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Original article

Predicting pesticide fate in the hive (part 2): development of a dynamic hive model

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Abstract – A new hive model is proposed for the assessment of the distribution and fate of pesticides in the hive ecosystem. Based on the chemical used, the model draws a dynamic picture of pesticide contamination in the hive, calculating contamination trends and concentration levels in the various hive components (e.g. bees, wax and honey). The proposed model is validated using empirical data on τ-fluvalinate residues in bees, wax and honey. It predicts with good approximation both the trends over time and the contamination levels of the pesticide in the various hive components. We have developed most of the parameters and equations used in this model. Although they will require further experimental testing, they provide realistic predictions that are consistent with the experimental data. The proposed model is a useful tool for predictive purposes and improves our understanding of contamination phenomena in the hive.

pesticide residues / hive contamination / fluvalinate / multi-compartimental models / fugacity

1. INTRODUCTION

Theoretical models are useful tools for evaluating the environmental distribution and fate of chemicals (Cowan et al. 1995; EC and European Commission 2003). By applying an appropriate model, the fate of contaminants can be predicted for a generic environment or for a specific geographical area. Models based on fugacity (Mackay and Paterson 1979; Mackay 2001) have been extensively applied to non-ionised organic chemicals at various scales: global (Wania and Mackay 1995), regional (Mackay et al. 1992), field (Di Guardo et al. 1994) and micro-ecosystem (Tremolada et al. 2004, 2009). The predictive capability of this approach has been proven through experimental validation procedures producing model predictions close to measured data.

A honeybee hive is a micro-ecosystem composed of several components (e.g. bees, wax, honey, air) that receive materials from and exchange materials with the outside. The primary inputs are derived from the foraging activity of bees, which carry nectar, pollen, water and resins into the hive. In nature, the primary outputs are the animals themselves (upon death at the end of the bees' life cycle) and new hives arising from the splitting of the colony. Various bee products (honey, wax, propolis and royal jelly) collected by beekeepers are additional outputs. Pesticides

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can enter into contact with bees by two primary means: (1) direct introduction into the hive to protect honeybees from parasites and (2) presence in the air or on surfaces with which bees come into contact (vegetation and food sources). Systemic neonicotinoid insecticides are currently under investigation as a possible cause of Colony Collapse Disorder (Johnson et al. 2010).

Theoretical models offer the opportunity to calculate the possible exposure of bees to agricultural pesticides and environmental contaminants to predict the contamination of bee products. Models make it possible to forecast the fate of a chemical and the resulting level of exposure before a compound is used. Moreover, theoretical models facilitate greater comprehension of the distribution and fate of chemicals in the hive (Tremolada et al. 2009).

In a previous study, we developed a very simple model for the hive ecosystem based on Level II of Mackay's Fugacity Model (Tremolada et al. 2004). This hive model was able to calculate the distribution of pesticide residues in the honey, wax and air within hives based on the physical and chemical properties of the chemicals and on the characteristics of the hive ecosystem. The model was applied to four acaricides (coumaphos, malathion, fluvalinate and bromopropylate) and showed adequate predictive capability. Its primary limitation was that it described a static picture of contamination rather than a dynamic situation in which concentrations may change over time. Another limitation was that the model did not consider the bees themselves, which is of primary importance because adult workers carry both the target of the toxic agents (i.e., *Varroa* mites) and the toxic agents themselves on their bodies, transferring contamination to other hive components.

To overcome these limitations, we here propose a level IV model (non-steady-state conditions) that provides a dynamic description of concentration trends in the various hive components. The new model takes into account three components (bees, bee products and air) and two sources of contamination (the distribution of the pesticides within the hive and contamination coming from outside the hive). We test the accuracy of the model with experimentally measured τ-fluvalinate data.

2. MATERIALS AND METHODS

2.1 The hive ecosystem

For a modelling approach, it is necessary to quantitatively characterise the various components of the hive ecosystem (e.g. bees, honey and wax) and the major fluxes to and from the hive (e.g. foraging materials, bee products and oxygen). Most of the information given here regarding typical productive honeybee colonies refer to *Apis mellifera ligustica* and are taken from Chauvin (1968), Groout (1973), Snodgrass (1984), Seeley (1985), Root (1990) and Goodman and Fisher (1991) or other authors specifically cited below. We have added further information based on data from the literature and expert judgements. The primary goal of this description is to quantify the major hive activities to obtain realistic values for use in the model application.

Adult bees are specialised for different tasks depending on their sex and age. The majority of bees are sterile females (worker bees, ranging in number from 10,000 in winter to 50,000 or even 100,000 in June). Worker bees 0–2 days old clean the cells (age caste I, cell cleaner); bees 2–11 days old care for the brood living in the central part of the nest (age caste II, broodnest caste); bees 11–20 days old constitute a food-storage caste, whose tasks occur in the peripheral, food-storage region of the nest and which are able to produce wax (age caste III) and bees 20 days old or more become forager bees whose work is mostly outside the nest (age caste IV). Approximately 20% of forager bees spend a day or two serving as guards at the nest entrance (guard bees). The biological cycle of worker bees varies from 40–45 days in summer to 6 months or more for wintering bees. The mean dimensions of a worker bee are 12 to 13 mm in length, 4 mm in width and 130 mg in weight. Given a volume of approximately 160 mm^3, the density of a bee is about 0.8 mg mm^{-3}.

Male bees are intermediate in size between workers and the queen. They are much less numerous than worker bees (numbering between 1,000 and 5,000 depending on the period) and are important in

reproductive activity, in hive thermoregulation and in food transfer and transport within the hive.

A colony is able to rear 150,000–200,000 bees annually (including 5,000–20,000 males and a few queens), consuming a total of 20–26 kg of pollen (130 mg of pollen are required to produce an adult bee) and 20–30 kg of honey (one honey cell is sufficient to rear several larvae). Larval dimensions range from 1.5 mm (near the egg size) to the size of the cell in the final stages (total worker development takes 21 days). Given a mean weight of 75 mg and an approximate volume of 90 mm^3 (1/4 of the cell volume), the density of a bee larva is about 0.83 mg mm^{-3}. In many temperate regions, brood rearing begins in January or February with around 1,000 cells containing brood (nearly 75 g of brood). Later, this number soars to a peak of 30,000–40,000 developing bees in May or June (2.5 kg of brood) and finally declines gradually throughout the remainder of the summer, ceasing in October. The total brood production is 15 kg/year.

A typical nest contains about 100,000 cells with 415 cells per dm^2 on each side of the comb. Each hexagon-shaped cell typically has a perimeter of 18 mm and an area of 24 mm^2, and the wall-to-wall distance is 5 mm. Cell depth is variable; considering a depth of 15 mm and an area of 24 mm^2, the volume of a typical cell is about 360 mm^3. Root (1990) reports cell volumes varying from 360 to 192 mm^3 depending on cell density (650–1,050 cells per dm^2 for both sides of the comb). In summer, two- to three-fifths of cells are filled with brood, two-fifths with honey and less than one-fifth with pollen. A typical nest is arranged in ten combs, each with external dimensions of 47×30 cm and wax foundation dimensions of 44×27 cm, giving a total surface area of 2.4 m^2. Cell walls and bases have only 0.073±0.008 and 0.176±0.028 mm of wax thickness, respectively. Based on these dimensions, rapid diffusion of pollutants is expected. The combs are composed of a total of about 1.4 dm^3 (1.35 kg) of wax. With the cap wax (0.15 kg), the total amount of wax in a typical nest is about 1.5 kg. Wax is a complex blend of straight-chain monohydric alcohols esterified with carboxylic acids and hydroxycarboxylic acids mixed with various straight-chain alkanes. The density of wax is 0.958–0.970 kg dm^{-3}.

