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Uptake, translocation and metabolism of imidacloprid in plants

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Abstract

The insecticide imidacloprid is mainly applied as a seed dressing formulation. After root uptake, imidacloprid is translocated acropetally within the xylem and degraded quickly in the plants. Three principal metabolic pathways have been identified in various plant metabolism studies with different crops and types of application showing a nearly uniform quantitative and qualitative pattern in all test systems.

Key words: imidacloprid, metabolism, nectar, pollen, residue analysis, sunflower, translocation, uptake.

Introduction

Imidacloprid is the first commercially available representative of a new chemical class, the chloronicotinyl or neonicotinoid insecticides. It was synthesized in 1985 and the first registration was achieved in France (1991) in sugar beet. It is a systemic broad-spectrum insecticide and acts as a contact and stomach poison against sucking and some biting insects (rice hoppers, aphids, thrips, whitefly, termites etc.). It can be applied for seed, soil or foliar treatment. The molecule exhibits a novel mode of action as it is an agonist of the nicotinic acetylcholine receptor leading to paralysis and death of pest organisms (Bai *et al.*, 1991; Nauen *et al.*, 1998; Schmuck *et al.*, 2003). The mammalian toxicity is about 700-fold lower than that of nicotine having a similar chemical structure and the same mode of action (Elbert *et al.*, 1998).

Experiments have been carried out to determine uptake, translocation and metabolic pathways of imidacloprid after different types of application (foliar, soil and seed treatment, stem injection, painting application) on various plant species including apples (Vogeler *et al.*, 1992), corn (Vogeler and Dräger, 1989), cotton (Vogeler and Brauner, 1993), eggplant (Yoshida, 1992), hop (Reckmann, 1998), potatoes (Dräger *et al.*, 1992; Vogeler *et al.*, 1991), rape (Clark, 1997), rice (Kurogochi and Araki, 1989; Kurogochi *et al.*, 1989; Sakamoto, 1991), sunflower (Stork, 1999; Schmuck *et al.*, 2001), tobacco (Clark and Brauner, 1994), tomatoes (Dräger *et al.*, 1989), wheat and sugar beet (Stein-Dönecke *et al.*, 1989). In addition, the behaviour in cell suspension cultures (Köster *et al.*, 1988; Köster, 1990) and rotational crops (Vogeler *et al.*, 1992) was investigated.

The objective of these investigations was to determine the nature of the residue in different crops and crop parts and to set up a uniform residue definition for all crops as a basis for succeeding pre-registration residue studies as well as for post-registration residue enforcement and monitoring purposes.

Materials and methods

As this article reviews a lot of metabolism studies performed with imidacloprid over a span of a decade, only general information about the used instrumentation, equipment and materials is given. During that time and depending on crop, nature of the residues and author different types of instrumentation and analytical procedures may have been applied. A more detailed insight can be taken from the individual studies cited above.

Radioactively labelled [methylene-^{14}C]imidacloprid was applied mainly as a spray, seed dressing or granular formulation in all greenhouse trials. The use of a ^{14}C-labelled active substance (a.s.) allowed very sensitive and selective analyses. Certified non-radiolabelled compounds were used as reference substances for metabolite identification by co-chromatography. Extraction of plant samples was performed with different solvents or solvent mixtures, containing methanol, acetone, acetonitrile, ethyl acetate or water. Radioactivity in extract solutions and solid samples was determined by liquid scintillation counting (LSC) and combustion/LSC, respectively. For separation, detection and quantitation of parent compound and metabolites within extracts, thin-layer chromatography (TLC) in combination with a Bio-Imaging Analyser or high-performance liquid chromatography (HPLC) with a radioactivity flow-through detector were used. Where identification with reference compounds was not possible, isolation, purification and subsequent structure elucidation was performed by means of mass and/or nuclear magnetic resonance spectroscopy.

Results and discussion

Following seed dressing or granular application of imidacloprid on cotton, eggplant, potato and rice, uptake of the applied activity ranged from 1.6 to 4.9% (Kurogochi *et al.*, 1989; Sakamoto, 1991; Vogeler and Brauner, 1993; Vogeler *et al.*, 1991; Yoshida, 1992; Kurogochi and Araki, 1989). After seed treatment of corn the uptake amounted to 20% (Vogeler and Dräger, 1989). The ratio of the activity in the reproductive organs compared to the activity of the whole plant was very low in corn, cotton, eggplant and rice ranging from

0.7 to 1.4%; in potato this ratio amounted to 12% (table 1).

Uptake and translocation have also been studied after spray application of imidacloprid 25 WP on apple and tomato (Vogeler *et al*, 1992; Dräger *et al.*, 1989). Fourteen days after the treatment of apple and tomato fruits 28 and 21% of the applied activity, respectively, was recovered in or on the fruits; between 65 and 76% of this recovered activity was located on the surface of the fruits (table 2). In additional translocation experiments the a.s. was applied only onto apple and tomato leaves, while the fruits were covered with plastic foil during the application to prevent contamination. At harvest, 14 days later, the amount of radioactivity in the fruit compared to the activity applied was 0.1% at maximum (Vogeler *et al.*, 1992; Dräger *et al.*, 1989).

Table 1. Uptake, distribution and main metabolites of imidacloprid after soil application/seed treatment (TRR: total radioactive residue, 6-CNA: 6-chloronicotinic acid, 6-CPA: 6-chloropicolyl alcohol, *Nitrosimine<<1% of TRR in reproductive organs).

