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# Urinary 3,5,6-Trichloro-2-pyridinol Levels of Chlorpyrifos in Nicaraguan Applicators and Small Farm Families

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Information on pesticide exposures for risk assessment in developing countries is lacking; cholinesterase monitoring is the only biological assessment technique commonly used (Wesseling et al. 1997). In addition, testing is performed almost exclusively on pesticide handlers and farmworkers. However, family members of small farm applicators also may be exposed to pesticides. In Nicaragua women and children sometimes participate in pesticide application and/or enter fields soon after application. Additionally, family members may experience indirect exposures as a result of storage and handling of pesticide containers and sprayers in the home, women's hand cleaning of contaminated work clothes, and direct contact with workers who commonly do not shower immediately after working with pesticides.

This pilot study examined insecticide use by small-scale basic grain farmers on the Pacific coastal plain of Nicaragua and the indirect exposures of their family members. These farmers have extremely low levels of training and commonly make multiple insecticide applications throughout the growing season, with little or no protective measures: bare hands and minimal clothing are common (Aragón et al., 2001). Economic constraints often dictate the use of less expensive organophosphate insecticides (methamidophos and chlorpyrifos) instead of less acutely toxic pyrethroids such as cypermethrin and deltamethrin (Dowling, personal observation). In 1998, organophosphates were responsible for 45% (110 cases) and 49% (82 cases) of pesticide-related illness diagnosed and reported by medical doctors in Chinandega and León, respectively (Ministerio de Salud, 1999). Overall rates were 58 per 100,000 in Chinandega (down from 100 per 100,000 the previous year, which the Ministry of Health attributes to a medical strike) and 46 per 100,000 in León (Ministerio de Salud, 1999). The present study measured the urinary clearance of the metabolite, 3,5,6-trichloro-2-pyridinol (TCP), by backpack applicators and their families over a three-day period following chlorpyrifos applications to small basic grain plots.

## MATERIALS AND METHODS

All participants were treated in accordance with the study protocol approved by the human subjects committee of the National Autonomous University of

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Nicaragua (UNAN) at León. Potential subjects were initially contacted through the León and Chinandega branches of the National Farmers and Ranchers Organization (UNAG) in northwestern Nicaragua. Members were asked to complete a short questionnaire to identify farmers who planned to apply chlorpyrifos at least once during the growing season and who had multiple family members at home. Due to the damage caused in this area by Hurricane Mitch in November 1998, no applicators had worked with pesticides for at least the previous six months.

The purpose, expected outcome, and possible inconveniences of the study, as well as sampling procedures and reimbursement were described at an introductory meeting with the heads of household. One head of household refused participation. An investigator accompanied each consenting volunteer to his home, where the study plan was again reviewed with his spouse, the informed consent form was read aloud, copies were provided for their examination, and all questions were answered. When both adults agreed to participate they were enrolled in the study, along with the children in their household over five years of age (Table 1). Concurrently, a physician evaluated subjects' fitness to provide a urine sample; all study participants were in good health except one female child who had recently received treatment for a chronic renal problem and anemia. The physician judged this child capable of providing a urine sample without ill effect, and the child was included in the study. Pre-application urine samples were collected for TCP analysis the morning following the introductory sessions, during May and June 1999. Subject body weights were measured, with the exception of Applicator 1's niece (whose weight was derived from standardized Nicaraguan age/weight tables).

**Table 1.** Characteristics of study participants.

| **Subject** | **N** | **Sex** | **Age** | **Weight** (kg) |
|---|---|---|---|---|
| Head of Household | 6 | Male | 25-44 | 51-77 |
| Spouse | 6 | Female | 19-35 | 58-81 |
| Children | 4 | Male | 9-13 | 26-34 |
| Children | 8 | Female | 7-14 | 18-40 |

Of the ten families enrolled, six heads of households applied chlorpyrifos between mid-May and mid-July of 1999; each application was the first of the year for each applicator. In all cases, the applicator indicated that Lorsban® was applied. In one case (Subject #2) a partially-used bottle labeled Lorsban® 48EC and a sealed bottle of Sumithion® 500E (fenitrothion) identified as chlorpyrifos were used. The remaining five applicators used formulations from refilled, unlabeled containers. To confirm chlorpyrifos use, a small amount of formulation applied by applicators 3 through 6 was sampled. Each was volumetrically diluted with acetone, and analyzed at the UNAN Pesticide Residue Analysis Laboratory using gas-liquid chromatography (GLC) with electron capture detection (Table 2). Chlorpyrifos formulation concentrations in the present study fell within the 22-

55% (w/v) range observed for a total of 14 other "Lorsban" formulations analyzed as part of a related pesticide study (Dowling, unpublished data).

