# Nature and Bioavailability of Nonextractable (Bound) Residues in Stored Wheat Treated with Chlorpyrifos-methyl†,‡

Kamal Singh, Shahamat U. Khan,* M. Humayoun Akhtar, Sam Kacew,§ and Noel D. G. White∥

Centre for Land and Biological Resources Research, Research Branch, Agriculture Canada, Ottawa, Ontario, Canada K1A 0C6

Wheat treated with chlorpyrifos-methyl [*O*,*O*-dimethyl *O*-[(3,5,6-trichloro-2-pyridyl) phosphorothioate] and stored for 28 months at 20 °C contained about 70% of the applied insecticide as nonextractable (bound) residue mainly in the form of parent compound. The stored wheat containing bound chlorpyrifos-methyl was fed to rats. The bound residue in wheat was released and metabolized predominantly by hydrolysis, as indicated by the presence of a major metabolite, 3,5,6-trichloro-2-pyridinol, in urine and feces. The major route of elimination was via urine, which contained 3,5,6-trichloro-2-pyridinol and *O*-methyl *O*-(3,5,6-trichloro-2-pyridyl) phosphorothioate. The acetone-extractable portion of feces contained both chlorpyrifos-methyl and 3,5,6-trichloro-2-pyridinol, whereas the nonextractable portion of feces contained only chlorpyrifos-methyl. The results show that bound residues of chlorpyrifos-methyl in stored wheat, which cannot be extracted by methods commonly used in residue analysis of grain, are highly bioavailable.

## INTRODUCTION

The use of several contact insecticides to protect stored grains against pests is a well-established practice (Snelson, 1987). In the past, information on the fate of grain protectant insecticides on storage was obtained only from the chemical analysis of the solvent-extractable residues from the substrate. However, analysis of the extractable residues alone does not provide a clear understanding of the grains' burden of total pesticide residues, as it does not include the formation of nonextractable residues which are generally referred to as bound residues. It is known that, after an extended storage period, these formerly unseen nonextractable (bound) residues can amount to a significant proportion of the applied dose (IAEA, 1990).

The meaning of nonextractable or bound residues may remain open to interpretation depending on the extraction system employed to remove pesticide or metabolites bound to constituents within the grain. However, in this study, the term "bound residues" is used according to the most widely accepted IUPAC definition (Kearney, 1982), which is interpreted as the residues which cannot be extracted by methods commonly used in residue analysis and metabolism studies. On the basis of this definition, an IAEA/FAO coordinated research program (IAEA, 1990) and other researchers (Snelson, 1987; Matthews, 1991, 1992; Khan *et al.*, 1990, 1992; Syed *et al.*, 1992; Neskovic *et al.*, 1992) have demonstrated the presence of bound pesticide residues in a variety of stored products. In these studies, as well as in the investigation reported here, the bound residue is the portion of the pesticide or metabolite residues in grains which is not extracted and detected by methods devised for routine residue analysis of grain samples.

Attempts have recently been made to quantify and identify the grain protectant insecticide residues which remain nonextractable (bound) after solvent extraction. The potential biological and toxicological consequences of such residues have also been evaluated (Akhtar *et al.*, 1992). In a recent study from our laboratory, the bioavailability to rats of bound residues of [$^{14}$C]deltamethrin in stored wheat was demonstrated (Khan *et al.*, 1990). In subsequent studies it was shown that stored wheat containing bound residues of pyrimiphos-methyl and malathion when fed to rats were bioavailable and produced toxicity symptoms, including a reduction in weight gain, an increase in alkaline phosphatase activity and blood urea nitrogen, and a decrease in serum cholinesterase activity (Khan *et al.*, 1992; Akay *et al.*, 1990a,b, 1992; Akay and Kolankaya, 1989; Neskovic *et al.*, 1991).

Chlorpyrifos-methyl [*O*,*O*-dimethyl *O*-(3,5,6-trichloro-2-pyridyl) phosphorothioate] is a broad spectrum insecticide that produces relatively little mammalian toxicity ($LD_{50}$) in rat is 2140 mg/kg). This insecticide is used extensively as a protectant for stored grains throughout the world and is considered to be moderately persistent. Although the efficacy, residual activities, and formation of bound chlorpyrifos-methyl residue in stored wheat are well documented (Bengston *et al.*, 1975; Quinlan, 1978; Rowlands, 1975; Matthews, 1991), the chemical nature and bioavailability of these bound residues were not established.

