



Department of Pesticide Regulation



Paul E. Helliker
Director

MEMORANDUM

Gray Davis
Governor
Winston H. Hickox
Secretary, California
Environmental
Protection Agency

TO: John Sanders, Ph.D.
Chief, Environmental Monitoring Branch
Department of Pesticide Regulation

FROM: Frank Spurlock, Ph.D.
Senior Environmental Research Scientist (specialist)
Environmental Monitoring Branch

DATE: January 13, 2002

**SUBJECT: STUDY SUMMARY: EVALUATION OF INTERFERENCES IN
ENZYME-LINKED IMMUNOSORBENT ASSAY (ELISA) FOR
DIAZINON**

BACKGROUND

In January and February, 2000, a Sacramento Valley surface water monitoring study was jointly conducted by the U.S. Geological Survey (USGS) and the California Department of Pesticide Regulation (DPR) (Dileanis et al., 2002). The purpose of the study was to characterize the rainy season occurrence and sources of diazinon in the Sacramento and Feather Rivers. Water samples were collected from 17 monitoring sites and analyzed for the presence of diazinon and other selected pesticides. Diazinon analysis on most samples was conducted using enzyme-linked immunosorbent assay (ELISA, 412 samples), while replicate splits from approximately 30 percent of those samples were also analyzed using gas chromatography/thermionic specific detection (GC/TSD, 107 samples) for confirmation. A small number of samples were analyzed using gas chromatography/mass spectrometry (GC/MS, 31 samples), but only 10 samples were also analyzed by ELISA and/or GC/TSD. Additional details on sampling locations, sampling procedures and analytical methods are discussed by Dileanis et al. (2002).

There were 87 split samples in which diazinon was detected in both the ELISA and GC/TSD methods above their respective limits of detection (20 ng/L for GC/TSD, 30 ng/L for ELISA). The ELISA method yielded higher concentrations than GC/TSD in every sample (Figure 1), with percent differences between ELISA and GC/TSD ($=\frac{[ELISA-GC/TSD]}{[GC/TSD]} * 100$) ranging from 7.5 to 429 percent, with a median of 81 percent (Figure 2). The ELISA method demonstrated a similar positive bias relative to the GC/MS method in nine of 10 samples in which detections were reported for both methods. The percent difference data were analyzed to determine if larger differences between the two analytical methods were associated with specific sampling sites, types of sampling sites (river vs. tributary), or varied systematically with concentration (Figure 3). No significant differences between sites, types of sites or concentration were evident.

The quality assurance/quality control (QA/QC) plan of the USGS/DPR winter 2000 Sacramento Valley diazinon study included rinse blanks, field blanks, reagent blanks, blank spikes, and matrix spikes (Dileanis et al., 2002). Diazinon was not detected in any rinse blank or field blank



samples. ELISA matrix spike recoveries were elevated, with an average recovery of 130% and a range in spike recoveries of 111-161% (n=14). These QC data are limited, but suggest some bias in the ELISA diazinon results for the Sacramento Valley samples due to matrix effects. The GC/TSD matrix spikes yielded a mean recovery of 87% (n=4), while GC/TSD analysis of American River water sample spikes demonstrated a mean recovery of 85% (n=11). Any possible matrix effect on GC/TSD is apparently smaller in magnitude than that observed for ELISA, and reduces instead of enhances GC/TSD analytical results.

Traditional GC-based methods for determination of diazinon in water have a demonstrated history of quantitative recoveries and reproducibility and so are usually considered to be the “gold standard” relative to newer methods such as ELISA. In addition, ELISA is also prone to matrix effects – either due to the presence of cross-reactants or nonspecific interferences. Sullivan and Goh (2000) reported that ELISA yielded elevated diazinon concentrations in storm runoff water samples relative to a gas chromatography/flame photometric detection method (GC/FPD). These researchers were unable to determine the specific cause of the apparently elevated ELISA results. Sullivan and Goh concluded “Before the diazinon kit can be employed routinely for regulatory compliance monitoring, particularly for quantifying runoff water from a storm event, further study is required to elucidate and quantify the factors responsible for its consistent overestimation of ELISA results.”

