



Ciência Rural

ISSN: 0103-8478

cienciarural@mail.ufsm.br

Universidade Federal de Santa Maria
Brasil

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Ciência Rural, vol. 45, núm. 4, abril, 2015, pp. 591-597
Universidade Federal de Santa Maria
Santa Maria, Brasil

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Estimation of herbicide bioconcentration in sugarcane (*Saccharum officinarum* L.)

Modelagem de bioconcentração de herbicidas em cana (*Saccharum officinarum* L.)

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ABSTRACT

Sugarcane is an important crop for sugar and biofuel production in Brazil. Growers depend greatly on herbicides to produce it. This experiment used herbicide physical-chemical and sugarcane plant physiological properties to simulate herbicide uptake and estimate the bioconcentration factor (BCF). The (BCF) was calculated for the steady state chemical equilibrium between the plant herbicide concentration and soil solution. Plant-water partition coefficient (sugarcane bagasse-water partition coefficient), herbicide dilution rate, metabolism and dissipation in the soil-plant system, as well as total plant biomass factors were used. In addition, we added Tebuthiuron at rate of 5.0kg a.i. ha⁻¹ to physically test the model. In conclusion, the model showed the following ranking of herbicide uptake: sulfentrazone > picloram > tebuthiuron > hexazinone > metribuzin > simazine > ametryn > diuron > clomazone > acetochlor. Furthermore, the highest BCF herbicides showed higher Groundwater Ubiquity Score (GUS) index indicating high leaching potential. We did not find tebuthiuron in plants after three months of herbicide application.

Key words: herbicide, bioconcentration factor, plant uptake, model, bagasse adsorption.

RESUMO

A cana de açúcar é uma cultura importante para produção de açúcar e biocombustíveis no Brasil e exige elevada utilização de herbicidas. Utilizamos modelo matemático para ajudar na compreensão da absorção de herbicida dessa cultura. Propriedades físico-químicas dos herbicidas e propriedades fisiológicas das plantas de cana foram usados para estimar a absorção e também o fator de bioconcentração, bioconcentration factor (BCF), calculado para o equilíbrio químico entre a concentração do herbicida na planta e na solução do solo. O coeficiente de partição planta/água, a taxa de diluição de herbicida, o metabolismo e a dissipação no sistema solo-planta e biomassa

total das plantas foram adicionados ao modelo. O herbicida tebuthiuron aplicado ao solo na dose de 5,0kg ha⁻¹ i.a. foi utilizado para testar o modelo. A absorção dos herbicidas mostrada pelo modelo indicou em ordem o seguinte: sulfentrazone > picloram > tebuthiuron > hexazinone > metribuzin > simazine > ametryn > diuron > clomazone > acetochlor. Esses herbicidas com alto índice (BCF) também apresentaram alto índice de potencial de lixiviação para água subterrânea "Groundwater Ubiquity Score" (GUS). Tebuthiuron não foi encontrado nas plantas após três meses de aplicação.

Palavras-chave: herbicida, fator de bioconcentração, absorção, estimativa, adsorção bagaço.

INTRODUCTION

In Brazil, sugarcane area encompasses more than 5 million hectares. Farmers produce more than 570 million tons of sugarcane yearly. The industry uses this crop to produce sugar, alcohol and other derivatives (ALCOPAR, 2012). This production level reflects the sugarcane favorable climate in many parts of Brazil. However, those same environmental conditions also favor the growth of several weed species, and growers use herbicides to manage them.

Sugarcane requires large amounts of water during its vegetative cycle and has a high transpiration rate. Normally water is also the vehicle of herbicide uptake by the plants. The herbicides used in sugarcane cultivation have high water solubility, long soil half-life, and high

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accumulation potential in plants (TRAPP, 1995; COUSINS & MACKAY, 2001).

