbofuran Methyl parathion *Ceriodaphnia* TIE/TRE Ambient

## INTRODUCTION

Patterns of pesticide use in the United States showed approximately 364 million kg of pesticides were used for agriculture, 45 million kg for home and garden use, and 114 million kg for industrial, commercial, and government use in 1982 [1]. It was also estimated that 2.2 million kg per year of active ingredients are lost to surface waters in runoff, but this represented less than 1% of the total amount of pesticides annually.

In 1982 about one million kg of herbicides were applied to rice in the Sacramento Valley to control aquatic weeds. Recent changes in the rice industry to a more productive variety of rice encouraged increased use of herbicides to control the weed competition [2]. Rice is cultivated using a flooding method of irrigation; the rice is kept partially submerged and receives irrigation during much of the growing season. Rice fields are treated with herbicides and pesticides to prevent the growth of water-grasses, broadleaf plants, algae and insects which reduce rice yields. Rice growers are required to hold the treated water on-site following application of some pesticides to promote dissipation before release into the agricultural drains. These discharge into the Sacramento River and Delta (Figure 1).

Rice irrigation comprises the largest single use of irrigation water in the Sacramento Valley and since rice return flows are the primary source of drain effluent during the spring and summer (up to 33% of the total flow) [2], these discharges can significantly affect drain water quality and resident aquatic organisms. Nearly all rice return flows in the Sacramento Valley are discharged

into the Sacramento River along a 90 km stretch from the city of Colusa and the confluence of the Sacramento and Feather River [2].

Recently, with reports of the decline of the striped bass population, the use and discharge of pesticides is frequently targeted as a possible cause [3]. Since 1986, biotoxicity studies to monitor the water quality of the Sacramento River and the major agricultural drains (Colusa Basin Drain (CBD) and Sacramento Slough) and tributary rivers have been undertaken by the Central Valley Region of the California Regional Water Quality Control Board, hereafter referred to as the Board. Numerous pesticides and chemicals can be identified as potential compounds to measure in the return and river water; such as bentazon, bromacil, carbofuran, cyhexatin, dacthal (DCPA), dicofol, malathion, MCPA and free acid, methoxychlor, parathion (-ethyl and -methyl), propanil, simazine, xylene and xylene range aromatics, carbaryl, captafol, molinate, thiobencarb [V. Connor, personal communication]. The uses of each pesticide are varied, for example applications of molinate (Ordram<sup>®</sup>) and thiobencarb (Bolero<sup>®</sup>) are made to control the water grasses. Carbofuran is soil incorporated to control the rice water weevil, and methyl parathion is added shortly after the carbofuran to control the tadpole shrimp. Broadleaf herbicides such as MCPA and bentazon are applied in June and July during the tillering phase of the rice development. Finlayson et al [4] reported that molinate and thiobencarb have been measured as high as 340 µg/l and 51 µg/l, respectively, and that the exposure period may last 40 to 60 d in the CBD. However, increase field holding times for both chemicals has reduced their concentrations to a maximum of 67 and 4.5 µg/l respectively in the drain [5].

In 1987, the toxicity to freshwater invertebrates was observed in the CBD water during the period that coincides with the initial draining of the rice fields. Early in 1988, the Environmental Protection Agency's (EPA) Region IX office requested the assistance of the Environmental Research Laboratory-Duluth (ERL-D) in a toxicity identification evaluation (TIE) on the Sacramento River and Delta. In 1988, the coupling of toxicity testing and effluent chemical characterization was initiated where the objective was to determine the specific toxicant(s) in the water. The toxicity tests were scheduled to coincide with the period of time immediately prior to, during, and after the initial release of the rice return water.