Previous investigations have demonstrated the formation of bound residues in stored grains and their bioavailability to experimental animals by using only radioisotope tracers ($^{14}$C) in pesticide molecules (Akay and Kolankaya, 1989; Akay *et al.*, 1992; Khan *et al.*, 1990; 1992; Neskovic *et al.*, 1991; Sandermann *et al.*, 1992). Any pesticide residues that are not extractable and detected by recommended methods of residue analysis but remain bound with the constituents of wheat grains under practical agriculture conditions may become a potential hazard to consumers, and, therefore, both the quantity and the nature of these residues must be determined. Thus, from a practical viewpoint, bound residue studies conducted with nonradiolabeled pesticides under conditions similar

† Centre for Land and Biological Resources Research Contribution 93-03.

‡ Centre for Food and Animal Research Contribution 2107.

§ Permanent address: Department of Pharmacology, University of Ottawa, Ottawa, ON, Canada K1H 8M5.

∥ Permanent address: Research Station, Research Branch, Agriculture Canada, Winnipeg, MB, Canada R3T 2M9.

0021-8561/93/1441-2421$04.00/0 

to those encountered in agriculture would be of considerable significance.

The current work describes our attempt to determine the nature and potential bioavailability in rats of bound chlorpyrifos-methyl residues in stored wheat. The insecticide-treated wheat was stored for more than 2 years with or without mineral oil on grains. The use of food grade mineral oil up to 200 ppm on stored grain for human consumption and up to 600 ppm for animal feed has been recommended for dust suppression in the United States (FDA, 1982).

## MATERIALS AND METHODS

**Chemicals.** An analytical reference standard of chlorpyrifos-methyl was a gift from Dow Chemical Co., Midland, MI. 3,5,6-Trichloro-2-pyridinol was purchased from Chem Service, Westchester, PA. All solvents used were of pesticide grade. White mineral oil AMOCO5-NF was obtained from Amoco Corp., Chicago, IL.

**Grain Treatment and Storage.** Newly harvested hard red spring wheat grains (*Triticum aestivum* cv. Katepwa), with moisture content of 14.2% wet weight, were spread in a fumehood in batches of 3 kg and sprayed with the appropriate solution for each treatment. Commercial formulation Reldan (43.2% chlorpyrifos-methyl) was diluted with acetone to achieve a final concentration of 6 ppm on the grain for the treatment CHME. For the treatment CHMEOIL, the commercial formulation of the insecticide was mixed with food grade white mineral oil AMOCO5-NF and acetone to obtain a final concentration of 6.5 ppm of chlorpyrifos-methyl and 600 ppm of oil on the grain. The grains used as control (CNTRL) were sprayed with an equivalent volume of acetone alone. Treated wheat grains were then allowed to dry for 24 h, mixed thoroughly, and stored at 20 ± 2 °C for 28 months in cotton bags. During the storage, the moisture content of the wheat gradually declined to 8.0%.

**Solvent Extraction of Stored Grains.** Wheat was ground in an Intermediate Mill (Thomas Wiley, Swedesboro, NJ) using an 850-μm screen. Ground wheat was extracted three times with acetone (5 mL/g) by mechanical shaking for 1 h. Further extractions of ground wheat with acetone did not remove any additional chlorpyrifos-methyl or metabolite as indicated by gas chromatographic (GC) analysis as described later. The extracts were pooled, evaporated to just dryness, and redissolved in methanol. A portion of the methanol solution was derivatized with freshly prepared diazomethane (prepared from diazald, Aldrich Co. Inc., Milwaukee, WI). Caution should be exercised in preparing and using diazomethane as it is a cumulative and an extremely toxic substance, is highly explosive, and could be easily detonated by any sharp point or edge including ground glass joints. It is absorbed through the skin, and its inhalation could produce chemical pneumonia. Samples of methanol solution were analyzed by GC before and after derivatization.

**Determination of Bound Residue.** The residual portion of acetone-extracted wheat material was dried at room temperature for 24 h and subjected to supercritical fluid extraction (SFE) as described by Khan *et al.* (1990). The extraction was carried out for 90 min at a pressure of 350 atm and temperature of 120 °C using carbon dioxide as a supercritical fluid and methanol as a modifier. The flow rates for $CO_2$ and methanol were maintained at 1.0 and 0.5 mL/min, respectively. SFE for a longer period (180 min) or SFE of the solid material after the initial 90-min extraction did not release any further residues of the insecticide or metabolites. The extracted material was concentrated to a small volume, and an aliquot was methylated with diazomethane as described above. Both methylated and nonmethylated SFE extracts were then analyzed by GC.