Mathematical models predict plant herbicide concentrations (TRAPP, 1995). Several models simulate any substance uptake by plants (TRAPP & MATTHIES, 1995; FUJISAWA et al., 2002; TRAPP et al., 2003; TRAPP, 2007; PARAIBA & KATAGUIRI, 2008). Some researchers developed models to simulate specific substance uptake by leaves (TRAPP, 1995), roots and tubers, (TRAPP et al., 2003; PARAIBA & KATAGUIRI, 2008) or by roots and leaves (FUJISAWA et al., 2002; TRAPP, 2007). However, none of these models estimate the herbicide bioconcentration factor and uptake in sugarcane.

The bioconcentration factor of a substance (*BCF*) in an organism is a coefficient that describes the increase in concentration of herbicides in the organism in relation to concentration in the medium, estimated by the limit in time in the chemical steady state equilibrium. In case of plants for food, the *BCF* permits scientists to evaluate the human's daily ingestion of pesticide establishing safe limits for concentration in the medium (PARAIBA & KATAGUIRI, 2008). This research used this model to estimate sugarcane herbicide absorption from soil solution. This work evaluated the *BCF* of herbicide in sugarcane through physical and chemical properties of herbicides and physiological characteristics of the crop. We chose tebuthiuron as an indicator because it is commonly used in sugarcane cultivation in Brazil and has high mobility (CERDEIRA et al., 2007).

MATERIALS AND METHODS

The *BCF* model

This paper's estimation of sugarcane herbicide bioconcentration relies on several assumptions: degradation in the soil and its metabolism and dilution in the plant are described by first order kinetic equation; the plant uses the water transpiration stream to uptake the herbicide from the soil solution; soil solution herbicide present in concentrations that are available for plant uptake; and the plant distributes the herbicide throughout itself by transpiration. These assumptions reflect the results of several studies (TRAPP, 2007; REIN et al., 2011; TRAPP & LEGIND, 2011; TRAPP & EGGEN, 2013).

We obtained the *BCF* for the steady state chemical equilibrium, which we estimated using the time limit of the quotient between the plant herbicide and the soil solution herbicide concentration. We

calculated the total balance of the herbicide's mass through the following equations:

$$\frac{M_p dC_p}{dt} = QTSCF_{soil} C_w - (k_E + k_G) M_p C_p - \frac{QC_p}{K_{pw}}$$

(TRAPP et al., 2003; PARAIBA, 2007; PARAIBA et al., 2010), where M_p (kg ha⁻¹) is the total plant fresh biomass, Q (L day⁻¹ ha⁻¹) is the plant transpiration rate, $TSCF_{soil}$ is the herbicide concentration factor in the transpiration stream in relation to the soil solution, C_w (mg L⁻¹) is the soil solution herbicide concentration, k_E (day⁻¹) is the herbicide transformation rate in the plant, k_G (day⁻¹) is the plant growth rate, C_p (mg kg⁻¹) is the plant herbicide concentration, and K_{pw} (L kg⁻¹) is the herbicide plant-water sorption coefficient or herbicide bagasse-water sorption coefficient measured as described in the sorption experiments.

The $TSCF_{soil}$ was estimated from the herbicide octanol-water partition coefficient using the equation, given by (BURKEN & SCHNOOR, 1998)

$$TSCF = 0.756 \exp\left[\frac{-(\log K_{ow} - 2.50)^2}{2.58}\right]$$

where $\log K_{ow}$ is the logarithm of herbicide octanol-water partition coefficient and $TSCF$ is the concentration factor of pesticide in the transpiration stream without interference of soil elements. The present paper calculates the $TSCF_{soil}$ is developed by

$$TSCF_{soil} = \frac{\theta TSCF}{\theta + K_{oc} f_{oc} \rho_s} \quad (\text{NICHOLLS, 1984}),$$

where K_{oc} (L kg⁻¹) is the herbicide sorption coefficient in the soil organic carbon, f_{oc} (g g⁻¹) is the soil organic carbon volumetric fraction, ρ_s (kg L⁻¹) is the total soil density, and θ (g g⁻¹) is the soil water volumetric content.