The toxicity tests conducted by the Board were those recommended by EPA for effluent monitoring [6]. With the development of these tests [6,7], EPA developed a program that requires an integrated approach, combining whole effluent testing and chemical specific analyses [8]. With the application of toxicity monitoring, a need to address what causes the toxicity in the point source discharges was apparent. The Duluth EPA laboratory was instrumental in developing a set of procedures that are designed to characterize [9], identify [10], and confirm [11] the toxicant(s) in acutely toxic complex mixtures, primarily effluents. This approach relies on the principals of chemistry to simplify and separate the toxicant(s), and toxicology by using living organisms to track the toxicity in a similar fashion that spiked samples are used to assess the analytical recovery for a chemical. This toxicity identification evaluation (TIE) procedure has generally been used on acutely toxic effluents, but more recently the application to sediment pore

water, ambient waters, and hazardous waste leachates has been initiated. The goal of any TIE is to identify the chemical(s) causing toxicity cheaply and quickly.

The objective of this paper is to describe the application and utility of the effluent toxicity identification procedures to ambient waters. Based on the experiences of previous years, chronic toxicity of the Basin water was expected to be more the rule than the exception. Therefore, the approach of this study was constrained to using TIE techniques that concentrate the toxicity. This paper describes the application of the TIE techniques to ambient water samples from the Colusa Basin Area.

## METHODS AND MATERIALS

### *Samples*

Two samples were received on 24 May 1988, one from the Colusa Basin Drain (CBD) collected 17 May and the Glen Colusa Canal (GCC) collected on 15 May. The GCC sample is water from above CBD before it is used on the rice fields, and it was considered as the control site. The Board had tested both samples and found the CBD sample to be acutely toxic to *Ceriodaphnia dubia* in 48 h, while the GCC was non-toxic [V. Connor, personal communication]. A later CBD sample (1 June) was tested at ERL-Duluth and it was not as toxic as the earlier CBD sample, as it caused only 20% mortality in 48 h. The Board shipped all samples air express on ice in polyethylene containers; and all samples were stored at ~4°C.

### *TIE Test Procedures*

Upon arrival of the samples, 48-h toxicity tests were conducted on each sample using  $\leq 24$  h *Ceriodaphnia dubia*. Tests were all conducted at  $25 \pm 1^\circ\text{C}$  with two replicates of five animals each and a photoperiod of 16 h light and 8 h dark. The CBD and GCC samples (100%) were diluted with a reconstituted softwater [10% diluted mineral water (DMW); 12] using a 0.5 dilution factor. The DMW was prepared using a 1:9 dilution of mineral water (Perrier®) and high quality organic free water from a Millipore® Super-Q System. This water mixture was then aerated for 24 h until the pH was 7.8-8.0. The DMW had dissolved oxygen concentrations of 8.0-8.2 mg/l, and hardness and alkalinity levels of approximately 40 and 30 mg/l as  $\text{CaCO}_3$ , respectively.

### *Fractionation*

Approximately 1,100 ml of the CBD and GCC samples were each vacuum filtered through a 1  $\mu$ m glass filter. The filtered sample was tested for toxicity, and then 1 L of the filtered sample was concentrated on 6 ml high capacity C18 solid phase extraction (SPE) columns (J.T. Baker Chemical Company, Phillipsburg, NJ), using a pump with the flow rate of 8 mL/min. The SPE column had been prepared with methanol, and appropriate blanks were collected; further details for the column preparation are given elsewhere [9]. Three 20 ml post-column samples were collected after 25, 500, and 950 ml had passed through the column, and each post-column sample was tested for toxicity. The SPE column was used to extract non-polar organic compounds from aqueous solutions and the compounds selectively removed off the column by eluting with methanol/water mixtures that were increasingly non-polar (i.e., 25, 50, 75, 80, 85, 90, 95, and 100%). As a series, the "fractions" resulting from column elution contain analytes that are decreasingly polar and decreasingly water soluble. A diagram of these procedures is given in Figure 2.

Each column was eluted with 3 ml of each of the eight methanol/water samples and collected for toxicity testing. Each fraction was toxicity tested separately at 5 $\times$  the original sample concentration without replication, and using a 0.5 dilution factor. Corresponding blanks of each methanol/water fraction were also tested at 5 $\times$ . The final methanol concentration in the test solution was 1.5% (v/v) which is below the *Ceriodaphnia* 48-h LC50 for methanol [9].