**Feeding Trials.** Male Sprague-Dawley rats weighing approximately 200 g were housed individually in metabolic cages to collect urine and feces separately. The rats were acclimatized for 2 days and were initially fed with solvent-extracted control wheat material. On the third day, rats were randomly assigned into sets of four for three treatment groups. Group A received extracted wheat flour from treatment CHME; group B rats were fed extracted wheat flour from treatment CHMEOIL, and group C rats continued on extracted wheat flour from treatment CNTRL. Rats were maintained on treated diets for 13 days followed by 2 days with CNTRL feed. All rats had *ad libitum* access to diet and tap water. Body weight and feed consumption for each animal were recorded daily. Feces and urine were collected every 24 h during the entire course of the experiment. On day 15, the animals were sacrificed by decapitation; kidney, liver, heart, testes, lung, and brain were excised and stored at −20 °C until analyzed.

**Analysis of Samples.** *1. Feces.* Feces were softened with water (4 mL/g) overnight at 4 °C, homogenized, and extracted first with acetone and then with methanol. The extraction was carried out three times for each solvent (8 mL/g of the original weight of feces). The residual feces were subjected to supercritical fluid extraction as described earlier for wheat samples. The acetone, methanol, and SFE extracts were dried to just dryness and then dissolved in a small volume of methanol; a portion of each extract was derivatized with diazomethane, evaporated to dryness, and dissolved in methanol. The derivatized and underivatized samples were then diluted with an equal volume of water and extracted three times with 2 volumes of hexane to remove the coextractive materials which may interfere with chromatographic analysis. The hexane layer was further extracted with acetonitrile and analyzed by GC.

*2. Urine.* Urine was adjusted to ≃3 N HCl with concentrated hydrochloric acid and refluxed for 7 h. The cooled reaction mixture was evaporated to dryness several times to remove HCl, the residue was dissolved in methanol and centrifuged for 10 min, and the supernatant was collected. A known volume of the supernatant was derivatized with freshly prepared diazomethane and allowed to react for about 3 h, the solvent was evaporated to dryness by a gentle stream of nitrogen, and the residues were redissolved in a known volume of methanol. Derivatized and underivatized materials were diluted with an equal volume of water and extracted with hexane as described earlier. Concentrated hexane along with authentic standards were applied on thin-layer chromatographic (TLC) plates (silica gel Whatman LK5DF, 20 × 20 cm, 250 μm thick), at about 1 cm below the preabsorbent line, and developed in hexane/acetone/ammonium hydroxide (80:18:2 v/v/v; containers were lined with paper and saturated prior to use) to approximately 15 cm from the origin. Plates were allowed to stand at room temperature overnight to remove solvent and then viewed under UV light (253 nm) to identify spots due to authentic standards (chlorpyrifos-methyl $R_f$ = 0.85; methylated 3,5,6-trichloropyridinol $R_f$ = 0.7). The regions corresponding to authentic standards were scraped off the plate, extracted with acetonitrile, and analyzed by GC after the volumes were adjusted.

*3. Tissues.* Tissues were cut into small pieces, suspended in acetone, and homogenized in a Polytron homogenizer (Brinkmann) for 2 min. Homogenized material was extracted with acetone by shaking for 1 h (3 × 10 mL of acetone/g of tissue). Combined acetone extracts were divided into two portions. One part was evaporated to just dryness and dissolved in a small volume of methanol, and an aliquot was derivatized with diazomethane. Samples before and after derivatization were subjected to cleanup by diluting with an equal volume of water and then extracting with hexane. The hexane extracts were analyzed by GC. A second portion of acetone extract was evaporated to dryness, dissolved in 3 N HCl, and refluxed for 4 h. The cooled hydrolyzed reaction mixture was evaporated to dryness to remove HCl, and the residue was dissolved in methanol. A portion of the sample was derivatized with diazomethane. The material in methanol before and after derivatization was further extracted with hexane, concentrated to a small volume, and analyzed by GC.