Consequently DPR designed a study in conjunction with the University of California (UC) with the primary objective of identifying any specific or non-specific interferences in Sacramento Valley dormant season runoff water that may be responsible for the high biased winter 2000 ELISA concentration data.

The study was performed under contract with Dr. B. Hammock and Shirley Gee of UC Davis; and detailed study data for this project are provided in the final report (Hammock and Gee, 2002). This memo is a summary of the main study conclusions and provides general recommendations for use of ELISA in future studies.

SUMMARY OF STUDY RESULTS

1. Cross-reactivity

Thirty different chemicals were tested for cross-reactivity in the laboratory using the brand of diazinon ELISA kit used to analyze the winter 2000 dormant spray runoff samples of Dileanis et al. (2002). These chemicals included structurally similar pesticides and degradates, other dormant-season high use organic pesticides, a variety of other organic pesticides, and inorganic pesticides. In certain cases a small degree of cross-reactivity was observed, but at levels too small to explain the consistent high bias in the winter 2000 dormant spray ELISA analytical results .

2. Recovery studies of spiked environmental water samples

Water samples were collected from two sampling sites in the Sacramento Valley in early December 2000, immediately prior to the 2001 dormant spray season diazinon applications. These samples were spiked with known amounts of diazinon and analyzed using ELISA; spike recoveries were variable, and there was no consistent bias in analytical recoveries relative to the known spike levels. The apparent bias that was observed in the previous year's sampling was not evident in these matrix spikes.

3. Comparison of ELISA to gas chromatography/flame photometric detection (GC/FPD) analysis of 2001 dormant season water sample splits

Water samples were collected during the 2001 dormant spray season and analyzed by GC/TSD and ELISA. Many of the 2001 sampling locations were either identical to or very close to those used during the 2000 dormant spray runoff sampling of Dileanis et al. (2002). There were 50 of the 2001 dormant season samples in which diazinon was detected by both the ELISA and GC/TSD methods. Among these data the median percent difference of the two methods was not significantly different than zero (Wilcoxon 1-sample test, $p=0.98$). No high bias in ELISA results relative to GC/TSD was evident. However, the percent differences between the two methods were highly variable, ranging from approximately -90% to 200% (Figure 4).

4. An additional observation

Shortly after the present study was initiated an additional possible cause for high bias in ELISA concentrations was discovered: use of expired ELISA kits. During analysis of diazinon samples from an unrelated DPR Environmental Monitoring study, the analyst discovered a strong high bias for the "expired" ELISA results (> 1 month past expiration) relative to GC/FPD (Figure 5, Appendix 1). It is possible that if expired or compromised ELISA kits were inadvertently used to analyze the winter 2000 dormant season samples, this would explain some or all of the apparent bias in those ELISA data. At this time there is no way to determine the status of the ELISA kits that were used to analyze the winter 2000 dormant spray data of Dileanis et al. (2002).

CONCLUSION

This study failed to identify a definitive cause for the (apparently) high-biased diazinon ELISA concentrations in Sacramento Valley water samples reported by Dileanis et al. (2002). It appears unlikely that a particular constituent was the cause of high biased ELISA concentrations in the winter 2000 monitoring study of Delineas et al. (2002) because (a) the high bias was apparent for ELISA-determined diazinon concentrations in all samples from every location in 2000, (b) 2001 ELISA samples displayed no such consistent bias, and (c) several pesticides with high use in the Sacramento Valley were shown to have no or little effect on the SDI immunoassay.

During the course of this study it was discovered that expired or compromised ELISA kits may yield data that are too high. While this is one possible explanation for the consistent bias observed between ELISA and GC/TSD in the 2000 data, there is no way to determine the status of the kits that were used to obtain those data.

The percentage differences between GC and ELISA results obtained on sample splits were highly variable in both 2000 and 2001: in 2000, the percentage differences ranged from 8 to 430 percent, whereas the range in 2001 was -92 to 196 percent. The inter-quartile range (25th to 75th centiles) was greater than 50 percentage points in both years: 54% - 107% in 2000, and -39% to 33% in 2001. Finally, the standard deviation of percent difference between GC and ELISA was 41 and 70% in 2000 and 2001, respectively. These and similar data (e.g., Holmes et al., 1998) illustrate the variability among analytical methods, and emphasize the need to thoroughly vet newer methods such as ELISA.

