

# THE EFFECTS OF WATER CHLORINATION ON THREE SPECIFIC ORGANOPHOSPHATE (OP) PESTICIDES

## Executive Summary

Three organophosphate (OP) pesticides [phorate, disulfoton, terbufos] were evaluated for their potential to undergo oxidation to their respective oxidative products [oxons, sulfoxides, sulfoxide oxons, sulfones, and sulfone oxons] in laboratory water simulating the chlorination process in drinking water facilities over a 72 hour exposure period. Samples were collected after 0, 0.25, 4, 24, and 72 hours of chlorination and analyzed by both gas chromatography-mass selective detection (GC-MSD) and liquid chromatography-tandem mass spectrometry (LC/MS/MS) to determine the presence of the pesticides and their oxidative products.

The results of the Phase II Experiment confirm the results of the Phase I Experiment that these three OP pesticides [phorate, disulfoton, terbufos] are unstable and degraded in the buffered water over the 72 hour exposure period. The results of the Phase II Experiment also confirm the results of the Phase I Experiment that the three OP pesticides did not undergo oxidation to their oxons under the experiment conditions. However, two of the OP pesticides [phorate and disulfoton] underwent oxidation to other oxidation products [sulfone oxons] which was present at the 72 hour exposure period. The phorate sulfone oxon was detected at the 15 minute exposure period at trace concentrations just at the detection limit during the 72 hour exposure period. The disulfoton sulfone oxon was detected at the 15 minute exposure period in significant concentrations which increased at the 4 hour exposure period and then gradually decreased during the 72 hour exposure period. This oxidative product was present at the 24 and 72 hour sampling periods. For brief periods of time early during the experiment, phorate sulfoxide oxon, disulfoton sulfoxide oxon, and terbufos oxon were detected; however, they were not stable and degraded.

Re-examination of the gas chromatographic/mass spectrometric chromatograms (GC-MSD) of the Phase I Experiment reveals the presence of these same oxidative products at approximately the same exposure periods and further confirms the findings of the Phase II Experiment.

Table 1. Results for pesticides and oxidation products examined in study.

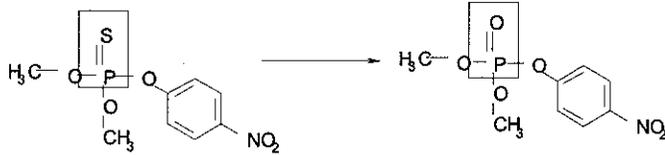
OP Pesticide	Stability in Water over 72 hours (no chlorination)	Oxon Formation after 24 hour (upon chlorination)	Oxon Stability in Water over 72 hours (upon chlorination)	Sulfoxide Oxon Formation after 24 hour (upon chlorination)	Sulfoxide Oxon Stability in Water over 72 hours (upon chlorination)	Sulfone Oxon Formation after 24 hour (upon chlorination)	Sulfone Oxon Stability in Water over 72 hours (upon chlorination)
Phorate	Poor	None	-	None	-	Yes	Yes
Disulfoton	Poor	None	-	None	-	Yes	Yes
Terbufos	Poor	None	-	None	-	None	-

In accordance with the Quality Assurance Project Plan (QAPP) for this phase of the study, there were two elements necessary for the strict qualitative interpretation whether the three OP pesticides underwent oxidative desulfonation during a 72 hour contact time in chlorinated laboratory water. This conclusion would be reached if the oxidative products are detected at any quantifiable level in either replication in the chlorinated laboratory water treatments at any sampling time and the OP pesticides are stable in non-chlorinated laboratory water. None of the three pesticides met those criteria.

## 1.0 Introduction

The application of pesticides in arable lands has resulted in the contamination of natural waters such as surface water and groundwater. The initial contamination at the application sites has spread via surface runoff to nearby lakes, rivers, and streams and through subsurface transport to aquifers. The contaminated surface waters and ground waters are eventually used as source or raw waters in some community drinking water systems. After subjecting the raw water to different treatment processes in the water purification facilities, the concentrations of the pesticides may change or remain essentially the same in the treated or final drinking water. Studies conducted by scientists at EPA's ORD in Cincinnati (Miltner et al, 1989) indicate that conventional treatment (coagulation/clarification, filtration, softening, recarbonation, and chlorination) are generally not effective in removing certain pesticides from raw water. However, other pesticides are unstable in the presence of chemical disinfectant such as chlorine. Previous studies in Japan (Magara et al, 1994) and United States (Tierney et al, 2001; Duirk and Collette, 2006) indicate that certain organophosphate pesticides can be transformed to their oxons during chemical disinfection by chlorine compounds. This chemical transformation process is shown in Figure 1.

### Figure 1: Oxidative Desulfonation Reaction of an Organophosphate Pesticide in Chlorinated Water



This transformation is a concern because chlorination is the most commonly used disinfection technique in many US drinking water treatment plants and the product oxons are generally considered to be more toxic than the parent compounds.

The Food Quality Protection Act of 1996 (FQPA) requires that all chemical pesticide residues in or on food be examined for any possible adverse health effects through exposure. Drinking water is one of the pathways for dietary exposure. Five organophosphate pesticides (diazinon, chlorpyrifos, methidathion, methyl parathion, and malathion) have been examined and have been found to transform during chlorination into their associated oxons. Three specific organophosphate pesticides [phorate, disulfoton, and terbufos] have other known oxidative products [sulfoxides, sulfoxide oxons, sulfones, and sulfone oxons] for which there is little or no data on their potential for oxidative transformation during these conditions. Consequently, data and additional information are needed on the probable oxidation of these organophosphate pesticides and the relative stability of oxidative products in chlorinated water. The three organophosphate pesticides and their degradation products considered in this study are listed in Table 1.

