Determination of Chlorpyrifos, Chlorpyrifos Oxon, and 3,5,6-Trichloro-2-Pyridinol in Rat and Human Blood

Kathy A. Brzak, Daniel W. Harms, Michael J. Bartels, and Richard J. Nolan
Health and Environmental Research Laboratories, The Dow Chemical Company, Midland, Michigan 48674

Abstract

Analytical methods to quantitate chlorpyrifos and two potential metabolites, chlorpyrifos oxon (oxon) and 3,5,6-trichloro-2-pyridinol (TCP), in human and rat blood are described. Chlorpyrifos and the oxon were extracted simultaneously with a methanol/hexane mixture from 0.5 mL blood that was deactivated with an acidic salt solution. The extract was then concentrated and analyzed by negative-ion chemical ionization gas chromatography–mass spectrometry (NCI-GC–MS). TCP was extracted from a separate 0.1-mL aliquot of blood, also deactivated by the addition of acid. The t-butyldimethylsilyl derivative of TCP was formed using MTBSTFA, and the analysis was performed by NCI-GC–MS. Stable isotope analogues of chlorpyrifos (-13C2-tSN), oxon (-13C2-tSN), and TCP (-t3C z) were used as internal standards. Oxon was observed to partially degrade to TCP during the sample analysis. Accurate oxon and TCP measurements were obtained with the use of oxon-t3Cz-tSN, TCP-t3C2, and TCP-t3C2-tSN internal standards, which compensated for both the degradation of oxon and the formation of artifactual TCP during analysis. The limits of quantitation were 1 ng/mL blood for both chlorpyrifos and oxon and 10 ng/mL for TCP. Calibration curves were linear over the concentration range of 2.5–2500 ng/mL solvent for chlorpyrifos and oxon and between 5 and 1060 ng/mL solvent for TCP. Taking concentration factors and extraction efficiencies into account, these linear ranges represent blood concentrations of approximately 0.3–300 ng/mL blood for chlorpyrifos and the oxon and 6–1300 ng/mL blood for TCP. Recoveries from rat blood were as follows: 106–119% for chlorpyrifos, 94–104% for oxon, and 85–102 % for TCP. In addition, chlorpyrifos and oxon were incubated with rat and human blood for various time intervals before deactivation to determine precautions that needed to be taken when collecting and handling specimens. No change in chlorpyrifos concentration was observed in rat blood up to 180 min at 37°C. In contrast, the oxon was rapidly hydrolyzed to TCP in both rat (t1/2 = 10 s) and human (t1/2 = 55 s) blood held at 37°C. The hydrolysis rate for the oxon was independent of whether a rat had been administered chlorpyrifos previously, the initial oxon concentration, the presence of chlorpyrifos, and the age or gender of the human volunteers. These results suggest rapid sample preparation is critical for accurate determinations of the oxon metabolite of chlorpyrifos. These methods provide excellent tools for use in chlorpyrifos pharmacokinetic modeling studies.

Introduction

Chlorpyrifos is the active ingredient in many commercial insecticide formulations (1). Chlorpyrifos is metabolized by oxidative desulfuration to the oxon, and both chlorpyrifos and the oxon are hydrolyzed to 3,5,6-trichloro-2-pyridinol (TCP) (2). Environmental or occupational exposure to chlorpyrifos is often estimated by quantitating the TCP metabolite in the urine (3). However, the toxicity associated with chlorpyrifos is due to the oxon that inhibits acetyl cholinesterase, whereas neither chlorpyrifos itself nor TCP inhibits acetyl cholinesterase activity or that of other serine-dependent esterases or proteases (4,5). The objective of this publication is to describe analytical methods to unambiguously quantitate chlorpyrifos, its oxon, and TCP in blood from the rat and humans at levels that are biologically relevant (1 to 1000 ng/mL of blood) in support of kinetic studies to model the oxon formation and degradation. A high-performance liquid chromatography (HPLC) method has been reported in which chlorpyrifos, its oxon, and TCP were extracted into ethyl acetate with quantitation limits of 10 to 40 ng per injection (6). However, this HPLC method does not have sufficient sensitivity and selectivity to unambiguously quantitate these analytes at physiological levels of less than 100 ng/mL. In addition, levels of chlorpyrifos and its oxon in the serum of grossly overexposed individuals as determined by gas chromatography–mass spectrometry (GC–MS) using the electron impact mode have also been reported (7). However, few details and no detection limits were presented for the analysis of either chlorpyrifos or the oxon. Moreover, this method followed the hydrolysis products of chlorpyrifos (diethylphosphorus metabolites) other than TCP, which is specific to chlorpyrifos.
Materials and Methods

