

1989

TOXICITY IDENTIFICATION EVALUATION RESULTS

71

C - 0 2 9 8 4 5

C-029845

NATIONAL EFFLUENT TOXICITY ASSESSMENT CENTER
TECHNICAL REPORT 09-89

November 1989

1989 Colusa Basin Drain Toxicity Identification Evaluation
Sacramento, California

by

Teresa J. Norberg-King and Elizabeth Durhan
U.S. Environmental Protection Agency
Environmental Research Laboratory
Duluth, Minnesota 55804

and

Elizabeth Makinen
ASCI Corporation
6201 Congdon Boulevard
Duluth, Minnesota 55804

Background

In 1989, we received samples from the Central Valley Region of the California Regional Water Quality Control Board (the Board) to identify the toxicants in the toxic water samples collected from the Colusa Basin Drain (CBD). The rice fields of the CBD were treated with a variety of pesticides, typically in mid-May. The water that is used to flood the fields is held for a period of time and released into the Sacramento River. Water samples were collected from above the drain, and at various locations in the river to evaluate the toxicity of the water. In 1989, the Board had chemical analyses for several pesticides being done by two laboratories, and conducted *Ceriodaphnia* and fathead minnow toxicity tests. Samples of the CBD water or the river water that exhibited acute toxicity were sent to Duluth for a toxicity identification evaluation (TIE) similar to the work done for the 1988 field season by NETAC (Norberg-King et al., 1988).

Samples

For the first set of samples, four samples selected based on the toxicity data generated by the Board (See Table 1) were shipped to Duluth and were received on 23 May. These consisted of the following: one control site (i.e., water before the pesticide application, collected above the drain May 6) and three CBD samples collected on May 15, May 17, and May 18. A second set of samples, two CBD samples (collected May 31 and June 6) and one downstream river sample (Walnut Grove site collected May 29) were received at Duluth on June 13, 1989. All samples were shipped in polyethylene containers on ice by overnight air delivery and held at 4°C.

Methods and Principle Approach

Samples were logged in at Duluth, and acute toxicity tests set up with both *Ceriodaphnia dubia* (48-h) and fathead minnows (*Pimephales promelas*) (96-h). All test animals were ≤ 24 h old. 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:8 h dark. All samples (100%) were diluted with a reconstituted softwater (DMW) using a 0.5 dilution factor. The DMW had a pH of 7.8-8.0, dissolved oxygen concentrations of 8.0-8.2 mg/l, and hardness and alkalinity levels of approximately 40 and 30 mg/l as CaCO_3 , respectively.

LC50 values for the effluent and concentration samples were calculated using the trimmed Spearman-Kärber method (Hamilton et al., 1977). The methods used for the toxicity identification evaluation follow those described by Phases I, II, and III (EPA, 1988;1989A;1989B).

Fractionation and Concentration

For this step, samples are passed over solid phase extraction columns to remove non-polar organics. Reduction in toxicity in the post-column effluent implies that toxicity is caused by non-polar compounds.

Approximately 1 L of each sample was vacuum filtered through a 1 μm glass filter and tested for toxicity. Each filtered sample (1 L) was concentrated on separate 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 4 mL/min. The column preparation followed the standard operating procedures used at NETAC (Attachment I). 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 (i.e., 25, 50, 75, 80, 85, 90, 95, and 100%) that were increasingly non-polar.

Concentration of the fractions and mass balance tests (add-backs) were done as described in the Phase II and III manuals, respectively. To each sample di-iodobenzene is added as the internal standard from which estimations of concentrations are calculated. The methanol fraction concentrates were injected onto a Hewlett Packard 5890 Gas Chromatograph (GC) interfaced with a Hewlett Packard 5970 Mass Selective (MS) Detector. A 30 m DB-5, 0.25 mm I.D. fused silica column was used. Identifications were made based on a comparison of the sample spectra to EPA/NBS library spectra. Upon identification of a likely toxicant, routine quantitative analyses of original sample was performed.