Bees explore about 300 ha of territory daily (with a foraging radius from one to more than 5 km) while searching for food resources. Bees need only four resources (nectar, pollen, water and resin) for their subsistence. The amounts of nectar, pollen, water and resin collected each year are 240, 30, 10 and 0.1 kg, respectively. Nectar is the base material for honey production. Nectar and honey are 20–40% and >80% sugar solutions, respectively. Honey has a density of 1.39–1.43 kg dm^{-3} (Crane 1976). Water is gathered for diluting honey for brood food and for evaporative cooling of the nest on hot days. Resins are the base materials for the production of propolis, which is composed of roughly 70% resin, 25% beeswax and 5% volatile oils. Resins are taken from trees and carried home in the pollen baskets. Propolis serves to plug unwanted openings and for hygienic purposes (because of its antimicrobial and antifungal activity) (Apimondia 1975; Simone-Finstrom and Spivak 2010).

Bees collect or produce five major products: honey, pollen, propolis, royal jelly and wax. The typical honey reserve in summer is 15 kg, and the total amount produced in a year is about 80 kg, of which 60 kg $year^{-1}$ is consumed for colony maintenance (25 kg in winter and 35 kg in summer) and 20 kg $year^{-1}$ is typically removed by the beekeeper. Pollen is carried to the hive and stored by mixing the dry pollen grains with liquid (honey, nectar or both). Pollen is generally composed by fresh weight of 10–20% water, 10–35% proteins, 15–40% sugars and 1–10% lipids. The typical pollen reserve in summer is 2 kg, and the total amount foraged in a year is 30 kg. Honey and pollen are the primary food sources; honey furnishes energetic support and pollen supplies proteins, vitamins, fats and minerals. Daily food consumption varies as a function of the ages and activities of bees. The daily food intake of larvae ranges from a few milligrams in the first days to tens of milligrams during the final stage. During the first 3 days, larvae are fed with about 3 mg day^{-1} of royal jelly. Later, the larval food changes in composition based on age. However, in order to define a mean value for the full larval period, we assume consumption of 12 mg day^{-1} of larval food composed of 50% honey and 50% pollen. An adult bee consumes food in proportion to its activity level. During flight, about 6.77 mg of sugar is consumed per hour. Because a foraging bee is able to carry 10–20 and 40–60 mg of pollen and nectar, respectively, bees must make efficient choices of the most promising foraging areas. We assume an average consumption of 10 mg day^{-1} of

honey and 1 mg day^{-1} of pollen for adult bees. Propolis is used as an antiseptic agent and is a permanent material of the hive system present in variable amounts depending on the needs of the hive. We assume an average quantity of 0.3 kg with a renewal rate of 0.1 kg $year^{-1}$. Royal jelly is used for high-quality nutrition (queens and first-stage larvae) and is not stored in the hive but produced upon demand. We estimate an overall annual consumption of about 0.55 kg (less than 10 and 540 g for the queen and larvae, respectively). These values are based on an average daily consumption of 20 and 1 mg for the queen and larvae, respectively. The queen (one individual) consumes this amount daily almost all year, while larvae (180,000 reared individuals) consume this amount on each of 3 days. Royal jelly is generally composed by fresh weight of 57–70% water, 6.4–17% proteins, 6.8–20% sugars, 1.3–7.1% of lipophilic substances (phospholipids, sterols, phenols, fatty acids, glycerides and waxes) and 0.75–1.1% minerals and other minor constituents and has a mean density of 1.1 g cm^{-3} (Lercker et al. 1992). Wax is the structural material of the hive, and its quantity is nearly constant throughout the year. The average quantity of wax is 1.5 kg, most of it remaining for a long time. Bees further minimise their colony's need for wax by constantly recycling it. New wax is produced in abundance when a colony needs storage space for a large crop of honey. Bees continuously remove part of the wax (uncapping activity) and rebuild it (capping, fixing and rebuilding activity). The amount of newly produced wax can be evaluated yearly based on the amount of wax cappings (0.15 kg). The surface area of the wax cells (empty or full) can be evaluated by multiplying the internal surface area of one cell (360 mm^2) by the total number of cells (100,000). The resulting surface area of the wax cells is 36 m^2. The exchange surface of honey, pollen and brood is equivalent to the proportion of the wax surface occupied by each component. The exchange surface between adult bees and wax can be approximated as that between wax and air because bees continuously work across the entire wax surface. This surface can be approximated as that of the ten combs (2.4 m^2) rather than that of the wax cells (36 m^2) because most of the cells are capped or filled with food or larvae. The exchange surface between an adult bee and air is the sum of the external bee surface and the internal respiratory surface, of which the latter is much larger. Bees are able to perform several highly energy-consuming activities for which a large oxygen flux is required (during flight, a bee consumes 5 mL of oxygen per hour, or 641 mL/kg of body weight per minute). In order to perform this efficient oxygen supply, the tracheal system and air sacs of bees are characterised by a high exchange surface (Snodgrass 1984). In the absence of specific data on the respiratory surface of bees, we tried to estimate it by a proportion with the exchange surface able to furnish oxygen to humans, being conscious that this extrapolation must to be considered an indicative datum waiting for specific measurements in this research area (Joos et al. 1997). During intense sporting activity, a man consumes 94 mL of oxygen per kilogram of body weight per minute using an oxygen exchange surface of 50–90 m^2; proportionally, we estimate a respiratory surface of 9.1 cm^2 per bee.

Environmental conditions within the hive are quite peculiar. In the proximity of brood, bees maintain a constant temperature between 34.5°C and 35.5°C. Far from brood or in its absence, temperatures fluctuate. These high temperatures in the brood nest accelerate the diffusion of contaminants within the hive. The intense exchange activity of bees for food distribution and social communication further increases the distribution of any contaminants that may be present.

Thermoregulation and other bee activities require a large energy supply and create a great oxygen demand. The oxygen consumption of a hive has been quantified as 30 kg $year^{-1}$ with the production of 52 and 34 kg $year^{-1}$ of CO_2 and H_2O, respectively. This oxygen consumption requires an air flux of 150 m^3 $year^{-1}$. With an air volume of about 0.2 m^3 inside the hive, this air flux results in a twice-daily exchange of the full air volume.

2.2 Model development and parameter estimation

Contaminants may enter the hive in several ways: (a) by air flux, if the contaminants are dispersed in this medium; (b) by the food supply, if these materials are contaminated (Villa et al. 2000); (c) by the foraging bees, if they come in contact with contaminated media (Decourtye et al. 2004) and (d) by chemical application within the hive (Wallner 1999). Once a pollutant reaches the hive, it can be

dispersed by contact if the contamination is on the bee surface or deposited in storage cells if the contamination is within foraged materials. Wax can be contaminated directly by contact or indirectly through the cell contents. Air is in contact with bees and wax and can act as a redistribution medium inside the hive, especially for volatile compounds. Once a pollutant is stored in honey and wax, it is unlikely to degrade because these compartments are microbiologically stable. The pollutant can be re-circulated when these products are used by the bees or collected by humans. On the other hand, bees are probably the most active hive compartment. Pollutants deposited on the bee surface are mostly distributed within the hive, while ingested pollutants are bioconcentrated, biotransformed or transferred again into bee products. Figure 1 shows a schematic diagram of the primary processes considered in the model.

The model takes into account three main compartments (bees, bee products and air), which differ in fugacity because of their time-dependent exchange kinetics. The bee products contains several different sub-components: the primary food supply (honey and pollen), the two construction materials (wax and propolis) and the growing larvae. Each of these sub-components maintains its specific properties (volume and affinity for the compound), but the sub-components are considered jointly in a single bulk components (bee products) to reduce the complexity of the model. In considering these sub-components jointly, we assume that the pesticide reaches near-equilibrium conditions among them (equal fugacity). Grouping sub-components into a single bulk component is a common mathematical approach to complex systems (Wania and Mackay 1995). Several facts support this choice: (a) all of these products (wax, propolis, honey, pollen and larvae) are produced by bee activity and stored (honey and pollen) or reared (larvae) inside the same supporting material (wax cells); (b) their large surface to volume ratio greatly decreases the time needed to reach equilibrium conditions; (c) honey, pollen, propolis and

