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Worker Exposures to Triclopyr: Risk Assessment Through Measurements in Urine Samples

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In the province of Quebec (Canada), the phytocide Garlon 4®, whose active ingredient is triclopyr, is often used to prevent trees from reaching electrical conductors. The object of this paper is to assess the potential health risks in workers coming into contact with Garlon 4®. Ten workers collected their urine during the 22 h following the beginning of a work shift. Measured urinary amounts of triclopyr varied between 1 and 13 mg. The absorbed daily doses were estimated from the amounts of triclopyr in urine through the use of a kinetic model that links the rates of triclopyr elimination to absorbed doses. These estimated doses were compared with the no-observed-effect level (NOEL) observed in rats: 5 mg per kg of body weight. The upper-bound estimations of the worker's daily absorbed doses were found to be 13.3% or less of the rat NOEL.

Keywords: biomonitoring; Garlon 4®; health risk assessment; occupational exposure; triclopyr

INTRODUCTION

Garlon 4® is a phytocide that is used to control the undesirable growth of woody plants and broadleaf weeds. The active ingredient of Garlon 4® is triclopyr (3,5,6-trichloro-2-pyridinyloxyacetic acid), which is present as a butoxyethyl ester form with a concentration of 61.6%. Its mode of action is similar to that of auxin, a natural growth-regulating hormone found in plants, which results in acceleration of the plant's maturation. Since the plant's energy supply is not sufficient to support this rapid growth, the plant dies prematurely. Garlon 4® is used on a variety of sites, in particular on power line rights-of-ways to reduce the risk of power outages and to allow safe access to transmission facilities. In the province of Quebec (Canada), this phytocide is, together with Tordon 101®, the principal product applied in summer for controlling the growth of trees capable of reaching electrical conductors. Concern for the workers applying the pesticides led the electric utility company to undertake a field study in 2001 to assess the potential occupational health risks from the seasonal application of Garlon 4®.

To evaluate occupational exposure to pesticides, biological monitoring through collection and analysis of urine to establish levels of the parent substance or its metabolites is a direct and accurate means. It integrates all routes of exposure and, compared with environmental monitoring, is independent of the absorption fractions of exposed surfaces, the protective equipments used and the climatic conditions. The use of biomarkers to assess health risks is, however, possible only if the link between their levels in urinary samples and the critical biological effects is established. This can be achieved by a two step procedure: first, relating absorbed dose to biomarkers levels with a validated toxicokinetic model; second, comparing this absorbed dose with a toxicity dose established from observed biological effects. This procedure, retained for the health risk assessment of triclopyr, was already being used for other pesticides (Carrier and Brunet, 1999; Bouchard *et al*., 2003; Gosselin *et al*., 2005). Since triclopyr, once absorbed by humans, is principally

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excreted without biotransformation (Carmichael *et al*., 1989), the suitable biomarker for the biological monitoring of Garlon 4® is triclopyr itself in urine. The object of this paper is to assess the health risks incurred by workers through measurements of the amounts of triclopyr in their urine.

METHODS

Field study

Studied workers. At the end of June 2001, 10 male workers were recruited to participate in a survey of biological monitoring. From June to August 2001, these workers sprayed Garlon 4® under high voltage transmission lines in the province of Quebec. Eight of them applied diluted Garlon 4® with a backpack unit directly on the stumps of recently cut trees. The diluted solution in the backpack unit was 20% Garlon 4® and 80% mineral oil. Dilution manipulations were carried out by the managers of the project; each worker loaded his backpack unit with this solution. The personal protective equipment of these eight workers were long pants, rubber boots and a helmet. Their work schedule was an 8 h day with two 15 min breaks in the morning and in the afternoon and a 30 min lunch break. The two other workers pulverized diluted Garlon 4® over the leaves under transmission lines from a tractor-mounted boom spray. The tractor reservoir was filled by these two workers with 12.6 l of Garlon 4® and 1800 l of water. These two workers wore rubber boots, gloves, an overall and a helmet. Their work schedule was an 11.5 h day with two 15 min breaks in the morning and a 30 min break in the afternoon.

Urinary sample collection. On the final day of a 5 day work week, the workers were instructed to collect all their urine into a plastic container from the start of their workday (8 o'clock) until the first micturition of the next day (6 o'clock). Thus the amounts of triclopyr measured in these samples represented the cumulative urinary excretion of triclopyr during the 22 h period from the beginning of that day's exposure. The body weight of each worker was recorded at the same time.