Chemicals

Chlorpyrifos, chlorpyrifos oxon, 3,5,6-trichloro-2-pyridinol, and the internal standards (chlorpyrifos-13C2-15N, chlorpyrifos oxon-13C2-15N, 3,5,6-trichloro-2-pyridinol-13C2, and 3,5,6-trichloro-2-pyridinol-13C2-15N) were obtained from Dow AgroSciences (Indianapolis, IN). The stable isotopes were located in the 2-(13C), 1-(15N), and 6-(13C) positions of the pyridinol ring for all of the internal standards. The silylation reagent (N-methyl-N-(t-butyldimethylsilyl)-trifluoroacetamide, MTBSTFA) was obtained from Pierce Chemical Company (Rockford, IL). Solvents were HPLC grade, and other chemicals were reagent grade. All were obtained from Fisher Scientific (Pittsburgh, PA).

Animals

Male Fischer 344 (CDF®) rats were obtained from Charles River Breeding Laboratories (Kingston, NY). The animals used in the in vivo experiment with chlorpyrifos were 10-weeks old, and those used in other incubation and method experiments varied in age. In order to investigate any effect of the oxon hydrolysis rate after in vivo exposure to chlorpyrifos, two rats were dosed by gavage with 1 mL of a corn oil solution containing 2.5 mg/mL chlorpyrifos (~11 mg chlorpyrifos/kg body weight). At approximately 3.3 h postdosing, the rats were euthanized with CO2, and blood was obtained via cardiac puncture. This blood was then used to determine the oxon hydrolysis rate described. Blood was obtained from control animals, via cardiac puncture, and used promptly for chlorpyrifos and oxon incubation experiments described subsequently or frozen at −20°C for later use.

Blood from male and female human volunteers (age 27–41) was obtained via venipuncture. All blood (rat and human) was drawn into a vial containing heparin to prevent clotting. Blood from rats or humans used to determine the oxon hydrolysis time course was collected and immediately placed in a water bath maintained at 37°C.

Chlorpyrifos and oxon

Sample preparation. In order to halt the hydrolysis of oxon to TCP, the samples were treated with an acidic solution containing approximately 2.5N acetic acid and a saturated amount of NaCl. The acidic saturated salt solution (175 μL) was added to a 0.5- mL aliquot of heparinized rat or human blood contained in a 4-mL glass vial, and the solutions were mixed well on a vortex mixer. Then, 10 μL of an acetonitrile solution containing approximately 40 ng each of both the chlorpyrifos and oxon internal standards were added, and the sample was mixed again briefly. Extraction of the analytes was accomplished by the addition of 2.5 mL ethyl acetate and mixing 5 min on a vortex mixer. Layers were separated by centrifugation (20 min at 1640 × g). An aliquot of the extract (0.5 mL) was dried under a gentle stream of nitrogen, and the residue was reconstituted in 50 μL toluene.

In order to determine relative recoveries from control rat or human blood, samples were prepared as described with the additional step of adding 1–10 μL of an acetonitrile solution containing 0.5–140 ng each of chlorpyrifos and oxon before addition of the internal standard solution.

Solvent standards were prepared in toluene containing varying amounts of chlorpyrifos and oxon (2–2500 ng/mL) and a constant amount of the internal standards at concentrations of approximately 220 ng/mL each.

