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May 11, 2006

MEMORANDUM

SUBJECT: Submission of a Report on the Study Entitled, **"Laboratory Study on the Effects of Chlorinated Water on OP Pesticides"**

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TO: James Hetrick, Study Director
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John Leahy
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Enclosed is a report on the results of the study entitled, "**Laboratory Study on the Effects of Chlorinated Water on OP Pesticides**". The report reflects the elements and data quality objectives of the signed quality assurance project plan, dated April 24, 2006.

If there are any questions regarding the results and conclusions presented, please do not hesitate in contacting Christian Byrne (228) 688 3213.

cc: Steve Knizner, Associate Director
OPP/BEAD

List of Study Participants

Laboratory Study on the Effects of Chlorinated Water on OP Pesticides

May 11, 2006

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THE EFFECTS OF WATER CHLORINATION ON ORGANOPHOSPHATE (OP) PESTICIDES

Executive Summary

Ten organophosphate (OP) pesticides [phorate, disulfoton, terbufos, methidathion, bensulide, chlorethoxyfos, phosmet, methyl parathion, phostebupirim, and temephos] were evaluated for their potential to undergo oxidation to their respective oxons in laboratory water simulating the chlorination process in drinking water facilities over a 72 hour exposure period. Samples were collected after 0, 1, 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 oxons.

The results show that only two of the ten OP pesticides [methidathion and methyl parathion] are stable in buffered water (without chlorination) over the 72 hour exposure period. The eight remaining OP pesticides [phorate, disulfoton, terbufos, bensulide, chlorethyoxyfos, phostebupirim, phosmet, and temephos] were unstable and degraded in the buffered water over the 72 hour exposure period.

The results also show that in chlorinated water, three of the ten OP pesticides [phorate, disulfoton, and terbufos] did not undergo oxidation to their oxons under the experiment conditions. Phosmet oxon was initially formed; however, it degraded and was not detected after 24 hours. Five of the remaining six OP pesticides [methidathion, bensulide, chlorethyoxyfos, methyl parathion, and phostebupirim] formed stable oxons over the 72 exposure period. The oxon of the last remaining OP pesticide, temephos, is not commercially available and its presence could not be confirmed under the experimental conditions. However, a full scan spectrum of the oxidation products from an exploratory LC/MS study revealed the presence of a compound with the same molecular ion profile as would be expected for the temephos oxon. Table 1 summarizes the results of the experiment.

Table 1. Results for parameters examined in study.

OP Pesticide	Stability in water over 72 hours (no chlorination)	Oxon formation after 1 hour (upon chlorination)	Oxon stability after 72 hours
Phorate	Poor	No	-
Disulfoton	Poor	No	-
Terbufos	Poor	No	-
Methidathion	Good	Yes	Good
Bensulide	Poor	Yes	Good
Chlorethoxyfos	Poor	Yes	Good
Methyl parathion	Good	Yes	Good
Phosmet	Poor	Yes	Poor
Phostebupirim	Poor	Yes	Good
Temephos	Poor	Possible (not confirmed)	n/a

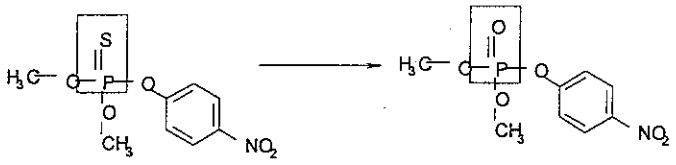
In accordance with the Quality Assurance Project Plan (QAPP) for study, there were two elements necessary for the strict qualitative interpretation whether the ten OP pesticides underwent oxidative desulfonation during a 72 hour contact time in chlorinated laboratory water. This conclusion would be reached if the oxons 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. Only methidathion and methyl parathion 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. Three organophosphate pesticides (diazinon, chlorpyrifos, and malathion) have been examined and have been found to transform during chlorination into their associated oxons. However, a number of other organophosphate pesticides have little or no data on their potential for transformation during these conditions. Consequently, data and additional information are needed on the probable oxidation of these organophosphate pesticides and the relative stability of oxons in chlorinated water. The ten organophosphate pesticides and their degradation products considered in this study are listed in Table 2.