The experiment measured the herbicide plant-water partition coefficient K_{pw} (L kg⁻¹) as follows (Table 1). Initially, it was determined the sorption coefficient of the herbicides in the bagasse (dry pulp that remains after juice extraction) for the development of the model that describes the mass balance of the herbicide in the soil-plant in sugarcane. We selected the following herbicides to evaluate the *BCF*: acetochlor, ametryn, clomazone, diuron, hexazinone, metribuzin, picloram, simazine, sulfentrazone and tebuthiuron. For this experiment we first washed and processed the sugarcane cane in a manual mill to separate the juice from the bagasse. We then dried the resulting bagasse in an oven with air circulation at 60°C for 72h. After complete drying, we grounded the residue using a knife mill. Then we

Table 1 - Partition (sorption) coefficients of herbicides in octanol/water, bagasse-water, and solubility.

Herbicide	$\log K_{OW^a}$	K_{PW^b}	Sol^c
Ametryn	2.63	31.5	0.2
clomazone	2.5	42.34	1.1
Diuron	2.85	88.14	0.0364
hexazinone	1.2	9.4	33
metribuzin	1.6	17.7	1.05
Simazine	2.1	21.13	0.0062
sulfentrazone	1.48	20.2	110
tebuthiuron	1.79	13.02	2.5

^a $\log K_{OW}$: partition coefficient octanol-water of herbicide ($L\ kg^{-1}$) (TOMLIN, 2000).

^b K_{PW} : partition coefficient bagasse-water of the herbicides (measured).

^c Sol : water solubility ($g\ L^{-1}$) (TOMLIN, 2000).

washed the bagasse with distilled water and filtered it through qualitative filter paper (Whatman n. 1), using a Büchner funnel. After washing, we again dried the residue under the same conditions described above and sieved in a 1mm sieve.

The soil solution herbicide concentration followed a first order kinetic equation given by $C_w(t) = C_w(0) \exp(-k_s t)$, where $C_{w(0)}$ ($mg\ L^{-1}$), $C_w = C_w(t)$ ($mg\ L^{-1}$) and k_s (day^{-1}) are the initial soil solution herbicide concentration, the current soil solution herbicide concentration, and the herbicide dissipation rate in the soil, respectively. The equation

$$C_p(t) = \frac{AC_w(0)}{(B - k_s)} [\exp(-k_s t) - \exp(-Bt)]$$

describes the plant herbicide concentration, where

$$A = \frac{QTSCF_{soil}}{M_p} \quad (L\ kg\ day^{-1}\ ha^{-1}) \text{ and}$$

$$B = (k_E + k_G) + \frac{Q}{M_p K_{PW}} \quad (day^{-1})$$

(day^{-1}). The A constant is the herbicide uptake rate by plants and the B constant is herbicide dilution and metabolic rate in the plants.

We calculated the bioconcentration factor for the steady state chemical equilibrium by the time limit of the quotient between the plant herbicide and soil solution herbicide concentrations, as follows

$$BCF' = \lim_{t \rightarrow \infty} \frac{C_p(t)}{C_w(t)} = \frac{QTSCF_{soil} K_{PW}}{Q + k_{EGS} K_{PW} M_p},$$

where BCF' ($L\ kg^{-1}$) is the plant herbicide bioconcentration factor and $k_{EGS} = k_E + k_G - k_s$ (day^{-1}) is the herbicide dilution rate, metabolism and dissipation in the soil-plant system (PARAIBA, 2007; PARAIBA et al., 2010).

Previous research provided the herbicide half-life time values and the soil sorption coefficients (HORNSBY et al., 1995). This experiment uses the half-life time to estimate the degradation rate, k_s values, through the expression $k_s = 0.693/t_{1/2}$, where $t_{1/2}$ (day) is the soil herbicide half-life (HORNSBY et al., 1995). Although polar herbicides K_{oc} varies with soil pH with geographical regions, normally crop soils pHs are adjusted to near 6 worldwide, thus validating the possible use of literature for this estimation (OLIVEIRA et al., 2001).