Urine samples were analysed at the Laboratoire du Centre de toxicologie humaine of the Institut national de santé publique du Québec which is accredited by the Canadian Association for Environmental Analytical Laboratories. Samples were kept frozen at −20°C until analysis. The sum of free and conjugated triclopyr was measured after acidic hydrolysis and extraction with a hexane/ether mixture. The extracts were evaporated to dryness and redissolved in a 25% acetonitrile:75% ammonium acetate solution. The samples were then analysed using a Micromass Quattro liquid chromatography–electrospray ionization–mass spectrometry (LC–ESI–MS) system operated in negative MRM mode. The LC system was equipped with a Waters Symmetry C18 column (5 μm, 50 mm × 2.1 mm). The limit of detection of triclopyr was 0.3 $\mu g\, l^{-1}$ and the limit of quantification was 0.9 $\mu g\, l^{-1}$. Average recovery of triclopyr from urine samples spiked with 10 $\mu g\, l^{-1}$ of a reference standard was 97%. Intra-day coefficient of variation for replicate analysis of the same urine sample spiked with 2 $\mu g\, l^{-1}$ of reference standard was 4% ($n = 10$ samples).

Health risk analysis

Determination of the daily absorbed dose. The daily absorbed dose of triclopyr for each worker was calculated from the following equation:

$$\text{Daily absorbed dose} = \frac{\text{Cumulative amount of triclopyr in the urine collected over } T \text{ hours}}{F_{\text{exc}}(T)} \quad (1)$$

where $F_{\text{exc}}(T)$ is the fraction of the daily absorbed dose of triclopyr recovered in the cumulative urinary sample during T hours following the onset of exposure.

The results of the pharmacokinetic study by Carmichael *et al*. (1989) were adapted to estimate $F_{\text{exc}}(22\text{ h})$. These authors exposed five healthy male volunteers to triclopyr via the oral and dermal routes. The dietary doses were 0.1 mg per kg of body weight (mg kg^{-1} b.w.) followed 3 weeks later by 0.5 mg kg^{-1} b.w. of triclopyr. The five volunteers were later exposed to a dermal dose equivalent of 3.7 mg kg^{-1} b.w. of triclopyr. After each administration, blood and urinary samples were collected over a period of 72 h. To fit these time-course data, Carmichael *et al*. (1989) used an open two-compartment pharmacokinetic model. A different set of model parameters was estimated for each administration and for each volunteer.

The original kinetic model of Carmichael *et al*. (1989) was modified in order to assess, for the gradual exposure occurring in typical work shifts, the absorbed dose starting from cumulative amounts of triclopyr excreted in urinary samples collected during the given period of time (see Fig. 1). Since the skin and the lungs are the significant route of entries for workers exposed to triclopyr (Middendorf *et al*., 1994), two functions were included in the model to describe the input dose per unit of time bioavailable at each site of absorption [$g_{\text{skin}}(t)$ and $g_{\text{lungs}}(t)$]. These two time dependent input functions allow one to simulate the different timetables of exposure encountered in occupational conditions. For skin absorption, an input compartment was necessary to account for the accumulation in skin followed by the slow release of triclopyr into the blood stream. On the other hand, simulations show that it is not necessary to add an input compartment for lungs, since the absorption is

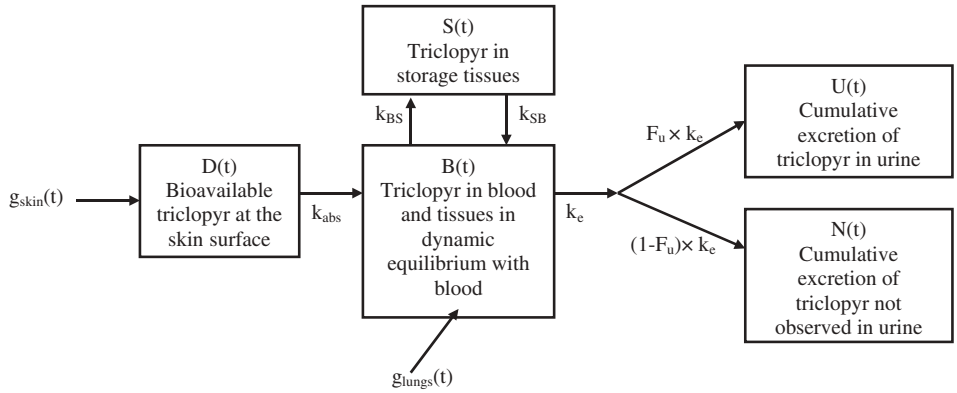

Fig. 1. Modified model of the kinetics of triclopyr developed by Carmichael *et al*. (1989). Symbols are described in Table 1.

too rapid to observe a sizable time delay to the blood stream; the pulmonary absorption is in fact similar to a drop-by-drop intravenous exposure. The differential equation system for this kinetic model is described in the Appendix. The model simulations were performed by solving this system numerically using the Runge–Kutta method incorporated in MathCad 2000 Professional.