GC-MS analysis. Extracts were analyzed on a Finnigan SSQ 710 (Finnigan MAT, San Jose, CA) or on a Hewlett Packard model 5989X GC-MS (San Jose, CA). Separations were achieved on a DB-17 capillary column (30 m × 0.25-mm i.d., 0.5-μm film thickness, J&W Scientific, Folsom, CA). Helium carrier gas was used with a head pressure of 10 psi. The GC oven temperature program included an initial hold at 80°C for 1 min followed by a 25°C/min ramp to 180°C and then a second ramp to 250°C at 20°C/min. The temperature was maintained at 250°C for 5 min then ramped at 40°C to a final temperature of 320°C with a 2-min hold. The injection port was at 210°C, and the transfer line was at 280°C. A 6-s splitless injection of 1 μL was made.

Additional representative instrumental conditions for low level quantitation of chlorpyrifos and oxon by negative ion chemical ionization include methane reagent gas at 9 torr, filament current at 400 μA, electron energy at 70 eV, electron multiplier at 1000–1400 volts, and dynode at 15 kV. Ions at m/z 297, 302, 313 (or 314), and 318 (or 319) were scanned at 30–50 ms/ion/scan.

3,5,6-Trichloro-2-pyridinol

Sample preparation. A saturated salt solution (150 μL) and 10 μL of concentrated HCl were added to a 0.1-mL aliquot of heparinized rat or human blood contained in a 4-mL glass vial, and the solutions were mixed well by vortex mixing. Then 10 μL of an acetonitrile solution containing approximately 50 ng of the TCP internal standard was added, and the solution was mixed briefly. Extraction of the analytes was accomplished by the addition of 2.5 mL ethyl acetate and mixing 5 min on a vortex mixer. Layers were separated by centrifugation (20 min at 1640 × g). An aliquot of the extract (1 mL) was dried under a gentle stream of nitrogen, and the residue was reconstituted in 75 μL toluene. Derivatizing reagent (MTBSTFA, 15 μL) was added, and the solutions were mixed and heated at 60°C for 1 h before analysis by GC-MS.

GC-MS instrumental conditions were similar to those used for chlorpyrifos and oxon analyses with the following exceptions: DB-5 GC column (30 m × 0.25-mm i.d., 0.25-μm film thickness, J&W Scientific); temperature program of 100°C initial temperature (1-min hold), 20°C/min ramp to 180°C, 6°C/min ramp to 210°C, 35°C/min ramp to 320°C; injection port 275°C; 6-s splitless injection of 0.5 μL; electron multiplier at approximately 1000; and scanning ions at m/z 161, 165, and 166 at 70 ms/ion/scan.

Solvent standards were prepared in toluene containing varying amounts of TCP (5–1060 ng/mL) and a constant amount of the internal standard at a concentration of approximately 250 ng/mL.

Full scan mass spectra. Negative ion chemical ionization mass spectra were obtained on an HP 5989X GC-MS. Solvent standards of the analytes at concentrations of 25–250 μg/mL were injected onto a DB-5ms column (30 m × 0.25-mm i.d., 0.25-μm film thickness, J&W Scientific) with a temperature...
program similar to that used for the low level chlorpyrifos/oxon analysis described here. The MS was operated in the negative ion mode using methane at 2.7 torr as the chemical ionization gas. The instrument was scanned from 10 to 450 amu at approximately 2 scans/s. TCP and the related internal standards were analyzed as the t-butyldimethylsilyl (t-BDMS) derivatives.

**Incubation experiments.** In order to follow the hydrolysis of chlorpyrifos and oxon, blood was acquired and used promptly (time 0 samples prepared within 5–15 min [rat] or 1–2 min [human] of acquisition). The time 0 point was prepared by adding the acidic saturated salt solution to an aliquot of blood and mixing well, then adding 7–10 μL of an acetonitrile solution containing chlorpyrifos, oxon, or both, and mixing again briefly. The internal standard was then added, and the sample was extracted as previously described. All subsequent samples (0.5, 1, 2, 5, or 10 min) were prepared by fortifying an aliquot of blood with the desired analytes, mixing, and incubating the sample in a 37°C water bath. Enzyme activity was halted with the addition of the acidic saturated salt solution, internal standards added, and the samples extracted as described here. Selected experiments were carried out with time points up to 180 min.