Sorption kinetics and chromatographic analysis of the herbicides

The equation $C_p = K_{PW} C_w$ determines the sorption coefficients, where C_p ($\mu g\ \mu g^{-1}$) is the concentration of the herbicides the bagasse sorbed, C_w ($\mu g\ mL^{-1}$) is the concentration in water solution, and K_{PW} is the partition coefficient bagasse-water of the herbicides measured. The equation $X/M = K_{PW} C_{fw}$ provides the coefficients, where X (μg) is the amount of herbicide sorbed, and M (μg) is the weight of bagasse and C_{fw} ($\mu g\ mL^{-1}$) is the final concentration of herbicide in solution. The formula $X = (C_{iw} - C_{fw})V$ measures the amount of herbicide sorbed, where C_{iw} ($\mu g\ mL^{-1}$) is the initial concentration of the herbicide in solution and V (mL) is the volume of solution (Table 1) (TRAPP et al., 2001).

We performed sorption kinetics and isotherms curves for each individual herbicide. We kept the rate of bagasse:solution at 1:15 (1g bagasse - 15mL of solution of $1\ \mu g\ mL^{-1}$ of herbicide in water), and maintained the samples at a temperature of $25^\circ C$, shaking at 185rpm. To quantify and determine the point on time of equilibrium of herbicide absorbed, took the aliquots and filtered in $0.45\ \mu m$ sieve for

herbicide at regular intervals. The initial time of the experiment ($t = 0$) was when we applied the herbicide to the media. We performed the analyses on HPLC using a UV-Vis detector (Shimadzu, model SPD 10AVvp), Supelco-Lichosorb RP-18.5 μ m (250mm-4.6mm) column, flow rate of 1.0mL min⁻¹, room temperature and injection volume of 20 μ L. Each herbicide had a specific chromatographic conditions (mobile phase and UV wavelength). All herbicides reached equilibrium within a period of 24 hours. For construction of the isotherms, seven standard concentrations, ranging from 0.1 to 8.0 μ g mL⁻¹, in duplicate were used.

Tebuthiuron sugarcane uptake experiment

To conduct the experiment we used containers of 40 \times 10³cm³ capacity with organic sugarcane so as to avoid interference with other chemicals watered to maintain at field capacity, IAC2480 variety. The experiment was conducted at pH 5.9 (CaCl₂), soil organic carbon of 0.012g g⁻¹, total soil density of 1.3kg L⁻¹; and soil moisture of 0.28g g⁻¹. The herbicide tebuthiuron was applied at of 5.0kg a.i. ha⁻¹, a rate that although higher than the recommended application rate did not cause phytotoxicity to the plant. We used higher rates to simulate a worst case scenario. We harvested the sugarcane every three months after application in order to quantify the herbicide until 20 months old.

RESULTS AND DISCUSSION

We applied the model to the sugarcane with the following soil and plant parameters: soil organic carbon=0.012g g⁻¹; total soil density = 1.3kg L⁻¹; soil humidity =0.28g g⁻¹; total plant fresh biomass = 80,000kg ha⁻¹; average plant transpiration rate = 32,000L day⁻¹ ha⁻¹; and average relative plant growth rate = 0.05 day⁻¹ (CABRAL et al., 2003). The plant herbicide metabolism rate k_E was calculated by $k_E = k_s/16$ (JURASKE et al., 2008).

The data (Table 1) were used to produce two curves correlation of herbicide properties such as water solubility, partition coefficient octanol-water, and sorption coefficient in bagasse. The equations $\log K_{PW} = 0.45 + 0.46 \times \log K_{OW}$, with $P < 0.001$, $n = 8$, correlation coefficient $R - s = 79.75\%$, standard deviation = 0.15 (Equation 1) and $\log K_{PW} = 0.29 + 0.046 \times \log Sol + 0.54 \times \log K_{OW}$, with $P < 0.001$, $n = 8$, correlation coefficient $R - s = 82.93\%$, standard deviation = 0.155 (Equation 2), were developed.

