Novartis Crop Protection, Inc.
and
Makhteshim-Agan of North America, Inc.

Investigation of Diazinon Occurrence, Toxicity, and Treatability in Southern United States Publicly Owned Treatment Works

EXECUTIVE SUMMARY

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Technical Report: 3-97

Environmental and Public Affairs Department
Greensboro, NC 27419-8300

This document has been prepared to provide technical information on insecticide occurrence and treatability in Publicly Owned Treatment Works (POTW) in the southern United States and the relationship to Clean Water Act biomonitoring requirements. This report is presented in 11 volumes including: Executive Summary; Project Report; Appendix A Raw Monitoring Data and Data Collection Forms; Appendix B Standard Operating Procedure for Analytical Characterization; Appendix C Batch Treatability Testing; Studies; Appendix D Continuous Flow Treatability Testing; Appendix E Auxiliary Process Enhancement; Appendix F Partitioning and Bench-Scale Fate Testing; Appendix G Work Plan; Appendix H Technical Memoranda No. 1 Literature Evaluation; and Appendix I Technical Memoranda No. 2 Evaluation of Data from Facilities Claiming Diazinon-Related Bioassay Failures.

Novartis Crop Protection, Inc. is dedicated to the development of insecticide products of economic value and to principles of good stewardship in product use and disposal. This document is produced in the spirit of cooperation to help address the various scientific, regulatory and educational issues retailers, product users and POTW managers will face in the 1990's to achieve compliance with the Federal Clean Water Act.

This study was directed and coordinated by Dennis Tierney, Ph.D., Novartis Crop Protection, Greensboro, NC. Field monitoring, bench testing and report preparation were conducted by Brian Christensen, Allison Martin Ph.D., P.E., James Robin P.E., Roberta Schlicher P.E., Steve Krueger, and Phil Heck Ph.D. Montgomery Watson, Wayzata, MN and Salt Lake City, UT. Laboratory assistance was provided by Environmental Analytical Solutions, Inc. (EASI), Kenner, LA and AQUA-SCIENCE, Sacramento, CA.

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INTRODUCTION

Diazinon is an organophosphorus insecticide widely used to control a variety of insects in agricultural crops and lawn and garden environments. In recent years (1989-present) diazinon has been the subject of regulatory and public scrutiny due to effluent biotoxicity at some Publicly Owned Treatment Works (POTW) primarily located in the southwestern United States. Starting in 1988 a biomonitoring requirement was added to the National Pollutant Discharge Elimination System (NPDES) permits of POTWs under the authority of the Clean Water Act (CWA). If a wastewater facility repeatedly fails the biomonitoring assay, it is required to identify the cause(s) of toxicity in the effluent through a process termed a Toxicity Reduction Evaluation (TRE) under Section 308 of the CWA.

The Environmental Protection Agency (EPA) has established 10 geographical regions in the United States. Region VI includes five states: Arkansas, Louisiana, New Mexico, Oklahoma, and Texas with the EPA regional office in Dallas, Texas. Within Region VI, the biomonitoring condition was required for all POTW with discharges greater than one million gallons per day (mgd) and was phased into POTW permit reissuance beginning in 1988. Thus, by 1992, 348 POTW in Region VI had conducted whole effluent toxicity (WET) tests under the standardized bioassay procedures. Forty-seven POTW had at least one bioassay failure and were required to conduct a follow-up TRE to identify and eliminate the cause of effluent toxicity. Fourteen POTW suspected diazinon in the effluent was the primary or partial cause of the toxicity.

During the same period (1988-91), the Texas Water Commission (TWC) now the Texas Natural Resource Conservation Commission (TNRCC), administered the CWA in Texas. At that time, TWC had partial delegation of the NPDES permit program. Thus, a POTW in Texas had two permits; one from the state and one from the EPA.

In June 1991 the TWC believed POTW effluent toxicity was caused by diazinon for at least two cities that had failed bioassay tests (EPA Region VI 1994). While other compounds (such as ammonia) can cause POTW effluent toxicity, diazinon is among the likely chemicals because of its widespread residential and commercial use in Texas cities. Several regional pests, including fire ants, grub worms, roaches, fleas and other insects, are commonly treated with diazinon. Data implicating diazinon in bioassay failures at some POTW in the region stirred public officials and private industry to further study the role of diazinon in the bioassay failures and, as needed seek ways to remedy the situation.

Based on ā review of all the issues, the TWC voted to postpone a proposed diazinon concentration criterion in a revision of the Texas Surface Water Quality Standard. Instead, the TWC in August 1991, passed a resolution asking the Texas Department of Agriculture to determine the feasibility of pursuing a limited-use designation for diazinon and to determine whether or not diazinon was a primary cause of toxicity at POTWs. If diazinon was determined to be a primary cause, public education and controls for the continued use of diazinon would be proposed. If diazinon was a cause of POTW toxicity, but the sources were not ubiquitous, the commission would pursue toxicity or concentration limits for the permits of specific POTW.

In October of 1991, Novartis (formerly Ciba) was contacted by EPA Region VI, Office of Water to participate in a joint state/federal meeting to review the POTW biomonitoring program in Region VI. As a result of that meeting, Novartis proposed a research plan to EPA to help develop information which could be used in developing guidance on the role of diazinon in POTW WET biomonitoring failures.

OBJECTIVES

This project was designed to answer a series of time-critical questions regarding the occurrence and treatability of diazinon-related toxicity in POTW in Texas, Oklahoma, and New Mexico. These questions led to four primary objectives:

I. Assess POTW Diazinon Occurrence and Relationship to Observed Bioassay Failures. This objective examined data linking diazinon to toxicity failures at 14 POTW in Texas, Oklahoma, and New Mexico from 1991 to 1994. In 1992 there were 348 POTW in EPA Region VI subject to biomonitoring requirements. Based on the biomonitoring results, 14 POTW identified diazinon as the primary agent in bioassay failures. Specifically, this work was designed to narrow the number of facilities to only those with biomonitoring failures strongly linked to diazinon. This was accomplished by a review of the TRE reports by each POTW along with the bioassay and chemical monitoring records of the 14 POTWs.

II. Determine Diazinon Concentration and Frequency of Occurrence in POTWs. This objective assessed the frequency and duration of diazinon and other lawn and garden insecticide's presence in the influent and effluent in selected POTW in Region VI.

III. Determine the Treatability of Diazinon in POTWs. This objective evaluated the relative effectiveness of conventional treatment process at POTW in reducing the concentration of diazinon in the effluent and/or reducing the bioavailability.

IV. Determine the Fate of Diazinon in POTWs. This objective assessed the binding of diazinon through physical partitioning to solids in the POTW treatment process.