In the current study, absorption of triclopyr occurs gradually during the work day and for five consecutive days; the input functions used to simulate this occupational exposure were based on the specified work schedules for each of the two groups of workers, those with a backpack unit and those using a tractor-mounted boom spray. Estimations of $F_{exc}(22\ h)$, specific to each work schedule, were obtained by solving the differential equation system with the appropriate input conditions (see the Appendix). For a unit of absorbed dose, under a given timetable of exposure, the numerical solution of the model predicts a cumulative amount of triclopyr to be observed in the urine collected during the 22 h following the beginning of exposure. From equation (1), the relevant $F_{exc}(22\ h)$ is then easily obtained.

Since the relative contributions of the dermal route and the pulmonary route cannot be determined by a simple measurement of triclopyr in urine, model simulations with input functions $g_{skin}(t)$ and $g_{lungs}(t)$ giving different fractions of the daily absorbed dose arising from the skin and from the lungs (f_{skin} and f_{lungs}) were carried out in order to determine their impact on outcome in urine. For each choice of f_{skin} and f_{lungs}, the daily absorbed dose, the peak of blood concentration and the area under the blood concentration–time curve were estimated by simulating an 8 h exposure to triclopyr without break time resulting in a 22 h cumulative urinary excretion of triclopyr equal to the average of triclopyr measured in the urinary samples of workers.

In model simulations, the sets of parameters describing the internal kinetics of triclopyr ($k_{SB}, k_{BS}, k_e, F_u, V_d$, see the descriptions in Table 1) were estimated from the time courses of triclopyr in each of the five volunteers orally exposed to 0.1 and 0.5 mg kg^{-1} b.w. of triclopyr in the study by Carmichael *et al*. (1989). The rates of dermal absorption (k_{abs}) used were estimated from the time courses of dermally absorbed triclopyr in the corresponding volunteers. An average set of parameters was also obtained from the average of these 10 individual datasets. Consequently, for each assumed value of skin versus lungs fractions contributing to daily dose (f_{skin} and f_{lungs}), the parameter sets derived from the individual sets presented by Carmichael *et al*. (1989) enabled 11 estimations of $F_{exc}(22\ h)$ for the workers with backpack units as well as for the workers with a tractor-mounted boom spray.

Determination of the toxicity dose. To assess the health risks associated with occupational exposure to triclopyr, the estimated daily absorbed dose was compared with the no-observed-effect level (NOEL) proposed by the US EPA for chronic exposure (US EPA, 1998). The critical effect used to define it is an increased incidence of proximal tubular degeneration of the kidneys in P_1 and P_2 parental rats observed in a two-generation dietary reproduction study in Sprague–Dawley rats (US EPA, 1998). In that experimental study, 30 rat couples per dosage were exposed to 0, 5, 25 and 250 mg kg^{-1} b.w. per day of triclopyr. The first and second generations P_1 and P_2 ingested triclopyr during 10 and 12 weeks before mating, respectively. The NOEL was found to be 5 mg kg^{-1} b.w. per day and the lowest-observed-effect level (LOEL) was 25 mg kg^{-1} b.w. per day. The reference dose (RfD) recommended by the US EPA is determined by dividing this NOEL by a safety factor of 100-fold to take into account the animal-to-human extrapolation and the inter-individual variability (US EPA, 1998).

RESULTS

Field study

The amounts of triclopyr were measured, for each of the 10 workers, in the cumulative urine collected

Table 1. Symbols used in the conceptual and functional representation of the modified kinetics model of triclopyr developed by Carmichael *et al*. (1989)