Equations 1 and 2 show the relationship of the sorption coefficient plant/water or bagasse/water

with the coefficient octanol-water. Due to the high solubility of the herbicides picloram and acetochlor, the analytical method did not work as for the other herbicides. For this reason, we used the equations to estimate sorption coefficient bagasse/water for the herbicides: acetochlor ($Sol = 0.233$ g L⁻¹ and $\log K_{OW} = 4.14$) and picloram ($Sol = 0.430$ g L⁻¹ and $\log K_{OW} = 19$). Sorption coefficient bagasse-water (K_{PW}) of acetochlor was 230 (Equation 1) and 313L kg⁻¹ (Equation 2). Picloram was 21 (Equation 1) and 19L kg⁻¹ (Equation 2). The Pesticide Manual provided the values of Sol (solubility) and $\log K_{OW}$ (octanol-water partition coefficient) of the herbicides (TOMLIN, 2000). The values obtained from Equation 1 for picloram and acetochlor were used to feed the model.

Sugarcane herbicide BCF varied between 0.0081L kg⁻¹ (acetochlor) and 0.8570L kg⁻¹ (sulfentrazone) (Table 2). Based on the BCF values measured (Table 2), we concluded that the herbicides that would most probably be found in plant are sulfentrazone > picloram > tebuthiuron > hexazinone > metribuzin > simazine > ametryn > diuron > clomazone > acetochlor. Herbicides with small K_{PW} and K_{OC} and high $TSCF_{soil}$ are the ones with the highest BCF , such as sulfentrazone, picloram, tebuthiuron, hexazinone, and metribuzin (Table 2). The herbicides ametryn, diuron, clomazone, and acetochlor have the lowest BCF and have in common high K_{OC} , K_{OW} , K_{PW} , and low $TSCF_{soil}$ (Table 2).

The BCF (Table 2) values permit an estimation of the herbicide daily intake (DI), per body weight, from sugarcane products consumption establishing acceptable limits. For example, a soil solution containing 1.0mg L⁻¹ (C_w) of tebuthiuron leads to a sugarcane concentration (C_p) of 0.4159mg kg⁻¹ ($C_p = BCF \times C_w$) and a daily herbicide intake of 0.003 mg kg⁻¹ (mg of tebuthiuron per kg body weight) considering a 70kg person with a daily sugarcane juice consumption (DI) of 0.5kg, measured by $DI = 0.5 \times C_p / 70$.

Besides being absorbed, herbicides could leach to groundwater. We can estimate the herbicide leaching potential through the GUS index (GUSTAFSON, 1989), given by $GUS = (4 - \log K_{OC}) \times \log t_{1/2}$. Depending on the GUS index numerical value, we may classify the herbicide as a high leaching potential ($GUS \geq 2.8$), non-leaching ($GUS \leq 1.8$) or with undetermined leaching potential (transient) ($1.8 < GUS < 2.8$). Therefore, herbicides with low soil sorption coefficients and high water solubility, are classified as leaching pesticides because of their GUS index values (Table 2). High soil herbicide sorption makes them adsorbed in soil matrix, and thus,

Table 2 - Herbicide physical-chemical characteristics and the bioconcentration factors determined in sugarcane.

