Use of a Pharmacokinetic Model to Assess Chlorpyrifos Exposure and Dose in Children, Based on Urinary Biomarker Measurements

Marc L. Rigas, Miles S. Okino, and James J. Quackenboss

National Exposure Research Laboratory, U.S. Environmental Protection Agency, Las Vegas, Nevada 89193–3478

Received October 4, 2000; accepted February 7, 2001

Chlorpyrifos is a common agricultural insecticide and has been used residentially in the United States until the year 2000 when this use was restricted by the U.S. Environmental Protection Agency (U.S. EPA). A chlorpyrifos metabolite, 3,5,6-trichloro-2-pyridinol (TCPy) has been found in urine samples collected during exposure field studies. In this work, we use urinary biomarker data and the inverse solution of a simple pharmacokinetic (PK) model for chlorpyrifos to estimate the magnitude and timing of doses. Three urine samples were collected on separate days from each of 15 children (ages 3–12) who were participants in the Minnesota Children’s Pesticide Exposure Study (MNCPES). The total volume of urine was noted and samples analyzed for TCPy. The urinary data was used along with constraints imposed on dose timing, based on responses of the individuals to pesticide-use surveys. We predicted the time and magnitude of multiple “event” exposures characterized by short-term, relatively high doses superimposed over a continuous background exposure. The average dose of chlorpyrifos predicted by the model was 1.61 μg/kg per reported event. Average background dose rate for these children that reported exposure events was 0.0062 μg/kg/h, or 0.15 μg/kg/day. In addition to predicting the total dose of chlorpyrifos received by an individual from urinary biomarker measurements, the model can then be run in a forward manner once the exposure regime is determined. This will allow the prediction of the total amount of TCPy eliminated in the urine over any time period of interest.

Key Words: chlorpyrifos; exposure; PK modeling; dose, 3,5,6-trichloro-2-pyridinol; biomarker.

Exposure to organophosphate (OP) and carbamate insecticides is of interest to the general public because of their ubiquity in residential environments and in food products. They are of interest to risk scientists and toxicologists as case studies, due to their common mechanism of neurotoxicity through anticholinesterase activity. Chlorpyrifos has been one of the most widely used of the OP insecticides. Over 20 million pounds of chlorpyrifos are used annually in the U.S., with almost an equal split between agricultural and nonagricultural uses (U.S. EPA, 2000). The top agricultural use is on corn. Chlorpyrifos has been one of the top 5 insecticides used in U.S. homes (U.S. EPA, 1997a). Registered nonagricultural uses include termiteicide, turf applications, indoor residential use, outdoor residential perimeter treatment, and pet tick and flea control (U.S. EPA, 2000). Subterranean termiteicide accounts for approximately 20% of total use. Its widespread use is reflected in the fact that urine samples collected from 82% of the individuals in the 3rd National Health and Nutrition Examination Survey (NHANES III) contained detectable levels of 3,5,6-trichloro-2-pyridinol (TCPy), a metabolite of chlorpyrifos (Hill et al., 1995).

Biomarkers of exposure such as chemical metabolites in urine or blood and parent compounds in blood can be used to assess exposure and risk from compounds such as chlorpyrifos. This exposure assessment method is attractive because biomarkers reflect the dose that actually entered the body. In addition, biomonitoring costs less than making measurements in multiple environmental media that would be necessary to characterize exposure. Despite its attractiveness, assessing exposure using only biomarkers also presents difficulties. A metabolite measured in urine must, for example, be specific to the parent toxic agent of interest. Further, the relationship between metabolite concentrations in urine and particular exposure events is often unclear. In field and clinical studies, biological samples are often included as part of a larger set of data collection that includes environmental measurements, but they have rarely been used alone.

The biomarker concentration measured in urine depends upon the magnitude and the time-profile of the exposure, as well as the rates of absorption, metabolism, and excretion. To completely characterize the pattern of excretion and estimate total absorbed dose by mass balance, total urine is often collected over a period of several days following an exposure (Nash et al., 1982; Vaccaro et al., 1996). Ideally, a collection protocol balances subject burden and cost of sample analysis against the uncertainty band associated with various design options. These issues are important in order to improve re-
spouse and compliance rates and to maximize the number of subjects in a study while maintaining the ability to estimate the absorbed dose.