Applicators prepared spray mixes (Table 2) based on their prior pest control experience, at times with the guidance of an elder/advisor. Bare-handed applicators mixed/loaded their backpack sprayers at the edge of the field to be treated. They transferred insecticide formulations into the application tank using small tin cans or plastic bottles (100 to 200 mL), then diluted with 10 to 20 L of water. Applicators treated crops at various growth stages, with the exception of applicator 1, who sprayed bare soil prior to sowing (a practice in which he alone chose to engage). They worked either alone or with the aid of helpers (two male children assisted applicator 1 and spouses aided applicators 5 and 6). Helpers commonly assisted in one or more of the following tasks: measuring pesticide, adding water, and lifting the backpack sprayer onto the back of the applicator.

**Table 2.** Application characteristics.

| **Application** | **Crop** | **Crop Height** (cm) | **Formulation** (% a.i.) | **Spray Mix** (mL/L) | **Formul. Sprayed** (L/ha) | **Chlorpyrifos Applied** (g) |
|---|---|---|---|---|---|---|
| 1 | bare soil | 0 | not analyzed* | 2.1 | 171 | 85 |
| 2 | sesame | 40 - 70 | not analyzed* | 5.0 | 36 | 87 |
| 3 | corn | 50 - 70 | 32.5 | 1.4 | 129 | 82 |
| 4 | corn | 50 - 160 | 42.9 | 5.0 | 71 | 213 |
| 5 | sesame | 120 - 150 | 32.2 | 15 | 29 | 196 |
| 6 | sesame | 60 - 160 | 29.1 | 15 | 43 | 131 |

* 34% assumed based on analysis of 3-6.

The fields of applicators 3 and 4 were located 3 to 5 km from the family house. The field of applicator 1 was about 500 meters from the house, and in the other three cases, the residence directly bordered the treated field. Fields were roughly one (applicators 1 and 6) to two *manzanas* (applicators 2-5) in size (1 *manzana* = 0.7 hectare). Applicators 2, 5, and 6 used 10-L motorized sprayers, whereas the others used 20-L manual sprayers. Other applications details appear in Table 2. The work period ranged from 1 to 2.5 hours, of which about half was actual application time. Mixing, loading, and problem solving (e.g. unclogging wands or nozzles, getting the motor started) consumed the remainder of the work period. Applicators 2-6 participated in additional exposure measurements not reported here (including dermal patch deposition, fluorescent tracer visualization, handwipes, air sampling and cholinesterase monitoring (Aragón, unpublished data).

Based on the 27-hr biological half-life of chlorpyrifos (Nolan et al. 1984), a three-day monitoring period was assumed to account for a clearance of 85% the absorbed dose of chlorpyrifos. Post-application urine samples were collected the first, second, and third mornings after the chlorpyrifos application. All urine samples were sub-sampled by transferring 25- to 30-mL to 35-mL polyethylene tubes in the field. Sub-samples were transported on blue ice in a cold box to the laboratory, where they were frozen in a –20°C freezer until transfer to the United States (packed both with blue ice containers and bags of wet ice in ice chests), where they were again stored frozen until analysis.

Laboratory personnel were blinded to the objectives of the study and participant identity. Conjugated and unconjugated TCP were analyzed from acid hydrolysates of urine (Pacific Toxicology Laboratories Standard Operating Procedure, 1998). The internal standard, $^{13}C_2{}^{15}N$-labeled-TCP, was added to a thawed, 5-mL urine specimen. Specimens plus 0.25-mL concentrated hydrochloric acid were held overnight at 60°C. TCP was extracted with 1-chlorobutane and derivatized in an autosampler vial with N-methyl-N-(tert-butyldimethyldimethylsilyl)-trifluoroacetamide reagent to produce tert-butyldimethylsilyl derivatives of TCP and the internal standard. The samples were analyzed by GLC with mass selective detection using five external calibration blanks. The detection limit for TCP in urine was 3 to 5 ppb.

TCP was reported as $\mu$g/g creatinine (Pacific Toxicology Laboratories Standard Operating Procedure, 1999). Daily urine volume was estimated using a creatinine correction (1.7 g and 1.0 g creatinine/day for adult males and females, respectively). Creatinine levels were assigned to children assuming an increase of 0.08 g creatinine/day for each year of age 2 though 12 (ICRP, 1975). Adult values were used for subjects aged 13 or more. Chlorpyrifos equivalent dosage ($\mu$g/kg · day) cleared during the three-day post-application monitoring period was estimated without adjustment for background levels and assuming no subsequent chlorpyrifos or TCP exposure:

chlorpyrifos equivalents ($\mu$g/kg BW · day) =
($\mu$g TCP/g creatinine) (FW chlorpyrifos/FW TCP) (g creatinine/day) / (kg BW),

where BW is body weight and FW is formula weight. Chlorpyrifos equivalents measured in the three-day post-application specimens were summed to estimate the exposures resulting from backpack applications. Exposure (primarily dermal) occurred over a short period during the morning of the spray day. We estimated that the monitoring period would represent about 2.7 half-life values (measured levels were multiplied by 1.0/0.85 = 1.2).