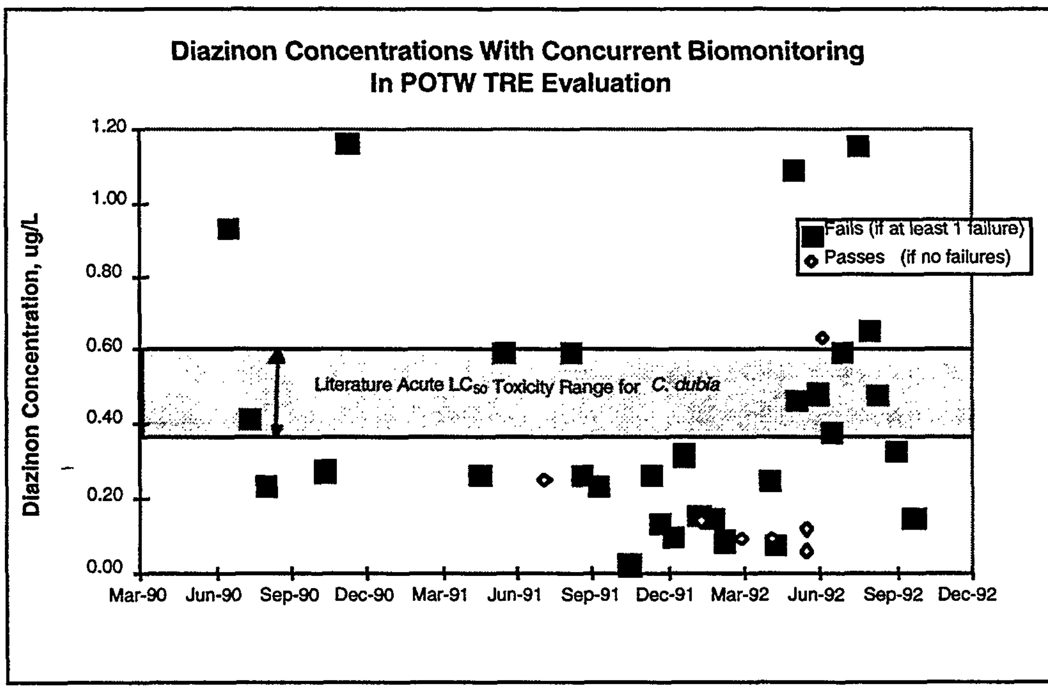

Figure 1
Range of Diazinon Influent and Effluent Concentrations for 14 POTW in EPA Region VI (1990-92)

STUDY OVERVIEW

Literature Review (1990-95)

The 14 POTW in EPA Region VI with suspected diazinon-related biomonitoring failures reported 131 monitoring events between May 1990 and September 1992 (Figure 1). Influent diazinon was monitored 25 times and effluent 106. Diazinon was detected in all influent samples and 101 effluent samples. Maximum concentrations were 0.96 µg/l in influent and 1.10 µg/l in effluent. Mean influent and effluent concentrations were 0.40 and 0.31 µg/l, respectively.

When the 25 influent samples were paired with an effluent sample collected during the same monitoring event, the diazinon mean concentration in the influent was 0.40 ppb and the effluent mean was 0.26 ppb for the 14 POTW with data.

The effluent diazinon concentration at the 14 POTWs fall above and below the laboratory acute toxicity LC_{50} results for *ceriodaphnia dubia* (*C. dubia*). Most diazinon LC_{50} acute effects occur at concentrations between 0.35 and 0.61 µg/l for *C. dubia*, the fresh water cladocern used as the invertebrate test organism in the WET biomonitoring procedure at POTWs (Amato et al. 1992; Ankley et al. 1991). EPA Region VI assessed POTW compliance with the whole effluent test (WET) monitoring requirement based on the 7-day chronic bioassay with survival as the measurement endpoint. Concurrent bioassay and diazinon monitoring data (Figure 1) indicates that many of the biomonitoring failures occurred at diazinon concentrations below the acute LC_{50} toxicity range. Toxicants other than diazinon were present.

Some of the 14 POTWs on some dates had diazinon concentrations in the effluent which could be expected to cause *C. dubia* acute mortality if a bioassay had been conducted. From the data, however, it is not possible to determine if the effluent diazinon concentrations were entirely or partially accountable for the expected or observed *C. dubia* mortality in the 7-day chronic survival bioassays. The proximity of commonly reported diazinon detection levels to toxicity thresholds for *C. dubia* makes attributing toxicity to diazinon in the Toxicity Identification Evaluation (TIE) process difficult (Figure 1).

Literature information concerning the potential for diazinon treatability in conventional POTWs was also limited. Other studies indicated the likely mechanisms for diazinon removal at POTWs were chemical and biologically catalyzed hydrolysis, biodegradation and biosorption (Adhya et al. 1991, Sethunathan and Pathak 1972, Tsezos and Bell 1987). Literature data also suggest that diazinon removal may be hindered if a facility was operating near or above its design capacity (Metcalf and Eddy, Inc. 1979).

I. Assess POTW Diazinon Occurrence and Relationship to Observed Bioassay Failure.

Overall, of 348 POTWs with biomonitoring requirements in Texas, Oklahoma, and New Mexico (Figure 2), 47 POTWs reported biomonitoring failures. The cause of toxicity identified for 33 facilities was clearly related to chemicals other than diazinon, such as ammonia and metals. However, 14 (4%) of the 348 POTW stated diazinon was the source of the effluent toxicity in the 1990-92 period (Figure 3).

In a detailed review of the POTW information submitted to EPA Region VI for these 14 facilities (during 1990-92), 6 were conducting a TRE and the associated TIE; thus, data and reports were not available at the time of this analysis (1993). Eight facilities had performed TREs which were completed in adequate detail to assess the relationship of diazinon to biomonitoring failures. The review of the TREs by the 8 POTWs is contained in Technical Memorandum No. 2 Evaluation of Data from Facilities Claiming Diazinon-Related Bioassay Failures (Appendix J).

Diazinon LC_{50} concentrations for toxicity to *C. dubia* were reported at 0.35 to 0.61 µg/l (Table 1). The other fresh water test organism (fathead minnow, *P. promelas*) routinely used in POTW has acute toxicity LC_{50} values between 3,700-10,000 µg/l. Mortality of the fathead minnow was not attributed to diazinon at the observed effluent concentrations in acute bioassays. Eight facilities reported acute *C. dubia* toxicity attributable to diazinon at concentrations <0.10 to 0.56 µg/l.

The inconsistent acute toxicity for the low-end diazinon concentrations (below 0.35 µg/l) also appeared in chronic tests (Table 1). This suggests that other toxicants were present in the wastewater effluent since the standard TRE/TIE process does not distinguish between diazinon and other O-P insecticides. Other insecticides and their reported toxicity to *C. dubia*, *D. magna*, and *P. promelas* are presented in Table 2.

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Table 1
Reported Toxicity to *C. dubia* Diazinon (µg/l)

	Acute Toxicity Range LC_{50}[1]	Chronic Toxicity Range IC_{25}[2]
Ankley et al. (1991)	0.43-0.61[3]	–
Amato et al. (1992)	0.35	–
EPA Region VI TIE	<0.10-0.561	<0.05-0.56[4]
TRAC Labs (1992)	–	0.12-0.34
APA and ENSR (1992)--	0.12	

[1] 7-day survival, [2] 7-day reproduction , [3] 48-hr, [4] 96-hr

Table 2
Reported Acute Toxicity (LC50) Selected Insecticides (µg/l)

Compound	*Ceriodaphnia dubia*	*Daphnia magna*	*P promelas*
Diazinon	0.35-0.61	0.56-1.0	3,700-10,000
Diazoxon	NRD	NRD	NRD
Chlorpyrifos	0.055	0.4	120-542
Malathion	2.12	0.27-33.0	8,650-14,100

NRD = No reported data

Overall, this analysis of 8 TREs did not result in unequivocal conclusions; however, the following observations were made: (1) Diazinon was sometimes reduced or removed in POTWs with extended hydraulic residence time (HRT) and extended mean cell residence time (MCRT). (2) A weak correlation existed between diazinon concentration and *C. dubia* toxicity, but the significance of the role of diazinon at the eight facilities was not clear. (3) It may be possible to enhance diazinon removal from wastewater at POTWs by optimizing processes already present at these facilities. (4) Chemical confirmation by ELISA or GC/MS for diazinon requires a simultaneous analysis for other O-P insecticides such as chlorpyrifos and malathion.

Specific findings of the data review were:

1. Diazinon concentrations in some effluent bioassays from the 14 POTWs was present at concentrations above the LC_{50} and would be expected to account for *C. dubia* acute mortality.

2. While *C. dubia* acute mortality was attributed to diazinon in some POTW tests, the diazinon concentration in the bioassayed effluent was at or below the no observed effect level (NOEL) of 0.080 ppb for diazinon obtained from laboratory LC_{50} studies. It appears the observed *C. dubia* acute mortality was not due exclusively to diazinon, but due to other unknown toxicants present with diazinon or were false positive results inherent in the test. The mortality could be due solely to the other unknown chemical(s) or, if other OP insecticides were present, and depending on concentrations, *C. dubia* mortality could be due to the additive effect from a common OP mode of action (Table 1, Table 2).