This paper proposes that a pharmacokinetic (PK) model can be used in conjunction with a well-designed field study to characterize exposure and reconstruct chlorpyrifos dose based on limited field urine measurements. We use data collected as part of the Minnesota Children’s Pesticide Exposure Study (MNCPEES), a field study of residential chlorpyrifos exposure in children, to develop and validate the approach. In brief, the PK model is solved in an inverse fashion, such that the solution is not the tissue concentrations or excreted metabolite concentrations, but the timing and magnitude of the initial dose. This solution is constrained using information from questionnaire responses by study participants. As another point of comparison between exposure assessment methods, the urinary output of metabolites measured in the MNCPEES study are compared with those that would be predicted using a chlorpyrifos PK model in conjunction with standard operating procedures and assumptions commonly used to estimate exposure and dose.

Dong et al. (1996) validated a PBPK model for malathion using data from clinical human dosing studies in the literature. They then used the model iteratively for consecutive time intervals between biomonitoring, adjusting the initial conditions at the beginning of each time interval to predict the total dermal absorption of malathion, as if the only information they were given were spot urine samples in time. The goal of our work is different in that we use a simple PK model for chlorpyrifos that was established in the literature for forward simulations in humans. We then implement it as an inverse simulation using constraints from supplementary questionnaire data to calculate not just the total absorbed dose, but the timing and magnitude of individual doses that gave rise to the observed urinary biomarkers.

The U.S. EPA revoked the registration for residential use of chlorpyrifos in 2000. Yet, this compound serves as a good demonstration of our method, because it has been well studied. The MNCPEES also provides a unique combination of biomarker, survey, and environmental information with which to test this method.

METHODS

Minnesota Children’s Pesticide Exposure Study

The 1997 Minnesota Children’s Pesticide Exposure Study (MNCPEES) was small, population-based, and designed to evaluate the feasibility of conducting a multiroute, multipathway study in children. This study was part of the National Human Exposure Assessment Survey (NHEXAS), a unique and comprehensive study using multiple measurement techniques to evaluate human exposure to a number of chemicals (Sexton et al., 1995). While studies such as the National Health and Nutrition Examination Survey (NHANES) and the National Human Adipose Tissue Survey (NHATS) have monitored the body burden of chemicals, no other studies have collected the myriad of associated environmental and exposure measurements being collected in NHEXAS studies. The 3 phases of the MNCPEES study were: (1) identify households with frequent pesticide use and with children of ages 3–12; (2) screen 308 households using a questionnaire and a pesticide use inventory, and finally (3) intensively monitor 102 households/children to estimate multipathway exposure (Quackenboss et al., 2000). Questionnaire responses regarding pesticide use were nonspecific for any particular compound or commercial formulation. However, the duration of an application event was reported in the questionnaire. Sample analysis emphasized the insecticides chlorpyrifos, diazinon, and malathion, selected for their frequent use and availability in multiple environmental media. In the intensive monitoring phase, the researchers measured personal air and other environmental samples. They also obtained hand rinses and duplicate diets for the 102 children. As a measure of internal dose, researchers collected first morning urine voids from the children on days 3, 5, and 7 of the study. They had study participants estimate the time since previous urine void occurred before the night before. The urine collections were analyzed for metabolites of the studied pesticides, including 3,5,6-trichloro-2-pyridinol (TCPy), a specific metabolite of chlorpyrifos. The details of this study are highlighted elsewhere (Quackenboss et al., 2000).

Study participants were not required to provide biological samples. Therefore, complete sets of urinary measurements were not available for all 102 children. In our analysis, we used data from 15 children for whom complete urine measurements were available and whose questionnaire data indicated that the participant was in the vicinity of a pesticide application during the study period, and whose urinary TCPy levels indicated possible chlorpyrifos exposure events.

Exposure Estimates Using Environmental Measurements

Environmental measurements collected in the residences of the MNCPEES were converted into personal exposures and doses using the U.S. EPA standard operating procedures (SOPs) for pesticides (U.S. EPA, 1997b) and other exposure factor assumptions as available.

For inhalation, pesticide concentration from “personal air samples” were used to calculate the daily inhalation dose as:

\[
R_i = C_a \times Q_i
\]

where \( R_i \) = potential dose rate (mass/day), \( C_a \) = ambient concentration (mass/m\(^3\)), and \( Q_i \) = inhalation rate (m\(^3\)/day). Inhalation rates were estimated using the correlations of D. L. Macintosh, J. Xue, and H. Ozkaynak (unpublished report; Buck et al., 2001).

Because chlorpyrifos was not detected in many of the outdoor air samples around the residence, inhalation was only considered to be an exposure route during times when the child reported being in the residence.

Duplicate diets were collected as part of the MNCPEES, and the food was analyzed for chlorpyrifos. The total amount of chlorpyrifos ingested from food was assumed to be the total amount reported from the analysis of the duplicate diets.