It is obvious that a robust QA/QC plan is imperative for all studies, and particularly the use of matrix spikes and control limits to confirm the veracity of data from each analytical set. If control limits are exceeded, analysis should always stop and diagnostic procedures should be used to identify problems in the analytical procedure. Finally, in those instances that the Environmental Monitoring Branch utilizes ELISA for diazinon analysis, we should continue to analyze splits of a substantial portion of ELISA samples using standard chromatographic methods for confirmatory purposes.

REFERENCES

- Dileanis, P.D., K. P. Bennett and J.L. Domagalski. 2002. Occurrence and transport of diazinon in the Sacramento River, California, and selected tributaries during three winter storms, January-February 2000. U.S. Geological Survey. Water Resources Investigations Report 02-4101.
- Hammock, B. and S. Gee. 2002. Evaluation of potential interferences for a diazinon ELISA test kit. Final Report for Agreement 00-0183S.
- Holmes, R., C. Foe, and V. de Vlaming. 1998. Sources and concentrations of diazinon in the Sacramento River watershed during the 1994 orchard spray season. June 1998 DRAFT Report, California State Water Resources Control Board. Data as obtained DPR's surface water database.
- Sullivan, J. and K.S. Goh. 2000. Evaluation and validation of a commercial ELISA for diazinon in surface waters. *J. Ag. Food Chem.* 48:4071-4078.