**Chlorpyrifos and oxon**

The isotopic overlap between the analytes and the internal standards was determined empirically by analyzing standard solutions of each compound separately. There was no detectable isotopic overlap from the oxon internal standard to the ion used to quantitate oxon (m/z 297); therefore, no correction to m/z 297 peak area was required. There was a contribution of approximately 5% of the m/z 297 peak area to m/z 302 (ion used for quantitation of oxon internal standard) from oxon. Chlorpyrifos was generally quantitated using m/z 313, and the chlorpyrifos internal standard used m/z 318. However, the method was also valid when using the less intense ions of m/z 314 and 319 for chlorpyrifos and the internal standard, respectively. From chlorpyrifos, there was a contribution of approximately 3.5% of the m/z 313 peak area to m/z 318. A contribution of 2% or less of the chlorpyrifos internal standard peak area to m/z 313 was observed. These isotopic overlap values were used to calculate corrected peak area for the analytes and internal standards according to the method of Barbalas and Garland (8).

**Calculations**

In this assay, analytes and internal standards were quantitated using major fragment ions for each compound. When using stable isotope-labeled internal standards, there is a possibility that isotopic contributions will occur between the ions used for quantitation of the unlabeled and labeled compounds. This isotopic overlap must be addressed for accurate determinations of concentrations. For example, the oxon internal standard was quantitated using m/z 302. The unlabeled oxon (quantitation ion m/z 297; Figure 1) also contributes to the peak intensity at 302 because of the presence of naturally occurring isotopes. This contribution to m/z 302 from oxon must be corrected in both standards and samples before quantitation to obtain accurate oxon response curves and concentrations (8). If the internal standards are not isotopically pure, there is the possibility that isotopic overlap will occur in the reverse, that is, contribution from the internal standard to the ion used to quantitate the unlabeled material. If this situation occurs, corrections must again be applied. As described subsequently, some isotopic overlap was observed from all three analytes and their corresponding internal standards.
TCP

Oxon was shown to partially degrade to TCP in the heated injection port of the GC. By using an isotopically labeled internal standard of oxon, as in the method described here previously, accurate quantitation of the oxon is possible. In order to quantitate TCP ($m/z$ 161), one must be able to distinguish TCP present in the blood from that which is formed from oxon degradation during preparation and injection of the extract. Figure 2 illustrates the metabolic pathway of chlorpyrifos, the structures of the internal standards (IS) used in this method, along with the demonstration of TCP formation from oxon degradation during sample preparation and analysis. The internal standard used for quantitation of TCP is dually labeled (F; quantitated using peak area at $m/z$ 165), and the internal standard for oxon quantitation is triply labeled (D). Any degradation of the oxon internal standard was evident by the appearance of the triply labeled TCP at $m/z$ 166 (G).

A second correction to the peak area (PA) at $m/z$ 161 was made after the initial correction for isotopic overlap (8) to account for thermally degraded oxon, which contributes to the TCP peak area at $m/z$ 161. For the second correction, the concentration ratio of oxon/oxon internal standard must be known. In standard solutions this ratio is known, but must be determined experimentally in samples by initially quantitating the oxon.

Final Corrected area $m/z$ 161 PA =

Isotopically corrected $m/z$ 161 PA - (Isotopically corrected $m/z$ 166 PA * [conc Oxon/conc Oxon IS])

or, referring to Figure 2:

Final Corrected area E =

Isotopically corrected area E - (Isotopically corrected area G * ([C] / [D]))

As shown in the equation, the peak area that represents oxon degradation is obtained by multiplying the concentration ratio of oxon/oxon internal standard (IS) by the peak area (PA) for degraded oxon internal standard ($m/z$ 166). Subtracting this area from the $m/z$ 161 peak area yields a final, corrected $m/z$ 161 area resulting from the TCP only. TCP concentrations in standards containing both TCP and oxon were calculated with and without the correction for oxon degradation to validate these corrections to the TCP peak area at $m/z$ 161.