It was assumed that daily inhalation exposure was averaged over the 24-h day to result in equal hourly exposures throughout the day. Dermal exposure was only assumed to occur during the time that the study subjects reported being awake.

The assumptions for the exposure estimates imply steady-state chronic exposure in this situation, the average absorption rate must be equal to the average elimination rate, accounting for mass differences between TCPy and chlorpyrifos. We used the assumption that 70% of an oral dose is absorbed (Nolan et al., 1984) and 3% of a dermal dose is absorbed (U.S. EPA, 1997b). Then, the average urinary excretion rate (UER) of TCPy in mg/h is related to the exposure assumptions as

\[
UER = 198.5/350.57(0.03D_p + 0.7R_p + 0.70I_p)/24,
\]

where the molecular weight of TCPy is 198.5 mg/mol and the molecular weight of chlorpyrifos is 350.57 mg/mol. \( D_p \) and \( I_p \) are the daily dermal and
Ingestion doses, respectively. The absorption fraction of 0.7 for respiratory exposures was taken from Buck et al. (2001).

**Dynamic Model**

If multiple urine measurements show widely variable levels of urinary TCPy, it is likely that chlorpyrifos exposure is not at a constant level, but rather that the observed biomarker concentrations are due to recent, nonrepetitive exposure events. A high coefficient of variation between daily urinary metabolite excretion can imply such variable exposure conditions. If these conditions exist, a pharmacokinetic (PK) model may be used to determine a possible dose or range of doses that would lead to the biomarker result observed. The solution of a PK model given an input dosing profile results in a uniquely determined excretion profile of urinary biomarkers. If one is examining urinary biomarkers and using the PK model in a reverse manner to estimate the dosing profile, the solution is not unique. Multiple dosing profiles via different routes could result in the same urinary excretion profile. If the dose profile is constrained by additional information, the inverse model may be solved to reach an optimum dosing profile given the imposed limitations.

PK models range in complexity from single compartment models that approximate the distribution of the chemical in the body, using a volume of distribution ($V_d$) to a model that uses a series of such compartments to represent different tissues in the body. While multicomartment, or physiologically based pharmacokinetic models (PBPK) are more representative of biological conditions and can more accurately predict the tissue distribution of a chemical, a single compartment model requires fewer parameters and often mathematically approximates the absorption and elimination of many chemicals from the body (Medinsky and Klaassen, 1996). The following method utilizes a single compartment model (Nolan et al., 1984) consisting of an absorption “reservoir” from which material is absorbed into the single body compartment. A single first-order elimination rate constant governs removal of material from the body. The parameters for the model were estimated using 6 human subjects (Nolan et al., 1984) and has been verified recently using additional human data (Vaccaro et al., 1996). If $k_a$ and $k_e$ represent the absorption and elimination rate constants, respectively, the model is represented by the following differential equations:

\[
\frac{dC_a}{dt} = -k_a C_a + \frac{SRI_{IA}}{V_d} \frac{M_{TFC}}{M_{TCP}}
\]

\[
\frac{dC_b}{dt} = k_a C_a - k_e C_b
\]

where $C_a$ is the concentration of TCPy in the absorption reservoir (in the stomach or on the skin), and $C_b$ is the body burden concentration of TCPy, or the concentration available systemically. $I_{IA}$ is the background dose rate, and $W$ is the body weight of the individual. $S$ (selectivity) refers to the amount on a molar basis of the absorbed material that can be collected as metabolite of interest. This is assumed to be 0.72, based on Nolan et al. (1984). $R$ is the stoichiometric ratio of chlorpyrifos to TCPy (1:1). $V_d$ is the “volume of distribution,” or the apparent volume that accounts for all the chlorpyrifos burden in the body. $M_{TFC}$ and $M_{TCP}$ are the molecular weights of chlorpyrifos and TCPy, respectively.

The initial conditions in both of these compartments are given by:

\[
\begin{align*}
\frac{C_a(t_0)}{C_a} &= \left(\frac{C_a(t_0) + \frac{SRFD_{bolus} W}{V_d \frac{M_{TFC}}{M_{TCP}}}}{C_a(t_0)}\right) \\
\frac{C_b(t_0)}{C_a} &= \left(\frac{C_a(t_0) + \frac{SRFD_{bolus} W}{V_d \frac{M_{TFC}}{M_{TCP}}}}{C_a(t_0)}\right)
\end{align*}
\]

where $F$ is the fraction absorbed through the oral route, assumed to be 70% based on Nolan et al. (1984) and $D_{bolus}$ is the dose ingested during each exposure event. The solution of a system of two differential equations results in a biexponential expression. In this case, by solving Equations 3 and 4, given Equation 5 results in the following solution, assuming that the background dose is zero, and letting $t_i$ be the time of dosing:

\[
C_a(t) = \frac{k_a D_{bolus} SFRW}{V_d (k_a - k_e) \left(\frac{M_{TFC}}{M_{TCP}}\right)} \left[ e^{-(k_a + k_e)t} - e^{-k_e t} \right]
\]