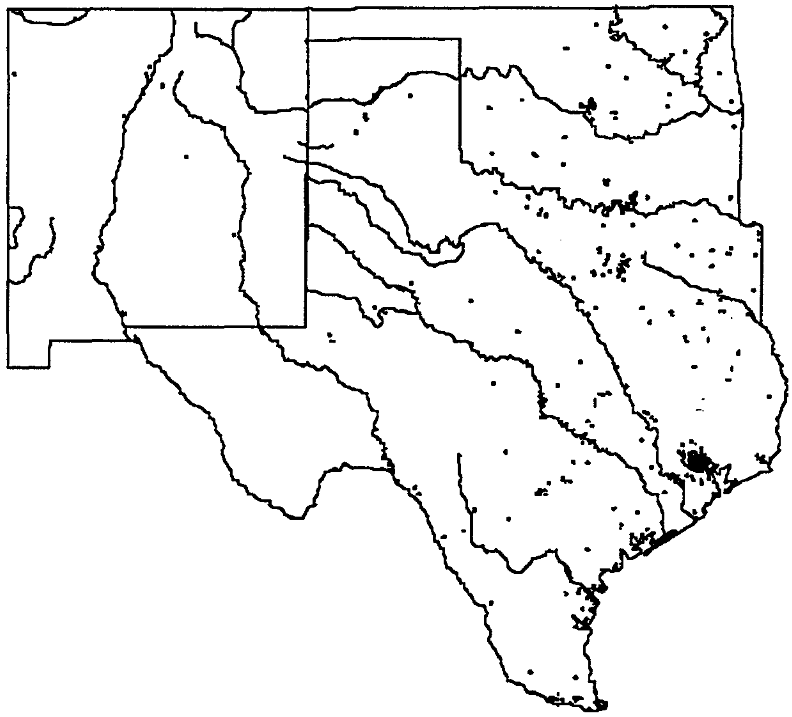

Figure 2
POTW with Biomonitoring Requirements

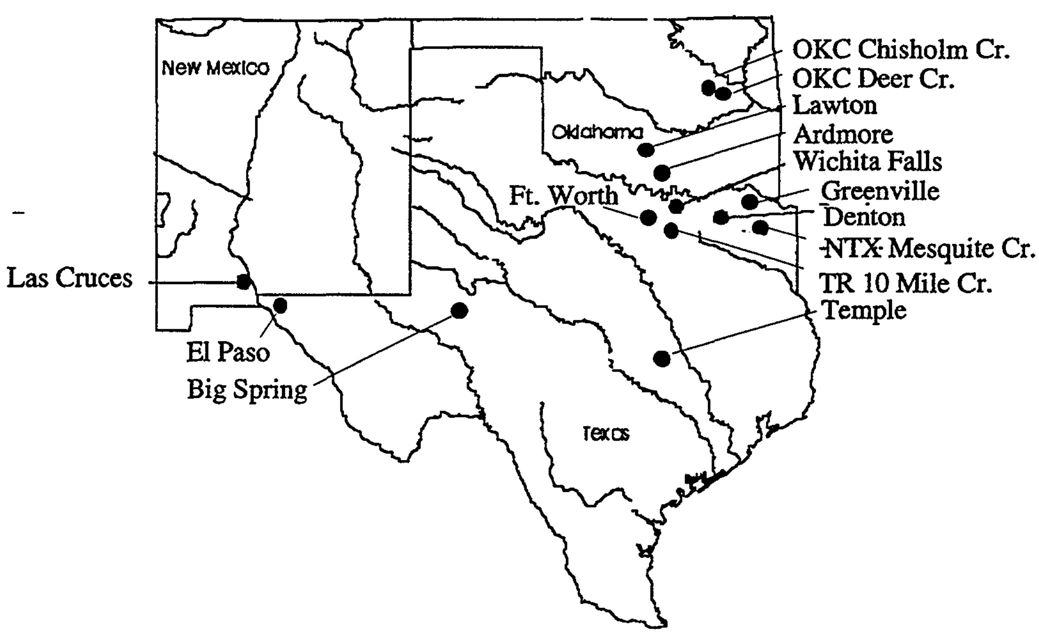

Figure 3
POTW Identified by EPA Region VI as Potentially Having Diazinon-Related Bioassay Failures

3. The diazinon acute and chronic LC_{50} concentrations for *C. dubia* reported from the TIE studies varied widely. For example, in one POTW bioassay, chronic mortality was observed at diazinon IC_{25} concentration of 0.56 ppb (above the acute LC_{50}) while the chronic IC_{25} in other bioassays was reported at diazinon concentrations of 0.12 ppb. This concentration is about 1/5 of the former study, as well as approximately 1/3 of the laboratory acute LC_{50} for *C. dubia* (Table 1). Therefore, the conclusion that diazinon should be implicated as the sole toxicant cannot be made.

   The differences in chronic toxicity have not been experimentally evaluated. However, some possible explanations are 1) the relative diazinon bioavailability to *C. dubia* varies among the effluents, 2) other unknown and unmeasured chemicals are present which have equal or greater toxicity to *C. dubia* than diazinon. These chemicals were not identified or escaped identification in the TIE characterization. And, 3) some bioassay failures are due to false positives inherent in the test.

4. The TIE *C. dubia* acute LC_{50} concentrations attributed to diazinon in the effluent varied more widely than the laboratory clean water LC_{50} for diazinon (Table 1). Some TIE reported acute LC_{50} at concentrations 1/3 (0.10 ppb) of the clean water LC_{50} (0.35 ppb). This leads one to conclude that toxicity attributed to diazinon is a) either enhanced in an effluent matrix or b) associated with other unknown toxicants simultaneously present.

II. Determine Diazinon Concentration and Frequency of Occurrence in POTWs.

The evaluation of the TRE/TIE reports for 8 of the 14 POTWs indicated the presence of diazinon as at least one cause of biomonitoring failures in some of the effluent bioassays. In many instances, however, the diazinon concentrations were below reported laboratory LC_{50} concentrations. To evaluate the potential connection between the presence of diazinon and NPDES biomonitoring failures at POTW, a one-year influent/effluent monitoring program was established.

Six POTW in Texas, Oklahoma, and New Mexico were selected and volunteered for the monitoring program (Figure 4). At the time of the study, three of these had historical NPDES biomonitoring failures attributed to diazinon and the remaining three had no historical biomonitoring failures. Companion facilities were matched based upon process type (activated sludge, fixed film, combination fixed film/activated sludge), size (10.7-6.3, 4.0-2.8 and 10.8-7.3 mgd), location (New Mexico, East Oklahoma, and Texas), and history of biomonitoring failures.