Table 1: Three Selected Organophosphate Pesticides from the Cumulative OP Assessment without Water Treatment Data on Chlorination Effects on Oxidation Product Formation	
OP Parent	OP Degradation Products
Phorate	phorate oxon phorate sulfoxide phorate sulfone phorate sulfoxide oxon phorate sulfone oxon
Disulfoton	disulfoton oxon disulfoton sulfoxide disulfoton sulfone disulfoton sulfoxide oxon disulfoton sulfone oxon
Terbufos	terbufos oxon terbufos sulfoxide terbufos sulfone terbufos sulfoxide oxon terbufos sulfone oxon

The objective of this study is to provide a qualitative screening level assessment on the potential for oxidation product formations in chlorinated laboratory water and the stability of the selected organophosphate pesticides in both un-chlorinated and chlorinated water and the stability of their respective oxidation products in the chlorinated laboratory water. There were approximately twenty organophosphate pesticides considered in the cumulative OP risk assessment. The three selected pesticides being tested in this study consist of the pesticides, which are capable of forming multiple oxidation products, have outdoor use patterns, and have no chlorination water treatment data available. These data will be used in the revised cumulative OP risk assessment to characterize the potential for human exposure to oxons in treated water.

## 2.0 Project Description

The project description is listed in the study protocol in Appendix 1. A brief summary description follows:

For each of the three OP pesticides to be tested, the experimental design will consist of:

- One replicate OP control [test water + OP pesticide, without chlorine]
- One replicate chlorine control [test water + chlorine]
- Two replicates of treatment [OP pesticide + test water + chlorine]
- One buffered water sample spiked with the three pesticides and fifteen oxidation products at a concentration of  $\frac{1}{2}$  of the spiking concentration (50 ppb) at each sampling time.

Chlorination experiments will be conducted in Fisher Environmental Grade reagent water to eliminate chlorine demand considerations. Similar testing conditions using laboratory waters are recommended as screening level testing for CCL water treatment studies and pesticide treatment studies at ORD. The chlorine dose in the laboratory water will be equivalent to the recommended maximum disinfectant residual (RMDL) of 4 mg/L free chlorine concentration  $\pm$  10%. The pH of the Fisher reagent water will be adjusted to pH 8 to represent typical water treatment conditions. The experiment will be conducted for 72 hours with sampling times immediately prior to chlorination (~2 minutes before pesticide dosing), 0.25 hour, 4 hours, 24 hours, and 72 hours post chlorination. The 24 and 72 hour sampling times were selected to represent the treatment system water residence and/or distribution transport times of approximately 24 hr or longer. The pesticide concentration in the experiment will be 100 ppb or below the solubility limit of the pesticide whichever is lower. The experiments will be conducted using a mixture of the OP pesticides delivered to the system with low co-solvent concentrations or in the absence of co-solvents. The chlorine demand from co-solvents and degradation processes will be determined by measuring free chlorine at each sampling interval.

## 3.0 Method and Materials

Fisher Scientific Certified Environmental Grade water will be used as the test water. Water quality parameters of the test water are:

Test	Value	Unit
Color	< 5	APHA

Residue after Evaporation	< 1	ppm
Fluorescence (as quinine)	< 100	ppt
Resistivity	> 18	MΩ
Total Organic Carbon	< 20	ppb

Water samples must be labeled clearly, and should include date, time, and name of the preparer(s). To preserve the integrity of the data, all samples must be stored at ~ 4°C until extraction to minimize the physicochemical changes in the samples. If sample extraction into a solvent is necessary, extracts must be stored below 0°C and also analyzed as soon as possible. All samples used and generated during the study should be properly disposed of.

Quality assurance samples shall consist of:

- 1) reagent water blank – analysis of reagent water (one time only);
- 2) method blank – analysis of buffered reagent water plus chlorine; [Carboy C, Table 1]; (time 0.25 hr);
- 3) non-chlorinated degradation check – analysis of buffered reagent water plus OPs [Carboy B, Table 2]; (time 0, 0.25, 4, 24, and 72 hrs);
- 4) matrix water blank – analysis of buffered reagent water (time 0);
- 5) matrix water spike – analysis of buffered reagent water plus 50 ppb of the OP parent(s) plus 50 ppb oxon(s) (one spike per analytical sample set);

These measures are classified as critical measurements and should be prepared and analyzed with each group of samples to monitor laboratory contamination and method performance. Addition of surrogate compounds to environmental samples is also recommended to measure the efficiency of the method. The surrogate compounds should not be normally found in the environment and should be selected such that the interference with elution of target analytes and the effect from sample matrix are minimal.

### **Analytical Procedures**

The analytical procedures used should be able to accurately identify and measure the presence of the target analytes in the samples. Identification and quantitation of residues will be by gas chromatography-mass selective detection (GC/MSD) and/or liquid chromatography/tandem mass spectrometry (LC/MS/MS) techniques.

A calibration curve shall be constructed with mixtures(s) of pure standards (target analytes) with the spiking level and method detection limit as the bounding concentrations. Complete initial calibration curves should be prepared monthly, and the individual calibration standards verified each day of operation.

In some cases, the analytical procedures may not be completely developed to allow for complete quantification of the parent OP and its degradation products. Nevertheless, the analytical method should be capable of providing clear separation of known pesticide residues on chromatograms to allow for residue identification.

### **Test Protocol**

These studies will be conducted at the OPP/BEAD/ACB Fort Meade and OPP/BEAD/ECB Stennis Space Center laboratories. A complete description of testing protocol can be found in the Appendix 1. Final studies will not be conducted for compounds unless the analytical method has been shown to be capable of detecting of the parent compounds and their oxons in chlorinated water during the preliminary testing stage.