The average UER of metabolite can be determined theoretically from a single urine collection by integrating the concentration of metabolite in the body from the time of last urination ($t_l$) and the time of current urine collection ($t_i$) as follows:

\[
UER = \frac{k_a V_d}{t_i - t_l} \int_{t_l}^{t_i} C(t) dt
\]

The model-predicted urinary excretion rate can now be calculated by substituting Equation 6 into Equation 7.

\[
UER = \frac{k_a k_e D_{bolus} SFRW}{(k_a - k_e)(t_i - t_l) \left(\frac{M_{TFC}}{M_{TCP}}\right)} \left[ \frac{1}{k_a} e^{-(k_a + k_e)t} - \frac{1}{k_e} e^{-k_e t} \right]
\]

At steady state, the dose rate is equal to the elimination rate. Therefore, the added urinary elimination due to the steady state background may be represented by adding the steady state dose rate to the expression above.

\[
UER = \frac{k_a k_e D_{bolus} SFRW}{(k_a - k_e)(t_i - t_l) \left(\frac{M_{TFC}}{M_{TCP}}\right)} \left[ \frac{1}{k_a} e^{-(k_a + k_e)t} - \frac{1}{k_e} e^{-k_e t} \right] + SRFI_{IA} W \left(\frac{M_{TFC}}{M_{TCP}}\right)
\]

In practice, UER is the total mass of the metabolite in a complete urine void divided by the time over which it accumulated in the bladder. Since this is based on the mass in the sample, variations in concentration due to changing urine water content are eliminated. The measured metabolite concentration in urine ($C_u$) is multiplied by the volume of the void (the entire sample delivered from the bladder, $V_u$) and divided by the duration of time that the void was accumulating in the bladder: collection time ($t_c$) – time of last urination ($t_i$).

\[
UER = \frac{C_u V_u}{(t_c - t_i)}
\]

Equation 10 can be used to estimate UER from the urine samples collected in MNCPES, as questionnaire data allowed the calculation of $t_i$. The results of Equation 8 for the 3 separate days were used as a constraint in Equation 9 to optimize the parameters $D_{bolus}$ and $I_{IA}$ as well as the timing of the dose. The time of exposure was constrained to sometime between 7:00 A.M. and 10:00 P.M. on the days of a reported application. We assumed that the event doses were through oral ingestion, which seems like the most reasonable route based on environmental chlorpyrifos concentrations in residences following non-broadcast treatment (Byrne et al., 1998). We constrained event duration based on the application durations reported in the MNCPES survey. Finally, we fit the data using a nonlinear optimization routine in the MATLAB computational software package (The Mathworks, Natick, MA), incorporating the constraints outlined above. The PBPK model and all of its parameter values can be found in Nolan et al. (1984). Briefly, it assumes that 70% of an oral dose is absorbed, with an absorption rate constant ($k_a$). The inverse methodology for calculating exposures will be presented in more detail by M. S. Okino et al. (manuscript pending).
in preparation), along with techniques for assessing the uncertainty in dose estimates.

As a point of comparison, UER was estimated using the model in a forward sense as in Nolan et al. (1984). The exposure estimates from the environmental measurements were used as inputs, and UER was calculated from the model as an average hourly UER.