*4. Standardization of Extraction Procedures.* All extraction procedures for wheat flour, feces, urine, and tissues were optimized by spiking the corresponding control samples with chlorpyrifos-methyl and 3,5,6-trichloro-2-pyridinol at two concentrations (0.1 and 3 ppm). The recoveries of these compounds in the extractable solvent as described in this study ranged from 87 to 95% (±7%).

**Gas Chromatography.** The gas chromatograph used was a Varian Model 3400 equipped with an electron capture detector (ECD) and a 15-m (0.522 mm i.d.) DB-17 megabore column with 1-μM film thickness. The oven temperature was programmed

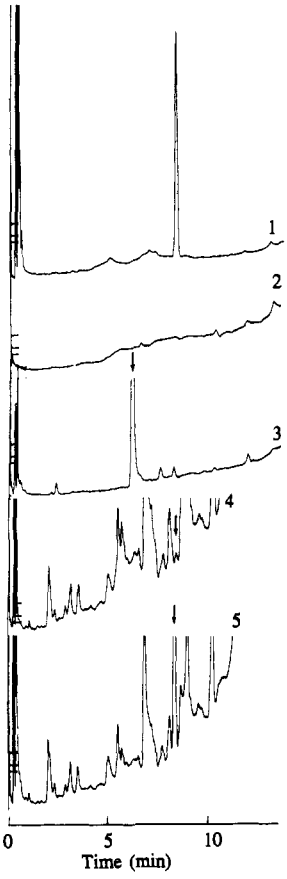


**Figure 1.** Gas chromatograms of (1) chlorpyrifos-methyl; (2) 3,5,6-trichloro-2-pyridinol; (3) derivatized (methylated) 3,5,6-trichloro-2-pyridinol; (4) acetone extract of control wheat; and (5) acetone extract of treated wheat.

at 8 °C/min from 80 to 220 °C. The detector and injector port temperatures were 310 and 180 °C, respectively. Nitrogen was the carrier gas at a flow rate of 30 mL/min. Under the GC conditions described, the retention times for chlorpyrifos-methyl and the methyl derivative of 3,5,6-trichloro-2-pyridinol were 8.3 and 6.4 min, respectively (Figure 1).

**Gas Chromatography–Mass Spectrometry (GC–MS).** The GC–MS analyses were carried out on a Finnigan mass spectrometer (Model MAT-312) fitted with a 20-m DB5-L column and connected to the INCOS data system. The column was operated between 90 and 270 °C with an increment of 10 °C/min. Spectra were recorded in electron impact mode at 70 eV.

## RESULTS AND DISCUSSION

The acetone extract of wheat grain, after 28 months of storage, showed the presence of unchanged chlorpyrifos-methyl at 0.5 and 0.9 ppm levels (air-dry weight basis) in the CHME and CHMEOIL treated samples, respectively. The extracted wheat material containing bound residues was subjected to supercritical fluid extraction. GC analysis of the supercritical extract from the treated samples showed only one major peak at a retention time of 8.3 min, which corresponded with the retention time of authentic chlorpyrifos-methyl (Figure 2). A GC–MS of this peak exhibited a peak at scan 390 at $m/z$ 321 ($M^+$, 5%) and a major peak at 286 ($M^+ - Cl$, 100%). The ions at $m/z$ 288 and 290 were due to a combination of various chlorine isotopes for a molecular formula $C_7H_7Cl_2NO_3PS$. The authentic chlorpyrifos-methyl under the same conditions had, at scan 390, peaks at $m/z$ 321 and a base peak at 286 with the two chlorine atoms pattern. This spectrum was consistent with the mass spectrum of the GC peak at retention time 8.3 min, thereby confirming the identity of

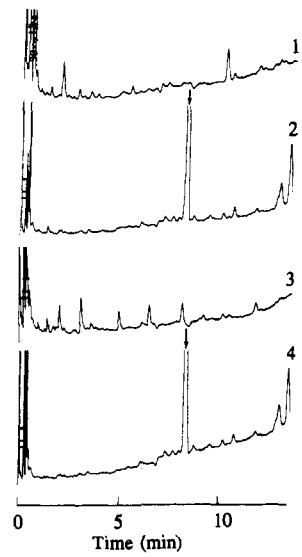


**Figure 2.** Gas chromatograms of (1) supercritical fluid extract (SFE) of control wheat; (2) SFE of treated wheat; (3) derivatized (methylated) SFE extract of control wheat; and (4) derivatized (methylated) SFE extract of treated wheat.