The control treatments will be used to assess whether the OP pesticide undergoes oxidation in non-chlorinated laboratory water and to assess whether OP pesticide or its degradation products are in the chlorinated water without pesticide dosing. Because the experimental design has minimal replication and the analytical methods are not fully vetted for all the OP pesticides and their oxon degradation products, there will be **strict qualitative interpretation** (i.e. presence or absence of oxon) on whether OP pesticides [phorate, disulfoton, and terbufos] undergo oxidative desulfonation during a 72 hour contact time in chlorinated laboratory water. This deduction will be reached if their oxidation products [sulfoxides and sulfones and their associated sulfoxide and sulfone oxons] are detected at any quantifiable level in either replication in the chlorinated laboratory water treatments at any sampling time and the OP pesticide is stable in non-chlorinated laboratory water. Additionally, the detection of oxidation products in chlorinated water at the 24 hour or 72 hour sampling times will suggest the oxon is stable enough in chlorinated water to have the potential for dietary exposure through drinking water

### **Assessment and Oversight**

A QA/QC laboratory audit will be performed at the conclusion of the water chlorination studies with OP pesticides and their sulfone, sulfoxide, sulfone oxon, and sulfoxide oxon.

## **4.0 Results**

### **(A) The Formation of Oxidation Products from Three Organophosphate Pesticides in Water**

Three organophosphate (OP) pesticides [phorate, disulfoton, terbufos] were evaluated for their potential to undergo oxidation to their respective oxidation products in laboratory water simulating the chlorination process in drinking water facilities. In these studies, the OP pesticides were dissolved into pH 8.0 buffered water and then chlorinated with a sodium hypochlorite solution. Over a 72 hour exposure period, water samples were collected, extracted whenever applicable, and analyzed by both gas chromatography-mass selective detection (GC-MSD) and liquid chromatography-tandem mass spectrometry (LC/MS/MS) to determine the presence of the pesticides and their oxons. The results are presented in Appendix 2 for both the GS-MSD and LC/MS/MS studies.

The results of both studies (GC-MSD & LC/MS/MS) showed that the three OP pesticides (phorate, disulfoton, and terbufos) did not undergo oxidation into their oxons under the experiment conditions. Two of the twelve remaining oxidation products [phorate sulfone oxon and disulfoton sulfone oxon] formed stable compounds over the 72 exposure period. The

phorate sulfone oxon was present at trace concentration at the minimum detection limit. For brief periods of time, phorate sulfoxide oxon, disulfoton sulfoxide oxon, and terbufos sulfoxide oxon were detected; however, they were unstable and degraded after the 4 hour exposure time.

A re-examination of the full scan of the gas chromatographic/mass spectrometric (GC-MSD) chromatograms from the Phase I Experiment confirmed these findings.

The analytical methods of GC-MSD and LC/MS/MS were complimentary to each other in the detection of the three OP pesticide parents and the majority of their oxidation products. The current GC/MSD conditions were not suitable for the detection of phorate sulfoxide, phorate sulfoxide oxon, terbufos sulfoxide, and terbufos sulfoxide oxon.

### **(B) The Stability of the Three Organophosphate (OP) Pesticides in Water**

The three organophosphate (OP) pesticides [phorate, disulfoton, terbufos] were evaluated in buffered laboratory water to act as a control to the separate studies of the pesticides in the buffered water during the chlorination process. In these studies the OP pesticides were dissolved into a pH 8.0 buffered water. Over a 72 hour exposure period, water samples were collected, extracted, and analyzed by both gas chromatography-mass selective detection (GC-MSD) and liquid chromatography-tandem mass spectrometry (LC/MS/MS) to determine the presence of the pesticides and their oxons without chlorination. The results are presented in Appendix 2 for both the GS-MSD and LC/MS/MS studies.

The results demonstrated and confirm that the three OP pesticides [phorate, disulfoton, and terbufos] are unstable and degrade in the buffered water without chlorination over the 72 hour exposure period. Trace concentrations of disulfoton sulfoxide were detected at the minimum detection limit.

A re-examination of the full scan of the gas chromatographic/mass spectrometric (GC-MSD) chromatograms from the Phase I Experiment confirmed these findings.

### **(C) The Stability of Free Chlorine Concentrations in Water**

The concentration of chlorine as free chlorine was evaluated in buffered laboratory water to act as a control to the separate studies of the pesticides in the buffered water during the chlorination process. In these studies chlorine as free chlorine was added to a pH 8.0 buffered water. Over a 72 hour exposure period, water samples were collected and analyzed to determine the stable concentration of this form of chlorine. In both studies the concentration of free chlorine remained stable within 25% of the initial concentration and neither the OP pesticides nor their oxidation products were detected at any time during the 72 hour exposure period.

### **(D) The Stability of the Three Organophosphate Pesticides and Their Oxidation Products as Laboratory Control Spike Samples**

Three organophosphate (OP) pesticides [phorate, disulfoton, terbufos] and their fifteen available

oxidation products were spiked into pH 8.0 buffered laboratory water to act as laboratory control spike samples. These samples were used to assess the detection of these compounds at the time of analysis. The water samples were collected, spiked, extracted whenever applicable, and analyzed by both gas chromatography-mass selective detection (GC-MSD) and liquid chromatography-tandem mass spectrometry (LC/MS/MS) to determine the concentration of the pesticides and their oxidation products. The results are presented in Appendix 2 for both the GS-MSD and LC/MS/MS studies.