Results and Discussion

Full scan negative ion chemical ionization mass spectra of the oxon, chlorpyrifos, and TCP are shown in Figures 1, 3, and 4, respectively. The major ion of chlorpyrifos is present at $m/z$ 313 (M-HCl)-. The peak area at $m/z$ 313 along with the corresponding area for the chlorpyrifos internal standard (chlorpyrifos-$^{13}$C$_2$-$^{15}$N) at $m/z$ 318 were used to quantitate chlorpyrifos. Likewise, concentrations of oxon were determined from the peak area at $m/z$ 297 (M-HCl)- and the internal standard (oxon-$^{13}$C$_2$-$^{15}$N) at $m/z$ 302. Following extraction from blood, TCP was treated with MTBSTFA to form the $t$-butyldimethylsilyl derivative and subsequently quantitated using $m/z$ 161 (M-Cl-$t$-BDMS) and $m/z$ 165 for the TCP internal standard (TCP-$^{13}$C$_2$). For each compound, the internal standard was quantitated using the $^{37}$Cl isotope ion (or higher) to minimize the isotopic overlap from the corresponding analyte.

Analytical method

Upon collection of a blood sample, enzymatic activity that leads to the hydrolysis of the oxon to TCP was halted with the addition of an acidified salt solution. Although the salt was added to aid in the extraction of analytes, the role of the acid was to inactivate the enzymes. Most enzymes have a characteristic pH at which their activity is maximal; the activity declines above or below this pH (9). Recently identified rat serum proteins with A-esterase activity towards chlorpyrifos oxon were found to be active at pH 4.5-5.6 with peak activity at pH 4.9-5.2 (10). It is apparent from the high sample recoveries that enzymes involved in oxon hydrolysis were inactivated at the low pH used in this method.

The mixed solvent of methanol/hexane resulted in an absolute extraction efficiency of 87% for chlorpyrifos and 84% for oxon from rat blood. An absolute extraction efficiency of 74% was obtained for TCP from rat blood with a single extraction using ethyl acetate.

Initial method development efforts were directed at developing one analytical method for the analysis of all three analytes, chlorpyrifos, the oxon, and TCP. However, significant degradation of the oxon (~30%) was evident in the presence of the MTBSTFA derivatizing reagent, making it necessary to develop separate methods for chlorpyrifos/oxon and TCP.

Standards of analytes and internal standards were prepared in toluene. Response was observed to be linear for concentration ranges of 2.5-2500 ng/mL for chlorpyrifos and the oxon...
Representative selected ion chromatograms of an extract of rat blood fortified with chlorpyrifos, oxon, and internal standards are shown in Figure 5A. A blood sample containing 1 ng/mL each of oxon and chlorpyrifos yields a peak for oxon at \(m/z\) 297, which is approximately 9x noise. The chlorpyrifos peak, also at 1 ng/mL blood, was significantly more intense (80x noise) but was only about 15x greater than the small interference seen in rat and human blood. Based on these data, the limit of quantitation (LOQ) for both chlorpyrifos and oxon was set at 1 ng/mL.

TCP quantitation was complicated by the degradation of the oxon to TCP. Corrections to the peak areas at \(m/z\) 161 of standards and samples to account for this degradation, as previously described, must be made before final quantitation of TCP. These correction calculations were verified, using standards containing both the oxon and TCP, by calculating concentrations with and without the correction for oxon degradation. The data, shown in Table I, indicate that approximately 30% of the oxon degraded to TCP. Without the \(m/z\) 161 peak-area corrections, the standard calculations averaged 100% of the actual concentrations. Also, corrected standards containing the oxon and TCP gave results comparable with standards containing only TCP.