Symbols	Description
Variables	
$g_{skin}(t)$	Dermal dose bioavailable per unit of time which can describe different timetables of exposure (mg kg^{-1} b.w. per hour)
$g_{lungs}(t)$	Inhaled dose bioavailable per unit of time which can describe different timetables of exposure (mg kg^{-1} b.w. per hour)
$D(t)$	Burden of triclopyr at the skin surface as a function of time (mg kg^{-1})
$B(t)$	Burden of triclopyr in blood and tissues in dynamical equilibrium with blood as a function of time (mg kg^{-1})
$S(t)$	Burden of triclopyr in storage tissues as a function of time (mg kg^{-1})
$U(t)$	Cumulative excretion of triclopyr in urine as a function of time (mg kg^{-1})
$N(t)$	Cumulative excretion of triclopyr not observed in urine as a function of time (mg kg^{-1})
Parameters	
k_{abs}	Dermal absorption rate constant of triclopyr (i.e. transfer rate from skin surface to blood) (h^{-1})
k_{BS}	Blood to storage tissues transfer coefficient of triclopyr (h^{-1})
k_{SB}	Storage tissues to blood transfer coefficient of triclopyr (h^{-1})
k_e	Elimination rate constant of triclopyr (h^{-1})
F_u	Total fraction of the absorbed dose of triclopyr eventually excreted in urine
V_d	Apparent volume of the blood compartment $B(t)$ (ml kg^{-1})
f_{skin}	Fraction of the daily absorbed dose from the dermal route
f_{lungs}	Fraction of the daily absorbed dose from the pulmonary route

Table 2. Amounts of triclopyr measured in the 22 h cumulative urinary samples of the workers and their body weights

Workers	Amount of triclopyr measured in the 22 h cumulative urine sample (mg)	Body weight (kg)
1[a]	2.71	77
2[a]	2.40	77
3[a]	4.23	68
4[a]	1.04	81
5[a]	12.98	75
6[a]	1.36	73
7[a]	3.04	75
8[a]	5.54	91
9[b]	3.61	73
10[b]	5.27	66

[a]Workers applying Garlon 4® with the backpack unit.
[b]Workers pulverizing Garlon 4® with the tractor-mounted boom spray.

during 22 h following the onset of a work-day exposure. These data and their body weights are presented in Table 2. The average of the measured urinary amounts is 56.4 μg kg^{-1} b.w.

Health risk analysis

Table 3 presents the exposure parameters estimated from model simulations where the contributions by the dermal absorbed route and the pulmonary route vary. Due to the small rate of dermal absorption, the estimation at the peak of blood concentration is lowest when the absorption occurs only via the skin whereas the estimations of the daily absorbed dose and the area under the blood concentration–time curve are highest.

The two exposure scenarios, the one for the eight workers with a backpack unit and the other for the two workers with tractor-mounted boom spray, were simulated with the kinetics model presented in Fig. 1 and the appropriate input conditions presented in the Appendix. For each exposure scenario, 11 time courses of triclopyr urinary excretion were calculated using each of the 10 individual sets of model parametric values and one set obtained from the average of these 10 individual sets. These simulations were carried out assuming that absorption occurred only through the skin, because as shown from Table 3, this assumption results in an estimated daily absorbed dose that is the highest for a given urinary excretion of triclopyr. This ensures that the worst-case scenario is retained. Figure 2 depicts the time courses of the cumulative urinary excretion of triclopyr expressed as the fraction of daily absorbed dose [i.e. $F_{exc}(t)$] predicted for the eight workers with a backpack unit. The curves shown in Fig. 2 are obtained from sets of model parametric values which give the lowest and highest values of F_{exc}(22 h) and from the average parameter set. Fig. 2 exhibits the three estimated values for the fraction of the absorbed dose recovered in the 22 h urinary samples: 0.259, 0.349 and 0.477. Similarly, by simulating the work timetable of the two workers who pulverised Garlon 4® with the tractor-mounted boom spray, the estimated values of F_{exc}(22 h) are: 0.230, 0.314 and 0.433.

The absorbed dose for each worker was calculated starting from their urinary biomarker level

Table 3. Effect of variations in the relative contributions of the dermal and pulmonary routes on model predictions of different exposure parameters following an 8 h exposure to triclopyr resulting in a 22 h cumulative urinary excretion of triclopyr equal to 56.4 μg kg^{-1}

Exposure parameters	Fraction of the daily absorbed dose from the dermal route (f_{skin}) and from the pulmonary route (f_{lungs})				
	$f_{skin} = 1$ $f_{lungs} = 0$	$f_{skin} = 0.75$ $f_{lungs} = 0.25$	$f_{skin} = 0.5$ $f_{lungs} = 0.5$	$f_{skin} = 0.25$ $f_{lungs} = 0.75$	$f_{skin} = 0$ $f_{lungs} = 1$
Daily absorbed dose of triclopyr (mg kg^{-1} b.w.)[a]	0.162	0.124	0.101	0.085	0.073
Peak of blood concentration of triclopyr (mg l^{-1})[b]	0.077	0.088	0.106	0.118	0.127
Area under the blood concentration–time curve of triclopyr, (mg $l^{-1} \times$ h)[c]	2.802	2.156	1.751	1.474	1.273