For the direct quantitation of methyl parathion and malathion, the samples were extracted with methylene chloride or hexane, and then injected onto a Hewlett Packard 5890 GC which was equipped with a flame photometric detector. A 3% OV-1, 1.8 m glass column was used and the detector had a phosphorus filter. Triphenyl phosphate is added to each sample as the internal standard. For direct quantitation of carbofuran, the appropriate standards were run on the GC-MS concurrently. It should be noted that carbofuran, a carbamate pesticide, is difficult to analyze underivatized on the GC as carbamates partially decompose upon injection, and these samples were not derivatized.

Results

First set of samples:

The May 6 sample had no toxicity to either of the test species, and when the sample concentrates were tested, no non-polar toxicity occurred at 5× the original sample concentration (Table 2). For the May 15, 17, and 18 samples none had toxicity to fathead minnows at 100% sample, and none of the fractions caused toxicity at concentrations 5× the original sample. However, for *Ceriodaphnia* only the May 17 sample had measurable toxicity in 48-h when tested on May 23 (LC50 of 83%); however *Ceriodaphnia* in the other two samples were spinning, and did not appear healthy. All three samples (May 15, 17, and 18) were fractionated, and toxicity was observed in each sample's fraction toxicity test (Table 2); the toxicity values (LC50s) have been expressed as toxic units (TUs) (at the original sample concentration) for all samples.

The toxic fractions were combined for the mass balance add-back toxicity test (Phase III) at original sample concentrations (1×). In these add-back tests, toxicity did not occur at 1× for any sample except the May 17, which had 1.16 TU in the original sample and the concentrates of the toxic fractions (which were subsequently analyzed on the GC-MS) had toxicity. Small amounts of toxicity (<1 TU) cannot be measured directly in the sample. For all three of the samples (May 15, 17, 18), the concentrates were analyzed on the GC-MS and the compounds identified for each sample date are given in Table 3. Only two compounds had toxicity values close to the measured concentrations, these were carbofuran and methyl parathion. [Note: The 48-h LC50 for *Ceriodaphnia dubia* is

2.6 µg/l for both methyl parathion and carbofuran.] Molinate levels were a factor of 100× too low to be a possible candidate for causing the toxicity (Norberg-King et al., 1988). The Board (V. Connor, personal communication), informed us that malathion had been detected by a contract laboratory. A specific search for malathion was then made and concentrations of about one-half the LC50 were found (e.g., the malathion 48-h LC50 for *Ceriodaphnia dubia* is 1.41 µg/l).

Concentrations of carbofuran, methyl parathion, and malathion from direct quantitations are given in Table 4.

Second set of samples:

The Walnut Grove river sample (May 29) did not have any acute toxicity for either the *C. dubia* or fathead minnows after it was received at Duluth. When the sample was fractionated using the C18 column, none of the fractions were toxic to either species at 5× the original sample concentration (Table 2).

The May 31 CBD sample was acutely toxic to *Ceriodaphnia* before and after vacuum filtration (48-h LC50 of 61%; 1.6 TU) but not to fathead minnows (Table 2). The toxicity in the fraction totalled 1.7 TUs, and no toxicity occurred in the post-C18 sample. When the mass balance add-back tests were done, toxicity occurred at whole effluent (1×) concentrations (TU=1.6) (Table 2).

For the June 6 CBD sample, the initial toxicity test indicated that the sample was acutely toxic to *Ceriodaphnia*, and not to fathead minnows. The 48-h LC50 was 35%; which was reduced slightly by vacuum filtration (LC50=50%). The fractions (at 5X) accounted for 1.5 TUs, and the mass balance tests indicated toxicity occurred at whole effluent concentrations.

Both of these samples had measurable levels of carbofuran, methyl parathion, and malathion (Table 4) as determined by direct quantitations. It should be noted that these levels of malathion are difficult to measure with the GC-MS used.