## RESULTS AND DISCUSSION

All participants completed the entire course of the study. No adverse effects were reported by either applicators or their family members. No subjects availed themselves of the free medical care offered by the medical doctors on the research team.

The mean pre-application chlorpyrifos equivalents (based upon TCP) were:

- 0.6 ± 0.3 μg/kg BW · day (n = 12) for children,
- 0.2 ± 0.2 μg/kg BW · day (n = 6) for female spouses, and
- 0.4 ± 0.5 μg/kg BW · day (n = 6) for the male handlers (Table 3).

These levels are similar to:

- the mean 0.1 μg/kg BW · day (n = approximately 1000) reported for the general adult population of the United States (Hill et al. 1995),
- the mean 0.1 ± 0.1 μg/kg BW · day (n = 42) reported for Italian adults (Aprea et al. 1999), and
- the mean 0.2 ± 0.3 μg/kg BW · day (n = 182) baseline levels of adults in Personal Chemical Exposure Program monitoring studies (Krieger, unpublished data).

When low amounts of TCP are cleared in urine and there is no history of chlorpyrifos (ethyl- or methyl-) exposure, caution should be exercised in expressing dosage as chlorpyrifos equivalents since it is likely that TCP itself contributes to the findings.

The apparent elimination half-life of TCP for applicators was roughly 36 hours (estimated graphically) when log percent remaining to be eliminated was plotted as a function of days post application. This half-life is greater than the 27 hours reported by Nolan et al. (1984) but less than the 41 hours measured by Griffin et al. (1999). Post-application chlorpyrifos equivalents presented in Table 3 indicate the backpack sprayers who used hand pumps and treated corn had higher exposures than others who used motorized sprayers to treat sesame. When corrected for the amount (g) of chlorpyrifos applied (from Table 2), exposure levels for applicators 3 and 4 were roughly an order of magnitude higher than for the others (Table 3). Further investigation of the work practices associated with manual spraying of corn may be merited.

In the worst case example, the work practices of Applicator 4 included overnight storage of prepared spray mix in his home (an unusual event prompted by a rain storm at the start of his application). This individual also applied the most chlorpyrifos of all. He engaged in nozzle cleaning and other equipment repair using his bare hands. Finally, his application technique thoroughly drenched his work clothes. Even so, these are relatively common occurrences with manual application equipment in Nicaragua.

In most cases, the chlorpyrifos exposures of the applicators' family members were unremarkable. Even applicator helpers (including the two children of applicator 1 and the spouses of applicators 5 and 6) had exposures similar to other persons who did not assist with the fieldwork. In contrast to the other persons in this study, family members of applicator 4 had substantially higher chlorpyrifos exposures. Since this family lived several kilometers from the application field, proximity (which was shown to elevate organophosphate urinary metabolite excretion in family members by Lu et al. 2000), could not have accounted for the exposures. Indeed, of the four families living adjacent to the treated fields, none

**Table 3.** Individual exposure values in chlorpyrifos equivalents.

| Application | Relationship to Applicator | Pre-Application TCP Levels* | Absorbed Dosage* | Absorbed Dosage per Chlorpyrifos Applied** |
|---|---|---|---|---|
| 1 | Self, age 44 | 0.26 | 2.7 | 0.03 |
| | Wife, age 32 | 0.45 | 0.25 | -- |
| | Son, age 9 | 1.04 | 0.85 | -- |
| | Son, age 10 | 0.83 | 0.67 | -- |
| | Daughter, age 11 | 0.59 | 0.72 | -- |
| | Niece, age 13 | 0.55 | 0.70 | -- |
| 2 | Self, age 34 | 1.11 | 4.1 | 0.05 |
| | Wife, age 30 | 0.16 | 0.42 | -- |
| | Daughter, age 7 | 0.33 | 1.2 | -- |
| | Daughter, age 10 | 0.69 | 1.2 | -- |
| 3 | Self, age 33 | 0.09 | 25. | 0.3 |
| | Wife, age 26 | 0.09 | 0.64 | -- |
| | Son, age 9 | 0.18 | 0.52 | -- |
| 4 | Self, age 35 | 0.15 | 130 | 0.6 |
| | Wife, age 35 | 0.18 | 16 | -- |
| | Daughter, age 7 | 0.54 | 48 | -- |
| | Daughter, age 11 | 0.24 | 14 | -- |
| | Son, age 13 | 0.30 | 20 | -- |
| 5 | Self, age 25 | 0.15 | 12 | 0.06 |
| | Wife, age 19 | 0.04 | 0.66 | -- |
| 6 | Self, age 34 | 0.13 | 5.8 | 0.04 |
| | Wife, age 35 | 0.06 | 0.62 | -- |
| | Daughter, age 12 | 0.40 | 0.65 | -- |
| | Daughter, age 14 | 0.16 | 1.0 | -- |