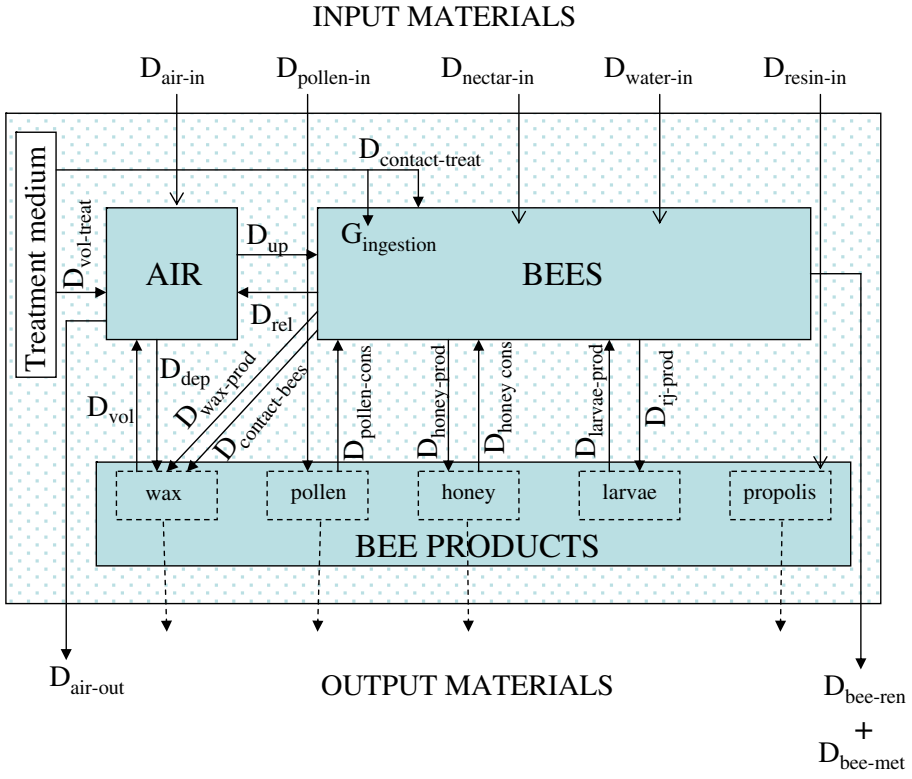

Figure 1. Schematic diagram of pesticide distribution in the hive ecosystem.

larvae remain in contact with wax for a long time, allowing the pesticide to reach the equilibrium conditions. In particular, wax and propolis are structural materials that remain in the hive for long time. Thus, the assumption of near-equilibrium conditions is acceptable.

The model uses as input parameters the physical and chemical properties of the compound and the major characteristics of the hive ecosystem. The former are necessary for calculating the capacities of the various compartments (Z values, units of moles per cubic metre per Pascal) and the latter are necessary for calculating advection, exchange and transformation processes (D values, unit of moles per day per Pascal). Formulas and basic parameters for calculating Z and D values are reported in Table I and II.

Several parameters shown in Table I (e.g. the affinity of the molecule for the air compartment, Z_{air}) are derived from the general formulation of this kind of model (Mackay 2001). Others (e.g. the affinity of the molecule for the wax compartment, Z_{wax}) are taken from the work of Tremolada et al. (2004). However, the majority of the formulas have been developed in this study using the framework of multicompartimental fugacity models (Cowan et al. 1995).

The affinities of the compound for bees (Z_{bees}), nectar (Z_{nectar}), pollen (Z_{pollen}), propolis (Z_{prop}), royal jelly ($Z_{r\text{-}j}$) and treatment medium (Z_{treat}; i.e., the medium in which the pesticide is delivered to the hive) are proposed ex novo based essentially on the lipid fraction of each matrix. Biological compartments, such as bees, have been previously treated on this basis (Mackay 2001); therefore, other biologically derived matrices, such as pollen and royal jelly, are considered analogously. Resin and propolis, which are mixtures of hydrophobic organic compounds, are considered to be similar to wax, whose affinity for the compound is defined on the basis of the organic carbon adsorption coefficient (K_{oc}). Propolis is partly composed of wax. Nectar, which is dilute sugar solution containing about 80% water, is considered to be similar to water. The affinity of this compartment for the compound is defined by Henry's constant (Mackay 2001). We must introduce these approximations to define the compound affinity of the various hive compartments because experimentally determined values are not yet available. Until more precise experimental data can be obtained, our confidence in the reliability of these approximations is based on the previously proposed affinities of honey and wax (Z_{honey} and Z_{wax}), which have been tested for several compounds in a previous study by Tremolada et al. (2004). The compound affinity of the bulk compartment (bee products) is calculated according to Wania and Mackay (1995).

For each of the three major components (bees, bee products and air), we define a non-steady-state mass-balance equation using fugacity (f, units of Pascal) as the unknown time-dependent variable (t=time, units of day). The dynamics of the three compartments are given by the following system of ordinary differential equations:

$$\frac{df_{bee}}{dt} = (D_{contact-treat}f_{treat} + G_{ingestion}C_{in} + D_{nectar-in}f_{nectar} + D_{water-in}f_{water} + D_{honey-cons}f_{prod} + D_{pollen-cons}f_{prod} + D_{larvae-prod}f_{prod} + D_{up}f_{air} - (D_{wax-prod} + D_{honey-prod} + D_{rj-prod} + D_{rel} + D_{bee-ren} + D_{bee-met})f_{bee})/V_{bee}Z_{bee} \quad (1)$$

$$\frac{df_{prod}}{dt} = (D_{contact-bees}f_{bee} + D_{wax-prod}f_{bee} + D_{honey-prod}f_{bee} + D_{r-j-prod}f_{bee} + D_{pollen-in}f_{pollen} + D_{resin-in}f_{resin} + D_{dep}f_{air} - (D_{larvae-prod} + D_{honey-cons} + D_{pollen-cons} + D_{vol})f_{prod})/V_{prod}Z_{prod} \quad (2)$$

$$\frac{df_{air}}{dt} = (D_{air-in}f_{out} + D_{vol-treat}f_{in} + D_{vol}f_{prod} + D_{rel}f_{bees} - (D_{air-out} + D_{dep} + D_{up})f_{air})/V_{air}Z_{air} \quad (3)$$

In Eqs. (1)–(3), f_{bee}, f_{prod} and f_{air} are the fugacity of the compound in bees, bee products and air, respectively (f, units of Pascal), while the various D values (D, units of mole per day per Pascal), volumes (V, units of cubic metres) and capacities of the compartments (Z, units of moles per cubic metre per Pascal) are defined in Tables I and II. For a treatment acting by contact (e.g., τ-fluvalinate), f_{treat} is the fugacity of the product in the treatment medium; for a treatment acting by ingestion (e.g., coumaphos), the pesticide input is obtained by multiplying the ingestion capacity of bees ($G_{ingestion}$, units of cubic metres per day) by the pesticide concentration in the treatment solution (C_{in}, units of moles per cubic metre). The concentration in the treatment solution becomes zero when the treatment ends. The ingestion capacity of bees can be calculated by multiplying the ingestion capacity of each bee (50 mg) by the number of bees taking care of the hive (25,000), assuming that the density of the pesticide suspension is about 1.0 g cm^{-3}. The ingestion time of a single bee is rapid (on the order of minutes), but all bees cannot ingest the treatment solution simultaneously. Therefore, the overall ingestion time is much longer, on the order of hours. In the model, an interval of 2 h is assumed as the time required to fill the overall ingestion capacity of bees, giving a value of 0.0006 m^3 day^{-1} as the overall ingestion capacity ($G_{ingestion}$).

The terms f_{nectar}, f_{pollen} and f_{water} are the fugacity of contaminated nectar, pollen and water, respectively. The term f_{out} is the fugacity of the air outside the hive when it is contaminated. These fugacity values (Pascal) can be calculated as the ratio between the concentration of the compound in each medium (moles per cubic metre) and its capacity (moles per cubic metre per Pascal) (Mackay 2001).