Herbicide	$\log K_{OW}$	$t_{1/2}^a$ (day)	K_{OC}^a (L kg ⁻¹)	K_{PW} (L kg ⁻¹)	$TSCF_{soil}$	GUS	BCF (L kg ⁻¹)
sulfentrazone	1.48	310	23	20.20	0.2214	6.57	0.8570
Picloram	1.9	90	16	21.14	0.3476	5.46	0.7539
Tebuthiuron	1.79	360	80	13.02	0.1139	5.36	0.4159
Hexazinone	1.2	90	54	9.40	0.0980	4.43	0.1883
Metribuzin	1.6	40	60	17.70	0.1272	3.56	0.1530
Simazine	2.1	60	130	21.13	0.0862	3.35	0.1424
Ametryn	2.63	60	300	31.50	0.0424	2.71	0.0719
Diuron	2.85	90	480	88.14	0.0260	2.58	0.0611
clomazone	2.5	24	300	42.34	0.0427	2.10	0.0347
acetochlor	4.14	13	261	229.70	0.0172	1.76	0.0081

$\log K_{OW}$: herbicide octanol-water partition coefficient; K_{OC} : herbicide sorption coefficient in the soil organic carbon; K_{PW} : plant-water partition coefficient; GUS : Groundwater Ubiquity Score; BCF: sugarcane herbicide bioconcentration factor; $TSCF_{soil}$: soil-plant transpiration stream concentration factor; ^aData from HORNSBY et al., (1995).

unavailable for lixiviation or plant uptake (TRAPP, 1995). Six of the ten studied herbicides (60%) are potentially leaching. One (10%) is a non-leaching herbicide and others transient (Table 2). Overall herbicides with the highest *BCF* or potential to be absorbed are also the ones with highest potential to leach to groundwater (Table 2).

Tebuthiuron bioconcentration

We applied Tebuthiuron to the soil at 5.0kg a.i. ha⁻¹, a rate higher than the recommended, without causing phytotoxicity to the plant. We harvested the plants every three months after application for herbicide quantification. We were able to measure and detect the herbicide only during the first harvesting date, three months after application, at a level of 0.5 mg kg⁻¹. Although, in our study, the herbicide had a significant *BCF* of 0.42, it also had a high *GUS* index rating, 5.36, indicating that most likely it would leach or break down (SILVA et al., 2010) before the plant uptakes the herbicide further.

Other authors found tebuthiuron residues in adult sugarcane plants cultivated in soils treated with 2.2kg. a.i. ha⁻¹ (recommended rate) and 11.2kg ha⁻¹ with herbicide residue concentrations of 0.074 and 0.026µg g⁻¹, respectively (CAUX et al., 1997). In this same experiment, the authors' found in their sugarcane bagasse, juice and syrup (11.2kg ha⁻¹) contained concentrations of 0.063, 0.076 and 0.193µg g⁻¹, respectively. They found no residues in the sugar obtained from these plants. However, researchers detected residues of three tebuthiuron metabolites in grasses and bushes grown in soils of a semiarid region of Arizona, 11 years after herbicide application (JOHNSON & MORTON, 1991). We found no other

literature regarding experiments on the application of tebuthiuron to sugarcane plants similar to those reported by CAUX et al. (1997) and JOHNSON & MORTON (1991).

Another practical information generated by this study is related to herbicide efficacy for weed control, since herbicides with high K_{PW} such as measured in this study (Table 2) can be less efficient for weed control. These herbicides are pre plant soil applied over a straw left by mechanical harvesting of sugarcane, are adsorbed by the straw residues left and therefore failing to reach the weed seeds that should be controlled (CORREIA et al., 2007).

CONCLUSION

This study presents a model equation to estimate the *BCF*. This equation depends directly on the herbicide concentration factor in the transpiration stream, the transpiration stream volume and the plant-water partition coefficient; and indirectly on transpiration stream volume, herbicide dilution rate, metabolism and dissipation in the soil-plant system, the plant-water partition coefficient and the plant biomass. This model allows identification of herbicides that might potentially bioconcentrate in sugarcane and sugar products. The herbicides with highest *BCF* that would most probably be found in plant are sulfentrazone > picloram > tebuthiuron > hexazinone > metribuzin > simazine > ametryn > diuron > clomazone > acetochlor. Overall herbicides with the highest *BCF* or potential to be absorbed are also the ones with highest potential to leach to groundwater.

ACKNOWLEDGEMENTS

This work was supported by The State of São Paulo Research Foundation (FAPESP), Brazil.

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