After these fraction tests were completed, "add-backs" tests were initiated to determine if all the toxicity in the original sample was accounted for in the SPE fractions. For the add-backs, there were three separate tests that were conducted: the "all fractions", the "toxic fractions", and the "non-toxic fractions". For the "all fraction tests", a portion of each fraction was added to the same dilution water volume and tested at 1× the original concentration. Assuming a complete recovery of all non-polar organics from the SPE column, this yielded a solution of non-polar organic compounds equal to the original sample concentrations. For the "toxic fractions" a portion (30 µl) of each fraction that exhibited toxicity was added to another 10 ml aliquot of dilution water; while the "non-toxic fractions" were tested by adding 30 µl of each of the remaining fractions that did not have any individual toxicity in 10 ml of dilution water.

#### *Concentration*

Next, the "toxic fractions" and their corresponding blanks were diluted (1:10) and then drawn through separate 1 mL C18 SPE columns under a pressure of 38 cm of Hg using a vacuum manifold. Each column was then dried for 5 min using nitrogen at a flow rate of 13 mL/sec. The post-column water/methanol sample was not tested due to the high percentage of methanol. After drying the column, three separate 100 µl aliquots of 100% methanol were forced through the sorbant at a rate of 4 mL/sec. The final volume of eluate was collected and measured for calculation of recoveries. This methanol concentrate (~200 µl) was approximately 5,000× the original concentration, and was tested at 20×, 10×, 5×, and 2.5×. When more concentrated samples were needed for GC/MS quantification, additional 1 L

samples were fractionated and tested. Toxic fractions were combined, concentrated, tested, and analyzed in the same manner as described above.

All concentrates were injected onto a gas chromatograph/mass spectrometer (GC-MS) for the identification of compounds present in the toxic concentrates. Mass spectral (MS) analyses were performed on a Hewlett Packard 5970 Mass Spectral Detector (MSD) interfaced to a Hewlett Packard 5890 Gas Chromatograph (GC). GC parameters used were a 30 m DB5 column (J&W Scientific, Folsom, CA) with a 0.25 mm I.D. and a 0.25  $\mu$  film thickness; helium was used as the carrier gas with a linear velocity of 40 cm/sec at 100°C, with an injection volume of 1  $\mu$ l. The injection port temperature was 250°C, and the GC was temperature programmed from 50°C to 250°C at 10°C/min with a 15 min hold at 250°C. The transfer line temperature was 270°C with a direct interface. The MSD acquisition parameter was used in a full scan mode from 50 to 550 m/z with 1 sec/scan. Compound identifications were made based on a comparison of sample spectra to EPA/NBS/NIH library spectra (37,000 compounds). When standards were available GC retention times were compared. Those constituents identified are roughly quantitated, by assuming that the identified constituents and the internal standard have the same response factor. Literature searches for toxicity information were performed on identified compounds to obtain any available toxicity information. Following the data analysis on the GC-MS, chemicals identified were checked for literature toxicity information (i.e., LC50s), and at this point strong candidates were tested for toxicity.

### Confirmation

Once chemicals were identified in the samples, *Ceriodaphnia* 48-h acute toxicity tests were conducted with methyl parathion, carbofuran, molinate and thiobencarb to determine their respective toxicities. Solutions of methyl parathion (0,0-dimethyl-0-4-nitrophenyl phosphorothioate; 99.7% pure, Research Triangle Park (RTP), NC), carbofuran (2,3-dihydro-2,2-dimethyl-7-benzofuranyl methylcarbamate; 98.1% technical grade, RTP, NC), molinate (Ordram<sup>®</sup> 8-3; s-ethyl hexahydro-1-H-azepine-1-carbothioate; 91.9% purity; Stauffer Chem. Co. Westport, CT), and thiobencarb (Bolero<sup>®</sup>; s-(4-chlorophenyl) methyl diethylcarbomothioate; Ortho) were prepared by dissolving small quantities of each in methanol. These stock solutions were used for subsequent testing and chemical analyses. The breakdown product of carbofuran, carbofuran phenol, was also tested for toxicity.