the compound as chlorpyrifos-methyl in the supercritical extract of the stored wheat grain containing bound residues. Derivatization of the supercritical fluid extract with diazomethane did not show any GC peak at the retention time corresponding to methylated 3,5,6-trichloro-2-pyridinol (Figure 2). Matthews (1991) reported the presence of this metabolite in a water/methanol extract of wheat treated with chlorpyrifos-methyl and stored for 20 weeks. The wheat samples were subjected to a sequential solvent procedure using hexane, chloroform, and acetonitrile prior to water/methanol extraction. The hydrolytic metabolite of chlorpyrifos-methyl, 3,5,6-trichloro-2-pyridinol, released by chemical solubilization using a water/methanol mixture from solvent-extracted wheat, was referred to as "non-solvent-extractable residue". However, under the experimental conditions used in our study, the presence of such a metabolite in either the acetone extracts of the stored wheat or the SFE extracts of the extracted wheat was not detected (Figures 1 and 2). Thus, both the CHME and CHMEOIL treated wheat contained primarily chlorpyrifos-methyl in the form of bound residues at concentrations of 5.1 and 5.3 ppm, respectively. It appears that oil had no effect on the nature and extent of formation of bound residues of chlorpyrifos-methyl in stored wheat. The SFE technique employed and the experimental procedure used in this study enabled us to determine the nature and amounts of bound residues in stored grains treated with the insecticide. These residues in the two treated samples were substantially greater than those determined by a conventional technique involving solvent (acetone) extraction. Therefore, when the total residue levels in stored grains are assessed, the presence of these bound, usually undetected, and chemically unidentified residues must be considered. This problem may be effectively addressed by SFE to isolate the nonextractable (bound) pesticides or their metabolites residues from these matrices. $CO_2$ was the fluid of choice in our study because of its low critical temperature (32 °C) and pressure (73 bar), which allowed SFE to be performed at relatively low temperature, avoiding thermal decomposition of the pesticide or metabolites.

Urine and feces samples were collected daily when rats were fed wheat material containing bound chlorpyrifos-methyl residues. These samples were then analyzed for determining the nature of the compounds excreted.

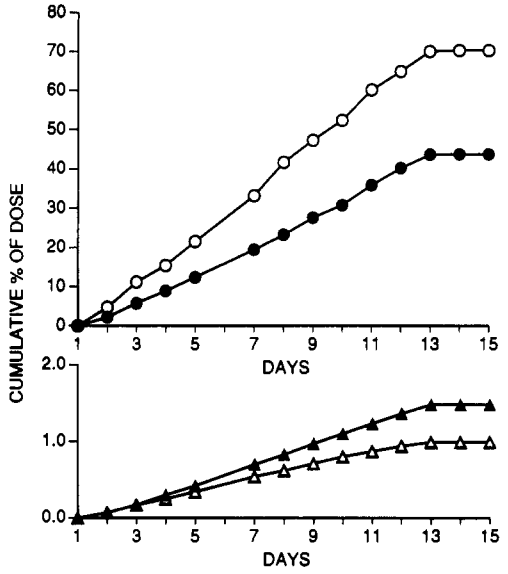


**Figure 3.** Elimination of metabolites in urine of rats fed stored wheat containing bound residues of chlorpyrifos-methyl: 3,5,6-trichloro-2-pyridinol, (○) CHME, (●) CHMEOIL; *O*-methyl *O*-(3,5,6-trichloro-2-pyridyl) phosphorothioate; (△) CHME, (▲) CHMEOIL.