RESULTS

There was insufficient environmental measurement information to predict exposure for 2 of the 15 children using the SOPs. For the other 13 children, the steady-state exposures predicted using the SOPs resulted in a generally lower UER than was indicated by the data and the model fit (Fig. 1). Note that the steady state UER predicted by the model is quite often related to the minimum measured UER in the children. This can be seen in Figure 2a where the model-predicted steady state UER (short dashed line) is close to but below any of the measured UER points. Figure 2 highlights an example of the model's predicted exposures and corresponding urinary TCPy excretion data. Figure 2a depicts data points corresponding to the urinary TCPy excretion rate (µg/h) that is calculated directly from the 3 urine samples. In addition, the model-predicted dynamic and steady state (background) urinary TCPy excretion rate is shown over time. This plot results from taking the exposure magnitudes and timing predicted by our inverse PK model (Fig. 2b) and reapplying these as inputs to the forward PK model to calculate urinary TCPy excretion rate. The study participant reported an application of pesticide on day 4. The magnitude of the event dose, determined by the inverse model optimization to be most likely to have caused the observed urinary TCPy measurements, is shown (Fig. 2b). In addition, Figure 2a shows what the inverse PK model predicts to be the urinary TCPy excretion rate resulting from steady-state background exposure as well as that which would result from the steady-state background exposures estimation using the environmental measurements and standard exposure assumptions. The exposure assumptions result in a lower exposure than that which the model predicted. The exposure is too low, in fact, to explain the TCPy excretion rate calculated from the observed data (using Equation 10). Several explanations for this are pursued in our Discussion. Figure 2c depicts the total mass of TCPy that would be present in each urine sample, based on the inverse PK model-derived exposures. These are superimposed upon the actual TCPy measured in the urine samples from the subject.

Figure 3 depicts an increasing trend in UER of TCPy over the study period. This could be attributed to multiple pesticide applications that were reported in this household on days 4 and 6. The predicted dose rates (Fig. 3b) lead to a total absorbed dose of 1.9 µg chlorpyrifos/kg body weight.

Table 1 shows, for all individuals, the model-predicted steady state UER as well as the number of exposure events, and the peak dose predicted by the model for each exposure event. The number of reported events will not necessarily match the number of predicted chlorpyrifos doses. Because questionnaire reporting was not specific to a single pesticide product, it is possible that the product being used did not contain chlorpyrifos. It is also possible that the application event did not lead to an exposure or a dose. The event doses shown in Table 1 for subjects 387 and 406 can be seen graphically in Figures 2b and 3b, respectively. Table 2 shows the model-predicted steady state (background) UER as well as the total model-predicted TCPy eliminated during the study period, beginning with the first event dose. Under only steady state conditions, the total mass absorbed should equal the total mass eliminated. The molecular weight ratio must also be applied such that:

\[
I_{bk} \Delta t = \frac{350.57}{198.5} M_{E,k,p} \]  

As an example, it was estimated that the levels of TCPy in the urine of Subject 427 resulted only from steady-state background exposure and that there were no specific exposure events during the study (Table 1). The body weight of this child was 36.54 kg. Based on the steady state dose of 0.0101 µg/kg/h, \( I_{bk} = 0.369 \) µg/h. Given the information that the total simulated time between the first urine sample (day 3) and the end of the study (day 7) was 96 h, one can solve for \( M \), obtaining 20.05 µg, which agrees closely with the value presented in Table 2 (19.08 µg).

DISCUSSION

The goal of this work was to use a pharmacokinetic model in conjunction with field exposure data to predict the absorbed dose of chlorpyrifos. Other work (Carrier et al., 1999; Nolan et
al., 1984) has used PK models in a “forward” sense to examine the resulting body burden and urinary elimination of metabolites. Here, the model was run in an “inverse” fashion with possible inputs constrained by questionnaire data and implementation of a nonlinear optimization algorithm to predict dosing profiles. The resultant applied dose predictions are similar to exposures and doses measured in clinical studies.

The doses predicted by the inverse PK model were generally higher than those predicted by the environmental measurements and standard assumptions (Fig. 1). This is surprising, as the assumptions are generally expected to be conservative in nature, overestimating exposure and dose. There are several possible explanations for this. It is possible that some of the TCPy measured in urine resulted from ingestion of TCPy directly and not chlorpyrifos. If this were the case, using total measured urinary TCPy to estimate chlorpyrifos dose would lead to an overestimate. While some studies have suggested that this is possible (Fenske et al., 2000), there is insufficient data to estimate the magnitude of this effect. Because of its hydrophilicity, it is unlikely that TCPy will be absorbed into the body following dermal exposure. TCPy would also likely dissolve in respiratory tract lining fluid and be transported out of the respiratory tract before reaching the breathing zone, preventing absorption there. Studies are needed to assess the pharmacokinetics of TCPy absorption from the gut to know whether or not oral TCPy exposures could lead to significant TCPy doses.

Another likely explanation for the low predictions from the direct environmental measurements relative to the model is that we did not attempt to estimate exposure via nondietary ingestion of chlorpyrifos on hands and toys, as none were documented for children in this age group (U.S. EPA, 1997b). It has been reported that nondietary ingestion can represent a significant exposure pathway for children (Hubal et al., 2000).