For methyl parathion and carbofuran acute mixture tests were conducted to determine their additivity in DMW using *Ceriodaphnia dubia*. These tests were done using both 1:3 and 3:1 ratios of each, as well as a 1:1 mixture. These tests were prepared by injecting microliter amounts of each chemical methanol stock into DMW, mixing well and testing for 48 h.

In addition, since chronic toxicity was observed in the ambient samples, chronic tests with *Ceriodaphnia dubia* with each chemical thought to be contributing the toxicity were conducted using DMW (see Test Procedures above). Chronic *Ceriodaphnia dubia* tests were conducted with carbofuran and methyl parathion separately to determine the sublethal toxicity of each generally following the method as described by EPA [12]. These tests were initiated with  $\leq 4$  h old neonates

collected from parents who were more than seven days old, each having produced greater than seven young each in the brood used for the test. Test solutions were prepared and 15 ml was distributed into each of 10 replicates per concentration, using block randomization [12]. Solutions were prepared and renewed daily and all animals received 100  $\mu$ l of a yeast-Cerophyll<sup>®</sup>-trout chow (YCT) mixture and 50  $\mu$ l of *Selenastrum capricornutum*. The amount of YCT fed gave a suspended solids concentration in each cup of 12-13 mg/l based on a suspended solids level of 1800 mg/l in the YCT. The algae was concentrated ( $35 \times 10^6$  cells/ml) and after the 50  $\mu$ l was added to each cup, the resultant cell concentration was 116,000 cells/ml. These tests also were conducted at  $25 \pm 1^\circ\text{C}$  as well, using a 16 h light: 8 h dark photoperiod. At each renewal, the adults were transferred to new solutions and the young counted. The test was terminated after at least 60% of the controls had their third brood (7-d).

For the methyl parathion chronic, a 1 L sample of the test solution was extracted using a C18 SPE column. The methanol extract was then injected on a Hewlett Packard 5880 GC with a Flame Photometric Detector (FPD). The GC was equipped with a 6 ft, 2 mm ID glass column packed with OV-1 on 80/100 Chromosorb W-HP. The carrier gas was nitrogen at 28 ml/min and the injection port temperature was  $250^\circ\text{C}$ , the detector temperature was  $300^\circ\text{C}$ , and the GC oven program was  $180^\circ\text{C}$  isothermal. Analytical techniques for measuring the carbofuran by this extraction technique were unsuccessful during the 7-d test; the carbofuran analysis needed was more time-consuming than possible to conduct during this study.

### *Statistical Analyses*

Acute LC50 values for the toxicity tests were calculated using the Trimmed Spearman-Kärber method [13]. Statistical analyses for the *Ceriodaphnia dubia* reproduction and survival data were performed using the procedure described by Hamilton [14] and modified by Rodgers [15]. The young production data was analyzed to obtain the mean number of young per female per treatment. Daily means were calculated and summed by a computer program to derive the seven-day mean young per female. By this technique, young produced by females which subsequently die during the test are included in the mean daily estimate of young. Mortalities of the original females are used to determine a separate mortality effect. Confidence intervals were calculated by a bootstrap procedure which subsamples the original data set 999 times to obtain a robust estimate of the standard error. The young production was then compared to the control young production using a Dunnett's two-tailed t-test [16]. The most sensitive endpoint was used to determine the Lowest Observable Effect Concentration (LOEC) and the No Observable Effect Concentration (NOEC).

### *Toxic Unit Calculation*

Toxicity values for the CBD and GCC samples are expressed as toxic units (TUs) which were calculated by dividing 100% by the LC50(%). The toxicity of each fraction can be expressed as TUs, but first a correction must be made for what concentration the original sample was tested at. For example, when fractions were tested at 5× the original concentration, the TUs were calculated by  $(100\% \div 5) \div \text{LC50}$ . Since the primary purpose of the C18 SPE fraction tests was

to assess whether or not acute toxicity is present or absent in the fractions, for any effluent to be toxic at the original sample levels, the fractions must have an LC50 of 20%. However, toxicity could elute into more than one fraction, and toxicity at 5x should not be disregarded. If there is more than one toxicant, and each has a different toxicity, the TUs and interactions of each must be determined before they can be summed.