1. diazinon concentrations - Elisa vs GC/TSD results - winter 2000 samples

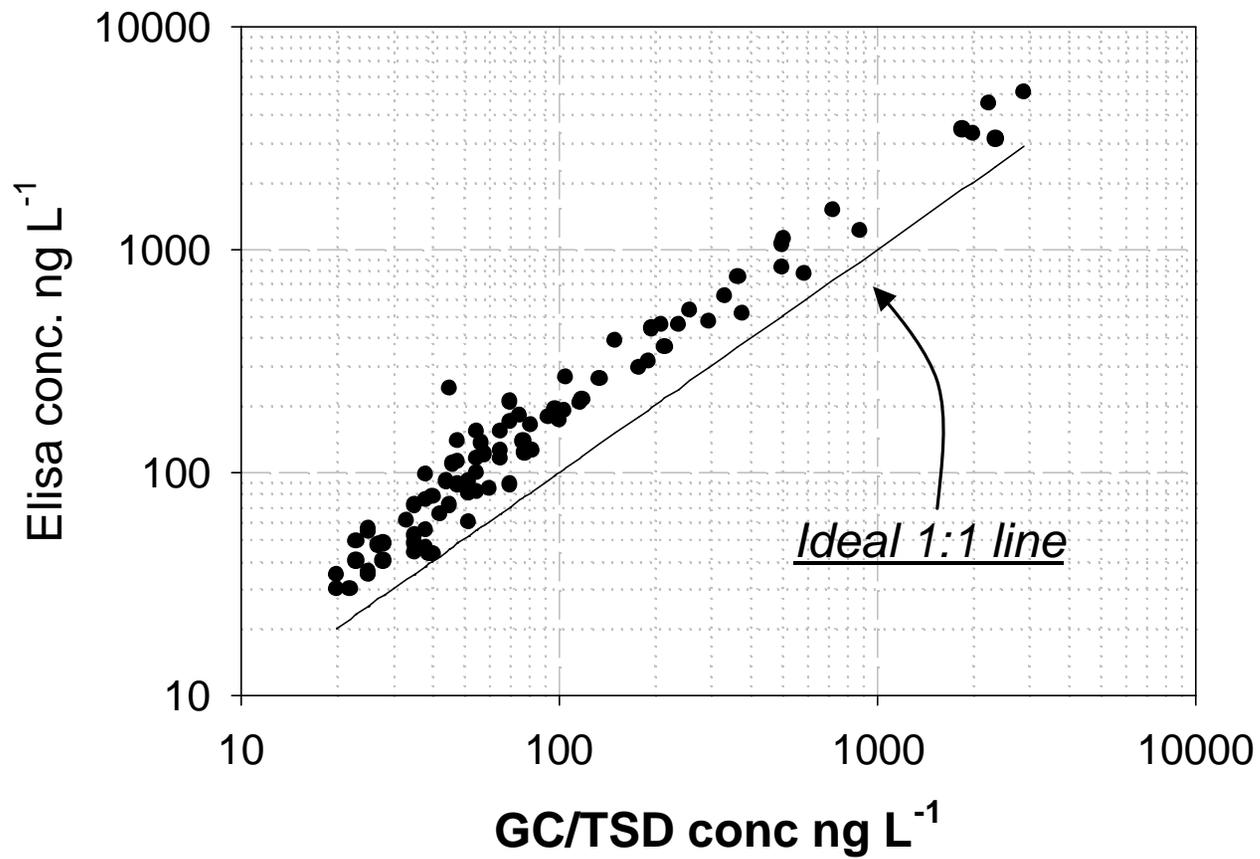
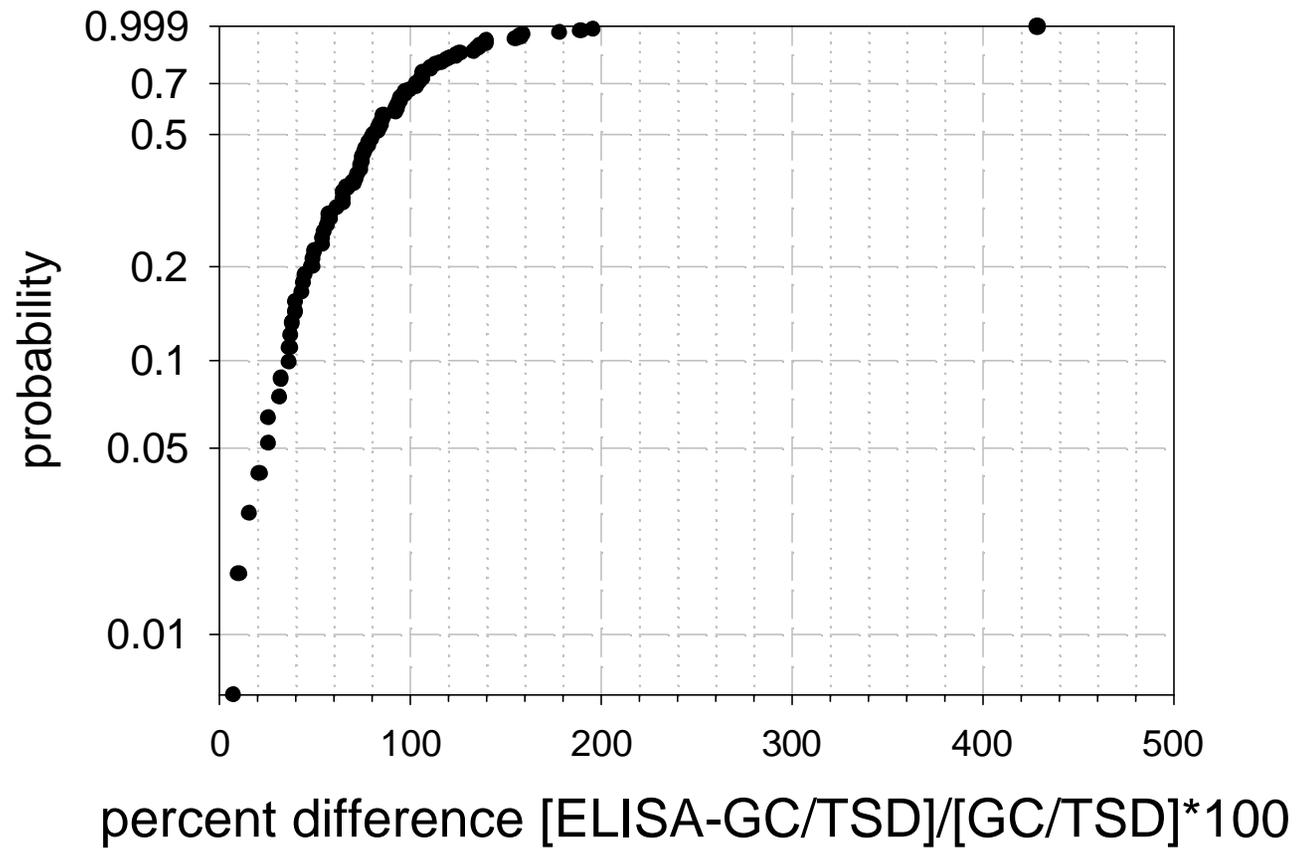