Our approach assumes that the event doses were a result of ingestion exposure (including the nondietary ingestion due to mouthing of contaminated objects and, perhaps, fingers). This seemed appropriate, as dermal absorption would be too slow to cause the changes in observed urinary TCPy carried over from one urine sample to the next. The steady-state (background) doses (Table 1) predicted by the model ranged from 0.0624 µg/kg/day to 0.24 µg/kg/day. These values are well below the acute oral NOAEL in rats of 0.5 mg/kg/day. They are between 100- and 1000-fold below the chronic oral and dermal NOAEL in rats of 0.03 mg/kg/day (U.S. EPA, 2000). If one considers the recommended 100-fold uncertainty factor applied to the animal-derived NOAELs, it would indicate that the model-

FIG. 2. (A) Model-predicted urinary excretion rate (UER) for one subject (solid line) superimposed over UER predicted from spot urine samples (data points). The model-predicted steady-state exposure (short dashes) is higher than that predicted by the exposure model (long dash). (B) The model-predicted dose rate, highlighting a single short exposure event above background. (C) The model-predicted simulation of the urinary TCPy concentration for each of the 3 urine samples collected in the MNCPE study, based on the model-predicted exposure events (connected by lines). The actual urinary TCPy concentration measurements are shown as single points.
predicted high-end background exposures in these children could approach a point of concern.

Vaccaro et al. (1996) examined exposures of adults to chlorpyrifos while they were actively moving around on freshly treated turf for 4 h. In their study, urine was monitored for TCPy concentrations for several days after exposure, and blood was monitored for cholinesterase inhibition. The surface area of body parts and other appropriate exposure factors were scaled down to values relevant for children, in order to extrapolate to a child’s exposure. Urinary TCPy measurements from Vaccaro et al. (1996) were 2 orders of magnitude higher than that measured in the MNCPES. Again, it should be recognized that the individuals in the study were adults. In addition, significant amounts of chlorpyrifos were measured in the air above the treated turf, suggesting that inhalation was a significant route of exposure. In the residential environment, chlorpyrifos is applied in smaller quantities than in the outdoors and is usually applied around cracks and crevices. Here, it can be expected there will be less volatilization and suspension, so inhalation will play a more minor exposure role.

Vaccaro and colleagues (1996) assumed that ingestion would be a minor route of exposure for adults during the 4-h period of their study, but this is relative to their high inhalation exposures in the outdoor environment following broadcast. Nonetheless, this group estimated, based on their study, that hand-to-mouth activity might result in orally ingested chlorpyrifos doses of 0.72–5.98 μg/kg. This corresponds reasonably well to the predicted doses of chlorpyrifos by children in the MNCPES, using our approach (as shown in Table 1). This at least suggests that the model assumptions of several oral dosing events (perhaps from hand-to-mouth activity) are reasonable and that the amount of chlorpyrifos that must therefore be ingested from a child’s hand is also reasonable. The mean amount of TCPy excreted (Mₑ₁₀₀) in the 8 subjects studied by Vaccaro et al. (1996) was 199 μg/kg. This was almost 3 orders of magnitude higher than the mean value for Mₑ₁₀₀ in this study of 0.55 μg/kg, suggesting that the deliberate exposure and scripted activities in the Vaccaro study resulted in higher exposure than that experienced by the 15 children in MNCPES during their normal daily routine.

Byrne et al. (1998) studied residents of 3 homes over a 10-day period following a crack and crevice application of chlorpyrifos. They attempted to assess the magnitude of respiratory, dermal, and oral exposure. They collected total urine samples over the 10-day period in each of their adult subjects. The excreted TCPy predicted by our model is about 4 times higher than that excreted by the adult subjects on a body weight basis (Byrne et al., 1998). This is probably due to the lower body weight of children (2–4 times lower) and the fact that children can experience higher internal body burdens resulting from the same external exposure (Hubal et al., 2000). Byrne et al. (1998) also estimated, based on environmental measurements, the absorbed dose in a child following the crack and crevice treatment. They estimated between 0.26 and 2.10 μg/kg. These estimates are similar to the values of 0.36–4.01 μg/kg predicted by our model over an approximate 4-day study period.

Using urinary biomarkers in a PK model to solve for the model inputs is one of a class of mathematical problems known as “inverse problems.” These are inherently difficult, as the solutions to such problems are not unique. Inputs via multiple routes and different temporal distributions of inputs could result in the same urinary biomarker concentration. Nonetheless, as we have demonstrated here, the problem can often be constrained to give plausible answers that explain and help interpret what has been observed. In more complex inverse problems, this has been done using Bayesian techniques, and it
may be possible to apply similar techniques to biomarker interpretation problems as well (Schmidt et al., 1999). If the absorption and elimination kinetic parameters are robust, the method we have applied here, utilizing a simple pharmacokinetic model and intuitive application of the prior data can estimate the applied and absorbed doses of a compound. Although we did not demonstrate this here, it would also be possible to differentiate exposure that is predominantly via a slow absorption route (dermal) vs. that via a rapidly absorbing route (inhalation or ingestion). Examining multiple urine samples over time and investigating the coefficient of variation between successive samples could do this.