## RESULTS AND DISCUSSION

### *Identification of Non-Polar Organic Toxicity*

The CBD sample of 17 May had an initial LC50 of 82% in 48 h, which agreed with the results obtained by the Board [C. Foe, personal communication] while the GCC sample had no initial toxicity. All results are presented as TUs in Tables 1-3 (cf., Methods). When the 17 May sample was concentrated on the C18 SPE column, and the fractions tested, toxicity occurred in three fractions, with the majority of the toxicity in the 80% (Table 1). No mortality occurred in the post-C18 samples, indicating that the chemical(s) causing the acute toxicity was retained on the column. For the GCC sample, since the original sample had no toxicity, it was expected that the fractions would not have any toxicity unless a compound(s) was made toxic by the concentration step and no fraction had any toxicity even at 5× (Table 3).

Add-back tests were conducted to ascertain whether toxicity in the toxic fractions was equal to that in the original sample. It is possible that some chemicals contributing to the toxicity in the original sample are not eluted from the C18 column, or that they may be partially eluted into two fractions, and toxicity not detected. The agreement of the add-back tests for the CBD 17 May sample are quite good (Table 1). For the CBD 17 May sample, the TUs of the original sample was essentially the same as that in the "all" and "toxic" add-back tests, and no toxicity was detected with the "non-toxic" fractions add-back test.

Once the toxic fractions were concentrated, toxicity was trackable, but the percent of toxicity detected was less. This is characteristic of this concentration

step where recoveries, based on toxicity, average 60% [Durhan et al., in press]. The absolute recovery is not as important as the fact that toxicity is retained in the concentrate to analyze. Typically the concentrates are further separated and concentrated using the HPLC, but since the CBD and GCC samples were less complicated in their make-up than effluents, we decided to go directly to the GC-MS for possible identifications. The samples were quite clean, with only five compounds identified in the CBD (17 May) and none in the GCC sample. In the CBD sample, the chemicals identified were: carbofuran, 2,3-dihydro-7-benzofuranol, carbofuran phenol (a metabolite of carbofuran), methyl parathion, and molinate.

From the first mass spectra data, the estimated concentrations of methyl parathion and carbofuran were about 1-2  $\mu\text{g/l}$ , while molinate was  $\sim 100 \mu\text{g/l}$ . As a result of these findings, toxicity tests with *Ceriodaphnia dubia* were conducted on molinate, methyl parathion, and carbofuran (Table 4). The results of these tests were encouraging since the LC50 values for the chemicals were within a factor of two of the estimated concentrations (Table 4). Typically if a chemical is within 1,000 $\times$  the available LC50 data, the chemical remains a suspect toxicant [10].

The next step was to obtain a larger quantity of CBD concentrated material to estimate the concentrations from another GC-MS analysis. For 2 L of fractionated sample, less toxicity was obtained in the fractions, but this time the toxicity occurred about equally in the 75% and 80% fractions. About two and one-half weeks had passed before the sample was fractionated and tested; during this time the sample apparently lost the acute toxicity, and the sum of the toxicity for one set of fractions (75% and 80%) equalled 0.59 TU, while another set of fractions

only had toxicity in the 80% fraction and 0.51 TU (Table 1). Both sets of toxic fractions were combined and concentrated. The 85% fraction had slight toxicity in one set of fractions, and it was also concentrated separately; this concentration step was effective, as a total of 0.52 TUs were recovered. The animals in the 75%, 80% and sometimes in the 85% fractions exhibited behavioral symptoms, such as rapidly swimming in circles. These symptoms were observed in the concentrate tests as well.

Attempts to quantify the level of methyl parathion and carbofuran in the concentrate sample on the HPLC indicated that the carbofuran in the 17 May sample was  $>0.33 \mu\text{g/l}$  while the methyl parathion was  $>1.8 \mu\text{g/l}$ . GC-MS analysis of the two liters of effluent on the 17 May sample 75% and 80% fractions indicated that levels were higher, around  $8.2 \mu\text{g/l}$  for carbofuran and  $4.1 \mu\text{g/l}$  for methyl parathion. However, as this was the first and only quantification on the GC-MS, these values should be viewed as estimates.