The following POTW participated in the monitoring program:

- **POTWs with historic NPDES biomonitoring failures**
  1. River Road WWTP, Wichita Falls, TX
  2. Las Cruces WWTP, Las Cruces, NM
  3. Pecan Creek WWTP, Denton, TX

- **POTWs without historic NPDES biomonitoring failures**
  4. Chickasaw WWTP, Bartlesville, OK
  5. Roswell WWTP, Roswell, NM
  6. Kings Creek WWTP, Terrell, TX

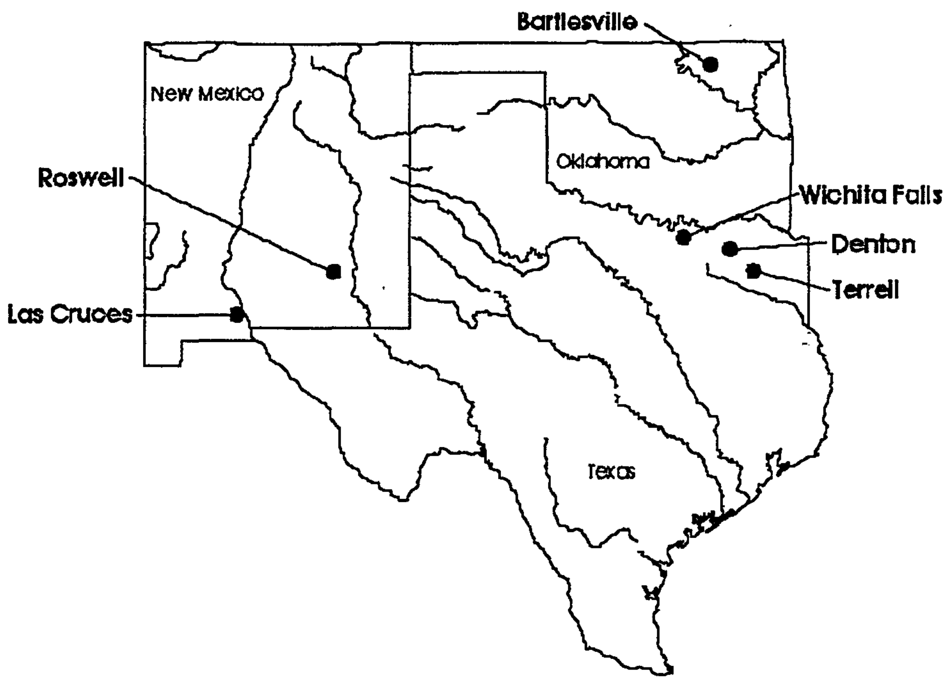

Figure 4
POTWs Participating in Monitoring Program

Each of the 6 POTW collected influent and effluent samples every 2 weeks for 27 biweekly periods in 1994. Samples collection dates were rotated between different days of the week over the 54-week period. Each facility also collected three samples during peak flow periods associated with stormwater runoff events. This schedule produced a total of 27 paired influent and effluent samples from each POTW. All samples were analyzed for diazinon, diazoxon, chlorpyrifos, and malathion. Table 3 presents a summary of the monitoring program.

The monitoring program was designed to answer a series of questions which, along with responses, follow:

Is the extent of diazinon occurrence represented by the limited data available before initiation of this program and by assumptions currently used in policy and decision making? The TRE/TIE reports evaluated in the Phase I of this study had mean influent (n=25) and effluent (n=106) diazinon concentrations above 0.200 µg/l. For the POTWs participating in this monitoring program, the influent diazinon mean concentration was 0.236 µg/l at facilities with and without historic failures. The effluent diazinon mean concentrations ranged from 0.041 to 0.128 µg/l. The overall maximum detected influent concentration was 4.940 µg/l at the Roswell WWTP. The maximum detected effluent concentration was 0.535 µg/l, again at the Roswell WWTP which coincided with the day of the maximum influent concentration. The influent mean diazinon concentration for this study was compared by a T-test with the historical TRE/TIE data and was found to be consistent within a 95 percent confidence interval (Figure 5). There was no historical monitoring for OP compounds other than diazinon for comparison to this study.

Table 3
Summary of Biweekly Sampling Results for the Six POTWs

	POTWs with Failures					POTWs without Failures				
	Concentration, µg/l			No.	No.	Concentration, µg/l			No.	No.
Compound	**Mean**	**Max**	**Min**	**Samples**	**Detects**	**Mean**	**Max**	**Min**	**Samples**	**Detects**
Influent										
Diazinon	0.232	3.186	<0.029	78	50	0.289	4.940	<0.029	74	43
Diazoxon	<0.181	0.589	<0.028	78	1	<0.181	0.268	<0.028	74	3
Chlorpyrifos	0.214	3.421	<0.054	78	48	0.229	1.050	<0.054	74	40
Malathion	0.117	3.476	<0.021	78	26	0.153	4.070	<0.021	74	27
Effluent										
Diazinon	0.052	0.350	<0.071	78	11	0.100	0.535	<0.071	76	33
Diazoxon	0.095	0.376	<0.056	78	20	<0.159	0.130	<0.056	75	3
Chlorpyrifos	<0.142	0.165	<0.039	78	4	0.060	0.170	<0.039	75	24
Malathion	<0.131	0.159	<0.034	78	1	<0.131	0.315	<0.034	75	4

Limits of Detection	Rounds 1-14		Rounds 15-27	
	Influent	Effluent	Influent	Effluent
Diazinon	0.089	0.091	0.029	0.071
Diazoxon	0.181	0.159	0.028	0.056
Chlorpyrifos	0.136	0.142	0.054	0.039
Malathion	0.052	0.131	0.021	0.034

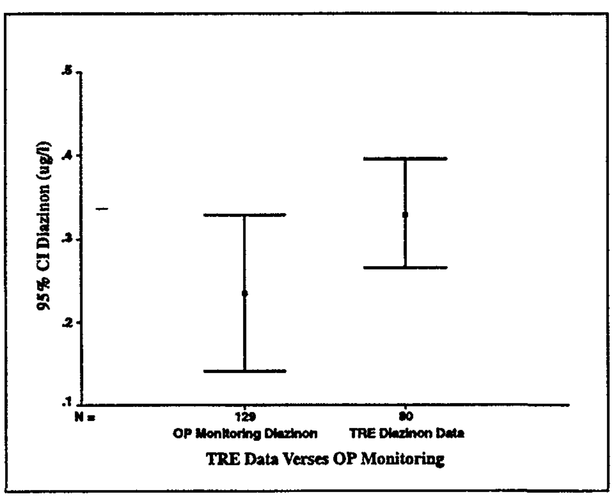

Figure 5
Error Bar for OP Monitoring and TRE Data

What is the extent of diazinon and other selected OP compounds in the influent and effluent of P OTWs in the study region? The influent occurrence of diazinon was 61 percent in the pooled data set from facilities with and without historic biomonitoring failures, respectively. Frequency of occurrence in effluent samples was 29 percent. Diazoxon was detected in 3 percent of the influent samples and 15 percent of the effluent samples. Chlorpyrifos was detected in 58 percent of the influent samples and 18 percent of the effluent samples. Malathion was detected in 35 percent of the influent samples and in 3 percent of effluent samples.

The pooled data from all the POTWsconcluded that there was no significant difference between the influent chlorpyrifos and diazinon concentrations.

Is there a notable difference between the occurrence of OP compounds at POTW historically with biomonitoring failures compared to those without historic failures? The mean effluent concentrations, with the POTW without past biomonitoring failures having slightly higher effluent OP compound concentrations (mean 0.535 µg/l) than POTW with past biomonitoring failures (mean 0.350 µg/l) (Table 3). However, all of the mean effluent data are very close to detection limits for the analytes; and cannot be reliably compared due to differences in those detection limits. Tables 4, 5 and 6 summarize frequency and extent of occurrence by failure history. Based on the pooled data from the POTWs, there was no significant differences in mean diazinon influent concentrations between facilities with past biomonitoring failures and those facilities without failures (Figure 6).