Table 2: Selected Organophosphate Pesticides from the Cumulative OP Assessment without Water Treatment Data on Chlorination Effects on Oxon 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
Methidathion	methidathion oxon
Bensulide	bensulide oxon
Chlorethoxyfos	chlorethoxyfos oxon
Methyl parathion	methyl paraoxon
Phosmet	phosmet oxon
Phostebupirim	phostebupirim oxon
Temephos	temephos oxon (not available)

The objective of this study was to provide a qualitative screening level assessment on the potential for oxon formation 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 oxons in the chlorinated laboratory water. There are approximately twenty organophosphate pesticides considered in the cumulative OP risk assessment. The ten selected pesticides being tested in this study consisted of the pesticides, which are capable of forming oxons, 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 ten OP pesticides to be tested, the experimental design consisted 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 ten pesticides and nine oxons at a concentration of ½ of the spiking concentration (50 ppb) at each sampling time.

Chlorination experiments were 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 was equivalent to the recommended maximum disinfectant residual (RMDL) of 4 mg/L free chlorine concentration ± 10%. The pH

of the Fisher reagent water was adjusted to pH 8 to represent typical water treatment conditions. The experiment was conducted for 72 hours with sampling times immediately prior to chlorination (~2 minutes after pesticide dosing), 1 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 was 100 ppb or below the solubility limit of the pesticide whichever is lower. The experiments were 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 was determined by measuring free chlorine at each sampling interval.

3.0 Method and Materials

Fisher Scientific Certified Environmental Grade water was used as the test water. Water quality parameters of the test water were:

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 were labeled clearly, and included date, time, and name of the preparer(s). To preserve the integrity of the data, all samples were stored at ~ 4°C until extraction to minimize the physicochemical changes in the samples. If sample extraction into a solvent was necessary, extracts were stored below 0°C and also analyzed as soon as possible. All samples used and generated during the study were properly disposed of.
Quality assurance samples consisted of:
1) reagent water blank – analysis of reagent water (one time only);
2) method blank – analysis of buffered reagent water plus chlorine; (time 1 hr);
3) non-chlorinated degradation check – analysis of buffered reagent water plus OPs; (time 0, 1, 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 were classified as critical measurements and were prepared and analyzed with each group of samples to monitor laboratory contamination and method performance. Addition of surrogate compounds to environmental samples was also recommended to measure the efficiency of the method. The surrogate compounds was not normally found in the environment and was selected such that the interference with elution of target analytes and the effect from sample matrix were minimal.

Analytical Procedures

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

A calibration curve was 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 were prepared monthly, and the individual calibration standards verified each day of operation.

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

Test Protocol

These studies were 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.

The control treatments were used to assess whether the OP pesticide undergoes oxidation in non-chlorinated laboratory water and to assess whether OP pesticide or its degradation products were in the chlorinated water without pesticide dosing. Because the experimental design had minimal replication and the analytical methods were not fully vetted for all the OP pesticides and their oxon degradation products, there was **strict qualitative interpretation** (i.e. presence or absence of oxon) on whether OP pesticides underwent oxidative desulfonation during a 72 hour contact time in chlorinated laboratory water. This deduction was reached if oxons were detected at any quantifiable level in either replication in the chlorinated laboratory water treatments at any sampling time and the OP pesticide was 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 was stable enough in chlorinated water to have the potential for dietary exposure through drinking water

The primary focuses of these studies were the OP parent pesticides and their associated oxons, degradation products. Later studies will address the measurements of the sulfone, sulfoxide, sulfone oxon, and sulfoxide oxon degradation products for selected OP pesticides. Method detection and reporting limits will be reported in revisions to this QAPP once the analytical methods have been assessed.

Assessment and Oversight

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

4.0 Results

(A) The Formation of Oxons from Ten Organophosphate Pesticides in Water

Ten organophosphate (OP) pesticides [phorate, disulfoton, terbufos, methidathion, bensulide, chlorethoxyfos, phosmet, methyl parathion, phostebupirim, and temephos] were evaluated for their potential to undergo oxidation to their respective oxons 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 three of the ten OP pesticides (phorate, disulfoton, and terbufos) did not undergo oxidation into their oxons under the experiment conditions. Phosmet oxon was initially formed; however, it degraded and was not detected after 24 hours. Five of the remaining six OP pesticides [methidathion, bensulide, chlorethyoxyfos, methyl parathion, and phosetebuprim] formed stable oxons over the 72 exposure period. The oxon of the last remaining OP pesticide, temephos, is not commercially available and its presence could not be confirmed under the experimental conditions. However, a full scan spectrum of the oxidation products in an exploratory LC/MS study revealed the presence of a compound with the same molecular ion profile as would be expected for the temephos oxon. This exploratory study was conducted at a concentration of 5 ppm of temephos in chlorinated laboratory water. The detected compound increased in concentration during a 24 hour exposure period, simultaneously, with the decrease of the parent OP temephos. The lack of an authentic standard of the temephos oxon limits the complete confirmation of this oxon.