Conclusions

Methyl parathion, carbofuran, and malathion were present in various concentrations in all five CBD samples collected after pesticides were applied. These compounds appear to account for the toxicity observed in the fractions, and where applicable, the original sample. Based on work during the 1988 CBD study, the toxicity of carbofuran and methyl parathion was shown to be additive. We have also found the toxicity of malathion and diazinon to be additive, therefore we have assumed additivity for carbofuran, methyl parathion and malathion. Thus, TUs (calculated on the measured concentrations and the LC50 for each compound) were summed. These values compare quite well with the toxicity TUs obtained from the fractions (cf., Tables 2 and 4). Agreement between TUs of toxic fractions and the chemical concentrations is high except for the Jun 6 sample. This could be due to degradation of the pesticides while in storage before the specific analysis was conducted. Usually the chemical TUs are compared to the original sample TUs but since there was low or no acute toxicity in some samples, the comparison of the TUs was made using the fraction TUs against the measured concentration TUs. The percentage that each compound contributed to the toxicity for each sample date is given in Table 5.

In addition, we conducted a 7-d *Ceriodaphnia dubia* mixture test with carbofuran and methyl parathion. From this test, we were able to determine that these compounds are additive on a chronic basis as well (Table 6). The chronic toxicity value for *Ceriodaphnia dubia* was again determined, and the IC25 for both compounds was lower than the LC50 from the acute tests. For example, for carbofuran the chronic value was 1.6× lower than the acute while for methyl parathion the chronic value was 3.3× lower.

Table 1. Background toxicity information on *Ceriodaphnia dubia* for the seven samples.

SAMPLE DATE	SAMPLES RECEIVED	TOXICITY INFORMATION ¹
<i>First Sample Set</i>		
May 5	Control site	No mortality
May 15	CBD	100% mortality in 72-h
May 17	CBD	100% mortality in 48-h
May 18	CBD	50% mortality in 96-h
<i>Second Sample Set</i>		
May 29	River Sample ²	50% mortality in 72-h
May 31	CBD	100% mortality in 24-h
Jun 6	CBD	100% mortality in 36-h

¹ Data provided by V. Connor, personal communication.

² Walnut Grove sampling station.

Table 2. Toxicity information generated on the CBD samples for the 1989 field season and all toxic unit (TU) values are expressed at 1x the original sample. All tests were performed using *Ceriodaphnia dubia*.

SAMPLE DATE	TOXICITY LC50(%)	TU	FRACTION TU	ADD-BACK TU
May 6	>100	<1	<1	--
May 15	>100 ¹	<1	1.14	<1
May 17	83	1.20	1.16	~1
May 18	>100 ¹	<1	0.72	--
May 31	61	1.6	1.7	1.6
Jun 6	50	2.0	1.5	1.6

¹ Spinning and unhealthy animals.

Table 3. GC-MS quantification estimates for the Colusa Basin Drain samples. Analysis was done using the combined toxic fractions for each of the three samples; these are not direct quantitations.

SAMPLE DATE	COMPOUND	CAS No.	ESTIMATED CONC. µg/L
May 15	Triamterene	396010	0.4
	1,2-benzenedicarbonitrile	91156	0.3
	carbofuran	1563662	1.6
	propanoic acid, 2-methyl-2-ethyl	74367310	0.6
	phenol, 2,4-bis (1,1-dimethyl ethyl)	96764	0.8
	methyl parathion	298000	0.5
	molinate	2212671	13.5
May 17	carbofuran	1563662	1.5
	propanoic acid, 2-methyl-2-ethyls	74367310	0.2
	phenol, 2,4-bis (1,1-dimethyl ethyl)	96764	0.9
	methyl parathion	298000	0.7
	molinate	2212671	16.6
May 18	carbofuran	1563662	1.8
	molinate	2212671	25.7
	methyl parathion	298000	0.4
	butyl citrate	77941	0.3
May 31	1,2 benzisothiazole	272162	0.36
	carbofuran	1563662	1.69
	acetamide, N (2-hydroxyphenyl)	614802	1.45
	molinate	2212671	54.1
	diethyl phthalate	84662	0.48
	unknown.	--	23.9
	malathion	121755	2.08
	benzyl butyl phthalate		0.54
Jun 6	carbofuran	1563662	1.22
	diethyl phthalate	84662	0.71
	unknown (same as 5/31)	--	31.6
	phenol, 2,4-bis (1,1-dimethylethyl)	96764	0.89
	BHT		0.35