Table 4
OP Insecticide Percent of Occurrence

	Influent		Effluent	
	BMF	NBMF	BMF	NBMF
Diazinon	64	58	14	43
Chlorpyrifos	62	54	5	32
Malathion	3	36	1	5
Diazoxon	1	4	26	4

BMF - Biomonitoring Failure
NBMF - Non-biomonitoring Failure

Table 5
OP Insecticide Annual Mean Concentrations

	Influent		Effluent	
(µg/l)	BMF	NBMF	BMF	NBMF
Diazinon	0.232	0.289	0.052	0.1
Chlorpyrifos	0.214	0.229	<0.142	0.06
Malathion	0.117	0.153	<0.131	<0.131
Diazoxon	<0.181	<0.181	0.095	<0.159

BMF - Biomonitoring Failure
NBMF - Non-biomonitoring Failure

Table 6
OP Insecticide
Maximum Detected Concentrations

	Influent		Effluent	
µg/l	BMF	NBMF	BMF	NBMF
Diazinon	3.186	4.94	0.35	0.535
Chlorpyrifos	3.421	1.05	0.165	0.17
Malathion	3.476	4.07	0.159	0.315
Diazoxon	0.589	0.268	0.376	0.130

BMF - Biomonitoring Failure
NBMF - Non-biomonitoring Failure

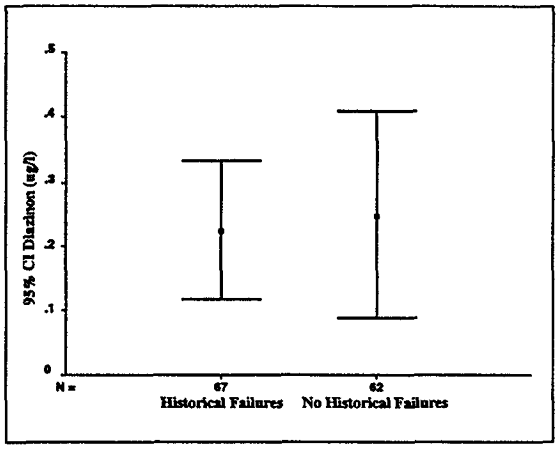

Figure 6
Error Bar Chart for Diazinon Effluent at 6 POTW

Is there a correlation between NPDES biomonitoring failures and high concentrations of OP compounds in the effluents of POTW in the monitoring program? A correlation between biomonitoring failures and the presence of OP compounds in POTW effluent could not be made. Twenty of the twenty-three concurrent samples resulted in passing biomonitoring requirements. Correspondingly, the OP compound concentrations were low, mostly below detection limits. Three concurrent samples resulted in bioassay failures. In two of these samples, OP compounds were all below detection limits, one sample had only diazinon present at 0.07 µg/l with the others below detection limits (Table 7).

Table 7
Comparison of Biomonitoring Results with Organophosphorus Compound Effluent Concentrations

Facility	Month	Biomonitoring Result	Concentration, µg/l Diazinon	Diazoxon	Chlorpyrifos	Malathion
River Road WWTP[a]	July	Pass	<0.091	<0.159	<0.142	<0.131
	October	Pass	<0.071	0.177	<0.039	0.159
Las Cruces WWTP[a]	January '94	Pass	<0.091	<0.159	<0.142	<0.131
	February	Pass	<0.091	<0.159	<0.142	<0.131
	March	Pass	<0.091	<0.159	<0.142	<0.131
	April	Pass	<0.091	<0.159	<0.142	<0.131
	May	Pass	0.135	<0.159	<0.142	<0.131
	October	Fail	<0.071	<0.056	<0.039	<0.034
	December	Fail	0.079	<0.056	<0.039	<0.034
	January '95	Fail	<0.071	<0.056	<0.039	<0.034
Pecan Creek WWTP[a]	February '94	Pass	0.120	0.160	<0.142	<0.131
	March	Pass	<0.091	<0.159	<0.142	<0.131
	April	Pass	<0.091	<0.159	<0.142	<0.131
	May	Pass	<0.091	<0.139	<0.142	<0.131
	June	Pass	<0.091	0.180	<0.142	<0.131
	July	Pass	<0.091	<0.159	0.155	<0.131
	August	Pass	<0.071	0.190	<0.039	<0.034
	September	Pass	<0.071	0.136	<0.039	<0.034
	October	Pass	<0.071	0.370	<0.039	<0.034
	November	Pass	<0.071	<0.056	<0.039	<0.034
	December	Pass	<0.071	<0.056	<0.039	<0.034
	January '95	Pass	<0.071	<0.056	<0.039	<0.034
Chickasaw WWTP	October	Pass	<0.071	<0.056	0.041	<0.034

[a] This plant has historically failed NPDES biomonitoring requirements

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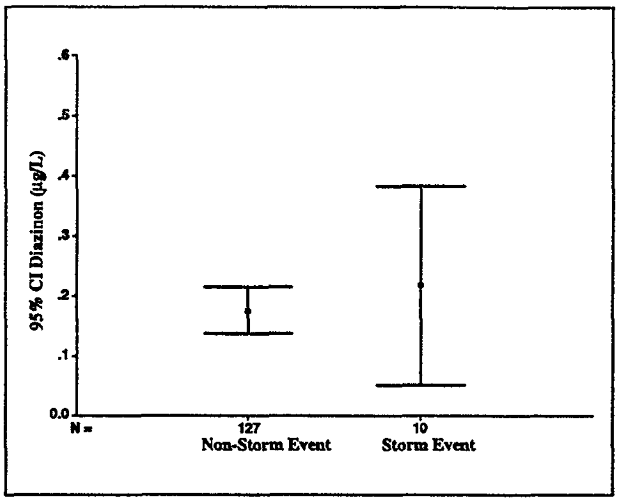

Figure 7
Error Bars for Storm Event and Non-Storm Event Monitoring at 6 POTW

Does a correlation exist between influent diazinon concentrations and significant rainfall events? The data generated during this study indicated that while there was increased variability in the storm event data, there was no significant differences in the mean diazinon concentrations for a storm event and non-storm event. The non-storm event concentrations were slightly lower, but the concentrations were well within the 95 percent confidence interval of the storm event (Figure 7).

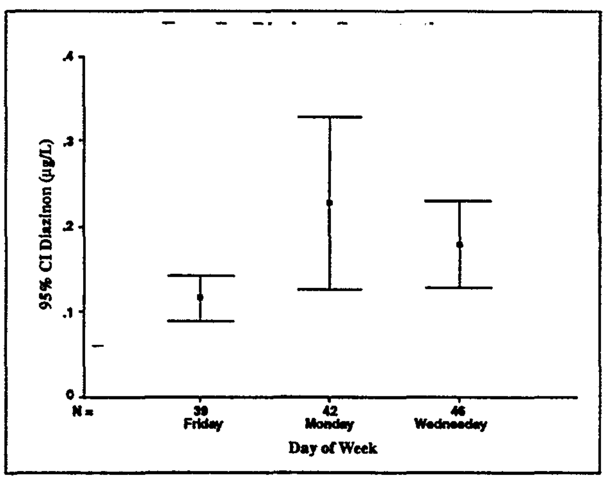

Figure 8
Error Bars for Diazinon Concentrations for Samples Collected at Varying Weekdays at 6 POTW

Does a correlation exist between influent diazinon concentration and day of week and season? The data generated during this study indicated that there was no significant differences in the influent diazinon concentrations on the various days of the week. The mean influent diazinon concentration was the highest on Monday, but the data are not strong enough to conclude that a statistically significant difference exists (Figure 8).

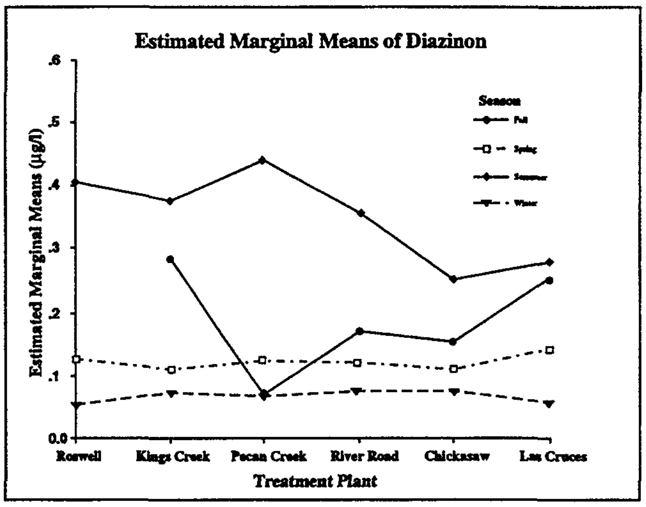

Figure 9
Seasonal Diazinon Effluent Concentrations at 6 POTW

Diazinon, chlorpyrifos, and malathion concentrations follow distinct seasonal trends. Influent maximums occurred in the second quarter, with minimums in the third. Maximum effluent concentrations occurred in the third quarter, with minimums in the fourth quarter. Diazoxon, showed no seasonal influent or effluent trends (Figure 9).