The analytical methods of GC-MSD and LC/MS/MS were complimentary to each other in the detection of all 10 OP pesticide parents and their oxons. The current GC/MSD conditions were not suitable for the detection of bensulide, while the LC/MS/MS conditions were not suitable for the detection of methyl parathion and chlorethoxyfos. However, their oxons were detectable under both method conditions.

(B) The Stability of the Ten Organophosphate (OP) Pesticides in Water

Ten organophosphate (OP) pesticides [phorate, disulfoton, terbufos, methidathion, bensulide, chlorethyoxyfos, phosmet, methyl parathion, phosetebuprim, and temephos] were evaluated in buffered laboratory water to act as a control to the separate studies of the pesticides in the

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 that two of the ten OP pesticides [methidathion and methyl parathion], are stable in the buffered water without chlorination over the 72 hour exposure period. The eight remaining OP pesticides [phorate, disulfoton, terbufos, bensulide, chlorethyoxyfos, phosetebuprim, phosmet, and temephos] were unstable and degraded in the buffered water over the 72 hour exposure period.

(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 10% of the initial concentration and neither the OP pesticides nor their oxons were detected at any time during the 72 hour exposure period.

(D) The Stability of the Ten Organophosphate Pesticides and Their Oxons as Laboratory Control Spike Samples

Ten organophosphate (OP) pesticides [phorate, disulfoton, terbufos, methidathion, bensulide, chlorethyoxyfos, phosmet, methyl parathion, phosetebuprim, and temephos] and their nine available oxons [temephos oxon is not available] 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 oxons. The results are presented in Appendix 2 for both the GS-MSD and LC/MS/MS studies.

The results demonstrated that these pesticides, with the exception of methyl parathion and methidathion, 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 at different time periods. For the D = 0 sample period the water sample was extracted within 1 hour, D = 4 sample period within 24 hours, and D = 72 within 1 minute. The results demonstrated that the longer the time

between collections and extraction the less stable were the pesticides in water.

On the other hand, all nine oxons were stable in the buffered water prior to analysis in both of the studies.

5.0 Summary

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

1) The oxons are detected at any quantifiable level in either replication in the chlorinated laboratory water treatments at any sampling time
   - **There were six quantifiable oxons detected in the chlorinated laboratory water within the seventy two hour exposure period [methidathion oxon, methyl paraoxon, phosmet oxon, bensulide oxon, phostebupirim oxon, and chlorethoxyfos oxon].**
   - **There was mass spectral evidence of the possible formation of a seventh oxon [temephos oxon]. However, there is, at present, no authentic temephos oxon standard to positively confirm this result.**

and

2) The OP pesticides are stable in non-chlorinated laboratory water.
   - **There were only two OP pesticides that were stable in the unchlorinated laboratory water [methidathion and methyl parathion].**

3) Additionally, the detection of oxons 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.
   - **Both of these oxons [methidathion oxon and methyl paraoxon] were stable at both the 24 hour and 72 hour sampling times.**

Only methidathion and methyl parathion 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.

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Appendix 1

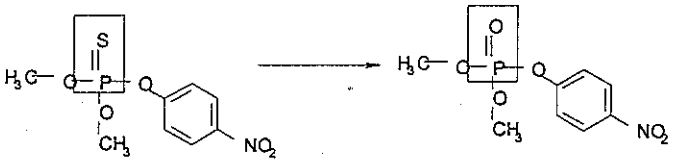
Procedures for the Preliminary Laboratory Study on the Effects of Chlorinated Water on OP Pesticides

April 24, 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. The organophosphate pesticides and their degradation products considered in this testing protocol are listed in Table 1.