Table 4. Concentrations and toxic units (TUs) in each sample of the CBD.

SAMPLE	CARBOFURAN		METHYL PARATHION		MALATHION		TOTAL TU
	$\mu\text{G/L}$	TU	$\mu\text{G/L}$	TU	$\mu\text{G/L}$	TU	
May 15	0.80	0.31	1.2	0.46	0.09	0.06	0.83
May 17	0.41	0.16	1.5	0.58	0.54	0.31	1.05
May 18	0.59	0.23	0.54	0.21	0.20	0.14	0.58
May 31	0.83	0.32	0.09	0.03	1.34	0.95	1.30
Jun 6	0.69	0.27	0.093	0.04	0.08	0.06	0.37

¹ Values for carbofuran are based on GC-MS quantitations. Since carbamates such as carbofuran partially decay upon injection on the GC, these values could be lower than values obtained when samples are derivatized.

Table 5. Percent of toxicity due to each compound in the 1989 Colusa Basin Drain samples. Values are based on analytical measurements and measured LC50 values.

SAMPLE DATE	CARBOFURAN	METHYL PARATHION	MALATHION
May 15	37	55	7
May 17	15	55	30
May 18	40	36	24
May 31	25	2	73
Jun 6	73	11	16

Table 6. Results of carbofuran and methyl parathion mixture 7-d chronic toxicity tests using *Ceriodaphnia dubia*. The IC25 or IC50 concentrations are based on the nominal values. The ICp value is the concentration that causes the 25% or 50% inhibition of reproduction or survival compared to the control response.

RATIO ^a	CARBOFURAN	METHYL PARATHION	TOTAL TU
	IC50 (µg/L)		
1 : 1	0.98	0.75	1.18
3 : 1	1.59	0.41	1.19
1 : 3	0.36	0.83	0.93
	IC25 (µg/l)		
1 : 1	0.77	0.59	1.28
3 : 1	1.25	0.32	1.21
1 : 3	0.18	0.41	0.66

^a For these ratio tests, the high concentration of the 1:1 was set at 2x the combined NOECs. For carbofuran alone, the IC50 and the IC25 were 1.96 and 1.6 µg/l, respectively. For methyl parathion alone, the IC50 and the IC25 was 1.0 and 0.74 µg/l, respectively. Total TU were based on the IC50 in the mixture divided by the IC50 of the respective compound and summed.

REFERENCES

- EPA, 1988. Methods for Aquatic Toxicity Identification Evaluations: Phase I Toxicity Characterization Procedures. EPA-600/3-88/034.
- EPA, 1989A. Methods for Aquatic Toxicity Identification Evaluations: Phase II Toxicity Identification Procedures. EPA-600/3-88/035.
- EPA, 1989B. Methods for Aquatic Toxicity Identification Evaluations: Phase III Toxicity Confirmation Procedures. EPA-600/3-88/036.
- Hamilton, M.A., R.C. Russo and R.V. Thurston. 1977. Trimmed Spearman-Kärber Method for Estimating Median Lethal Concentrations in Toxicity Bioassays. Environ. Sci. Technol. 11:714-719; Correction 12(4):417 (1978).
- Norberg-King, T.J., E. J. Durhan, G.T. Ankley, and E. Robert. 1988. Application of Toxicity Identification Evaluation Procedures to the Ambient Waters of the Colusa Basin Drain, California. NETAC Technical Report 04-89.