Hydrolysis of urine samples and subsequent analysis of the methylated material by TLC and GC, as described earlier, indicated the presence of 3,5,6-trichloro-2-pyridinol (**III**) as a major metabolite in addition to a low concentration of *O*-methyl *O*-(3,5,6-trichloro-2-pyridyl) phosphorothioate (**II**). In other studies, the principal urinary metabolite of chlorpyrifos-methyl administered to rats orally was also found to be 3,5,6-trichloro-2-pyridinol (FAO/WHO, 1973). The GC–MS of the methylated extract of urine acid hydrolysate exhibited a base peak with scan 289 and had peaks at *m/z* 211, 213, and 215 (a three chlorine atoms pattern). The scan number and fragmentation patterns were identical with that for the authentic methyl derivative of 3,5,6-trichloro-2-pyridinol. Calculations of percentage recovery of 3,5,6-trichloro-2-pyridinol were based on the assumption that 1 mol of chlorpyrifos-methyl on hydrolysis will give 1 mol of 3,5,6-trichloro-2-pyridinol. Similarly, 1 mol of *O*-methyl *O*-(3,5,6-trichloro-2-pyridyl) phosphorothioate will form from demethylation of 1 mol of the parent compound. Figure 3 shows the elimination pattern of 3,5,6-trichloro-2-pyridinol (**III**) and *O*-methyl *O*-(3,5,6-trichloro-2-pyridyl) phosphorothioate (**II**) in urine. After 15 days, the cumulative excretions of 3,5,6-trichloro-2-pyridinol from CHME and CHMEOIL treated material were 70% and 44%, respectively. The corresponding values for *O*-methyl *O*-(3,5,6-trichloro-2-pyridinol) phosphorothioate were 1% and 1.5%, respectively, of the total bound chlorpyrifos-methyl consumed by rats. The oil in CHMEOIL treated grain samples may make the digestible residues more lipophilic, thereby resulting in lower excretion of the polar compounds such as **III**. Unhydrolyzed urine was analyzed in a similar way, but it did not show the presence of 3,5,6-trichloro-2-pyridinol, thus indicating that this metabolite was present in urine in the conjugated form.

Residues in bile may enter entero-porto-hepatic circulation. Since the bile duct of the experimental rats was not cannulated, the residue in bile, if not reabsorbed in the intestine, will be eliminated in feces. Extraction of residues from moistened feces samples with acetone removed chlorpyrifos-methyl (**I**) and 3,5,6-trichloro-2-pyridinol (**III**). Extraction with methanol did not result in any significant increase in recovery of these compounds.

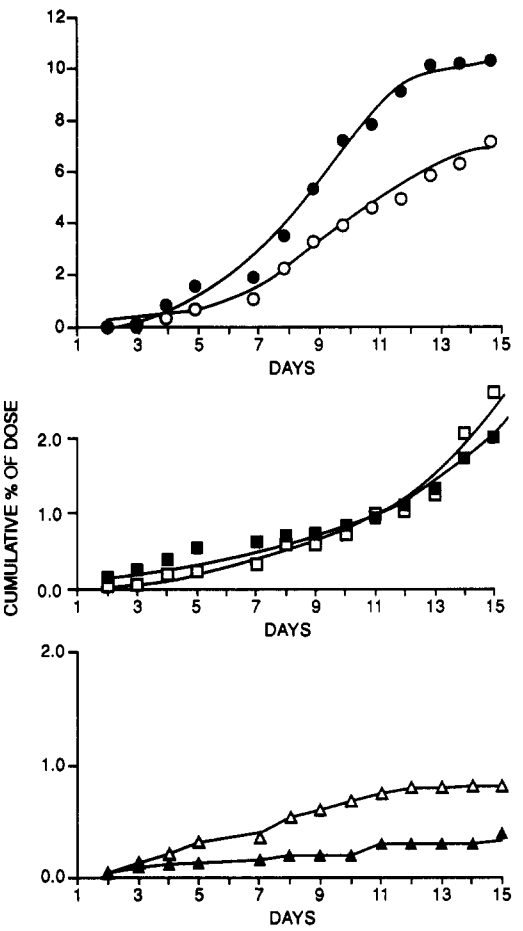


**Figure 4.** Elimination of extractable and bound residues in feces of rats fed stored wheat containing bound residues of chlorpyrifos-methyl: solvent-extractable 3,5,6-trichloro-2-pyridinol, (○) CHME, (●) CHMEOIL; chloropyrifos-methyl, (□) CHME, (■) CHMEOIL; bound chlorpyrifos-methyl extracted with supercritical fluid extraction; (△) CHME, (▲) CHMEOIL.