Figure 2. distribution of percent difference for winter 2000 dormant season runoff data (Dileanis et al. ,2002) (n=87)



**Figure 3. percent difference vs GC/TSD diazinon conc. (ng/L)
for winter 2000 runoff (Dileanis et al., 2002)**

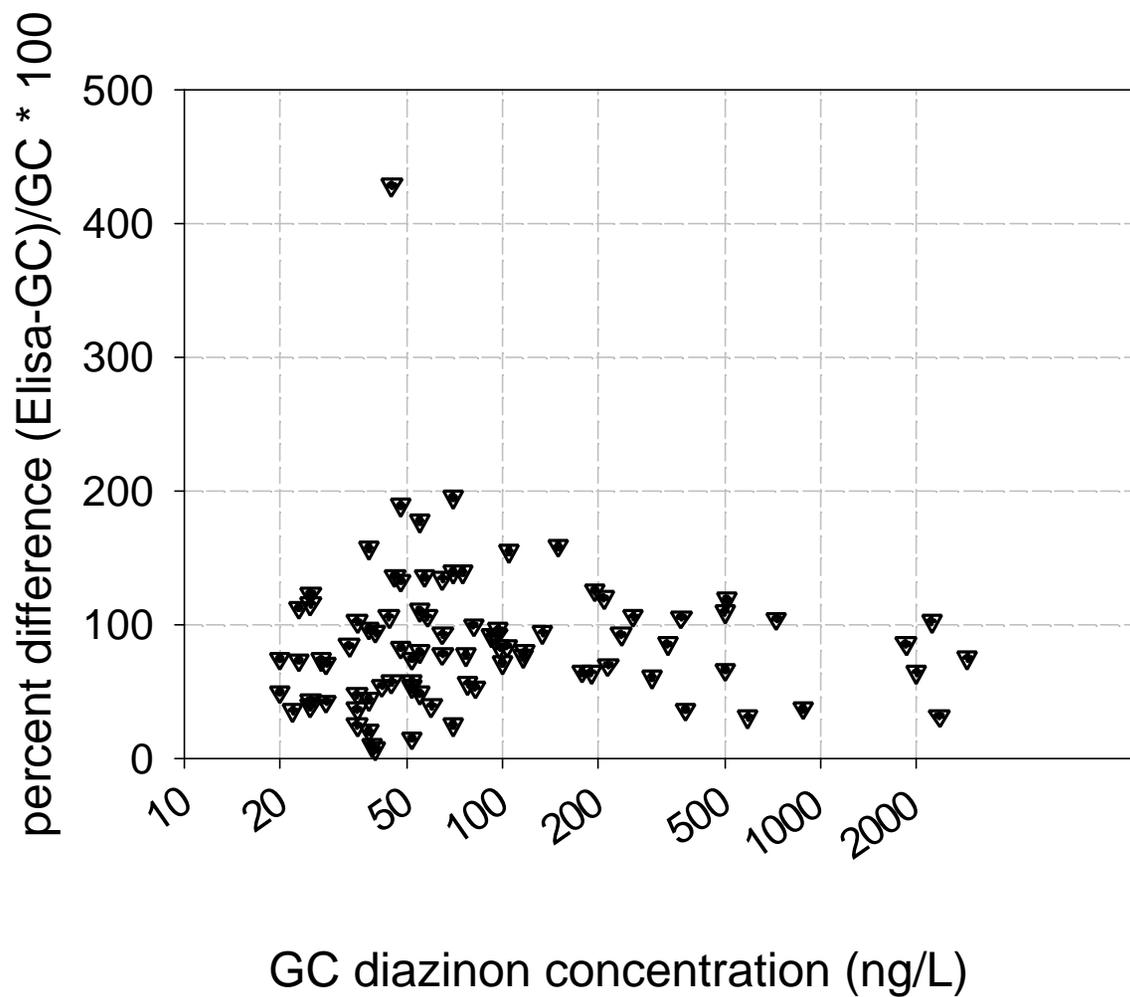


Figure 4. Distribution of percent difference for winter 2001 Sacramento Valley dormant season samples (n=56)