Each of the three Eqs. (1)–(3) is a balance between input flows (D values with positive algebraic signs) and output flows (D values with negative algebraic signs). Pesticide contamination can be derived from many pathways, each represented by a specific D value. Each D value is reported as an input flow only for the specific compartment that initially receives the pesticide. For example, $D_{nectar\text{-}in}$ and $D_{water\text{-}in}$, which represent the eventual pesticide inputs from the foraging of nectar and water, are considered as input flows only for the bee compartment. In fact, nectar and water are effectively ingested by bees and not simply carried by them in external structures (pollen baskets), in contrast to pollen and resin. Pesticides present in the latter materials are deposited by bees directly into the wax compartment (inside cells or above the wax). For this reason, $D_{pollen\text{-}in}$ and $D_{resin\text{-}in}$, which represent the eventual pesticide inputs from the foraging of pollen and resin, are considered as input flows for the bee products compartment, which contains wax.

Pesticide inputs from anti-*Varroa* treatments are represented by the two groups of terms ($D_{contact\text{-}treat}f_{treat}$ and $G_{ingestion}C_{in}$) described above. In the case of τ-fluvalinate, $D_{contact\text{-}treat}f_{treat}$ is a positive term given by the calculated fugacity in the treatment medium (f_{treat}), while $G_{ingestion}C_{in}$ is equal to zero because no pesticide ingestion is expected (pesticide concentration in the ingested solution, C_{in}, is set to zero). The value of f_{treat} is obtained from the amount of pesticide in the strips and the volume and capacity of the strips. The strip volume is obtained geometrically, while the strip capacity is defined in Table I (Z_{treat}). The total quantity of pesticide is 1.6 g, and the resulting fugacity is 0.00172 mPa.

All D values present in the three Eqs. (1)–(3) represent advection and exchange flows among the three compartments. When a given process determines the transfer of a certain amount of pesticide from one compartment to another (output flow for the first compartment), the same process determines an input flow for the second (receiving) compartment. For example, D_{vol}, which represents the eventual pesticide volatilisation from wax to air (D_{vol} with a negative algebraic sign in Eq. (2)), determines an input flow for the air compartment (D_{vol} with a positive algebraic sign in Eq. (3)).

For the bee compartment, the major input flows come from contamination of nectar and water outside the hive ($D_{nectar\text{-}in}$ and $D_{water\text{-}in}$), from the consumption of contaminated honey and pollen ($D_{honey\text{-}cons}$ and $D_{pollen\text{-}cons}$), from residues derived from the larvae ($D_{larvae\text{-}prod}$) and from uptake from the air through the respiratory surfaces (D_{up}). The major output flows are derived from the production of wax, honey, and royal jelly ($D_{wax\text{-}prod}$, $D_{honey\text{-}prod}$ and $D_{r\text{-}j\text{-}prod}$), from the release of the compound to the air through the respiratory surfaces (D_{rel}), from bee

Table I. Compartment capacities expressed as Z values (mol m^{-3} Pa^{-1}) and transport, reaction and advection parameters expressed as chemical flux parameters or D values (mol day^{-1} Pa^{-1}).

Parameter	Symbol	Formula	Units
Capacity of compartment			
Capacity of air	Z_{air}	$1/RT$	mol m^{-3} Pa^{-1}
Capacity of bees	Z_{bees}	$K_{ow}\text{lip}_{bees}/H$ or BCF$/H$	mol m^{-3} Pa^{-1}
Capacity of water	Z_{water}	$1/H$	mol m^{-3} Pa^{-1}
Capacity of nectar	Z_{nectar}	$1/H$	mol m^{-3} Pa^{-1}
Capacity of honey	Z_{honey}	$1/H \times 0.9998 + Z_{wax} \times 0.0002$	mol m^{-3} Pa^{-1}
Capacity of wax	Z_{wax}	$K_{oc}\text{ocf}_{wax}\rho_{wax}/H$	mol m^{-3} Pa^{-1}
Capacity of pollen	Z_{pollen}	$K_{ow}\text{lip}_{pollen}/H$	mol m^{-3} Pa^{-1}
Capacity of propolis	Z_{prop}	$K_{oc}\text{ocf}_{propolis}\rho_{propolis}/H$	mol m^{-3} Pa^{-1}
Capacity of royal jelly	Z_{r-j}	$K_{ow}\text{lip}_{r-j}/H$	mol m^{-3} Pa^{-1}
Capacity of bulk hive products	Z_{prod}	$Z_{honey}vf_{honey} + Z_{wax}vf_{wax} + Z_{pollen}vf_{pollen} + Z_{propolis}vf_{propolis} + Z_{bees}vf_{larvae}$	mol m^{-3} Pa^{-1}
Capacity of treatment medium	Z_{treat}	$K_{oc}\text{ocf}_{treat}\rho_{treat}/H$	mol m^{-3} Pa^{-1}
Exchange			
Volatilisation/deposition from/to hive surfaces	$D_{vol} = D_{dep}$	$D_{vol} = \frac{A_{wax}}{\frac{1}{\text{MTC}_{air}Z_{air}} + \frac{1}{\text{MTC}_{wax}Z_{wax}}}$	mol day^{-1} Pa^{-1}
Volatilisation from treatment surfaces	$D_{vol-treat}$	$D_{vol-treat} = \frac{A_{treat}}{\frac{1}{\text{MTC}_{air}Z_{air}} + \frac{1}{\text{MTC}_{treat}Z_{wax}}}$	mol day^{-1} Pa^{-1}
Uptake/release between air and bees	$D_{up} = D_{rel}$	$D_{up} = \frac{A_{bees}}{\frac{1}{\text{MTC}_{air}Z_{air}} + \frac{1}{\text{MTC}_{bees-resp}Z_{bees}}}$	mol day^{-1} Pa^{-1}
Transfer by contact between the treatment surface and bees	$D_{contact-treat}$	$D_{contact-treat} = \frac{A_{treat}}{\frac{1}{\text{MTC}_{treat}Z_{treat}} + \frac{1}{\text{MTC}_{bees-kitin}Z_{bees}}}$	mol day^{-1} Pa^{-1}
Transfer by contact between bees and wax	$D_{contact-bees}$	$D_{contact-bees} = \frac{A_{wax}}{\frac{1}{\text{MTC}_{bees-kitin}Z_{bees}} + \frac{1}{\text{MTC}_{wax}Z_{wax}}}$	mol day^{-1} Pa^{-1}
Reaction			
Biotransformation in bees	$D_{bee-met}$	$k_{bee-met}V_{bees}Z_{bees}$	mol day^{-1} Pa^{-1}
Advection			

Air inflow and outflow	$D_{\text{air-in}} = D_{\text{air-out}}$	$G_{\text{air-in-out}} Z_{\text{air}}$	mol day^{-1} Pa^{-1}
Nectar inflow	$D_{\text{nectar-in}}$	$G_{\text{nectar-in}} Z_{\text{nectar}}$	mol day^{-1} Pa^{-1}
Pollen inflow	$D_{\text{pollen-in}}$	$G_{\text{pollen-in}} Z_{\text{pollen}}$	mol day^{-1} Pa^{-1}
Resin inflow	$D_{\text{resin-in}}$	$G_{\text{resin-in}} Z_{\text{propolis}}$	mol day^{-1} Pa^{-1}
Water inflow	$D_{\text{water-in}}$	$G_{\text{water-in}} Z_{\text{water}}$	mol day^{-1} Pa^{-1}
Larvae production	$D_{\text{larvae-prod}}$	$G_{\text{larvae-prod}} Z_{\text{bees}}$	mol day^{-1} Pa^{-1}
Wax production	$D_{\text{wax-prod}}$	$G_{\text{wax-prod}} Z_{\text{wax}}$	mol day^{-1} Pa^{-1}
Honey production	$D_{\text{honey-prod}}$	$G_{\text{honey-prod}} Z_{\text{honey}}$	mol day^{-1} Pa^{-1}
Royal jelly production	$D_{r-j-\text{prod}}$	$G_{r-j-\text{prod}} Z_{r-j}$	mol day^{-1} Pa^{-1}
Honey consumption	$D_{\text{honey-cons}}$	$G_{\text{honey-cons}} Z_{\text{honey}}$	mol day^{-1} Pa^{-1}
Pollen consumption	$D_{\text{pollen-cons}}$	$G_{\text{pollen-cons}} Z_{\text{pollen}}$	mol day^{-1} Pa^{-1}
Bee renewal	$D_{\text{bee-ren}}$	$G_{\text{bee-ren}} Z_{\text{bees}}$	mol day^{-1} Pa^{-1}