The results demonstrated that these pesticides were unstable and degrade in the buffered water if they were allowed to remain for any prolonged period prior to extraction and/or analysis. In the LC/MS/MS studies the laboratory control spike samples remained in the buffered water until analyzed. That time period could be as much as 4 hours. This resulted in varying degrees of degradation of the pesticides. In the GC-MSD studies the laboratory control spike samples were extracted immediately after equilibration, ensuring minimum degradation.

## 5.0 Summary

There were two elements necessary to the **strict qualitative interpretation** whether these three OP pesticides underwent oxidative desulfonation during a 72 hour contact time in chlorinated laboratory water. This conclusion could be reached if:

- 1) The oxidation products of the three OP pesticides are detected at any quantifiable level in either replication in the chlorinated laboratory water treatments at any sampling time.
  - **There were five quantifiable oxidation products detected in the chlorinated laboratory water during the seventy two hour exposure period [phorate sulfoxide oxon, phorate sulfone oxon, disulfoton sulfoxide oxon, disulfoton sulfone oxon, and terbufos sulfoxide oxon].**
  - **There was mass spectral evidence from the previous study in Phase I to confirm the possible formation of these oxidation products.**

and

- 2) The OP pesticides are stable in non-chlorinated laboratory water.
  - **All three OP pesticides were unstable and degraded in the unchlorinated laboratory water.**
- 3) Additionally, there were detection of oxidation products in chlorinated water at the 24 hour or 72 hour sampling times would suggest the oxon is stable enough in chlorinated water to have the potential for dietary exposure through drinking water.
  - **Two of the oxidation products [phorate sulfone oxon and disulfoton sulfone oxon] were stable at both the 24 hour and 72 hour sampling times.**

**None of the three OP pesticides meet the criteria as established in the QAPP to conclude that they underwent oxidative desulfonation.**

## References

- Duirk, S. Collette, T. 2006. Degradation of Chlorpyrifos in Aqueous Solutions: Pathways, Kinetics, and Modeling. *Environ. Sci. Technol.* 40: 546-551.
- Magara, Y. et al. 1994. Degradation of pesticides by chlorination during water purification. *Water Sci. Technol.* 30(7): 119-128.
- Miltner, R.J., D.B. Baker, T.F. Speth, and C.A. Fronk. 1989. Treatment of seasonal Pesticides in Surface waters. *Jour. Amer. Water Works Assoc.* 81: 43-52.
- Tierney, D.P. et al., 2001. Chlorine degradation of six organophosphorus insecticides and four oxons in a drinking water matrix. Syngenta Crop Protection Center, Greensboro, NC.
- USEPA, Laboratory Study on the Effects of Chlorinated Water on OP Pesticides, Quality Assurance Project Plan, OPP/EFED/WTEWG, April 24, 2006.
- USEPA, Laboratory Study on the Effects of Chlorinated Water on OP Pesticides, Final Report, OPP/EFED/WTEWG, May 15, 2006.

## Appendix 1

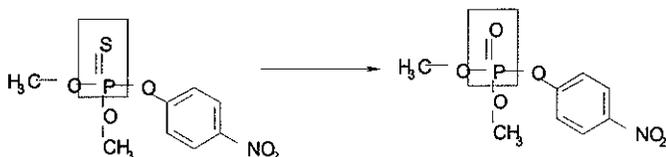
**Procedures for the Preliminary Laboratory Study on  
the  
Effects of Chlorinated Water on OP Pesticides,  
Phase II**

*June 8, 2006*

Water Treatment Effects Work Group  
Environmental Fate and Effects Division  
U.S. EPA Office of Pesticide Programs

(A) **Introduction:** Previous studies in Japan (Magara et al, 1994) and United States (Tierney et al, 2001) indicate that certain organophosphate pesticides can be transformed during disinfection by chlorine compounds to oxons. This chemical transformation process is shown in Figure 1.

**Figure 1: Oxidative Desulfonation Reaction of an Organophosphate Pesticide in Chlorinated Water**



This transformation is a concern because chlorination is widely used in many drinking water treatment plants and the product oxons are generally considered to be more toxic than the parent compounds. Consequently, data and additional information are needed on the probable oxidation of selected organophosphate pesticides and the relative stability of oxons in chlorinated water. In the Laboratory Study on the Effects of Chlorinated Water on OP Pesticides, Phase I, ten organophosphate (OP) pesticides were examined and it was determined that five OP pesticides [methidathion, bensulide, chlorethoxyfos, methyl parathion, and phostebupirim] formed stable oxons in chlorinated water. However, three of those pesticides [phorate, disulfoton, and terbufos] have additional oxidation products [sulfoxides, sulfoxide oxons, sulfones, and sulfone oxons] that can be formed. The organophosphate pesticides and their oxidation products considered in this testing protocol are listed in Table 1.

<b>Table 1: Selected Organophosphate Pesticides from the Cumulative OP Assessment without Water Treatment Data on Chlorination Effects on Oxon Formation</b>	
<b>OP Parent</b>	<b>OP Degradation Products</b>
Phorate	phorate oxon phorate sulfoxide phorate sulfone phorate sulfoxide oxon phorate sulfone oxon
Disulfoton	disulfoton oxon disulfoton sulfoxide disulfoton sulfone disulfoton sulfoxide oxon disulfoton sulfone oxon