Figure 6A illustrates selected ion chromatograms from rat blood fortified with TCP and TCP internal standard. This sample, fortified at 10 ng/mL TCP, yields a peak at \(m/z\) 161, which is about 160x noise, with approximately 10% of the peak area resulting from isotopic overlap and oxon degradation (oxon concentration of 9.5 ng/mL blood). The peak at \(m/z\) 166 results from contributions of TCP-\(^{13}\)C\(_2\) (~7% of corrected \(m/z\) 165 peak area) and from degradation of the oxon internal standard (oxon-\(^{13}\)C\(_2\)-\(^{15}\)N). The corresponding chromatograms from a control blood sample are shown in Figure 6B. A small interference, which was equivalent to less than 1 ng TCP/mL blood, was present in the control sample. The LOQ was set at 10 ng/mL based on these results.

To determine recovery, aliquots of rat blood were fortified with 1–282 ng chlorpyrifos and oxon/mL blood in triplicate and analyzed. The recoveries were...
106–119% for chlorpyrifos and 94–104% for the oxon (Table II). Likewise, triplicate control rat blood samples were fortified with TCP at levels of 10 to 425 ng/mL blood. The recoveries were 96–114%, also shown in Table II. The precision of the methods for all three compounds was excellent, with coefficients of variation ranging from 0.3 to 4.9%.

Interday variability of 5–6 separate analyses is shown in Table III. For the concentration ranges previously given, average recoveries were as follows: chlorpyrifos, 103–121%; oxon, 78–97%; and TCP, 100–120%. The precision of these analyses was good, with relative standard deviations ranging from 0.8 to 19.6% (average, 6.7%).

**Rat and human blood experiments**

Previous studies have reported that the oxon, when present at 3–25 µg/mL blood, degrades in rat blood with a half-life of 4–6 min (11). To examine the stability of this analyte and chlorpyrifos at the lower concentrations used in this method, a series of incubation experiments was performed in rat and human blood.

When chlorpyrifos and the oxon were incubated simultaneously in blood from naïve rats (~100 ng/mL each; 37°C), the concentration of chlorpyrifos remained constant for at least 10 min, whereas the concentration of the oxon decreased rapidly with an apparent t½ of ~10 s (Figure 7). Incubations of chlorpyrifos alone (30 ng/mL) showed that the concentration after 180 min was 98% of the initial concentration. In separate incubation experiments with the oxon at 100 and 1000 ng/mL blood, samples from selected time points (1 and 10 min) were analyzed for TCP concentrations. TCP was found to be the major product of oxon hydrolysis, which accounted for 102 ± 8% of the metabolized substrate.

In order to determine if prior exposure to chlorpyrifos would affect the degradation of the oxon, blood was collected from rats approximately 3.3 h after they had been given a single 11 mg/kg oral dose of chlorpyrifos. Following collection, the blood was immediately spiked with oxon (100 ng/mL) and incubated as previously described. The concentration of the oxon decreased rapidly at a rate similar to that observed with blood from a naive animal indicating that, even though this dose was expected to cause a large decrease in plasma and erythrocyte cholinesterase activity, it had no effect on the disappearance of the oxon.

When chlorpyrifos and oxon were incubated in human blood, the results were similar to those obtained with rat blood (Figure 8). The chlorpyrifos concentration remained constant for at least 40 min, whereas the oxon was hydrolyzed with a t½ of 28–89 s (average, 55 s). No differences were observed based upon age and gender of the volunteers (age 27–41, four female, four male). Initial method development work showed diminished esterase activity in human blood samples that were greater than 10-min old.