Z_i capacity of the compartment i (mol m^{-3} Pa^{-1}) calculated according to the formula proposed in Mackay 2001; R gas constant: 8.314 Pa m^3 mol^{-1} °K^{-1}; T absolute temperature (273.15+t°C); H Henry's constant, depending on the compound physical-chemical properties (0.000044 Pa m^3 mol^{-1} for τ-fluvalinate, Tomlin 1997); K_{ow} octanol water partition coefficient, depending on the compound physical–chemical properties (19,953 for τ-fluvalinate, Tomlin 1997); K_{oc} organic carbon adsorption coefficient (Log K_{oc}=Log K_{ow} − 0.21); BCF bioconcentration factor on fresh weight, depending on the compound physical–chemical properties; ocf_{wax} organic carbon fraction of wax: 0.57; $ocf_{propolis}$ organic carbon fraction of propolis: 0.57; ocf_{treat} organic carbon fraction of treatment medium: 0.57; P_{wax} density of wax: 0.97 g cm^{-3}; $p_{propolis}$ density of propolis approximated to 1 g cm^{-3}; p_{treat} density of treatment medium approximated to 1 g cm^{-3}; lip_{bees} lipid fraction of bees on fresh weight: 0.02; lip_{pollen} lipid fraction of pollen on fresh weight: 0.05; lip_{r-j} lipid fraction of royal jelly on fresh weight: 0.04; vf_i volume fraction of the compartment i; D_i fluxes parameters of type i (mol day^{-1} Pa^{-1}) calculated according the formula proposed in Mackay 2001; $K_{bee\text{-}met}$ biotransformation rate constant in bees (day^{-1}), calculated from the biotransformation half life in bees (30 days) by the formula: ln 2 × half life^{-1}; V_i volume of the compartment i (m^3); A_i area of the compartment i in contact with air (m^2); MTC_i compartment i -side Mass Transfer Coefficient (m day^{-1}); G_i mass flux of the compartment i (m^3 day^{-1})

renewal ($D_{bee\text{-}ren}$) and from the biotransformation process ($D_{bee\text{-}met}$).

For the bee products compartment, the major input flows come from contact with bees ($D_{contact\text{-}bees}$), from the deposition of contaminated wax, honey, and royal jelly as larval food ($D_{wax\text{-}prod}$, $D_{honey\text{-}prod}$ and $D_{r\text{-}j\text{-}prod}$), from contaminated pollen and resin brought into the hive from outside ($D_{pollen\text{-}in}$ and $D_{resin\text{-}in}$) and from deposition from the air (D_{dep}). The major output flows result from the production of new bees from larvae ($D_{larvae\text{-}prod}$), from the consumption of honey and pollen ($D_{honey\text{-}cons}$ and $D_{pollen\text{-}cons}$) and from volatilisation of the compound to the air compartment (D_{vol}).

For the air compartment, the major input flows come from contaminated air entering the hive from outside ($D_{air\text{-}in}$), from volatilisation of the compound from the surface of the treatment medium ($D_{vol\text{-}treat}$), from volatilisation of the compound from wax (D_{vol}) and from the release of the compound from bees to air through the respiratory surfaces (D_{rel}). The major output flows result from air flux to the outside ($D_{air\text{-}out}$),

Table II. Parameters used in Table I.

Symbol	Description	Units	Value
V_{air}	Volume of air [a]	m^3	0.2
V_{bees}	Volume of bees [b]	m^3	0.0064
V_{honey}	Volume of honey [c]	m^3	0.0106
V_{wax}	Volume of wax [d]	m^3	0.00155
V_{pollen}	Volume of pollen [e]	m^3	0.002
$V_{propolis}$	Volume of propolis [f]	m^3	0.0003
V_{larvae}	Volume of larvae [g]	m^3	0.0036
vf_{honey}	Volume fraction of honey [h]		0.587
vf_{wax}	Volume fraction of wax [h]		0.086
vf_{pollen}	Volume fraction of pollen [h]		0.111
$vf_{propolis}$	Volume fraction of propolis [h]		0.017
vf_{larvae}	Volume fraction of larvae [h]		0.199
A_{wax}	Area of wax in contact with air [i]	m^2	2.4
A_{bees}	Area of bees in contact with air [j]	m^2	36.4
A_{treat}	Treatment surface [k]	m^2	0.024
MTC_{air}	Air-side Mass Transfer Coefficient [l]	$m\ day^{-1}$	1
MTC_{wax}	Wax-side Mass Transfer Coefficient [m]	$m\ day^{-1}$	0.001
$MTC_{bees\text{-}resp}$	Bee-respiration-side Mass Transfer Coefficient [n]	$m\ day^{-1}$	0.01
$MTC_{bees\text{-}kitin}$	Bee-kitin-side Mass Transfer Coefficient [o]	$m\ day^{-1}$	0.0001
MTC_{treat}	Treatment-medium-side Mass Transfer Coefficient [p]	$m\ day^{-1}$	0.0001
$G_{air\text{-}in\text{-}out}$	Air flux into the hive and out of it [q]	$m^3\ day^{-1}$	0.41
$G_{nectar\text{-}in}$	Nectar flux into the hive [r]	$m^3\ day^{-1}$	0.00133
$G_{pollen\text{-}in}$	Pollen flux into the hive [s]	$m^3\ day^{-1}$	0.000167
$G_{water\text{-}in}$	Water flux into the hive [t]	$m^3\ day^{-1}$	0.0000555
$G_{resin\text{-}in}$	Resin flux into the hive [u]	$m^3\ day^{-1}$	0.000000555
$G_{larvae\text{-}prod}$	Larvae production [v]	$m^3\ day^{-1}$	0.0000675
$G_{wax\text{-}prod}$	Wax production by bees [w]	$m^3\ day^{-1}$	0.000000863
$G_{honey\text{-}prod}$	Honey production by bees [x]	$m^3\ day^{-1}$	0.000317
$G_{r\text{-}j\text{-}prod}$	Royal jelly production by bees [y]	$m^3\ day^{-1}$	0.00000231
$G_{honey\text{-}cons}$	Honey consumption by adult bees [z]	$m^3\ day^{-1}$	0.000286

Table II (continued)

Symbol	Description	Units	Value
$G_{\text{pollen-cons}}$	Pollen consumption by adult bees [aa]	$m^3\ day^{-1}$	0.00004
$G_{\text{bee-ren}}$	Bee renewal [ab]	$m^3\ day^{-1}$	0.000142

[a] The volume of air was geometrically calculated

[b] The volume of bees was calculated from a number of 40,000 adult bees and a individual volume of 160 mm^3

[c] The volume of honey was calculated from an average stored amount of 15 kg of honey and a density of 1.41 g cm^{-3}

[d] The volume of wax was calculated from an average amount of 1.5 kg of wax and a density of 0.965 g cm^{-3}

[e] The volume of pollen was calculated from an average stored amount of 2 kg of pollen and a density of 1 g cm^{-3}

[f] The volume of propolis was calculated from an average amount of 0.3 kg of propolis and a density of 0.97 g cm^{-3}

[g] The volume of larvae was calculated from a number of 40,000 larvae and a individual volume of 90 mm^3

[h] The volume fractions of honey, wax, pollen, propolis and larvae were calculated from the volume of each matrix divided by the sum of them

[i] The area of wax in contact with air was calculated from the area of the wax foundation of each honeycomb (44×27 cm) multiplied for the two sides and the number of honeycombs; for the evaluation of the contact area between wax and bees, one third of this geometric value was considered

[j] The area of bees in contact with air was calculated from a number of 40,000 adult bees and a individual respiratory surface of 9.1 cm^2

[k] The area of treatment was calculated from the two sides of the two strips containing fluvalinate with the dimension of 30×2 cm; for the evaluation of the effective contact area between treatment medium and bees, half of this geometric value was considered

[l] The air-side Mass Transfer Coefficient value was evaluated according to those proposed by Mackay (2001)

[m] The wax-side Mass Transfer Coefficient value was evaluated according to those proposed by Mackay (2001)