Terbufos	terbufos oxon terbufos sulfoxide terbufos sulfone terbufos sulfoxide oxon terbufos sulfone oxon
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Chlorination experiments will be conducted in Fisher certified environmental grade test water. Although the experiments will be conducted in environmental grade water, water pH (pH=8) will be altered to represent water treatment plant conditions. The chlorine dose in the laboratory water will be equivalent to the recommended maximum disinfectant residual (RMDL) of 4 mg/L free chlorine. Because the laboratory water will have extremely low chlorine demand, the free chlorine concentration and total chlorine concentration should be similar. The pH of the laboratory water will be adjusted to pH 8 to represent typical water treatment conditions. The experiment will be conducted for 72 hours with sampling times immediately prior to chlorination and 0.25 hour, 4 hours, 24 hours, and 72 hours post chlorination. The 24 and 72 hour sampling times were selected to represent the treatment system water residence and/or distribution transport times of approximately 24 hr or longer. The pesticide concentration in the experiment will be 100 µg/L or below the solubility limit of the pesticide whichever is lower. The experiments will be conducted using a mixture of the OP pesticides. The experiments will be conducted with low co-solvent concentrations or in the absence of co-solvents. The chlorine demand from co-solvents and degradation processes will be determined by measuring free chlorine at each sampling interval.

The experimental plan will consist of a series of preliminary studies and final studies. These studies will be conducted by EPA personnel at the Biological and Economic Analysis Division Fort Meade Analytical Laboratory and Stennis Space Center Environmental Chemistry Laboratory. The chlorination study protocol and QAPP will be reviewed by Richard Miltner, P.E. from the ORD/NRMRL/Water Supply and Water Resources Division/ Treatment Technology Evaluation Branch.

Final chlorination studies for selected OP pesticides will be conducted once analytical methods are developed with reliable identification of the OP pesticide and their oxon degradation products in chlorinated test water. These studies will be conducted using a factorial experimental design [5 sampling times x 2 replicates pesticide(s), chlorination treatments x 1 pesticide(s), non-chlorinated water treatment (control) + 1 chlorinated water (control) + 1-3 buffered water spiked with a intermediate level of parent(s) and oxon(s)].

**(B) Objectives:** The objective is to qualitatively determine oxon formation and stability in chlorinated, laboratory water for selected OP pesticides. These data will be used in the revised cumulative OP risk assessment to characterize the potential for human exposure to oxons in treated water.

**(C) Glassware, Pipets, and other containers:** Glassware, pipettes, and other devices used in the study should be chlorine-demand free. Soak dark or amber incubation bottles in detergent (Fisher FL-70, 4%, Fair Lawn, NJ or comparable) overnight, rinse four times with hot tap water, and

then two times with distilled and deionized water. Place in 10 - 20 mg/L chlorine solution for 24 hr. After rinsing four times with distilled and deionized water and one to two times with laboratory clean water, dry in 140<sup>0</sup> C oven overnight. Clean pipettes may need to be stored in ~ 50 mg/L Cl<sub>2</sub> solution and rinsed three times with dosing solution before use. Store in same chlorine solution after use.

**(D) Materials:** The following solutions will be prepared for this study:

- (1) pH 6.7 borate buffer: 1.0 M boric acid [ACS grade] and 0.11 M NaOH (ACS grade) prepared in boiled laboratory reagent water;
- (2) pH 8 borate buffer: 1.0 M boric acid (ACS grade) and 0.26 M NaOH (ACS grade) prepared in boiled laboratory reagent water;
- (3) Chlorine solution (1000 - 3000 mg/L Cl<sub>2</sub>): Dilute reagent-grade stock solution of sodium hypochlorite (5 - 13%) with laboratory reagent water. Check the exact concentration using Standard Methods (1998) or a commercial chlorine measurement kit that can detect down to 0.1 mg/L Cl<sub>2</sub>.
- (4) pH 8 hypochlorite-buffer solution: Add about 4 - 5 volume of chlorine solution (~ pH 11) to one volume of pH 6.7 borate buffer. The resulting solution gives a pH 8. About a 20% decrease in chlorine strength is expected. About 2.5 mL of this combined dosing hypochlorite-buffer solution can be added to a 1-L test water (<0.5% water sample volume change)

**(E) Test Waters:** Fisher Environmental Grade water will be used in the water chlorination studies. Laboratory reagent water will be used for cleaning and reagent preparation.

**(F) Chlorine Residuals Measurement:** Free chlorine residuals will be measured using a Hach pocket colorimeter analysis system and Hach Methods 8021 for free chlorine in water. This DPD method is equivalent to USEPA Method 330.5 for wastewater. It can measure free chlorine at reasonable detection limits (at least 0.1 mg/L free chlorine).

**(G) Preliminary and Final Study:** Preliminary studies with one replication will be conducted to provide sufficient experience in measuring analytes in chlorinated water as well as an exercise in sequencing/timing the laboratory operations for the chlorination experiments. Once the preliminary studies have been conducted, final water chlorination studies will be done using two replicates for the test water. Appropriate OP pesticide and chlorine residual controls will be prepared and monitored during the chlorination tests.

**(H) Chlorine Dosing Study:** Before the chlorination experiments are started, the chlorine demand of the test waters has to be established to determine the dose of chlorine solution that provides the target  $4.0 \pm 0.4$  mg/L free chlorine residual. Chlorine demand of the Fisher environmental grade water will be determined. Chlorine demand is operationally defined as chlorine dose (applied free chlorine) - free remaining chlorine residual under a specified contact or incubation period, pH and temperature. For the preliminary study, only one replicate is desirable. The unchlorinated Fisher Environmental Grade water can be used for this purpose, but it must include appropriate concentrations of co-solvents that will be used to introduce OP pesticides into solution as well as similar reaction vessels used in the experiment.