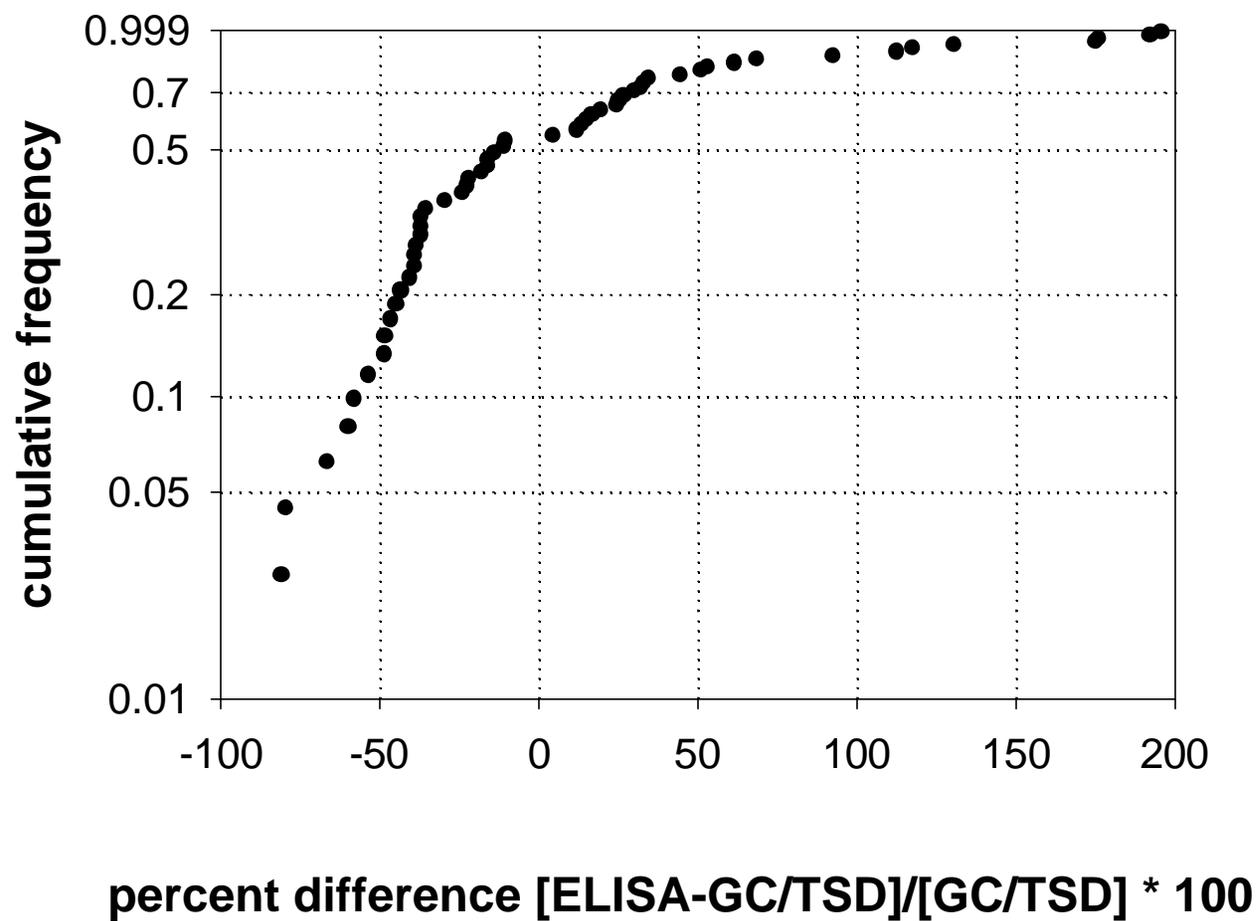
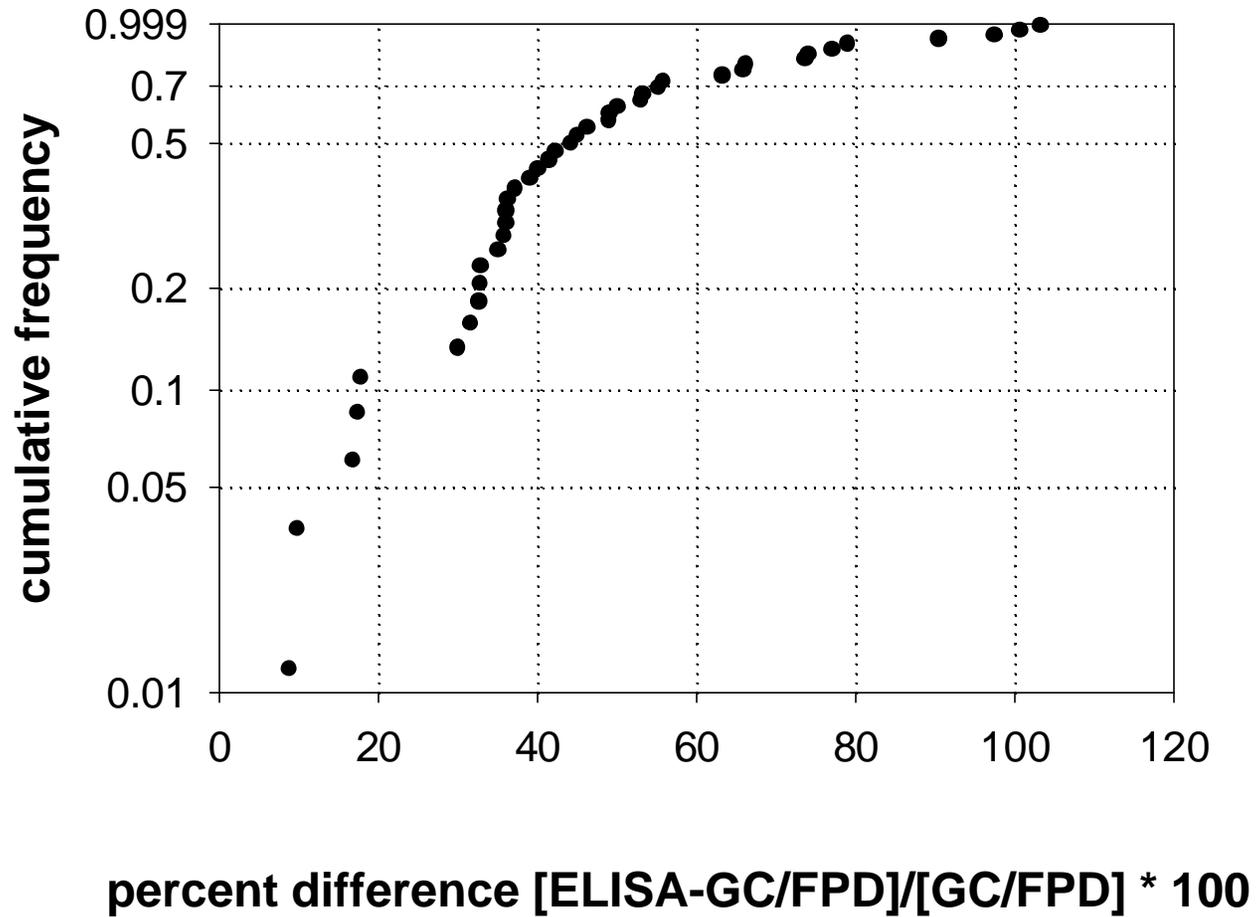
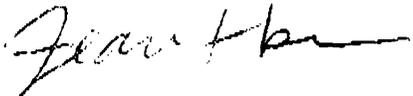