The cumulative eliminations after 15 days of extractable chlorpyrifos-methyl were 2.6% in CHME and 2.0% in CHMEOIL, and those of 3,5,6-trichloro-2-pyridinol were 7.1% in CHME and 10.3% in CHMEOIL groups of the total bound chlorpyrifos-methyl ingested by the animals. Extraction of residues in the feces containing nonextractable (bound) residues by SFE indicated that these residues were not bioavailable. Cumulative eliminations of the nonextractable chlorpyrifos-methyl from feces at the end of experiment were 0.8% for CHME and 0.4% for CHMEOIL groups of the total bound chlorpyrifos-methyl consumed by the rats (Figure 4). The data indicate that bound chlorpyrifos-methyl (**I**) in stored wheat grain released on digestion was metabolized by dealkylation and hydrolytic cleavage to compounds **II** and **III** (Figure 5). Furthermore, a portion of the bound residues of **I** remained unchanged and was excreted in feces.

In tissues, neither the parent compound nor any of its metabolites were detected. The total recoveries of bound chlorpyrifos-methyl after 15 days were 81.4% for CHME and 58.2% for CHMEOIL treated groups. The lower recoveries could be due to either extensive metabolism and finally expiration in the form of carbon dioxide or storage of residue in fat or certain tissues which were not analyzed in this study. During the feeding trials, none of the rats showed any apparent clinical signs of toxicity. During the course of this study, feed consumption per rat was 94.5% for CHME and 93.6% for CHMEOIL treatment groups compared to CNTRL (100%). There was no significant difference in body weight gain or organ weights

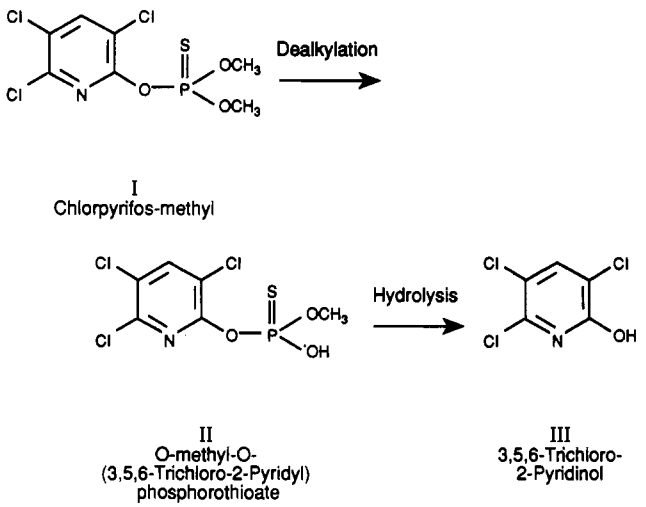


**Figure 5.** Metabolism of bound chlorpyrifos-methyl in stored wheat fed to rats.

of animals among treatment groups, suggesting an apparent lack of toxicity in the rat.

The results of this study suggest that a substantial amount of chlorpyrifos-methyl may not be extracted from stored grain when methods commonly used for residue analysis of this insecticide are employed. Although this nonextractable (bound) residue in wheat grains when fed to rats did not exert any visible direct toxic effects, it was found to be highly bioavailable. This was indicated by the elimination via urine of some of the metabolized products of the released insecticide on digestion. In the present study, the bound residues in wheat grains, after 28 months of storage, amounted to 6–10 times more than the extractable residues determined by the recommended methods used in routine analysis. On the basis of toxicological and residue data on chlorpyrifos-methyl, determined by routinely devised methods, a maximum residue limit for the insecticide in stored wheat of 10 mg/kg has been recommended (FAO/WHO, 1976). In view of the likely formation of substantial amounts of bound residues of chlorpyrifos-methyl in stored grain at the recommended level and their potential bioavailability and toxicity, the bound insecticide residues need to be taken into consideration when the toxic hazard of this insecticide is evaluated.

## ACKNOWLEDGMENT

The expert technical assistance of Rod MacDowell is greatly appreciated. This study was supported by Food Safety Research Funds, Agriculture Canada.

## LITERATURE CITED

Akay, M. T.; Kolankaya, D. Bioavailability of bound residue of $^{14}$C-malathion in stored wheat grains to rats. *Doga: Turk. J. Zool.* **1989**, *13*, 47–53.

Akay, M. T.; Yilmazoglu, G.; Gokce, D.; Kolankaya, D. The effects of bound residues of malathion in stored wheat grains on rat brain acetylcholinesterase and pseudocholinesterase activities. *Doga: Turk. J. Zool.* **1990a**, *14*, 162–166.

Akay, M. T.; Yilmazoglu, G.; Keskin, P.; Kolankaya, D. Biological activity in rats of bound residues of malathion in stored wheat grains. *Doga: Turk. J. Zool.* **1990b**, *14*, 167–172.