Table 1: Selected Organophosphate Pesticides from the Cumulative OP Assessment without Water Treatment Data on Chlorination Effects on Oxon 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
Methidathion	methidathion oxon
Bensulide	bensulide oxon

Chlorethoxyfos	chlorethoxyfos oxon
Methyl parathion	methyl paraoxon
Phosmet	phosmet oxon
Phosetebuprim	phosetebupirim oxon
Temephos	temephos oxon

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 1 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 140^0 C oven overnight. Clean pipettes may need to be stored in ~ 50 mg/L Cl_2 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_2): 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_2.
(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 ± 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_2SO_4 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 ± 0.4 mg/L Cl_2 and a > 1.0 ± 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_2SO_4 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 ± 0.4 mg/L 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), 1 hr, 4 hr, 24 hr, 72 hr
Transformation products (oxon, sulfoxide, sulfone, sulfone oxon, sulfoxide oxon) – 0 (prechlorination), 1 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 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 H_2SO_4 or dilute NaOH. Dose with OP pesticide(s) to achieve a concentration of 100 µg/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, 1, 4, 24 and 72 hours. The samples should be 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 H_2SO_4 or dilute NaOH.
(3) Add pH 8 hypochlorite-buffer solution to give an initial free chlorine residual of 4.0 ± 0.4 mg/L Cl_2 and a subsequent free chlorine residual of > 1.0 ± 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 1 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 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 1 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_2SO_4 or dilute NaOH.
(3) Collect 100 ml samples of the unchlorinated, buffered water at each sampling interval of 0, 1, 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 will be stored for possible analysis with sample set batches. The samples should be 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**			
A1 OP Cl_2 **A2** OP Cl_2	**0**	**1 hr**	**4 hrs**	**24 hrs**	**72 hrs**
	OP	OP	OP	OP	OP

	Oxon[1]	Oxon	Oxon	Oxon	Oxon
		Cl	Cl	Cl	Cl
B OP H_2O	OP	OP	OP	OP	OP
	Oxon	Oxon	Oxon	Oxon	Oxon
C Cl_2 H_2O		OP			
		Oxon			
		Cl			
D H_2O	Spiked OP	Spiked OP	Spiked OP	Spiked OP	Spiked OP
	Spiked Oxon	Spiked Oxon	Spiked Oxon	Spiked Oxon	Spiked Oxon

1- Sulfone, sulfoxide, sulfone oxon, and sulfoxide oxon will be analyzed if appropriate for the test pesticide. This assumes analytical methods and analytical standards are available for the various degradation products.

(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

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APPENDIX I

Standards Availability for Drinking Water Project
February 6, 2006

STANDARD	PARENT	METABOLITES
Bensulide	Yes	Oxon
Chlorethoxyfos	Yes	Yes
Disulfoton	Yes	Oxygen analogue
Methidathion	Yes	Oxon
Methyl Parathion	Yes	Oxon
Phorate	Yes	Oxon
Phosmet	Yes	Oxon
Phostebupirim (tebupirimofos)	Yes	Oxygen Analogue
Temephos[1]	Yes	No
Terbufos	Yes	Oxon

1- The registrant indicates that temephos does not form an oxon. Review of the chemical structure indicates that two oxons might result from chlorination.

APPENDIX 2

Results of the LC/MS/MS Analyses of OP Pesticides and Oxons in the Water Chlorination Studies

PARENTS		A1	A2	B	D-spike[1]
Methidathion	0	67	64	72	39
	1 hour	ND	ND	73	44
	4 hours	ND	ND	73	45
	24 hours	ND	ND	74	47
	72 hours	ND	ND	77	NA
Methyl parathion2	0	NA	NA	NA	NA
	1 hour	NA	NA	NA	NA
	4 hours	NA	NA	NA	NA
	24 hours	NA	NA	NA	NA
	72 hours	NA	NA	NA	NA
Phosmet3	0	NA	NA	NA	NA
	1 hour	NA	NA	NA	NA
	4 hours	NA	NA	NA	NA
	24 hours	NA	NA	NA	NA
	72 hours	NA	NA	NA	NA
Phorate	0	22	23	26	18
	1 hour	ND	ND	24	19
	4 hours	ND	ND	20	18
	24 hours	ND	ND	11	22
	72 hours	ND	ND	8	NA
Bensulide	0	15	15	18	14
	1 hour	2	2	21	15
	4 hours	2	3	16	14
	24 hours	ND	ND	15	15
	72 hours	ND	ND	17	
Chlorethoxyphos2	0	NA	NA	NA	NA
	1 hour	NA	NA	NA	NA
	4 hours	NA	NA	NA	NA
	24 hours	NA	NA	NA	NA
	72 hours	NA	NA	NA	NA
Disulfoton	0	ND	ND	ND	10
	1 hour	ND	ND	ND	9
	4 hours	ND	ND	ND	10
	24 hours	ND	ND	ND	11
	72 hours	ND	ND	ND	NA
Terbufos	0	11	11	13	11
	1 hour	ND	ND	12	11
	4 hours	ND	ND	8	11
	24 hours	ND	ND	3	12
	72 hours	ND	ND	6	NA
Phostebupirim	0	19	19	23	18
	1 hour	2	3	22	17
	4 hours	1	1	14	18
	24 hours	ND	ND	8	18
	72 hours	ND	ND	10	
Temephos	0	55	54	67	10
	1 hour	3	3	46	9
	4 hours	3	3	49	9
	24 hours	ND	ND	40	10
	72 hours	NA	NA	NA	NA