On June 21, we received a second CBD sample from 1 June. This sample had no measurable toxicity to *Ceriodaphnia* in 48 h, but had 0.56 TUs when fractionated (Table 2), and the toxicity occurred in the 75% and 80% fractions. These toxic fractions were combined, and analyzed on the GC-MS for carbofuran and methyl parathion only. Levels of methyl parathion and carbofuran were 25% and 58% less than the earlier sample (17 May).

#### *Testing of Potential Toxicants*

For the chemicals identified as possible causes of toxicity, the toxicity test results are given in Table 4. Molinate was not present at toxic levels in any of

these samples, and the toxicity of molinate to *Ceriodaphnia* was low ( $>605 \mu\text{g/l}$ ). The Board [V. Connor, personal communication] reported a measured concentration of molinate in the 17 May sample of  $62 \mu\text{g/l}$ , which compared quite well with the GC-MS estimate of  $104 \mu\text{g/l}$ . The 48-h LC50 of thiobencarb to *Ceriodaphnia* was  $510 \mu\text{g/l}$ , but no thiobencarb was detected in any samples. Because the metabolite of carbofuran, carbofuran phenol, was detected in the sample, it was tested and found not to be toxic at  $\leq 20 \mu\text{g/l}$ . The suspect compounds, carbofuran and methyl parathion had identical 48-h LC50s of  $2.6 \mu\text{g/l}$  (Table 4). Since these compounds may be additive, synergistic, or antagonistic, it was important to evaluate their combined toxicity. When any mixture (1:3, 3:1, or a 1:1) was tested, the combined toxicity were strictly additive for carbofuran and methyl parathion (Table 5).

In addition to the acute single toxicant tests conducted, chronic tests with *Ceriodaphnia dubia* and methyl parathion and carbofuran (separately) were completed to determine the sublethal no effect concentrations for each. The NOEC for carbofuran was  $1.3 \mu\text{g/l}$  (based on survival) while the NOEC for methyl parathion was  $0.99 \mu\text{g/l}$  (based on survival and reproduction) (Table 6). The acute and chronic toxicity is quite similar, which we have observed for other organophosphates and *Ceriodaphnia* such as diazinon [J. Amato, personal communication].

Through toxicity tracking, methyl parathion and carbofuran were identified as the most likely candidates for causing the acute toxicity, while the herbicides molinate and thiobencarb were not. These compounds were present in both CBD

samples at concentrations that could be causing the chronic toxicity for the *Ceriodaphnia*.

#### *Species Sensitivity, Pesticide Application and Use*

Both methyl parathion and carbofuran have been in use for a long time, and consequently numerous species have been tested with each, but little chronic data exists for either. The section of the Sacramento River downstream of the city of Sacramento and the subsequent downstream estuary serve as the spawning and nursery habitats for striped bass (*Morone saxatilis*) [18]. Other resident fish species of concern are the American shad (*Alosa sapidissima*), the white sturgeon (*Acipenser transmontanus*), chinook salmon (*Oncorhynchus tshawtscha*), and steelhead trout (*Salmo gairdneri*). Several fathead minnow (*Pimephales promelas*) 96-h LC50s have been reported for methyl parathion, ranging from 4460 to 9500  $\mu\text{g/l}$  [19,20]; the only chronic data available was with the fathead minnow, and the NOEC for methyl parathion was 310  $\mu\text{g/l}$  [19].

For species that are of concern in the Sacramento River and Delta area, the 96-h LC50 for methyl parathion was 790  $\mu\text{g/l}$  using striped bass [21], 2800  $\mu\text{g/l}$  for the rainbow trout (*Oncorhynchus mykiss*) [22] and 5300  $\mu\text{g/l}$  the coho salmon, (*Oncorhynchus kisutch*) [23]. The freshwater invertebrates appear to be more sensitive, with the scud (*Gammarus fasciatus*) 96-h LC50 of 3.8  $\mu\text{g/l}$  [23], and 48-h EC50s reported for *Simocephalus serrulatus* of 0.37  $\mu\text{g/l}$  [23], and *Daphnia magna* values of 7.8-9.1  $\mu\text{g/l}$  [24]. One chronic value for *Daphnia magna* was reported, and the NOEC was 1.2  $\mu\text{g/l}$  [24] which is essentially the same as for determined for *Ceriodaphnia dubia* in this paper.