Akay, M. T.; Yilmazoglu, G.; Yasacan, S.; Turk, H.; Kolankaya, D. Bioavailability and toxicologial of potential wheat-bound primiphos-methyl residues in rats. *Xenobiotica* **1912**, *22*, 293–302.

Akhtar, M. H.; Khan, S. U.; Kacew, S. Bioavailability of bound pesticide residues and potential toxicological consequences. *Proc. Soc. Exp. Biol. Med.* **1992**, *199*, 13–21.

Bengston, M.; Cooper, L. M.; Grant-Taylor, F. J. A comparison of bioresmethrin, chlorpyrifos-methyl and primifos-methyl as grain protectants against malathion resistant insects in wheat. *Queensl. J. Agric. Anim. Sci.* **1975**, *32*, 51–78.

FAO/WHO. "1975 Evaluation of some pesticide residues in food"; *AGP*:**1975**/M/12:WHO Pesticide Residue Series 5; FAO, Rome, 1976.

FAO/WHO. "1972 Evaluation of some pesticide residues in food"; *AGP*/**1972**/M/9/1:WHO Pesticide Residue Series 2; FAO, Rome, 1973.

FDA Food additives permitted for direct addition to food for human consumption white mineral oil. *Fed. Regist.* **1982**, *47* (41), P8763.

IAEA. "Studies of the magnitude and nature of pesticide residues in stored products using radiotracer techniques"; IAEA Panel Proceeding Series, STI/PUB/822; IAEA, Vienna, 1990.

Kearney, P. C. IUPAC Pesticide Commission Report. *J. Assoc. Off. Anal. Chem.* **1982**, 1030–1032.

Khan, S. U.; Kacew, S.; Akhtar, M. H. Bound $^{14}$C residues in stored wheat treated with [$^{14}$C]deltamethrin and their bioavailability in rats. *J. Agric. Food Chem.* **1990**, *38*, 1077–1082.

Khan, S. U.; Kacew, S.; Matthews, W. Bioavailability to rats of bound [$^{14}$C]primiphos-methyl in stored wheat. *J. Environ. Sci. Health* **1992**, *B27*, 355–367.

Matthews, W. A. An investigation of the non-solvent extractable residues of [$^{14}$C]chlorpyrifos-methyl in stored wheat. *Pestic. Sci.* **1991**, *31*, 141–149.

Matthews, W. A. The biological activity of bound residues of [$^{14}$C]chlorpyrifos-methyl and $^{14}$C-malathion on treated wheat in a stored product insect. *J. Environ. Sci. Health* **1992**, *B27*, 419–426.

Neskovic, N. K.; Karan, V. Z.; Vitorovic, S. L.; Vojinovic, V. D. Toxic efffects of bound malathion residues in rats. *Biomed. Environ. Sci.* **1991**, *4*, 423–432.

Neskovic, N. K.; Karan, V.; Budimir, M.; Vojinovic, V.; Gasic, S.; Vitorovic, S. L. Bioavailability and biological activity of wheat-bound chlorpyrifos-methyl residues in rats. *J. Environ. Sci. Health* **1992**, *B27*, 387–397.

Quinlan J. K. Chlorpyrifos-methyl and malathion applied as protectants for high moisture stored wheat. *Proc. Entomol. Soc. Am.* **1978**, *33*, 27.

Rowlands, D. G. The metabolism of contact insecticides in stored grains 1970–1974. *Residue Rev.* **1975**, *58*, 113–155.

Sanderman, H., Jr.; Musick, T. J.; Aschbacher, P. W. Animal bioavailability of a 3,4-dichloroaniline-lignin metabolite fraction from wheat. *J. Agric. Food Chem.* **1992**, *40*, 2001–2007.

Snelson, J. T. *Grain Protectants*; ACIAR Monograph Series 3; Australian Centre for International Agricultural Research: Canberra, 1987; 448 pp.

Syed, M. A.; Arshad, J. H.; Mat, S. Biological activity and bioavailability of grain bound $^{14}$C-malathion residues in rats. *J. Environ. Sci. Health* **1992**, *B27*, 347–354.

Received for review February 3, 1993. Revised manuscript received August 19, 1993. Accepted September 13, 1993.[⊗]

[⊗] Abstract published in *Advance ACS Abstracts*, October 15, 1993.