Does a correlation exist between typical measured diazinon concentrations and area diazinon use rates? No data exist regarding diazinon usage or application rates within the subject urban areas. However, it is assumed that diazinon use would be highest during the predominant pest seasons in the South. The occurrence of diazinon in POTW influent coincides with the fire ant season (March to September with peaks in April to June).

Can process variables such as plant design and operating parameters related to OP compound removal from POTW influent? The combination fixed film/activated sludge facilities were more effective at diazinon removal than the other process types. For example, the combination fixed film activated sludge plants at River Road and Las Cruces were able to remove or degrade 68 and 57% of the diazinon entering the plants. This is in comparison to the 1 and 9% removal at the activated sludge plants in Pecan Creek and Chickasaw. Similarly, diazoxon formation was greater for these "combination" facilities. Figure 10 shows the overall removal percentages for diazinon and chlorpyrifos for the three process types. Due to the limited number of facilities representing each process design type, the incomplete submission of process information from each of the participating POTW, and the variability in the OP compound occurrence data, it was not possible to relate OP compound removal to specific operating parameters.

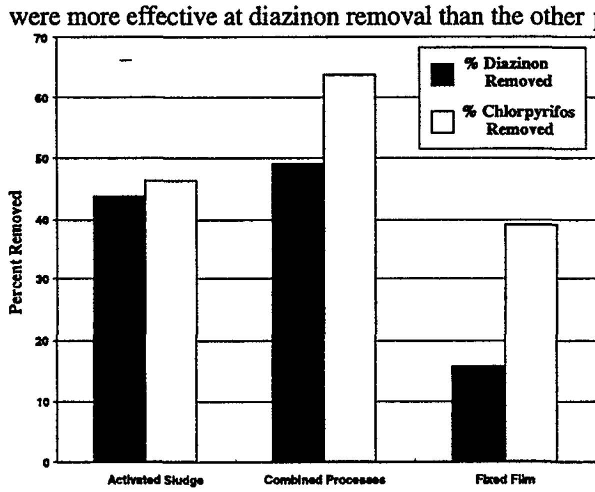

Figure 10
Diazinon and Chlorpyrifos Removal at 6 POTW

D-040369

III. Determine the Treatability of Diazinon in POTW.

Information gained from the TRE/TIE review and the annual monitoring of six POTWs indicated that diazinon reduction through conventional POTW unit processes appeared feasible. Bench-scale testing was conducted to assess the effect of WWTP design and operating parameters on organophosphorus compound removal and toxicity reduction.

By controlling operating conditions and utilizing the same wastewater source, effluents were compared and contrasted to determine how different design and operating parameters affected both diazinon and toxicity removal. Two independent approaches to the bench-scale treatability study were implemented: batch testing and continuous flow testing.

Batch Testing. Two series of batch treatability tests were performed in parallel; one series involved four reactors without powder activated carbon (PAC) addition, and the other series involved four reactors with PAC addition. The general approach to the batch treatability testing was to set up the reactors, add an appropriate mixture of return activated sludge (RAS) and primary clarifier effluent from a nearby POTW, spike in a fixed amount of diazinon, and observe reactor performance in terms of diazinon removal and traditional operating parameters over a 28-day period.

The results were intended to frame conditions for follow-on continuous flow testing and answer two basic questions on treatability:

How does the mixed liquor suspended solids (MLSS) concentration affect diazinon removal? Results indicated that increasing either mixed liquor suspended solids or reaction time increased diazinon removal (Figure 11). Diazinon at concentrations of 1.6 µg/l was removed to below detection limits between 5 and 6 days after it was introduced into the system.

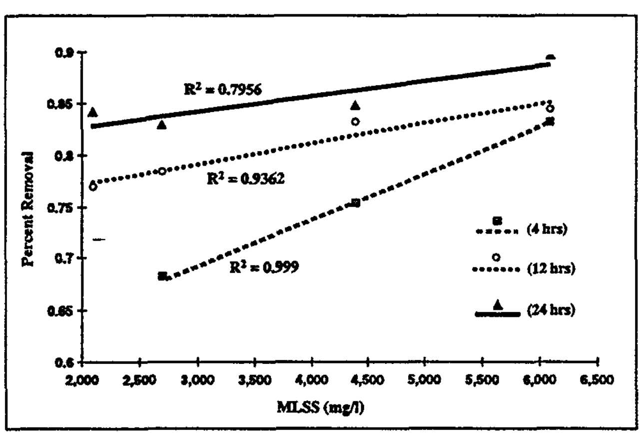

Figure 11
Diazinon Removal with Varying Mixed Liquor Suspended Solids and Hydraulic Residence Times

How does PAC addition to activated sludge affect diazinon removal? PAC is effective at removing diazinon, but dosages were not optimized in this study. Data reveal that at 1,000 mg/l an excess of adsorption capacity was present. From an initial diazinon concentration of 1.6 µg/l, all PAC reactors dropped to below detection limits between 10 minutes and 4 hours.

Continuous Flow Treatability Testing. In order to determine variables affecting diazinon removal and toxicity in POTW effluents, flow-through reactors were constructed to meet the following attributes:

- The reactors needed to represent actual POTW processes.
- The reactors should simulate POTW processes of those facilities that participated in the monitoring program.
- The reactors needed to have enough differences between them so that effective mechanisms for treatment could be identified.

To meet these requirements and from knowledge of POTW biological processes, both fixed film and suspended growth systems were examined. Testing was carried out in three distinct phases (low diazinon level, intermediate, and high level concentrations), each phase having an acclimation period followed by a steady-state intensive testing period. During the first two phases, all of the above seven reactors were used. For the third phase, only four of the reactors were used, with adjusted operating parameters as performance of the other three reactors had diminished. Physical/chemical parameters, OP concentration, and bioassays were monitored routinely during the 29-39 day acclimation period and 7-12 day testing period for each reactor.

Is toxicity linked to diazinon concentrations in the effluent? When the effluent diazinon level was below 0.25 µg/l no toxic effects are measurable. When the diazinon level was greater than 0.40 µg/l toxic effects are seen; however, chlorpyrifos and malathion were also present in nearly all reactor effluents. At diazinon levels between 0.25 and 0.40 µg/l increasing diazinon did not always result in increasing toxic responses. Bioassay results could not be shown to correlate well with the diazinon levels for all the reactor systems. It was determined after consideration of the effluent concentration and reported toxic effects of all the insecticides monitored, together diazinon and chlorpyrifos accounted for 90 percent of the chronic toxicity effects observed.

What are the key operating variables controlling diazinon removal and effluent toxicity in POTWs? Data from the four activated sludge reactors indicate that a correlation exists between SRT, HRT and MLSS, and diazinon removal. This link was apparent in all Phase I and Phase II intensive testing and in one of two tests in Phase III (Figures 12, 13, and 14).