- (1) Add 2 ml pH 8 borate buffer to 1 L (or proportional volumes) of unchlorinated Fisher Environmental Grade water.
- (2) Check the pH. If necessary, adjust to pH 8 with dilute H<sub>2</sub>SO<sub>4</sub> or dilute NaOH.
- (3) Fill each incubation bottle (300 - 500 ml) three quarters full with the unchlorinated Fisher Environmental Grade water. Two bottles will be needed. Addition of co-solvent, in the appropriate concentration as would be employed in (I) below, may be necessary to mimic co-solvent additions through pesticide dosing procedures. The doses should be set up in duplicate to determine if the initial dosing at 4 mg/L will result in a > 1 mg/L free chlorine residual after 24 hours in the Fisher Environmental Grade water containing the co-solvents. Initial dose of 4.0 mg/L free chlorine is appropriate.
- (4) Add pH 8 hypochlorite-buffer solution through a pipette held just above water surface. Dose the appropriate volume of hydrochlorite-buffer solution to give the required dose in full bottles.
- (5) Cap the bottle and invert twice.
- (6) Fill to top of bottle with pH 8 borate buffered unchlorinated Fisher Environmental

Grade water and cap head space-free.

- (7) Invert 10 times
- (8) Incubate for 24 hr in the dark at room temperature.
- (9) After incubation, measure the free chlorine residual, pH, and temperature. (Note: Addition of hypochlorite-buffer solution should be sequenced and timed to provide allowance for measurement of free chlorine residual and pH for each test water)
- (10) The initial chlorine dose that yields an initial free chlorine residual of  $4.0 \pm 0.4$  mg/L Cl<sub>2</sub> and a  $> 1.0 \pm 0.4$  mg/L at 24 hours will be selected and used in the chlorination and product stability assessment discussed in (I).

**(I) Chlorination and Product (Oxon) Stability Experiments:** The study will be conducted in 4L low density polyethylene reaction vessels that can be covered with black plastic to simulate dark condition. For this final study, the chlorination experiment at pH=8 should be done in duplicate, along with one replicate OP control [test water + OP pesticides, without chlorine], one replicate chlorine control [test water + chlorine], and one buffered water control [test water for spiking with immediate concentrations of OPs and oxons] indicated as A1, A2, B, C, and D solutions in Table 2, respectively.

**For Treatment A:**

- (1) Put 2L of unchlorinated Fisher Environmental Grade water and add 4 ml of pH 8 borate buffer in a dark, 4L polyethylene reaction vessel. This will require five 4L vessels.
- (2) Measure pH and adjust, if necessary, to pH 8 with dilute H<sub>2</sub>SO<sub>4</sub> or dilute NaOH.
- (3) Dose with OP pesticide(s) to achieve a concentration of 100 µg/L or below the water solubility limit, whichever is lower.

- (4) Collect the unchlorinated, pesticide spiked OP sample.
- (5) Add pH 8 hypochlorite-buffer solution to give an initial free chlorine residual of  $4.0 \pm 0.4$  mg/L  $\text{Cl}_2$  and a subsequent free chlorine residual of  $> 1.0 \pm 0.4$  mg/L at 24 hours. Dose the appropriate volume of hypochlorite-buffer solution to give the required dose in the 2L sample. The time of chlorination is  $T = 0$ .
- (6) Prior to taking water samples, stir solution with the aid of magnetic stirring bar for two minutes.
- (7) Take samples at the time intervals for analysis summarized in Table 2:  
OP pesticide – 0 (prechlorination), 0.25 hr, 4 hr, 24 hr, 72 hr  
Oxidation products (oxon, sulfoxide, sulfone, sulfone oxon, sulfoxide oxon) – 0 (prechlorination), 0.25 hr, 4hr, 24 hr, 72 hr
- (8) The samples are immediately withdrawn from the reaction vessel and then quenched stoichiometrically with sodium thiosulfate (with slight excess) based on the free chlorine residual [1.25 mg per 100 ml aliquot]. The samples should be extracted and the extract stored in the dark at 0-4°C, if they cannot be analyzed right away.
- (9) Separate samples will be taken to measure the free chlorine residual, pH, and temperature.
- (10) Analyze the quenched samples for the parent compound, primary product (oxon) by appropriate analytical method (GC/MS or LC/MS/MS). Other transformation products will be identified, when possible, and described as tentatively identified compounds.

#### **For Treatment B:**

- (1) Put 2L of unchlorinated Fisher Environmental Grade water and add 4 ml of pH 8 borate buffer in a dark, 4L polyethylene reaction vessel.
- (2) Measure pH and adjust, if necessary, to pH 8 with dilute  $\text{H}_2\text{SO}_4$  or dilute NaOH. Dose with OP pesticide(s) to achieve a concentration of 100  $\mu\text{g/L}$  or below the water solubility limit, whichever is lower.
- (3) At approximately the same time as the collection of the chlorinated samples in Treatment A, collect the unchlorinated, pesticide spiked OP samples at 0, 0.25, 4, 24 and 72 hours. The samples should be extracted and the extract stored in the dark at 0-4°C, if they cannot be analyzed right away.
- (4) Separate samples will be taken to measure the pH and temperature.
- (5) Analyze the samples for the parent compound, primary product (oxon) by appropriate analytical method (GC/MS or LC/MS/MS). Other transformation products will be identified, when possible, and described as tentatively identified compounds.

#### **For Treatment C:**

- (1) Put 2L of unchlorinated Fisher Environmental Grade water and add 4 ml of pH 8 borate buffer in a dark, 5L polyethylene reaction vessel.
- (2) Measure pH and adjust, if necessary, to pH 8 with dilute  $\text{H}_2\text{SO}_4$  or dilute NaOH.
- (3) Add pH 8 hypochlorite-buffer solution to give an initial free chlorine residual of  $4.0 \pm 0.4$  mg/L  $\text{Cl}_2$  and a subsequent free chlorine residual of  $> 1.0 \pm 0.4$  mg/L at 24 hours. Dose the appropriate volume of hypochlorite-buffer solution to give the required dose in

the 2L sample.