The model simulations were carried out with the parametric set obtained from the average of each individual parametric set of Carmichael *et al*. (1989) (i.e. $k_{abs} = 0.04\ h^{-1}$; $k_{BS} = 0.14\ h^{-1}$; $k_{SB} = 0.28\ h^{-1}$; $k_{BU} = 0.30\ h^{-1}$; $F_u = 0.81$, $V_d = 194.1$ ml kg^{-1}).

[a]$$\text{Daily absorbed dose} = \frac{0.56\ \mu g\ kg^{-1} \text{b.w.}}{\text{Simulated fraction of the daily absorbed dose recovered in the 22 h urinary samples}}.$$

[b]The blood concentration–time curve of triclopyr $C_{blood}(t)$ is obtained by the following equation:

$$C_{blood}(t) = \frac{B(t)}{V_d}.$$

[c]The area under the blood concentration–time curve of triclopyr (AUC) is obtained by the following equation:

$$\text{AUC} = \int_0^\infty C_{blood}(t)dt.$$

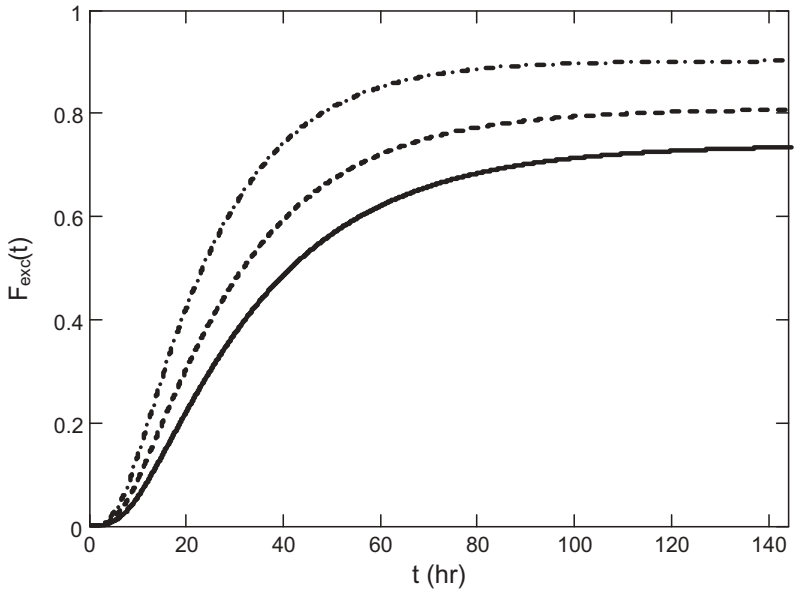

Fig. 2. Model simulations of the time course of cumulative excretion of triclopyr in urine (expressed as a fraction of the daily absorbed dose) following the onset of an 8 h day with two 15 min breaks in the morning and in the afternoon and a 30 min break for lunch with absorption only through the skin: (——) $k_{abs} = 0.04\ h^{-1}$, $k_{BS} = 0.13\ h^{-1}$, $k_{SB} = 0.46\ h^{-1}$, $k_e = 0.19\ h^{-1}$, $F_u = 0.74$; (—·—·—) $k_{abs} = 0.06\ h^{-1}$, $k_{BS} = 0.13\ h^{-1}$, $k_{SB} = 0.23\ h^{-1}$, $k_e = 0.35\ h^{-1}$, $F_u = 0.90$; (– – –) $k_{abs} = 0.04\ h^{-1}$, $k_{BS} = 0.14\ h^{-1}$, $k_{SB} = 0.28\ h^{-1}$, $k_e = 0.30\ h^{-1}$, $F_u = 0.81$.

presented in Table 2 and the above estimations of F_{exc}(22 h). Table 4 gives the lower- and upper-bound estimates of the daily absorbed dose for each worker in addition to the value obtained from the average parameter set. For each worker, these three estimations of the daily absorbed dose were divided by their body weight, for comparison with the rat NOEL. Table 5 presents the results of this comparison. The upper-bound estimations of the workers daily absorbed doses were found to be 13.3% or less of the rat NOEL. On the other hand, only worker number 4 showed an absorbed daily dose lower than the RfD, since there is a 100-fold safety factor included in this criteria.