* Expressed in units of $\mu$g chlorpyrifos equivalents/kg BW · day
** Units of $\mu$g chlorpyrifos equivalents/kg BW · g chlorpyrifos applied

showed elevated levels in family members. Additionally, none of the members of family 4 visited the site or participated as helpers during the application.

overnight presence in the home of the fully loaded backpack sprayer. When questioned, Applicator 4 and his wife stressed that they did not use spray mix indoors during the period of this study, although such a practice is not uncommon among persons in the region. It is interesting to note that one of the household members was a 13-year-old male, and pesticide application among rural male Nicaraguans is considered a rite of passage.

Given that Nicaragua does not promulgate pesticide exposure standards, U.S. Environmental Protection Agency guidelines are presented as a reference. A route-specific short-term dermal No Observed Adverse Effect Level (NOAEL) of 5 mg/kg/day for cholinesterase inhibition is 5 mg/kg/day (Smegal, 2000). In the most extreme case (applicator 4), the absorbed dosage resulting from a single day's work was 0.13 mg/kg, roughly 2.5 times above 0.05 mg/kg/day (100-fold less than the NOAEL, a common regulatory goal). For all other applicators, calculated margins-of-exposure are greater than 100-fold. Only the family members of Applicator 4 exceeded an additional Food Quality Protection Act ten-fold safety factor; the worst case was the youngest daughter, nearly ten times higher.

These findings in Nicaragua provide evidence that poor work practices, coupled with manual sprayer applications of chlorpyrifos, may cause applicators to exceed the United States regulatory safety margin. In addition, the apparent use of agricultural chlorpyrifos formulation in the home environment elevated household members above U.S. regulatory levels for children and women of child-bearing age. Based on observations by the research team of additional applications that did not form part of this study, the storage of pesticide formulation in the house, application of agricultural formulations in and around the house, drenching of work clothing during manual sprayer operations, and manual handling of pesticide formulation and spray mix are common in Nicaragua. Further investigation of these practices is needed to determine which practices are most implicated, and the extent to which they elevate family member exposure.

Many developing countries do not restrict use of higher toxicity products, require training in their safe application, or mandate use of personal protective equipment (which is difficult to use in tropical conditions and costly to obtain). However, applicators who customarily accept the risks of pesticide application for themselves may be less willing to do so, knowing that their practices may expose more vulnerable family members. With further information of the type generated by this study, valuable risk communication tools could be developed to protect the entire family, including the applicator.

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## REFERENCES

Aprea C, Betta A, Catenacci G, Lotti A, Magnaghi S, Barisano A, Passini V, Pavan I, Sciarra G, Vitalone V, Minoia C (1999) Reference values of urinary 3,5,6-trichloro-2-pyridinolin in the Italian population—validation of analytical method and preliminary results (multicentric study). J AOAC Int 82:305-312

Aragón A, Aragón C, Thorn A. Pests, peasants, and pesticides on the Northern Nicaraguan Pacific Plain. Int J Occup Environ Health. 2001 Oct-Dec; 7: 295-302

Griffin P, Mason H, Heywood K, Cocker J (1999) Oral and dermal absorption of chlorpyrifos: a human volunteer study. Occup Environ Med 56:10-3

Hill RH Jr, Head SL, Baker S, Gregg M, Shealy DB, Bailey SL, Williams CC, Sampson EJ, Needham LL (1995) Pesticide residues in urine in adults living in the United States: reference range concentrations. Environ Res 71:99-108

International Commission on Radiological Protection (1975) Report of the task group on reference man. ICRP publication 23, Pergamon, Oxford, UK, p 356

Knishkowy B, Baker EL (1986) Transmission of occupational disease to family contacts. American J Industr Med 9:543-550

Ministerio de Salud (1999) Abordaje Integral de la Exposición a Plaguicidas desde los Departamentos, Programa de Plaguicidas, Nicaragua, 8:4-22

Lu C, Fenske RA, Simcox NJ, Kalman D (2000) Pesticide exposure of children in an agricultural community: evidence of household proximity to farmland and take home exposure pathways. Environ Res A 84:290-302

Nolan RJ, Rich DL, Frehour NL, Saunders JH (1984) Chlorpyrifos: pharmacokinetics in human volunteers. Toxicol Appl Pharmacol 73:8-15

Smegal DC (2000) Human health risk assessment: chlorpyrifos, phase 4. U.S. Environmental Protection Agency, Office of Pesticide Programs, Health Effects Division, Washington

Wesseling C, McConnell R, Partanen T, Hogstedt C (1997) Agricultural pesticide use in developing countries: health effects and research needs. Int J Health Serv 27:273-308