(4) Prior to taking water samples, stir solution with the aid of magnetic stirring bar for two minutes.

(5) Collect a sample after about 0.25 hour for OP pesticides and for oxons.

(6) The sample is withdrawn from the reaction vessel and then quenched with the selected reducing agent (with slight excess) based on the free chlorine residual [1.25 mg per 100 ml aliquot]. The aliquots should be extracted and the extract stored in the dark at 0-4° C, if they cannot be analyzed right away.

(7) A separate sample will be taken to measure the free chlorine residual, pH, and temperature at 0.25 hour.

(8) Analyze the sample for the parent compound, primary product (oxon) by appropriate analytical method (GC/MS or LC/MS/MS). Other transformation products will be identified, when possible, and described as tentatively identified compounds.

**For Treatment D:**

(1) Put 2L of unchlorinated Fisher Environmental Grade water and add 4 ml of pH 8 borate buffer in a dark, 5L polyethylene reaction vessel.

(2) Measure pH and adjust, if necessary, to pH 8 with dilute H<sub>2</sub>SO<sub>4</sub> or dilute NaOH.

(3) Collect 100 ml samples of the unchlorinated, buffered water at each sampling interval of 0, 0.25, 4, 24, and 72 hours.

(4) These samples will be spiked with the OP pesticide(s) and oxon(s) at a spiking level of 50 ppb, as necessary.

(6) The samples should be extracted immediately and the extract stored in the dark at 0-4° C, if they cannot be analyzed right away.

(7) A separate sample is taken to measure the pH and temperature.

(8) Analyze the samples for the parent compound, primary product (oxon) by appropriate analytical method (GC/MS or LC/MS/MS). Other transformation products will be identified, when possible, and described as tentatively identified compounds.

**Table 2. Proposed Sampling and Analysis Regime**

Treatment Condition (Treated Water Samples and Controls: OP pesticide)	Sampling Times				
	Prechlorination	Postchlorination			
	0	0.25 hr	4 hrs	24 hrs	72 hrs
A1 OP Cl <sub>2</sub> H <sub>2</sub> O	A2 OP Cl <sub>2</sub> H <sub>2</sub> O	OP	OP	OP	OP
	Oxidation Products <sup>1</sup>	Oxidation Products <sup>1</sup>	Oxidation Products <sup>1</sup>	Oxidation Products <sup>1</sup>	Oxidation Products <sup>1</sup>
		Cl	Cl	Cl	Cl

<b>B</b> OP H <sub>2</sub> O	OP	OP	OP	OP	OP
	Oxidation Products <sup>1</sup>				
<b>C</b> Cl <sub>2</sub> H <sub>2</sub> O		OP			
		Oxidation Products <sup>1</sup>			
		Cl			
<b>D</b> H <sub>2</sub> O	Spiked OP				
	Spiked Oxon				

1- Oxon, sulfone, sulfoxide, sulfone oxon, and sulfoxide oxon.

**(J) Data Reduction and Reporting:** Report detections of parent OP and its degradation products. Calculate concentrations, when possible, of OP pesticides and their stability products. Report identities and structural formulas of transformation products.

**(K) Interpretation of Results:** The interpretation of study results will be dependent on the detection of oxon degradation products in the chlorinated test water treatments. The control treatments will be used to assess whether the OP pesticide undergoes oxidation in non-chlorinated test water and to assess whether OP pesticide or its degradation products are in the chlorinated water without pesticide dosing. Because the experimental design has minimal replication and the analytical methods are not fully vetted for all the OP pesticides and their oxon degradation products, there will be **strict qualitative interpretation** on whether OP pesticides undergo oxidative desulfonation during a 72 hour contact time in chlorinated laboratory water. This deduction will be reached if oxons are detected in either replication in the chlorinated laboratory water treatments at any sampling time and the OP pesticide is stable in non-chlorinated laboratory water. Additionally, the detection of oxons in chlorinated water at the 24 hour or 72 hour sampling times will suggest the oxon is stable enough in chlorinated water to have the potential for dietary exposure through drinking water.

## References

Magara, Y. et al., 1994. Degradation of pesticides by chlorination during water purification. *Water Sci. Technol.* 30(7): 119-128.

Tierney, D.P. et al., 2001. Chlorine degradation of six organophosphorus insecticides and four oxons in a drinking water matrix. Syngenta Crop Protection Center, Greensboro, NC.

Summers, R.C., et al., 1996. Assessing DBP yield: uniform formation conditions. *J. Amer. Water Works Assoc.* 88(6): 80-93.

USEPA, Laboratory Study on the Effects of Chlorinated Water on OP Pesticides, Quality Assurance Project Plan, OPP/EFED/WTEWG, April 24, 2006.

USEPA, Laboratory Study on the Effects of Chlorinated Water on OP Pesticides, Final Report, OPP/EFED/WTEWG, May 15, 2006.

## **Appendix 2**

### **Results for the Laboratory Study on the Effects of Chlorinated Water on OP Pesticides, Phase II**

Results of the LC/MS/MS Analyses of the OP Pesticides Terbufos, Phorate and Disulfoton and Degradation in Chlorinated and Unchlorinated Wa

Sample	Sample Time	Terbufos						Phorate						parent	oxon s
		parent	oxon	sulfoxide	sulfone	oxon sulfoxide	oxon sulfone	parent	oxon	sulfoxide	sulfone	oxon sulfoxide	oxon sulfone		
MDL		5	5	10	25	4	4	5	5	10	25	4	4	5	5
AI	0	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
	15 min	N.D.	N.D.	N.D.	N.D.	35	N.D.	N.D.	N.D.	N.D.	N.D.	73	N.D.	N.D.	N.D.
	4 h	N.D.	N.D.	N.D.	N.D.	4	N.D.	N.D.	N.D.	N.D.	N.D.	35	5	N.D.	N.D.
	24 h	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	8	N.D.	N.D.
	72 h	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.