In the case of extremely young children and infants, hand-to-mouth activity may result in multiple exposures via ingestion of material collected on the skin. Hubal et al. (2000) reviewed some of the literature regarding the frequency of hand-to-mouth activity and other types of dermal contacts that result in exposure. Activity patterns of children, using frame-by-frame videotape analysis, has begun, in the appropriate age group, to lead to estimates of the duration of this activity. Zartarian et al. (1997) videotaped each of 4 children separately for approximately 8 h each. There were 2 males (ages 2 and 4) and 2 females (ages 2 and 4). Activities leading to dermal exposure and hand-to-mouth activities resulting in nondietary ingestion were quantified by an operator using a cataloguing software package developed by the authors. Among the 4 children, the amount of time spent in a hand-to-mouth activity ranged from 0.2 to 2.3% of the total time. The minimum and maximum were in 2 children of the same age. Further work is needed to see if it is possible to establish a distribution of frequency and duration of hand-to-mouth activity that might occur in children of various age groups, in order to assess whether this could be incorporated realistically into an exposure model. One can assume a periodic oral dosing.

### TABLE 1
**Exposure and Dose of Chlorpyrifos Predicted by Inverse PK Model**

<table>
<thead>
<tr>
<th>Subject</th>
<th>BW</th>
<th>Uses reported</th>
<th>Modeled background</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>Total dose</th>
</tr>
</thead>
<tbody>
<tr>
<td>344</td>
<td>38.4</td>
<td>2</td>
<td>0.0026</td>
<td>0.2337</td>
<td>2.07</td>
<td></td>
<td></td>
</tr>
<tr>
<td>359</td>
<td>15.8</td>
<td>2</td>
<td>0.0032</td>
<td>0.69</td>
<td>1.22</td>
<td></td>
<td></td>
</tr>
<tr>
<td>361</td>
<td>38.3</td>
<td>1</td>
<td>0.0020</td>
<td>0.02</td>
<td>0.36</td>
<td></td>
<td></td>
</tr>
<tr>
<td>363</td>
<td>25.8</td>
<td>1</td>
<td>0.0049</td>
<td>0.82</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>367</td>
<td>21.8</td>
<td>3</td>
<td>0.0037</td>
<td>0.41</td>
<td>0.34</td>
<td>1.17</td>
<td></td>
</tr>
<tr>
<td>369</td>
<td>22.5</td>
<td>1</td>
<td>0.0073</td>
<td>2.79</td>
<td></td>
<td></td>
<td>4.01</td>
</tr>
<tr>
<td>373</td>
<td>31.5</td>
<td>2</td>
<td>0.0061</td>
<td>0.0144</td>
<td>1.20</td>
<td></td>
<td></td>
</tr>
<tr>
<td>374</td>
<td>32.8</td>
<td>3</td>
<td>0.0037</td>
<td>0.05</td>
<td>0.72</td>
<td>1.06</td>
<td></td>
</tr>
<tr>
<td>376</td>
<td>35.3</td>
<td>1</td>
<td>0.0035</td>
<td>0.43</td>
<td></td>
<td></td>
<td>1.44</td>
</tr>
<tr>
<td>387</td>
<td>48.3</td>
<td>1</td>
<td>0.0016</td>
<td>0.33</td>
<td></td>
<td></td>
<td>0.59</td>
</tr>
<tr>
<td>406</td>
<td>23.5</td>
<td>2</td>
<td>0.0056</td>
<td>0.59</td>
<td>0.39</td>
<td>1.92</td>
<td></td>
</tr>
<tr>
<td>415</td>
<td>21.2</td>
<td>1</td>
<td>0.0113</td>
<td></td>
<td></td>
<td></td>
<td>1.9</td>
</tr>
<tr>
<td>420</td>
<td>27.6</td>
<td>1</td>
<td>0.0156</td>
<td>0.18</td>
<td></td>
<td></td>
<td>2.8</td>
</tr>
<tr>
<td>427</td>
<td>36.5</td>
<td>1</td>
<td>0.0101</td>
<td>1.69</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>431</td>
<td>20.4</td>
<td>1</td>
<td>0.0111</td>
<td>0.0001</td>
<td>1.86</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Note.** BW, body weight (kg). Modeled background values given in µg/kg/h. All doses are stated in µg/kg. Each event dose corresponds to a 48-h time period between urine sample collection. Event dose 1 occurred between days 1 and 3, event dose 2 between days 3 and 5, etc. Each reported application does not necessarily lead to an exposure and dose.