[n] The bee-respiratory-side Mass Transfer Coefficient value was evaluated according to those proposed by Mackay (2001)

[o] The bee-kitin-side Mass Transfer Coefficient value was evaluated according to those proposed by Mackay (2001) considering that kitin is the principal constituent of the insect skeleton

[p] The treatment-medium-side Mass Transfer Coefficient value was evaluated according to those proposed by Mackay (2001)

[q] The air flux into the hive and out of it was calculated from an average year oxygen request of 30 kg (150 m^3 of air)

[r] The nectar flux into the hive was calculated by an average flux of 240 kg $year^{-1}$ of nectar (density approximated to 1.0 g cm^{-3}) considering a collection period of 180 days

[s] The pollen flux into the hive was calculated by an average flux of 30 kg $year^{-1}$ of pollen (density approximated to 1.0 g cm^{-3}) considering a collection period of 180 days

[t] The water flux into the hive was calculated by an average flux of 10 kg $year^{-1}$ of water (density of 1.0 g cm^{-3}) considering a collection period of 180 days

[u] The resin flux into the hive was calculated by an average flux of 0.1 kg $year^{-1}$ of resin (density approximated to 1.0 g cm^{-3}) considering a collection period of 180 days

[v] The larvae production was calculated from the yearly produced larvae (180,000), the individual volume of 90 mm^3 and the production period of 8 months (from February to September)

[w] The wax production into the hive was calculated from an amount of cap wax (0.15 kg $year^{-1}$), a density of 0.965 kg dm^{-3} and a production time of 6 months

[x] The honey production was calculated from the yearly produced amount (80 kg $year^{-1}$), a density of 1.4 kg dm^{-3} and a production time of 6 months

[y] The royal jelly production was calculated from the yearly produced amount (0.61 kg $year^{-1}$), a density of 1.1 kg dm^{-3} and a production time of 8 months

[z] The honey consumption by adult bees was calculated considering the number of 40,000 adult bees, the individual daily honey consume of 10 mg and the honey density of 1.4 kg dm^{-3}

[aa] The pollen consumption by adult bees was calculated considering the number of 40,000 adult bees, the individual daily pollen consume of 1 mg and the approximated pollen density of 1 kg dm^{-3}

[ab] The bee renewal was calculated considering the number of 40,000 adult bees, the lifetime of 45 days and the individual volume of 160 mm^3

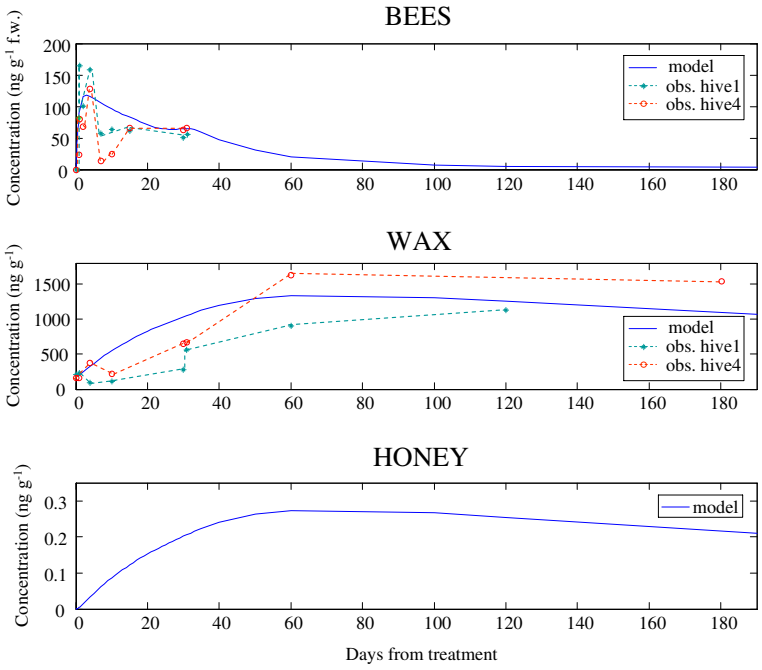

Figure 2. Comparison of predicted and measured concentrations of τ-fluvalinate in bees, wax and honey.

from deposition of the compound from air to wax (D_{dep}) and from uptake of the compound by the respiratory surfaces of bees (D_{up}).

We numerically solve the equation system (1)–(3) using the procedure ode45 in MATLAB (Matworks).

3. RESULTS

The hive model described in the previous section was applied to hives treated with τ-fluvalinate. All model parameters were set according to the previously determined physical and chemical properties of the compound (Table I) and the typical hive parameters described above (Table II). The pesticide was administered in two treatment strips, from which the compound passes to bees by direct contact and to other compartments by contact with bees or indirectly by contamination of bee products. If bees are contaminated with τ-fluvalinate, they tend to produce contaminated products or to contaminate products by contact during handling. Once τ-fluvalinate residues are transferred to bee products (wax, honey, pollen, larvae and propolis), the products themselves became secondary sources of contamination for bees. All of these exchanges are mathematically quantified by the D values listed in Table I. By simultaneously balancing all of these values in the equation system (1)–(3), we can calculate the resulting quantity of pesticide in each compartment as a function of time (Figure 2).

Figure 2 compares the predicted and measured concentrations in bees, wax and honey following treatment with τ-fluvalinate. Concentrations in bees and wax were measured in two experimental hives (hives 1 and 2) for up to 30 days (bees) and 180 days (wax). A complete description of the treatment modalities and the analytical techniques used for residue analyses is given in the part 1 of this work. Experimental data were consistent between the two hives. Bees were not contaminated before treatment; thereafter, their contamination level rose to a peak and later decreased to a nearly constant

value (50 ng g^{-1} f.w.) until the end of the treatment (30 days). In contrast, measured concentrations in wax gradually increased after treatment, reaching a plateau slightly above 1,000 ng g^{-1} at 60 days after treatment. Measured contamination levels in honey were always below the limit of quantification (2.5 ng g^{-1}). In general, the predicted concentrations (blue lines) closely follow the measured data, showing contamination levels near or intermediate between the two measured data trends. The ability of the model to forecast residue concentrations was tested by means of correlation analysis between observed and predicted data. Significant correlations were obtained for both bees (n=11; r=0.71; P<0.05) and wax (n=9; r=0.82; P< 0.01). Predicted concentrations for honey were always below the limit of quantification, as found in the empirical results.

Modelled concentrations predict a rapid increase in concentration just after treatment, as suggested by the measured data. Apistan strips are placed in between frames where a limited space is available. This causes a lot of inadvertent contacts, making their working activity more difficult. For this reason, bees are probably induced to reduce the strip interference for example by gluing them down to one frame with wax, to make for easier passage. The potential capacity of the strips to release the pesticide by contact is probably constant during the treatment, but its efficacy probably declines over time because of the reduced actual contact between bees and the treatment area. This would also reduce pesticide transfer from the strips to the bee compartment. Mathematically, this behaviour can be modelled by an exponential relationship vs. time (t) of the contact area between strips and bees ($A_{treat}=0.012\times e^{-1.26\times t}$). Initially, high variability in the measured concentrations of individual bees is expected because only a fraction of the bees working inside the hive can simultaneously visit the treatment strips. Later, the general contamination of all hive compartments (secondary sources) and the reduced visitation of bees to the treatment strips (primary source) tends to make the concentrations in bees more uniform. Predicted concentrations in bees peak during the first days of treatment and then slowly decrease because of the reduced contact area between strips and bees. After 30 days, when the treatment strips are removed, concentrations in bees tend to decrease more rapidly. The primary pesticide source (the strips) is no longer present, but secondary sources (contaminated hive products) remain. Therefore, bees tend to remain contaminated.