OXONS		A1	A2	B	D-spike
Methidathion oxon	0	ND	ND	ND	44
	1 hour	80	81	ND	48
	4 hours	87	81	ND	50
	24 hours	80	76	ND	51
	72 hours	58	50	ND	NA
Methyl paraoxon	0	ND	ND	ND	52
	1 hour	58	46	ND	53
	4 hours	79	72	ND	55
	24 hours	81	72	ND	58
	72 hours	78	75	ND	NA
Phosmet oxon3	0	NA	NA	NA	NA
	1 hour	NA	NA	NA	NA
	4 hours	NA	NA	NA	NA
	24 hours	NA	NA	NA	NA
	72 hours	NA	NA	NA	NA
Phorate oxon	0	ND	ND	ND	45
	1 hour	ND	ND	ND	43
	4 hours	ND	ND	ND	45
	24 hours	ND	ND	ND	50
	72 hours	ND	ND	ND	NA
Bensulide oxon	0	ND	ND	ND	43
	1 hour	58	57	ND	44
	4 hours	57	52	ND	46
	24 hours	55	49	ND	48
	72 hours	60	56	ND	NA
Chlorethoxyphos oxon	0	ND	ND	ND	40
	1 hour	17	15	ND	38
	4 hours	25	23	ND	38
	24 hours	24	20	ND	40
	72 hours	17	13	ND	NA
Disulfoton oxon	0	ND	ND	ND	49
	1 hour	ND	ND	ND	47
	4 hours	ND	ND	ND	50
	24 hours	ND	ND	ND	51
	72 hours	ND	ND	ND	NA
Terbufos oxon	0	ND	ND	ND	33
	1 hour	ND	ND	ND	30
	4 hours	ND	ND	ND	32
	24 hours	ND	ND	ND	39
	72 hours	ND	ND	ND	NA
Phostebupirim oxon	0	ND	ND	ND	48
	1 hour	49	ND	ND	48
	4 hours	55	51	ND	48
	24 hours	59	54	ND	51
	72 hours	56	52	ND	NA
Temephos oxon4	0	ND	NA	ND	NA
	1 hour	Detected	NA	ND	NA
	4 hours	Detected	NA	ND	NA
	24 hours	Detected	NA	ND	NA
	72 hours			ND	NA

[1] Sample D-spike 72 hr was not included

[2] Methyl parathion and chloroethoxyfos are not suitable for the current LC/MS conditions

[3] Phosmet and phosmet oxon are unstable in methanol and water, however, phosmet oxon was detected after 1 hr, and its amount declined until only traces remained after 24 hr.

[4] Temephos oxon standard is not available, however a full scan spectrum revealed the presence of a compound with the same $(M+1)^+$ as temephos oxon after 1 hr and its amount increased after 4 and 24 hr.