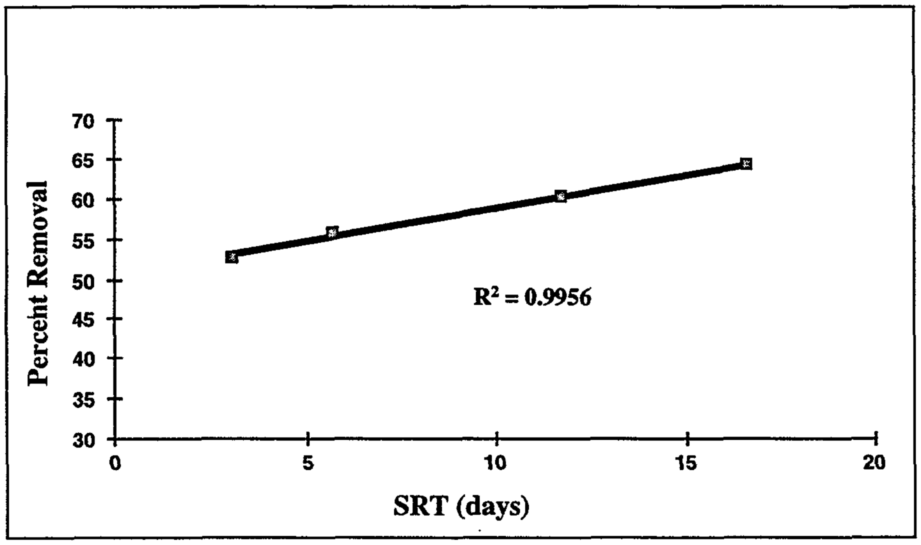

Figure 12
Diazinon Removal as a Function of SRT
(Phase II)

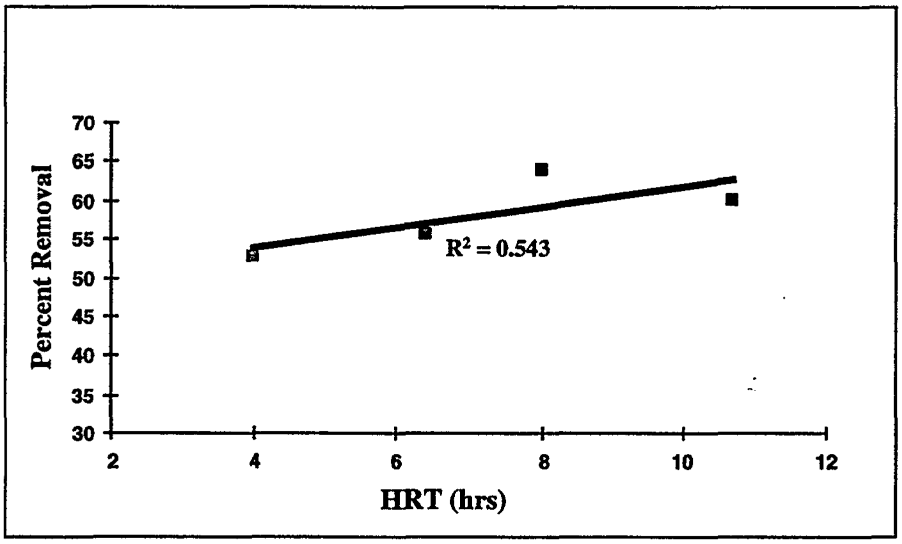

Figure 13
Diazinon Removal as a Function of HRT
(Phase II)

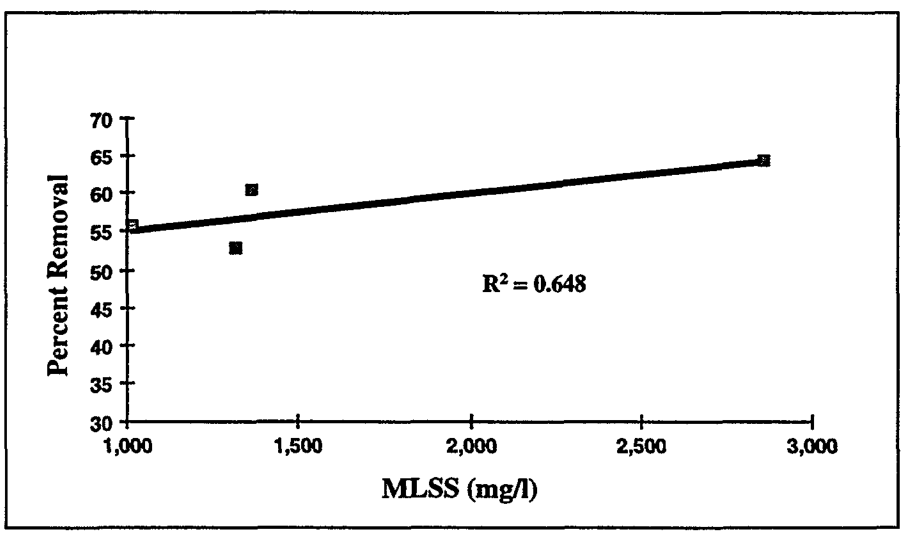

Figure 14
Diazinon Removal as a Function of MLSS
(Phase II)

Is there a relationship between effluent toxicity and any reactor operating parameters? The data suggest that there may be a link between reactor hydraulic residence time and reduced toxicity for the activated sludge systems. This link was confirmed in Phase I and II testing (Table 8), however, the Phase III data did not confirm this correlation.

Can effluent toxicity be reduced through changes in the operating parameters? Correlations are apparent with SRT, HRT and MLSS and effluent toxicity. Since adsorption appears to be a dominant removal mechanism, enhanced removal through an increase in the three operating parameters is an attractive possibility for reducing effluent toxicity.

Table 8
Summary of Bioassay
(*C. dubia*) Results as of % Mortality[1]

	Phase I		Phase II	
Reactors	**Acute**	**Chronic**	**Acute**	**Chronic**
Conventional	50%	0%	75%	1%
PAC	15%	0%	0%	0%
Nitrifying No. 1	75%	0%	85%	10%
Nitrifying No. 2	5%	0%	30%	0%
Extended Air	0%	0%	5%	0%
A/O	50%	0%	95%	100%
Trickling Filter	100%	0%	100%	100%

[1] Diazinon dosing rates were different for the 3 phases

Auxiliary Process Enhancement Studies. Studies utilizing the continuous flow reactors from the treatability study were conducted to evaluate other operational procedures to enhance OP removal. Procedures considered were:

- Ferric chloride/polymer addition to primary clarifier influent
- Polymer addition to secondary clarifier influent
- Chlorination/dechlorination of secondary clarifier effluent
- Post aeration of secondary clarifier effluent

The results suggested the following:

What are the effects of ferric chloride and anionic polymer addition at the primary clarifier on diazinon removal? The data showed that moderate amounts of $FeCl_3$ (10-15 mg/l) in combination with moderate amounts of anionic polymer (0.25-0.50 mg/l) resulted in a minor decrease in diazinon concentrations. At a higher dosage of both chemicals (25 mg/l $FeCl_3$ plus 1.0 mg/l anionic polymer), no decrease in diazinon concentration was observed (Table 9).

Table 9
Ferric Chloride and Anionic Polymer Addition to Primary Clarifier Influent

$FeCl^3$/ Anionic Polymer	Diazinon Concentration, µg/l	% Diazinon Removal	Turbidity, NTUs	% Reduction of Turbidity
Control	974		8.9	
Trial #1 (10 mg/l/0.25 mg/l)	862	115	8.7	2.2
Trial #2 (15 mg/l/0.50 mg/l)	836	14.2	9.1	2.2
Trial #3 (25 mg/l/1.00 mg/l)	998	2.5	9.4	5.6

What are the effects of polymer addition at the final clarifier on diazinon removal? The cationic polymer used in the study did not appear to reduce the amount of diazinon in the secondary clarifier influent. However, an increase in toxicity was observed, most likely due to the polymer itself (Table 10).