Figure 5. Percent difference between "expired" ELISA kit data and GC/FPD (n=41)



Appendix 1

Date: December 22, 2000
To: Catherine Cooper
From: Jean Hsu 
Subject: The Results of **Diazinon** Analysis in Water by **ELISA**

The Strategic Diagnostics **Inc. (SDI) ELISA** plate kit was used for the determination of **Diazinon** in this study. The kits **expired in 3 1/2000**. Since I observed that **one** of the reagent (substrate) had color change from colorless to light blue, I became **concerned** the **accuracy** of the test results.

For samples (194-61 to 194-101), the **results** by **ELISA** were **much higher** than the results by GC method. See attached result **table**.

Since all the samples **have** been diluted 1: 10,000 times before analysis, there **should** not be any background interferences. Even after I tried to use a fresh substrate prepared in-house to substitute the **reagent** of the kit, the results were still unacceptable. The **color** turned out to be **too pale** to generate a good standard curve.

In order to have reliable results by **ELISA**, **expired kits** should never be used. In addition, we should not substitute **any components** of the kits with in-house reagent.

①

Appendix 1

The Diazinon results for water by SDI ELISA				
Rec'd	12/14/2000			
Allquoted	12/14/2000			
Diluted:	12/18/2000	All samples have been diluted 1:10,000 and not been filtered.		
Analysis:	12/18-21/00			
Sample	CDFA#	ELISA result ug/L	GC Result ug/L	%Different
194-61	2000-2620	6260	5707	9.24%
194-62	2000-2621	6510	3926	49.52%
194-63	2000-2622	5900	4215	33.32%
194-64	2000-2623	5160	3915	28.01%
194-65	2000-2624	4660	3584	26.52%
194-66	2000-2625	4850	3429	34.33%
194-67	2000-2626	3720	3168	18.09%
194-68	2000-2627	3970	2895	31.32%
1 9463	2000-2628	4090	3007	30.52%
1 w-70	2000-2629	3870	2187	55.57%
194-71	2000-2630	3960	2214	56.56%
194-72	2000-2631	3650	2359	42.97%
194-73	2000-2632	3550	2045	53.80%
194-74	2000-2633	3100	2357	27.23%
194-75	2000-2634	3450	2599	28.14%
194-76	2000-2635	2560	1882	30.53%
194-77	2000-2636	2320	1989	15.41%
194-78	2000-2637	2320	1669	21.53%
194-79	2000-2638	3480	1753	65.49%
194-80	2000-2639	2770	1779	43.57%
194-81	2000-2640	3100	2180	34.85%
194-82	2000-2641	7830	4715	49.89%
194-83	2000-2642	5030	3441	37.52%
194-84	2000-2643	4720	2713	54.00%
194-85	2000-2644	5060	2523	66.91%
194-86	2000-2645	3630	2436	39.37%
194-87	2000-2646	3710	2474	39.97%
194-88	2000-2647	3740	2442	41.99%
194-89	2000-2648	4000	2962	29.82%
194-90	2000-2649	4170	2160	62.26%
194-91	2000-2650	2810	2400	8.38%
194-92	2000-2651	2830	2179	25.99%
194-93	2000-2652	2560	2183	15.90%
194-94	2000-2653	3330	2464	30.29%
194-e	2000-2654	3360	231%	36.66%
194-a	2000-2655	3050	1987	42.21%
194-97	2000-2656	3170	2127	39.33%
194-98	2000-2657	3450	1698	58.04%
194-99	2000-2658	2700	1766	41.83%
194-100	2000-2659	2370	1784	28.21%
194-101	2000-2660	2810	1960	36.13%
BK				
Spike	1ppm	1.36	1.36%	
Spike	1ppm	1.38	1.38%	
Spike	1ppm	1.19	1.19%	
Spike	1ppm	0.981	95.1%	
Spike	1ppm	1.37	1.37%	
Spike	1ppm	1.18	1.18%	
Spike(PE)	175ppt	201	115%	
Spike(PE)	175ppt	209	119%	

% Different: (ELISA result - GC result)/Average of ELISA & GC result X 100

2