Table 4. The lower bound, the mean and the upper-bound estimates of the daily absorbed dose for each worker assuming that the absorption occurs only via the skin

Workers	Estimates of the daily absorbed dose (mg)[a]		
	Lower-bound	Mean	Upper-bound
1[b]	5.69	7.77	10.46
2[b]	5.03	6.87	9.24
3[b]	8.88	12.13	16.32
4[b]	2.19	2.99	4.02
5[b]	27.23	37.18	50.04
6[b]	2.85	3.89	5.24
7[b]	6.37	8.70	11.71
8[b]	11.63	15.87	21.36
9[c]	8.33	11.49	15.69
10[c]	12.16	16.78	22.91

[a]The estimations were calculated using the following equation:

$$\text{Daily absorbed dose} = \frac{\text{Amounts of triclopyr measured in the 22 h urinary samples}}{\text{Fraction of the daily absorbed dose recovered in the 22 h urinary samples}}$$

[b]The lower-bound, the mean and the upper-bound estimates of the absorbed dose were calculated with three values of the simulated fraction of the absorbed dose recovered in the 22 h urinary samples: 0.477, 0.349 and 0.259.
[c]The lower-bound, the mean and the upper-bound estimates of the absorbed dose were calculated with three values of the simulated fraction of the absorbed dose recovered in the 22 h urinary samples: 0.433, 0.314 and 0.230.

Table 5. Comparison of the lower-bound, mean and upper-bound estimates of daily absorbed dose (mg kg^{-1} b.w.) with the dose corresponding to the NOEL in rats: 5 mg kg^{-1} b.w.

Workers	Daily absorbed dose[a]/NOEL		
	Lower-bound	Mean	Upper-bound
1	0.015	0.020	0.027
2	0.013	0.018	0.024
3	0.026	0.036	0.048
4	0.005	0.007	0.010
5	0.073	0.099	0.133
6	0.008	0.011	0.014
7	0.017	0.023	0.031
8	0.026	0.035	0.047
9	0.023	0.032	0.043
10	0.037	0.051	0.070

[a]See Table 4 for the estimates of the daily absorbed dose of each worker.

DISCUSSION

A novel strategy was presented in this study for biomonitoring occupational exposure to triclopyr and for assessing the associated human health risks. While it is already well known that the kinetics of a substance is necessary for reconstruction of the absorbed dose starting from excreted biomarkers (Carmichael, 1989; Lauwerys and Hoet, 2001), biomonitoring studies that consider the gradual exposure occurring in typical work shifts are rare. When the work schedule is known, the system of differential equations with the appropriate input function allows simulations of the time course of the gradual absorption of the substance and the biomarker excretion rate corresponding to that specific exposure scenario. The health risk assessment can consequently be determined more accurately by taking full advantage of this mathematical tool. That is, if, instead of employing the specific input function governed by the given timetable of exposure, the daily absorbed dose had been estimated from simulations of a bolus administration in the skin at the beginning of the workday, the daily absorbed dose estimations presented in Table 4 would have been reduced by a factor of 1.3.

For the reconstruction of the daily absorbed dose, some assumptions were made to avoid underestimations. For one, only the dermal route was considered, even if, according to the study of Middendorf *et al*. (1994) on occupational exposure to triclopyr, the pulmonary route accounts, on average, for 13.8% of the daily absorbed dose and the dermal route for 86.2%. Model simulations with this combination show that the estimates of the daily absorbed dose would be ~1.2 times lower than the estimates based on dermal absorption only. Given that in the study at hand it is impossible to know the relative contributions of the two entry routes, the safest dose estimates arise from the assumption that 100% of absorption is through the skin. Moreover, the estimates of daily absorbed dose carried out assume single-day exposure. However, as a result of the slow urinary elimination arising from the low dermal absorption rate, an accumulation of triclopyr is expected to occur in the bodies of the workers. This slow elimination was noticed by Carmichael *et al*. (1989) where the volunteers still excreted amounts of triclopyr 3 days after dermal administration of triclopyr. Therefore, the amounts recovered in the urinary samples collected on the last day of a 5 day workweek stem not only from the amounts of triclopyr absorbed during that day, but also from the remains of amounts absorbed during the previous days. Had the model simulations incorporated this bioaccumulation phenomenon by simulating 5 days of exposure instead of a single day, the estimates of the dose absorbed during the last day would have been about two times lower.