Table 10
Polymer Addition to Secondary Clarifier Influent

Sample ID	Turbidity	Diazinon, µg/l	Chronic Toxicity IC25 Survival	Chronic Toxicity IC25 Reproduction
Control	5.1	NA	NA	NA
Trial #1 (0.50 mg/l polymer)	4.7	NA	NA	NA
Trial #2 (1.50 mg/l polymer)	5.0	NA	NA	NA
Trial #3 (5.00 mg/l polymer)	8.4	NA	NA	NA
Control #2	5.8	381	66.3	83.3
Trial #4 (3.00 mg/l polymer)	7.4	418	48.4	65.9

Note: NA = the sample was not analyzed.

What are the effects of effluent chlorination/ dechlorination on diazinon removal and effluent toxicity? Chlorination was effective in the removal of diazinon from the secondary clarifier effluent. At the lowest chlorine dosage, approximately 50% of the diazinon originally present was removed. However, the toxicity of the effluent was not changed.

What are two effects of effluent post aeration on diazinon removal and effluent toxicity? Post aeration resulted in removal of nearly 10% of the diazinon. However, the effluent toxicity did not change.

IV. What is the Fate of Diazinon in a POTW?

The continuous flow bench-scale study revealed that their may be a link between diazinon removal and Sludge Retention Time and/or Hydraulic Residence Time. A partitioning study was conducted to determine if and to what extent diazinon partitioned to influent wastewater solids, activated sludge mixed liquor solids, and biomonitoring sample container types.

Do diazinon and chlorpyrifos adsorb to solids in the primary influent or to activated sludge mixed liquor suspended solids? Diazinon and chlorpyrifos both strongly adsorb to primary influent solids. Data show that nearly 65% of the diazinon partitions immediately increasing to nearly 75% over 7 days (10,000 minutes). Adsorption is greater for the 30 day SRT than for the 15 day SRT sludge (Figure 15).

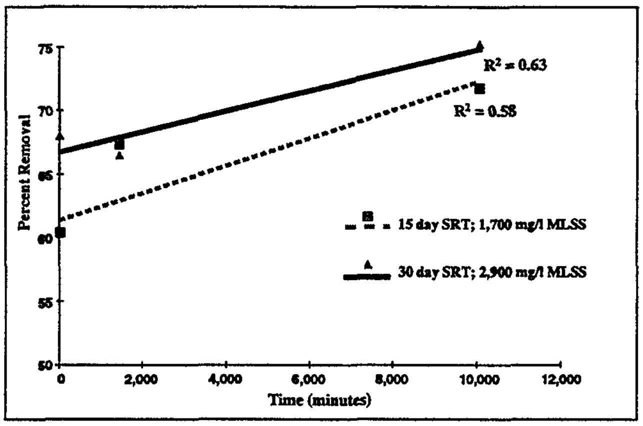

Figure 15
Diazinon Partitioning to Primary Influent Solids Over Time

Was adsorption the primary removal mechanism in reactors that exhibited diazinon removal? A major pathway for diazinon and/or chlorpyrifos removal is through adsorption. This adsorption may be onto primary influent solids, mixed liquor or other substances that the wastewater comes in contact with. It is suspected, for biological treatment alone, the major removal mechanism is adsorption followed by biological degradation.

V. CONCLUSIONS

Review of the 14 TRE/TIEs, the conducting of annual monitoring of 6 POTW, bench-scale batch and continuous flow treatability, and partitioning studies has confirmed diazinon occurrence and its relationship to biomonitoring failures, determined the extent of diazinon occurrence and related toxicity, and determined the treatability of diazinon in POTW.

The overall findings of the work are presented below:

Review of 14 TRE/TIEs

1. *dubia* acute mortality occurred in some effluent bioassays from the 14 POTW under TRE/TIE order. In some cases, diazinon was present in these effluents at concentrations where it could be expected to be the sole toxicant. In other cases, however, the diazinon concentration in the bioassayed effluent was at or below the diazinon no observed effect level (NOEL). While *C. dubia* acute mortality was attributed to diazinon, it is apparent that toxicity was due to other unknown toxicants present in the effluent. Full characterization to determine other potential toxicants was not conducted; thus, the complete cause of toxicity cannot be conclusively determined.

2. The TIE *C. dubia* acute LC_{50} concentrations attributed to diazinon varied widely. Some TIE reported acute LC_{50} at concentrations 1/3 (0.12 µg/l) of the clean water LC_{50} (0.35 µg/l).

3. Five facilities reported other toxicants including ammonia, metals and 2,4,5-T.

Extent of Occurrence of Diazinon in POTW

4. Low end POTW TIE reported diazinon concentrations for acute and chronic toxicity do not agree with literature reported values. Toxicants such as ammonia were also identified as sources of toxicity.

5. There is no significant difference in the influent diazinon concentration and chlorpyrifos concentrations. In general, diazinon and chlorpyrifos concentrations were significantly higher than diaxozon and malathion.

6. The influent diazinon concentrations observed during the OP monitoring study was consistent with historical TRE data. This conclusion suggests that the data used in this study is consistent with past experiences at the POTWs.

7. Influent diazinon concentrations were not significantly higher than chlorpyrifos concentrations with the exception of Chickasaw WWTP. At Chickasaw WWTP the influent chlorpyrifos concentration was significantly higher than the diazinon concentrations. This would suggest that diazinon may not be the only OP compound affecting the biomonitoring results of these POTWs.

8. There were no significant differences between the influent diazinon concentrations during a storm event and those concentrations during the non-storm events. There were no significant differences between the influent diazinon concentrations between the day of the week.

9. There was a significantly higher influent diazinon concentration in the POTWs during the summer months. This may be related to the fire ant season which occurs in March through September with peaks in April and June.

10. There is little evidence suggesting that the six POTWs participating in this study had biomonitoring failures during 1994 attributable to OP compounds in the effluent. Only one of the six participating POTWs experienced biomonitoring failures; the corresponding diazinon concentrations for those samples were either nondetect or below 0.08 µg/l. All other OP compounds were nondetect.

11. The POTWs participating in the organophosphorus compound one year (1994) monitoring program were not significantly different from each other in terms of organophosphorus compound occurrence in the influent or effluent.

12. The POTWs which were most effective in removing diazinon, chlorpyrifos, and malathion during the OP compound monitoring program were also the ones which produced the most diazoxon. POTWs with fixed film sludge process had a significantly lower percent removal of diazinon and chlorpyrifos compared to the combined activated sludge/fixed film and strictly fixed film processes.

Treatability of Diazinon

13. Diazinon, at a concentration of 1.6 µg/l, was removed to below detection limits in 5-6 days in all reactors, regardless of hydraulic residence time, sludge retention time, or mixed liquor total suspended solids.

14. When diazinon levels were below 0.25 µg/l no toxic effects were measurable. When diazinon concentrations were above 0.40 µg/l toxic effects were seen. At diazinon levels between 0.25 and 0.40 µg/l increasing diazinon did not always result in increasing toxic responses.

15. For activated sludge systems data indicated a correlation between a longer solids retention time and increased diazinon removal and a link between a longer hydraulic residence time and reduced toxicity.

16. Effluent toxicity is potentially linked to SRT (and sometimes HRT). An increase in solids retention time resulted in a decrease in effluent toxicity. Increasing SRT to 15 or more days may result in reduced effluent toxicity.

17. PAC was effective in removing diazinon. Diazinon concentrations of 1.6 µg/l were reduced to below detection limits within 10 minutes to 4 hours in reactors where PAC was >1,000 µg/l. PAC dose was not optimized as a part of this study.

18. Additions of ferric chloride and an anionic polymer resulted in a minor decrease in diazinon concentrations of 13 percent. Cationic polymer addition did not reduce diazinon concentrations but did increase toxicity approximately 50% and chlorination removed the diazinon but had no impact on toxicity.

Fate of Diazinon

19. Partitioning onto wastewater solids accounted for 60 to 70 percent of diazinon and 100 percent of chlorpyrifos during laboratory tests.

20. A major pathway for diazinon and chlorpyrifos removal is through adsorption onto either primary influent solids, mixed liquor, or other substances in wastewater.

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