<b>A2</b>	<b>0</b>	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
	<b>15 min</b>	N.D.	N.D.	N.D.	N.D.	<b>31</b>	N.D.	N.D.	N.D.	N.D.	N.D.	<b>82</b>	N.D.	N.D.	N.D.
	<b>4 h</b>	N.D.	N.D.	N.D.	N.D.	<b>5</b>	N.D.	N.D.	N.D.	N.D.	N.D.	<b>47</b>	<b>4</b>	N.D.	N.D.
	<b>24 h</b>	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	<b>6</b>	N.D.	N.D.
	<b>72 h</b>	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
<b>B</b>	<b>0</b>	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
	<b>15 min</b>	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
	<b>4 h</b>	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
	<b>24 h</b>	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
	<b>72 h</b>	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
<b>C</b>		N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
<b>D</b>	<b>0</b>	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.
	<b>15 min</b>	N.D.	<b>41</b>	<b>37</b>	<b>42</b>	<b>52</b>	<b>55</b>	N.D.	<b>45</b>	<b>69</b>	<b>33</b>	<b>49</b>	<b>50</b>	N.D.	<b>42</b>
	<b>4 h</b>	N.D.	<b>34</b>	<b>32</b>	<b>41</b>	<b>49</b>	<b>54</b>	N.D.	<b>41</b>	<b>56</b>	<b>35</b>	<b>44</b>	<b>42</b>	N.D.	<b>40</b>
	<b>24 h</b>	N.D.	<b>34</b>	<b>33</b>	<b>47</b>	<b>50</b>	<b>48</b>	N.D.	<b>42</b>	<b>56</b>	<b>37</b>	<b>49</b>	<b>45</b>	N.D.	<b>40</b>
	<b>72 h</b>	N.D.	<b>38</b>	<b>34</b>	<b>39</b>	<b>51</b>	<b>53</b>	N.D.	<b>47</b>	<b>83</b>	<b>39</b>	<b>53</b>	<b>47</b>	N.D.	<b>38</b>

Amounts are in

ppb

N.D. = Not detected

N.A. = Not analyzed

Results of the ECB GC-MSD Analyses of the OP Pesticides Terbufos, Phorate and Disulfoton and Degradation in Chlorinated and Unchlorinated

Sample	Sample Time	Terbufos						Phorate						parent	oxon s	
		parent	oxon	sulfoxide	sulfone	oxon	oxon	parent	oxon	sulfoxide	sulfone	oxon	oxon			
		5	5	10	25	sulfoxide	sulfone	4	4	5	5	10	25			sulfoxide
A1	0	51	N.D.		N.D.		N.D.	56	N.D.		N.D.		N.D.		58	N.D.
	15 min	N.D.	N.D.		N.D.		N.D.	N.D.	N.D.		N.D.		1	N.D.	N.D.	
	4 h	N.D.	N.D.		N.D.		N.D.	N.D.	N.D.		N.D.		4	N.D.	N.D.	
	24 h	N.D.	N.D.		N.D.		N.D.	N.D.	N.D.		N.D.		2	N.D.	N.D.	
	72 h	N.D.	N.D.		N.D.		N.D.	N.D.	N.D.		N.D.		1	N.D.	N.D.	
A2	0	59	N.D.		N.D.		N.D.	63	N.D.		N.D.		N.D.	66	N.D.	
	15 min	N.D.	N.D.		N.D.		N.D.	N.D.	N.D.		N.D.		1	N.D.	N.D.	
	4 h	N.D.	N.D.		N.D.		N.D.	N.D.	N.D.		N.D.		4	N.D.	N.D.	
	24 h	N.D.	N.D.		N.D.		N.D.	N.D.	N.D.		N.D.		2	N.D.	N.D.	
	72 h	N.D.	N.D.		N.D.		N.D.	N.D.	N.D.		N.D.		1	N.D.	N.D.	
B	0	N.A.	N.A.		N.A.		N.A.	N.A.	N.A.		N.A.		N.A.	N.A.	N.A.	
	15 min	73	N.D.		N.D.		N.D.	78	N.D.		N.D.		N.D.	77	N.D.	
	4 h	23	N.D.		N.D.		N.D.	29	N.D.		N.D.		N.D.	33	N.D.	
	24 h	7	N.D.		N.D.		N.D.	14	N.D.		N.D.		N.D.	2	N.D.	
	72 h	2	N.D.		N.D.		N.D.	8	N.D.		N.D.		N.D.	3	N.D.	
C		N.D.	N.D.		N.D.		N.D.	N.D.	N.D.		N.D.		N.D.	N.D.	N.D.	
D	0	N.A.	N.A.		N.A.		N.A.	N.A.	N.A.		N.A.		N.A.	N.A.	N.A.	
	15 min	N.A.	N.A.		N.A.		N.A.	N.A.	N.A.		N.A.		N.A.	N.A.	N.A.	

4 h	29	36	47	52	27	31	47	46	31	31
24 h	44	52	56	59	43	48	57	50	44	44
72 h	33	35	48	42	35	32	49	42	36	35

Amounts are in

ppb

N.D. = Not detected

N.A. = Not available