### TABLE 2
**TCPy Elimination Predicted in Subjects in the Minnesota Children’s Pesticide Exposure Study (MNCPES) between Days 3 and 7**

<table>
<thead>
<tr>
<th>Subject</th>
<th>Model-predicted UER</th>
<th>Total eliminated TCPy (Mₑₛₑₜ)</th>
<th>Eliminated TCPy/W</th>
</tr>
</thead>
<tbody>
<tr>
<td>344</td>
<td>0.0450</td>
<td>21.95</td>
<td>0.7206</td>
</tr>
<tr>
<td>359</td>
<td>0.0300</td>
<td>9.01</td>
<td>0.5356</td>
</tr>
<tr>
<td>361</td>
<td>0.0539</td>
<td>5.61</td>
<td>0.1175</td>
</tr>
<tr>
<td>363</td>
<td>0.0637</td>
<td>6.18</td>
<td>0.2665</td>
</tr>
<tr>
<td>367</td>
<td>0.0403</td>
<td>7.85</td>
<td>0.4110</td>
</tr>
<tr>
<td>369</td>
<td>0.0748</td>
<td>29.34</td>
<td>1.6136</td>
</tr>
<tr>
<td>373</td>
<td>0.1575</td>
<td>16.64</td>
<td>0.3661</td>
</tr>
<tr>
<td>374</td>
<td>0.0767</td>
<td>16.11</td>
<td>0.4429</td>
</tr>
<tr>
<td>376</td>
<td>0.0813</td>
<td>15.17</td>
<td>0.3708</td>
</tr>
<tr>
<td>387</td>
<td>0.0613</td>
<td>14.85</td>
<td>0.2177</td>
</tr>
<tr>
<td>406</td>
<td>0.0714</td>
<td>13.04</td>
<td>0.5738</td>
</tr>
<tr>
<td>415</td>
<td>0.0930</td>
<td>8.93</td>
<td>0.6136</td>
</tr>
<tr>
<td>420</td>
<td>0.3500</td>
<td>36.44</td>
<td>0.9215</td>
</tr>
<tr>
<td>427</td>
<td>0.1967</td>
<td>19.08</td>
<td>0.5522</td>
</tr>
<tr>
<td>431</td>
<td>0.1281</td>
<td>12.30</td>
<td>0.6014</td>
</tr>
</tbody>
</table>

Mean ± SD 15.50 ± 8.58 0.55 ± 0.36

**Note.** Model-predicted UER, steady-state urinary excretion rate (µg/h). Total eliminated TCPy (Mₑₛₑₜ) is given in µg. Eliminated TCPy/W is given in µg/kg.
pattern resulting from hand-to-mouth activity if one has an idea of how often this occurs. Then, the inverse PK model can be solved to determine what the magnitude of those doses must be to lead to either the background or event doses observed in urine.

**Conclusion**

Typically, PK models are only used to calculate the internal dose of a chemical or the amount of chemical or metabolite eliminated over a certain time period. We have demonstrated a method by which a PK model may be used to back-estimate the magnitude and timing of exposure doses in environmental public health field studies. In such studies, resource constraints as well as difficulties maintaining participant compliance limited the extent of monitoring that could be done. It is also unrealistic to expect subjects (especially children) to collect total urine over a study period, the method that would be required to estimate exposures with the least uncertainty. Methodology, as we have described here, will help researchers analyze the limited data collected in field studies to estimate timing and magnitudes of exposures. While simply knowing body burden is useful from a toxicological standpoint when trying to mitigate the exposures, it is necessary to know where and when the significant exposures occurred. This method can be used not only for pesticides but for other chemicals and situations as well. In fact, we have plans to test this method in future studies, which we will design a priori to collect the time of supplemental activity information that might be needed to properly apply this methodology; for example, the approximate time of use of the products containing the chemicals of interest. In this work, we tested the method using data from a previously designed and completed field study. There is no way to directly verify the estimates of exposure magnitude and timing that we predicted. However, as we have discussed, our predictions are similar to measurements of exposure during intentional or forced exposure scenarios in adults.

**ACKNOWLEDGMENT**

The U.S. Environmental Protection Agency, through its Office of Research and Development, funded the research described here.

**REFERENCES**