It is interesting to compare the level of triclopyr exposure measured in the current field study with other studies. In particular, the study by Middendorf *et al*. (1994) estimated the daily absorbed doses of 21 workers who applied a diluted form of Garlon 4® with a backpack unit. These workers collected all their urinary samples during 4 days following the onset of a workday. Middendorf *et al*. (1994) calculated their daily absorbed doses by dividing the amounts of triclopyr measured in the urinary samples

by a factor of 0.789. This value is equal to the mean fraction of the absorbed dose found in urine (F_u) after a long time as determined from the volunteers exposed orally to 0.5 mg kg^{-1} of triclopyr in the study by Carmichael *et al*. (1989). According to these dose estimates, the workers in Middendorf *et al*. (1994) study absorbed between 0.207 and 7.690 mg of triclopyr in the monitoring day, with a geometric mean value of 1.1 mg. The comparison between these daily absorbed doses and the ones presented in Table 4 reveals that the workers in the current study were much more exposed; this is so even from comparison using the lower-bound estimates of Table 4. This important difference is possibly due to the different procedures used to reconstruct the absorbed daily doses as well as from the different field conditions (e.g. mixing procedures, equipment maintenance, work practices).

One of the advantages of simulating the time course of the biomarker excretion rate for a given exposure scenario is the opportunity to choose any convenient period of urinary collection. In practice, it is important to specify a period which suits the workers as well as the employers. Sensitivity tests carried out on the model parameters (k_{abs}, k_{SB}, k_{BS}, k_e, F_u) show that the longer the urinary collection period is, the smaller is the impact of the variability of human kinetics on the estimation of the daily absorbed dose. Of course, for a collection period of 5 days or longer following the beginning of a single exposure day, only the total fraction of the absorbed dose eventually excreted in urine, F_u, affects the daily dose reconstruction. In addition to being very demanding for workers, this long period is unfortunately not suitable for employers because the workers would have to cease exposure to triclopyr during the 4 days of the collection period following their workday. Alternatively, a unique micturition at the end of the work shift, as proposed by ACGIH® for biological exposure indices (ACGIH, 2002), or a 12 h cumulative urinary excretion following the onset of exposure are not suitable because, in both instances, estimations of the daily absorbed dose are too dependent on the wide variability in the dermal absorption rates, which are known to vary according to the anatomical region of the skin exposed, the skin condition and the environmental temperature and humidity (Bronaugh and Maibach, 1999). The biomarker urinary concentration measured in a single micturition depends on the degree of urinary dilution. The use of adjustment on the creatinine is also subject to intra- and inter-individual variations as well as day-to-day variations (Boeniger *et al*., 1993). Consequently, the estimates from a single urinary sample are less reliable. For the biomonitoring of triclopyr, urinary collections of 24, 48 or 72 h following the onset of the last day of a week of exposure appear to be the more appropriate periods.

For future field studies, biological reference values (BRVs) should be established from the present results to prevent adverse health effects at an early stage in workers exposed to triclopyr. The BRV could take the form of amounts of triclopyr recovered in urinary samples over a given period that correspond to an absorbed dose that is considered safe. For the convenient collection periods proposed, 24, 48 and 72 h following the onset of a workday of exposure, the BRV amounts in urine corresponding to a NOEL dose can be obtained by simulating a typical worker exposure scenario and using the set of model parametric values which results in the lowest cumulative urinary values. The typical exposure scenario retained is an 8 h work shift, without break time, where the NOEL daily dose (5 mg kg^{-1} b.w.) is assumed to be entirely absorbed through the skin (See Appendix for the input conditions). This dose would result in a cumulative urinary excretion of triclopyr equal to 1.45 mg kg^{-1} b.w. for a 24 h collection, 2.63 mg kg^{-1} b.w. for a 48 h collection and 2.83 mg kg^{-1} b.w. for a 72 h collection. However, since there is no observed effect in humans exposed to triclopyr, there is no proof that the NOEL established for rats corresponds to a safe dose for humans. Thus, the future biomonitoring studies can also be based on the above BRVs divided by appropriate uncertainty factors.