For carbofuran, EC50s reported for *Daphnia magna* and *Daphnia pulex* are quite similar, at 48 and 35 µg/l respectively [25,26]. Both of these values are considerably higher than the LC50s obtained for *Ceriodaphnia dubia* (Table 4). The 96-h LC50s for several fish species were also much higher, for rainbow trout the LC50 was 380 µg/l [23], for coho salmon the reported LC50 was 530 µg/l [23], and for the fathead minnow the LC50 was 872 µg/l. No invertebrate or fish chronic values appear in the literature. Another invertebrate, the midge (*Chironomus tentans*) was more sensitive than the fish with a 96-h LC50 of 1.6 µg/l [27].

The use of methyl parathion is estimated to be 19,068 ha in the Central Valley of California [28] with the major use occurring in May and June. The counties in California with the highest density of rice fields treated with it were the Colusa and Sutter Counties, which bracket the Colusa Basin Drain. The use of carbofuran has increased since another carbamate insecticide (bufencarb) was withdrawn [29]. In fact the use of carbofuran has increased from 15,238 ha in 1978 to 39,378 ha in 1985. Waterfowl mortality has been reportedly caused by the ingestion of the carbofuran granules [29]. Carbofuran is applied at a rate of 10 kg per ha or 0.5 kg of active ingredient per ha for controlling the rice weevil.

Recently, Galassi et al [30] used XAD resins to extract organic compounds from surface waters in Italy and combined with 24-h *Daphnia magna* immobilization tests. Atrazine and butylphosphates were identified in the extracts from below the tributary where maize cultivation and a herbicide farm are situated, but they were not found at acutely toxic levels either individually or as a mixture.

Most organophosphate and carbamate insecticides are regarded as non-persistent but they have been found as residues persisting in organic soils used for vegetable production, and the surrounding drainage systems. The persistence of twelve insecticides in water was investigated by Sharom et al [31] who found that carbofuran degraded in a natural surface water in 12 weeks, but 50% degradation occurred in about three weeks. Carbofuran was one of the least stable of the twelve tested [31], similar to parathion, carbaryl, and p,p'-DDT. Also, they [31] determined that the less persistent compounds, i.e., diazinon, mevinphos, carbaryl, carbofuran, parathion, are not strongly absorbed by sediment.

In summary, the TIE procedures for effluents can be effectively used on the acutely lethal ambient waters, and have potential for chronically toxic samples with toxicants exhibiting non-polar characteristics. The valuable step for the CBD samples, is the isolation of the toxicant from the drain water, and tracking the toxicity with the test species that first triggered the request for the TIE. The use of a freshwater invertebrate such as *Ceriodaphnia* may be useful for predicting the toxicity to resident species, such as the striped bass, but the use of several species with several toxic samples for the toxicant identification procedure would be beneficial for confirmation of the toxicant.

Because of the concern about the striped bass population [C. Foe, personal communication] and their main food source, the opossum shrimp (*Neomysis mercedis*), acute and chronic tests with carbofuran and methyl parathion should be conducted. Although the striped bass are notoriously sensitive to handling in the

egg-to-fry stage acute and chronic toxicity tests on both methyl parathion and carbofuran would be important to determine the contribution of toxicity of each.

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Table 1. Toxic units for each test conducted on the 17 May sample of the Colusa Basin Drain.

TEST TYPE	TEST DATE	TOXIC UNIT
Original sample	5/24	1.22
Fractions Sum of toxicity (75%, 80%, 85%)	5/25	1.61
Post-column	5/25	<1.0
Add-backs	5/31	
All fractions		1.30
Toxic fractions		1.41
Non-toxic fractions		<1.0
Original sample	5/31	<1.0 <sup>a</sup>
Fraction concentrates	6/1	
75%		0.14
80%		0.56
85%		0.18
Original sample	6/29	<1.0
Fractions	6/30	
1st 1 L: 75% and 80%		0.59
2nd 1 L: 80%		0.51
Combined fractions and concentrates of 6/30	7/8	
75% and 80%		0.45
85%		0.07
SUM of 75%, 80%, 85%		0.52

<sup>a</sup> The survival was 60% at 48 h.