### Table I. TCP Standard Recovery Data Demonstrating Oxon Degradation

<table>
<thead>
<tr>
<th>Actual TCP standard conc. (ng/mL)</th>
<th>TCP/Oxon combination standards* no correction</th>
<th>TCP/Oxon combination standards* with correction</th>
<th>TCP-only standards</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>TCP (ng/mL)</td>
<td>Recovery (ng/mL)</td>
<td>TCP (ng/mL)</td>
</tr>
<tr>
<td>14.5</td>
<td>19.5</td>
<td>134%</td>
<td>15.1</td>
</tr>
<tr>
<td>29.1</td>
<td>37.4</td>
<td>128%</td>
<td>18.6</td>
</tr>
<tr>
<td>145.4</td>
<td>184.5</td>
<td>127%</td>
<td>142.0</td>
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<tr>
<td>290.8</td>
<td>377.1</td>
<td>130%</td>
<td>292.5</td>
</tr>
</tbody>
</table>

* These standards contain equal concentrations of TCP and oxon.

![Figure 6](https://academic.oup.com/jat/article-abstract/22/3/203/789122)
Conclusion

An analytical method was developed for determination of low levels of chlorpyrifos and its potential metabolites chlorpyrifos oxon (oxon) and 3,5,6-trichloro-2-pyridinol in rat and human blood. The quantitation limits for chlorpyrifos and the oxon were 1 ng/mL blood for each, whereas the TCP quantitation limit was 10 ng/mL blood. Response of solvent standards was linear over 2–3 orders of magnitude. Isotopically labeled internal standards of each analyte were used to obtain excellent recoveries. The oxon was observed to degrade during sample preparation and analysis to TCP. A novel use of isotopically labeled internal standards made it possible to correct for this degradation and obtain accurate quantitation of metabolite TCP in the presence of the oxon.

It was determined, by incubating various amounts of chlorpyrifos and oxon in rat and human blood, that the chlorpyrifos concentration remained constant up to 180 min while the oxon hydrolyzed rapidly (t_{1/2} rat blood = 10 s, t_{1/2} human blood = 55 s). Because of the rapid oxon hydrolysis rate, blood must be acquired and extracted quickly to obtain accurate oxon concentrations.

These methods require the use of a GC–MS system capable of operating in the chemical ionization mode. Even though this technique is quite routine, some laboratories may desire to use an electron impact (EI) GC–MS instrument. It has been shown that NCI analysis provides much better sensitivity and selectivity for the detection of urinary TCP than does EI or positive CI GC–MS (3). Therefore, a change in ionization technique may result in significantly higher detection limits for the analytes.

References


| Table II. Recoveries of Chlorpyrifos, Oxon, and TCP from Fortified Rat Blood |
|-----------------------------|------------------|-------|-------|
| Concentration (ng/mL) | Recovery (%) Mean C.V. n |
| Chlorpyrifos   | 1.0 | 119 | 1.1 | 3 |
|                | 24.7 | 117 | 0.6 | 3 |
|                | 282.2 | 106 | 0.4 | 3 |
| Oxon          | 1.0 | 104 | 1.8 | 3 |
|                | 24.0 | 95 | 0.3 | 3 |
|                | 268.7 | 94 | 1.8 | 3 |
| TCP           | 10.4 | 102 | 4.9 | 3 |
|                | 50.7 | 96 | 3.7 | 3 |
|                | 424.9 | 114 | 0.9 | 3 |

| Table III. Interday Precision in the Determination of Chlorpyrifos, Oxon, and TCP from Fortified Rat Blood |
|-----------------------------------------------|------------------|-------|-------|
| Concentration (ng/mL) | Recovery (%) Mean C.V. n |
| Chlorpyrifos   | 1.0 | 118 | 6.5 | 5 |
|                | 24.7 | 121 | 5.9 | 5 |
|                | 282.2 | 103 | 2.0 | 5 |
| Oxon          | 1.0 | 78 | 19.6 | 5 |
|                | 24.0 | 97 | 2.1 | 5 |
|                | 268.7 | 93 | 0.8 | 5 |
| TCP           | 10.4 | 120 | 9.2 | 5 |
|                | 50.7 | 100 | 9.5 | 6 |
|                | 424.9 | 109 | 4.9 | 6 |


Manuscript received May 29, 1997; revision received September 22, 1997.