Comparisons between the estimated daily doses absorbed by the workers in this study and the RfD show that there is a potential health risk for these workers under the current conditions. The RfD used here was proposed by the US EPA to prevent significant adverse health effects, assuming repeated daily exposure in the general population over a lifetime (US EPA, 1998). The default uncertainty factors of 100-fold were used to establish this RfD from the rat NOEL. However, according to WHO (1994), it is often suitable for an occupational exposure to use lower safety factors than the proposed default factors, since the occupational population does not include the more vulnerable persons (i.e. children, sick and elderly). In the current study, to bypass the interspecies uncertainty and the variability in the kinetics of triclopyr, the model simulations were designed to result in the highest estimate of the daily absorbed dose corresponding to a given cumulative amount in urine. Moreover, since the workers in this field study are exposed only for 3 months per year, Renwick (1999) judged that it may be more appropriate to assess their health risks with a guidance value derived from a NOEL established for sub-chronic exposure rather than a NOEL established for chronic exposure.

The results of this study were reported to the managers supervising these workers so that tighter security measures may be implemented during manipulations of the product in order to minimize worker contact with the product. Worker education and serious supervision were also recommended to ensure that neoprene gloves and rubber boots or boot covers are

worn at all times. The company should ensure that these recommendations will be implemented.

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APPENDIX

Functional representation of the kinetics of triclopyr

Differential equation system. From Fig. 1, the following differential equations are obtained (see Table 1 for the description of each symbol):

$$\frac{dD(t)}{dt} = g_{skin}(t) - k_{abs} \times D(t)$$

$$\frac{dB(t)}{dt} = g_{lungs}(t) + k_{abs} \times D(t) + k_{SB} \times S(t) - (k_e + k_{BS}) \times B(t)$$

$$\frac{dS(t)}{dt} = k_{BS} \times B(t) - k_{SB} \times S(t)$$

$$\frac{dU(t)}{dt} = F_u \times k_e \times B(t)$$

$$\frac{dN(t)}{dt} = (1 - F_u) \times k_e \times B(t)$$

Input conditions. The following equations describe the input conditions of the time tables simulated by the kinetic model (see the description of variables and parameters in Table 1).

Dose input rate for the exposure scenario of workers with backpack units (amount of the unit daily absorbed dose per hour):

$$g_{skin}(t) = \begin{cases} \dfrac{1 \times f_{skin}}{7 \times \text{hr}} & \text{for } (0 < t < 2\text{ h}), (2.25\text{ h} < t < 4\text{ h}), \\ & (4.5\text{ h} < t < 6\text{ h}), (6.25\text{ h} < t < 8\text{ h}) \\ 0 & \text{otherwise} \end{cases}$$

$$g_{lungs}(t) = \begin{cases} \dfrac{1 \times f_{lungs}}{7 \times \text{hr}} & \text{for } (0 < t < 2\text{ h}), (2.25\text{ h} < t < 4\text{ h}), \\ & (4.5\text{ h} < t < 6\text{ h}), (6.25\text{ h} < t < 8\text{ h}) \\ 0 & \text{otherwise} \end{cases}$$

with the initial conditions: $D(0) = Q(0) = U(0) = N(0) = 0$.

Dose input rate for the exposure scenario of workers with tractor-mounted boom spray (amount of the unit daily absorbed dose per hour):

$$g_{skin}(t) = \begin{cases} \dfrac{1 \times f_{skin}}{10.5 \times \text{hr}} & \text{for } (0 < t < 2\text{ h}), (2.25\text{ h} < t < 4 \cdot \text{h}), \\ & (4.25\text{ h} < t < 8.75\text{ h}), (9.25\text{ h} < t < 11.5\text{ h}) \\ 0 & \text{otherwise} \end{cases}$$

$$g_{lungs}(t) = \begin{cases} \dfrac{1 \times f_{lungs}}{10.5 \times \text{hr}} & \text{for } (0 < t < 2\text{ h}), (2.25\text{ h} < t < 4 \cdot \text{h}), \\ & (4.25\text{ h} < t < 8.75\text{ h}), (9.25\text{h} < t < 11.5\text{ h}) \\ 0 & \text{otherwise} \end{cases}$$

with the initial conditions: $D(0) = Q(0) = U(0) = N(0) = 0$.

Dose input rate for the determination of the BRV (mg kg^{-1} b.w. per hour):

$$g_{skin}(t) = \begin{cases} \dfrac{1 \times f_{skin}}{8 \times \text{hr}} & \text{for } (0 < t < 8\text{ h}) \\ 0 & \text{otherwise} \end{cases}$$

$$g_{lungs}(t) = \begin{cases} \dfrac{1 \times f_{lungs}}{8 \times \text{hr}} & \text{for } (0 < t < 8\text{ h}) \\ 0 & \text{otherwise} \end{cases}$$

with the initial conditions: $D(0) = Q(0) = U(0) = N(0) = 0$.