APPENDIX 2

Results of the GC-MSD Analyses of OP Pesticides and Oxons in the Water Chlorination Studies - ECB

PARENTS		A1	A2	B	D-spike[2]
Methidathion	0	106	64	107	51
	1 hour	ND	ND	110	NA
	4 hours	ND	ND	109	47
	24 hours	ND	ND	103	NA
	72 hours	ND	ND	101	59
Methyl parathion	0	95	94	96	47
	1 hour	12	ND	89	NA
	4 hours	ND	ND	98	46
	24 hours	ND	ND	83	NA
	72 hours	ND	ND	88	53
Phosmet	0	80	81	81	20
	1 hour	ND	ND	103	NA
	4 hours	ND	ND	17	2
	24 hours	ND	ND	ND	NA
	72 hours	ND	ND	ND	49
Phorate	0	62	61	62	38
	1 hour	ND	ND	68	NA
	4 hours	ND	ND	52	25
	24 hours	ND	ND	17	NA
	72 hours	ND	ND	11	45
Bensulide[1]	0	NA	NA	NA	NA
	1 hour	NA	NA	NA	NA
	4 hours	NA	NA	NA	NA
	24 hours	NA	NA	NA	NA
	72 hours	NA	NA	NA	NA
Chlorethoxyfos	0	41	43	41	29
	1 hour	12	12	42	NA
	4 hours	ND	ND	32	26
	24 hours	ND	ND	3	NA
	72 hours	ND	ND	ND	51
Disulfoton	0	64	64	64	36
	1 hour	ND	ND	68	NA
	4 hours	ND	ND	56	34
	24 hours	ND	ND	24	NA
	72 hours	ND	ND	16	45
Terbufos	0	63	63	65	39
	1 hour	ND	ND	64	NA
	4 hours	ND	ND	46	34
	24 hours	ND	ND	9	NA
	72 hours	ND	ND	ND	49
Phostebupirim	0	79	79	78	41
	1 hour	2	2	71	NA
	4 hours	ND	ND	65	42
	24 hours	ND	ND	30	NA
	72 hours	ND	ND	14	51
Temephos	0	93	94	94	50
	1 hour	ND	ND	121	NA
	4 hours	ND	ND	93	41
	24 hours	ND	ND	43	NA
	72 hours	ND	ND	11	50

OXONS		A1	A2	B	D-spike[2]
Methidathion oxon	0	ND	ND	ND	48
	1 hour	90	98	ND	NA
	4 hours	81	97	ND	47
	24 hours	72	77	ND	NA
	72 hours	66	67	ND	59
Methyl paraoxon	0	ND	ND	ND	48
	1 hour	90	69	ND	NA
	4 hours	72	85	ND	47
	24 hours	66	70	ND	NA
	72 hours	68	66	ND	59
Phosmet oxon	0	ND	ND	ND	20
	1 hour	57	69	ND	NA
	4 hours	7	9	ND	2
	24 hours	ND	ND	ND	NA
	72 hours	ND	ND	ND	43
Phorate oxon	0	ND	ND	ND	38
	1 hour	ND	ND	ND	NA
	4 hours	ND	ND	ND	32
	24 hours	ND	ND	ND	NA
	72 hours	ND	ND	ND	45
Bensulide oxon[1]	NA	NA	NA	NA	NA
	NA	NA	NA	NA	NA
	NA	NA	NA	NA	NA
	NA	NA	NA	NA	NA
	NA	NA	NA	NA	NA
Chlorethoxyfos oxon	0	ND	ND	ND	39
	1 hour	26	30	ND	NA
	4 hours	41	55	ND	38
	24 hours	26	30	ND	NA
	72 hours	23	23	ND	54
Disulfoton oxon	0	ND	ND	ND	40
	1 hour	ND	ND	ND	NA
	4 hours	ND	ND	ND	37
	24 hours	ND	ND	ND	NA
	72 hours	ND	ND	ND	48
Terbufos oxon	0	ND	ND	ND	38
	1 hour	ND	ND	ND	NA
	4 hours	ND	ND	ND	31
	24 hours	ND	ND	ND	NA
	72 hours	ND	ND	ND	46
Phostebupirim oxon	0	ND	ND	ND	44
	1 hour	44	43	ND	NA
	4 hours	42	50	ND	43
	24 hours	44	43	ND	NA
	72 hours	47	50	ND	52
Temephos oxon[3]	0	NA	NA	NA	NA
	1 hour	NA	NA	NA	NA
	4 hours	NA	NA	NA	NA
	24 hours	NA	NA	NA	NA
	72 hours	NA	NA	NA	NA

[1] Bensulide is not suitable for the current GC-MSD conditions

[2] Only the D-Spike Samples at 0, 4, and 72 hours were analyzed.

[3] Temephos oxon standard is not available.