Predicted concentrations in wax and honey follow a slowly rising trend. Concentrations increase after the end of the treatment (30 days) until about 60 days, according to the measured data. After removal of the strips, the hive products still receive the pesticide through contact with bees until their fugacities became equal to that of bees. Thereafter, concentrations in the hive products begin to slowly decline because, in the absence of new pesticide input, various loss processes begin to reduce the overall contamination. Concentrations decrease more rapidly in bees than in bee products (wax and honey) because the former are the only metabolically active compartment and because bees are renewed more rapidly than the other compartments (especially wax and propolis). Biotransformation and advection out of the hive by dead bees are the primary processes by which the pesticide is lost from the hive system. The slow decline of contamination levels in wax is consistent with the presence of the pesticide 1 year later at lower but still detectable levels. In fact, a concentration of about 200 ng g^{-1} was measured in wax before treatment, presumably derived from previous treatments. Predicted concentrations in wax and honey are consistent with the measured data (for honey, only indirect confirmation of the model prediction is possible), confirming the predictive capability of the proposed model for these compartments.

4. DISCUSSION

The application of the hive model to the τ-fluvalinate treatment was successful; the model

was able to closely predict both the trends over time and the absolute contamination levels of the pesticide in the various hive compartments. The model predictions result from the input parameters and of the methods used to simulate the biological and physical processes occurring in the hive (Cowan et al. 1995). The primary input parameters are the physical and chemical properties of the compound and the environmental characteristics of the hive ecosystem. Although some variability and uncertainty exist in defining the exact values of the physical and chemical properties (Tomlin 1997) and in establishing the mean values of the hive characteristics (Seeley 1985), both classes of parameters are relatively well known. In contrast, the various processes by which the pesticide is taken up by, exchanged within, and finally lost from the hive ecosystem are more poorly known, and their mathematical description has been attempted only by Tremolada et al. (2004). Therefore, the most important and innovative contribution of this study is the attempt to mathematically describe the pesticide distribution in the hive ecosystem.

The affinities of the compound for the different compartments are essential for evaluating the tendency of the compound to partition into each phase, but do not determine whether the compound will actually reach a particular compartment or in what amount. This depends on the degradation, advection and exchange fluxes of the compound among the compartments and on their balance. In the framework of multi-compartimental fugacity models (Mackay 2001), these fluxes are defined by the *D* values listed in Table I. The *D* values are readily compared for a specific chemical since they have the same unit, even if they refer to different processes: mass fluxes (advection processes), diffusion events (exchange processes) or degradation (reaction processes) (Mackay 2001). Each of these categories is reported in Table I. Each *D* value is specific for a particular process and for a given molecule: a particular process can be important for one molecule but negligible for another, depending on the physical and chemical properties of the compound. Although *D* values may refer to different processes (e.g. reactions inside bees

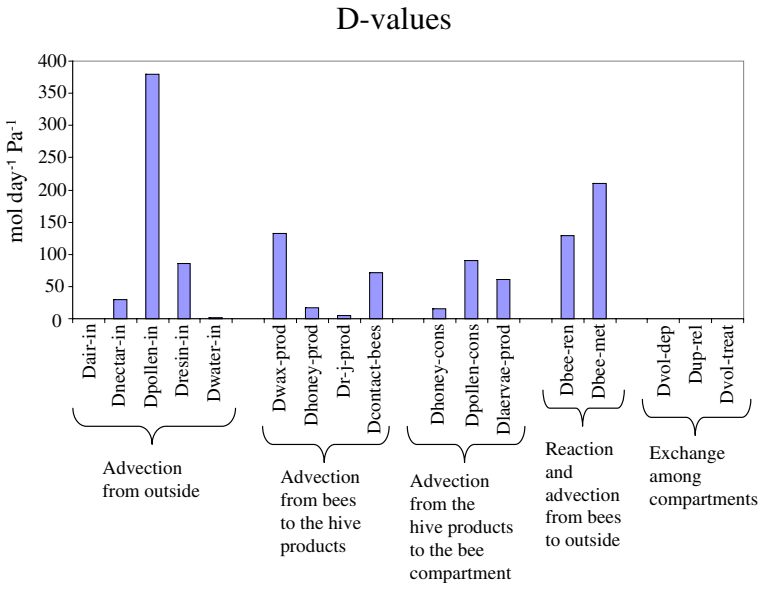

Figure 3. Advection, reaction and exchange processes calculated by the model for τ-fluvalinate in the hive ecosystem.

or volatilisation from bees to air via respiratory surfaces), they have the same units (moles per day per Pascal) because they are flux parameters (with units of moles per day) relative to the same fugacity level (per Pascal) (Mackay 2001). Therefore, for a given compound (in this case, τ-fluvalinate), all *D* values are comparable for a given system (in this case, the hive ecosystem). All *D* values reported in Table I have been adapted or were originally proposed to describe hive ecosystem fluxes and thus the actual movement and distribution of the compound.

In Figure 3, the various *D* values included in the hive model for τ-fluvalinate are grouped into five categories. The first refers to pesticide inputs from outside through the collection of resources and materials (nectar, pollen, resin, air and water). In the present model application, the pesticide input through these pathways is considered negligible; the respective fugacities were set to zero (see model description). However, it is possible to quantify their role as potential contamination sources through their respective *D* values. These *D* values are introduced in the model as options to evaluate the possibility of contamination coming from outside, as in the case of agricultural pesticide application (Barmaz 2009). Examination of Figure 3 shows that for molecules having physical and chemical properties similar to those of τ-fluvalinate, the pollen is the most important external contamination pathway, with a value nearly five times greater than that of resin, ten times greater than that of nectar, 300 times greater than that of water and 1,000 times greater than that of air. Because of the low vapour pressure of τ-fluvalinate, its presence in air in the vapour form is negligible. However, because of its high affinity for lipids, this compound may be present in large quantities in materials with a consistent lipid fraction, such as pollen. For this kind of compound, pollen may be a consistent contamination source in the hive ecosystem, as emphasised by Villa et al. (2000). Foraging bees have marginal contact with contaminated pollen because they carry it externally on the legs; however, once the pollen is used inside the hive (principally as larval food), it becomes a possible source of contamination.

Anti-*Varroa* treatments inside the hive are targeted toward the mites on the bee surface and are, therefore, aimed specifically at bees (Wallner 1999). From bees, pesticides can diffuse easily to other hive compartments (Bogdanov 2006). The *D* values that describe the transfer and distribution of the pesticide from bees to other hive components (wax, honey, pollen, propolis and larvae) are found in the second group in Figure 3. Larvae are included in the bee products because they grow inside the wax cells, are produced by adult bees and are fed by adult bees using typical hive products (honey, pollen and water). Not all bee products are strictly produced by bees; for example, pollen is collected outside and is mixed with honey only for storage purposes. The resin portion of propolis is also collected outside. If they are not previously contaminated by outside sources, these products are not contaminated by bees. In contrast, other products (newly secreted wax, honey and royal jelly) are directly produced by bees and thus, more easily, directly contaminated by them. Therefore, they are included in the pesticide flux from bees to the bee product components. Another contamination pathway from bees to bee products is determined by contact between contaminated bees and the products themselves. Examination of Figure 3 shows that the most effective contamination pathway is wax production, even though it may be limited in quantity, because of the high affinity of τ-fluvalinate for this product. Transfer of the compound by direct contact is an important pathway; this kind of transfer may occur when bees walk on the wax surface or handle hive products. Contaminant transfer through honey production and royal jelly production is of lesser importance, the former because of the low affinity of τ-fluvalinate for honey and the latter because of the small amount produced.

Once the compound is transferred to the bee products, it becomes a secondary source of diffusion within this compartment and of secondary bee contamination (third group of *D*

values). Bees receive the pollutant by consuming contaminated food (honey and pollen) and by inheriting the residues accumulated by larvae when metamorphosis occurs. Among these pathways, pollen consumption is the most important process, similar to the contribution inherited from the larvae, while honey (the primary food supply) is less relevant. Again, the physical and chemical properties of the compound explain these relative transfer rates.

Loss pathways through bee renewal and bee metabolism (fourth group of D values) are also important. Their relative importance is determined by the duration of the life cycle (45 days) and by the biotransformation susceptibility of the compound in bees (half life of 30 days). These pathways appear to be of similar importance and both are responsible for decreasing pesticide concentrations in bees once the treatment is finished.