Table 2. Toxic units for each test conducted on the 1 June sample of the Colusa Basin Drain.

TEST TYPE	TEST DATE	TOXIC UNIT
Original sample	6/22	<1.0
Fractions Sum of toxicity (75% + 80%)	6/22	0.56
Concentrates (75% + 80%)	7/8	0.29

Table 3. Toxic units for each test conducted on the 15 May sample of the Glen Colusa Canal.

TEST TYPE	TEST DATE	TOXIC UNIT
Original sample	5/24	<1.0
Fractionation Sum of toxicity	5/27	-- <sup>a</sup>
Concentrates	5/31	-- <sup>a</sup>

<sup>a</sup> No toxicity in sample; therefore the TU = 0.

Table 4. Acute toxicity values for *Ceriodaphnia dubia* for several chemicals tested at ERL-D.

Toxicant	Test Date	LC50 ( $\mu\text{g/l}$ )	
		24 h	48 h
Carbofuran	6/13	3.4 (2.8-4.3)	2.6 ( -- )
Methyl parathion	6/15	>2.9	>2.9
	6/17	5.5 (4.3-7.2)	2.6 (2.1-3.1)
Carbofuran phenol	6/17	>20	>20
Molinate (Ordram <sup>®</sup> )	6/22	>5,000	>5,000
	7/18	>605	>605
Thiobencarb (Bolero <sup>®</sup> )	6/22	580 (430-790)	510 (400-650)

Table 5. Results of carbofuran and methyl parathion mixture 48 h acute toxicity tests using *Ceriodaphnia dubia*. The LC50 concentration of each compound is based on the nominal values.

Ratio <sup>a</sup>	LC50 (µg/l) (C.I)	
	Carbofuran	Methyl parathion
1 : 1	1.3 (1.0-1.6)	1.3 (1.0-1.6)
3 : 1	2.1 (1.69-2.59)	0.70 (0.57-0.86)
1 : 3	0.65 (0.53-0.81)	2.0 (1.6-2.4)

<sup>a</sup> For these ratio tests, the high concentration of the 1:1 was set at 2× the combined LC50s; each chemical was at 2.6 µg/l and 0.5 dilutions were made. For the 1:3 mixture, the concentration of carbofuran was 3.9 µg/l and the methyl parathion level was 1.3 µg/l (0.5× LC50) with 0.5 dilutions made, conversely 3:1 mixture was set up the same way changing the ratios for each compound.

Table 6. Results of the *Ceriodaphnia dubia* 7-d chronic toxicity tests with carbofuran and methyl parathion.

Concentration µg/l	Mean Young per Original Female	95% Confidence Limit	Percent Survival
<b>CARBOFURAN<sup>a</sup></b>			
Control	15.8	12.5-19.1	100
0.16	14.3	10.6-18.4	90
0.33	12.9	9.3-16.5	90
0.65	14.2	11.2-19.2	100
1.3	12.8	9.4-16.2	100
2.6	10.7	4.8-16.6	60 <sup>b</sup>
<b>METHYL PARATHION<sup>c</sup></b>			
Control	19.6	17.6-21.4	100
0.364	16.9	16.1-21.7	100
0.443	18.0	15.8-20.0	90
0.994	18.8	15.1-21.9	100
1.37	6.4 <sup>b</sup>	--	0 <sup>b</sup>
2.67	0.8 <sup>b</sup>	--	0 <sup>b</sup>

<sup>a</sup> Nominal concentrations.

<sup>b</sup> Statistically different at  $P < 0.05$ .

<sup>c</sup> Concentrations are based on measured concentrations.

Figure 1. The rice growing regions in the Sacramento Valley.

Figure 2. Flow diagram for concentrating and identifying compounds in the CBD and GCC samples.