The fifth group of D values deals with diffusion exchanges via air. These D values are of negligible importance compared to the others because of the low volatilisation potential of τ-fluvalinate. Their contribution to the distribution of the pesticide within the hive is limited. Only the uptake and release from the respiratory surfaces of bees is relatively greater (0.015 mol day^{-1} Pa^{-1}) than the volatilisation from the wax surface (0.00097 mol day^{-1} Pa^{-1}) or from the treatment surface (0.0000097 mol day^{-1} Pa^{-1}).

The overall balance of these fluxes in Eqs. (1)–(3) determines the actual distribution of the pesticide among the hive compartments and its trends over time. Some parameters are based on characteristics that are relatively well known, such as the quantity of food storage, but others are more poorly known, such as the amount of pesticide transferred from the treatment strips to the bee compartment per unit of time. Even among the well-known parameters, there is a certain level of variability or uncertainty. For example, what is the average food intake of an adult bee? How much honey and pollen do bees consume in a day? Of course, consumption depends on food availability, bee age and primary activity of the bee. For example, bees that are preparing royal jelly require more pollen (as **a** protein source) than those that are producing new wax. Even this relative simple parameter cannot be easily described. However, for modelling purposes, it is sufficient to define an average amount for the overall bee diet.

All of the parameters listed in Table II are not easily defined (their calculation is explained in the footnotes), but their quantitative evaluation is essential for the model. Much more experimental work is needed to better define these parameters, which are estimated here on the basis of the authors' knowledge. This modelling study suggests many interesting areas for further research, such as the actual diffusion of the pesticide by contact, the amount of pesticide ingested during the self-cleaning procedures of bees, the actual transfer kinetics of the contaminant between wax and newly deposited honey and pollen, and the contributions of food intake and contact with contaminated wax to larval contamination. We believe that the proposed model is a useful contribution to the comprehension of contamination phenomena in the hive ecosystem.

5. CONCLUSION

The proposed hive model correctly predicts τ-fluvalinate concentration levels and trends over time in bee and wax compartments and (presumably) in honey (indirectly confirmed by empirical data). The model considers pesticide inputs from outside *via* nectar, pollen, resin, water and air and from inside via pesticide treatment against bee pests to calculate the levels of pesticide residues in bee products (honey, pollen, royal jelly, wax and propolis). A priori prediction of the possible contamination levels of bee products is important because contaminated products may threaten both bee health and human health (when the products are collected by beekeepers). The model is able to calculate predicted concentrations in all hive products over time, giving expected contamination levels 1 month or 1 year after treatment, for example.

Based on our validation of the model using empirical data on τ-fluvalinate residues, we consider the model reliable, once the amount of pesticide input is correctly defined. In fact, the model focuses in part on defining the exact amount of pesticide transferred from the pesticide source. In the case of τ-fluvalinate, our empirical data suggest that only a small amount of the pesticide present in the strips is actually transferred into the hive system (less than 1%) and that this transfer occurs at two different rates. The more rapid initial rate is limited to the first days, during which bees presumably actively explore the newly introduced material, thus readily coming into contact with the pesticide. The much slower second rate depends probably on reduced contact between the bees and the strips as the strips are touched inadvertently during normal bee activities. The difference in the transfer rate of the pesticide during treatment is modelled using an exponential expression for the actual contact area between the strips and the bees, which provides a reliable estimate of the transfer of the pesticide from the strips to the hive system over time, resulting in good predictions for bees and wax. This calibration is specific for the application method used for τ-fluvalinate and can be applied only for similar conditions. Other application methods will require different modelling parameters to be added to the general equations in the proposed hive model. The general framework of the model is designed to evaluate every possible source of contamination from outside or inside the hive by contact or ingestion of any non-ionised compound. However, more calibration tests are needed to obtain precise predictions. In particular, the model should be tested with other commonly used anti-*Varroa* pesticides (coumaphos, malathion and bromopropylate), for which a large amount of empirical data is available for comparison.

Prédire le devenir des pesticides dans la ruche (2° Partie): développement d'un modèle dynamique de la ruche.

Résidus de pesticides / contamination de la ruche / fluvalinate / modèles à compartiments multiples / fugacité

Zusammenfassung – Vorhersage der Verteilung von Pestiziden im Bienenvolk (Teil II): Entwicklung eines dynamischen Bienenvolk-Modells. Wir stellen ein neues Bienenvolk-Modell für die Abschätzung der Verteilung und des Verbleibs von Pestiziden im „Ökosystem Bienenvolk" vor. Das Modell berücksichtigt den Pestizideintrag von außen über Nektar, Pollen, Harze, Wasser und Luft sowie von innen über Bekämpfungsmaßnahmen gegen Bienenkrankheiten; daraus kann die Höhe der Pestizidrückstände in den Bienenprodukten (Honig, Pollen, Geleé Rojale, Bienenwachs und Propolis) abgeschätzt werden. Die Eingabeparameter sind die chemischen und physikalischen Eigenschaften des Wirkstoffes und die Charakteristika des Bienenvolk-Ökosystems wie sie in Table I und 2 definiert sind. Die Validierung des vorgeschlagenen Modells erfolgte auf der Grundlage empirisch gewonnener Rückstandsdaten zu tau-Fluvalinat in Bienen, Bienenwachs und Honig. In Figure 2 werden die prognostizierten und gemessenen Konzentrationen in Bienen, Wachs und Honig verglichen. Insgesamt gleichen die prognostizierten Konzentrationen (blaue Linien) recht gut den gemessenen Werten. Die meisten Parameter und Gleichungen in diesem Modell wurden von den Autoren entwickelt. Obwohl weitere Tests in der Praxis notwendig sein werden, liefern sie bereits jetzt Vorhersagen, die im Einklang zu den experimentellen Ergebnissen stehen. Das Modell soll u. a. die exakte Pestizidmenge definieren, die von einer bestimmten Wirkstoffquelle übertragen wird. Im Fall von tau-Fluvalinat wird der Wirkstoffeintrag während der Behandlung durch eine exponentielle Gleichung für die Kontaktfläche zwischen Streifen und Bienen über die Zeit modelliert ($A_{treat} = 0.012 * e^{-1.26 * t}$). Diese Gleichung liefert eine brauchbare Schätzung des zeitabhängigen Wirkstofftransfers vom Streifen in das Bienenvolk, wodurch eine gute Vorhersage für die Belastung von Bienen und Wachs möglich ist. Ein anderes wichtiges Ergebnis des Modells ist die Möglichkeit, die in 5 Kategorien eingeteilten D-Werte (Figure 3) zu vergleichen. Die erste Kategorie bezieht sich auf den Wirkstoffeintrag von außen durch das Sammeln von Nektar, Pollen, Harzen, Luft und Wasser. Die zweite Kategorie beschreibt den Transfer des Wirkstoffes von den Bienen zu den Bienenstock-Kompartimenten (Wachs, Honig, Pollen, Propolis und Brut). Die Larven wurden in das „Produkt-Kompartiment" mit einbezogen, da sie sich innerhalb der Wachszellen entwickeln und von den Bienen aufgezogen und gefüttert wurden. Ist der Wirkstoff erst einmal im „Bienenprodukt-Kompartiment" angekommen, wird er innerhalb dieses Kompartiments zu einer zweiten Diffusionsquelle, unter anderem durch sekundäre Bienenkontaminationen (dritte Gruppe der D-Werte). Die vierte Gruppe der D-Werte steht für den primären Abbauweg der Pestizide (Erneuerung der Bienenpopulation und Metabolismus der Bienen). Schließlich beschreibt die fünfte Gruppe der D-Werte den Dif-

fusionsaustausch über die Luft. Diese D-Werte sind wegen der geringen Flüchtigkeit von tau-Fluvalinat von geringer Bedeutung im Vergleich zu den anderen Kategorien. Die Gesamtbilanz dieser Wiorkstoff-Ströme in den Gleichungen (1) – (3) bestimmt die jeweilige Verteilung des Pestizids in den Bienenstock-Kompartimenten und deren zeitlichen Verlauf. Das vorgeschlagene Modell ist ein hilfreiches Vorhersageinstrument und verbessert unser Verständnis von Kontaminationsabläufen im Bienenvolk.

Pestizidrückstände / Beutenkontamination / Fluvalinat / Multikompartiment-Model / Flüchtigkeit

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