Crop	Harvest [days after treatment/seeding]	Uptake of applied activity [%]	Activity in reprod. organ relative to the activity in whole plant [%]	Nature of the Residue (Compounds >1% of TRR)
Corn	134	20	1.2	Imidacloprid, Guanidine, 5-Hydroxy, Olefine, Nitrosimine*, Ring-open-guanidine, 6-CNA, 6-CPA, Dihydroxy
Cotton	211	4.9	1.4	6-CPA-conj., Guanidine, 6-CPA-Glucoside, Imidacloprid, 6-CNA, 6-CPA, Olefine, Nitrosimine*
Eggplant	69	1.6	1.0	Guanidine, Imidacloprid, , 6-CPA-Glucoside, 5-Hydroxy, Olefine, 6-CNA
Potato	129	2.5	12	Imidacloprid, 6-CNA, Guanidine, 5-Hydroxy, Olefine, Nitrosimine*, 6-CPA-Glucoside
Rice	79	4.5	1.1	Guanidine, Imidacloprid, 6-CNA, 5-Hydroxy, Nitrosimine*
Rice	124	4.4	0.7	Guanidine, Imidacloprid, 5-Hydroxy, 6-CNA, Olefine

Table 2. Uptake and main metabolites after spray application of imidacloprid.

Crop	Harvest [days after treatment]	Uptake of applied activity [%]	% TRR on surface	Nature of the Residue (Compounds >1% of TRR)
Apple fruit	14	28	65	Imidacloprid, Olefine, 5-Hydroxy, Guanidine, 6-CPA-Glucoside, Urea, Dihydroxy
Tomato fruit	14	21	76	Imidacloprid, Guanidine, Urea, 5-Hydroxy

TRR: total radioactive residue

Table 3. Residues of imidacloprid, hydroxy- (4- and 5-hydroxy) and olefine-metabolites in nectar and pollen samples of corn, sunflower and summer rape (field trial results).

Crop	Formulation	Sample Material	Residues in treated and control samples [mg/kg]		
			Imidacloprid	Hydroxy-metab.	Olefine-metab.
Corn 1 trial (France)	WS 70 1 mg a.s./seed	Pollen	<<0.005	<<0.005	<<0.01
Sunflower	WS 70	Nectar	<<0.005	<<0.005	<<0.01
2 trials (Germany)	0.7 mg a.s./seed	Pollen	<<0.005	<<0.005	<<0.01
Summer Rape 3 trials (France, Sweden, UK)	FS 500 0.04 mg a.s./seed	Nectar Pollen	<<0.005 <<0.005	<<0.005 <<0.005	<<0.01 <<0.01

a.s.: active substance (=imidacloprid)

Translocation experiments examine the mobility and distribution of chemical compounds within the vascular system and the tissues of plants. The results of these experiments for imidacloprid with different application types show, that there is a good acropetal translocation of the a.s. to shoots and leaves (excellent xylem mobility) on the one hand and on the other hand a poor basipetal translocation to sinks, i.e. storage organs, roots and fruits (negligible phloem mobility). Consequently, highest residues are expected to occur in the older leaf parts of the plants. The systemic properties of a molecule are a function of its physicochemical properties, mainly water solubility, octanol/water-partition coefficient (log P_{OW}) and dissociation constant (pK_a) determining e.g. its ability to penetrate through biomembranes. These properties are responsible for the kinetics of root uptake and translocation into the xylem, which is a prerequisite for entering the phloem and in turn to be taken up by sucking insects. According to the BRIGGS model (Briggs *et al.*, 1982) the loading of the xylem by organic compounds due to root-systemic uptake can be described by a bell-shaped curve with maximum xylem mobility between log P_{OW} of 1.0 and 2.5. As a measure of xylem mobility the transpiration stream concentration factor (TSCF) has been introduced relating the concentration of the compound in the transpiration stream (xylem) to the exposure concentration. This model predicts for imidacloprid with log P_{OW} = 0.51 a considerable high xylem mobility of about TSCF = 0.6, which was qualitatively confirmed in the translocation experiments mentioned above.

Xylem and phloem have different pH values of about 5 and 8, respectively. Therefore, especially weak acids with pK_a values of about 5.0-5.5 and log P_{OW} between 1.0 and 2.5 (molecules with this lipophilicity properties can cross membranes very easily), e.g. 6-chloronicotinic acid, tend to accumulate in the phloem sieve tubes (ion trap mechanism). Imidacloprid with its high pK_a of 14 is nonionized and therefore these pH differences do not affect the distribution between the compartments and the a.s. moves freely between phloem and xylem according to its biomembrane permeability. However, since there is no active loading of imidacloprid to the phloem and due to the far greater water flow (50- to 100-fold) imidacloprid is predominantly transported within the xylem vessels (Bromilow and Chamberlain, 1989). Once imidacloprid has entered the leaves it will be trapped by this counter current principle in the leaf and not re-transported into the plant stem.

Despite the wide variety of crops and application types having been investigated a rather uniform picture of the metabolic behaviour of imidacloprid in plants was found consisting of three principal biotransformation pathways (figures 1-3). Especially after soil application or seed treatment a quick degradation of the a.s. was observed after root uptake of the a.s. In the case of spray application only a part of the a.s. is translocated into the plant and metabolized there, so the degree of metabolism tends to be lower in this case.

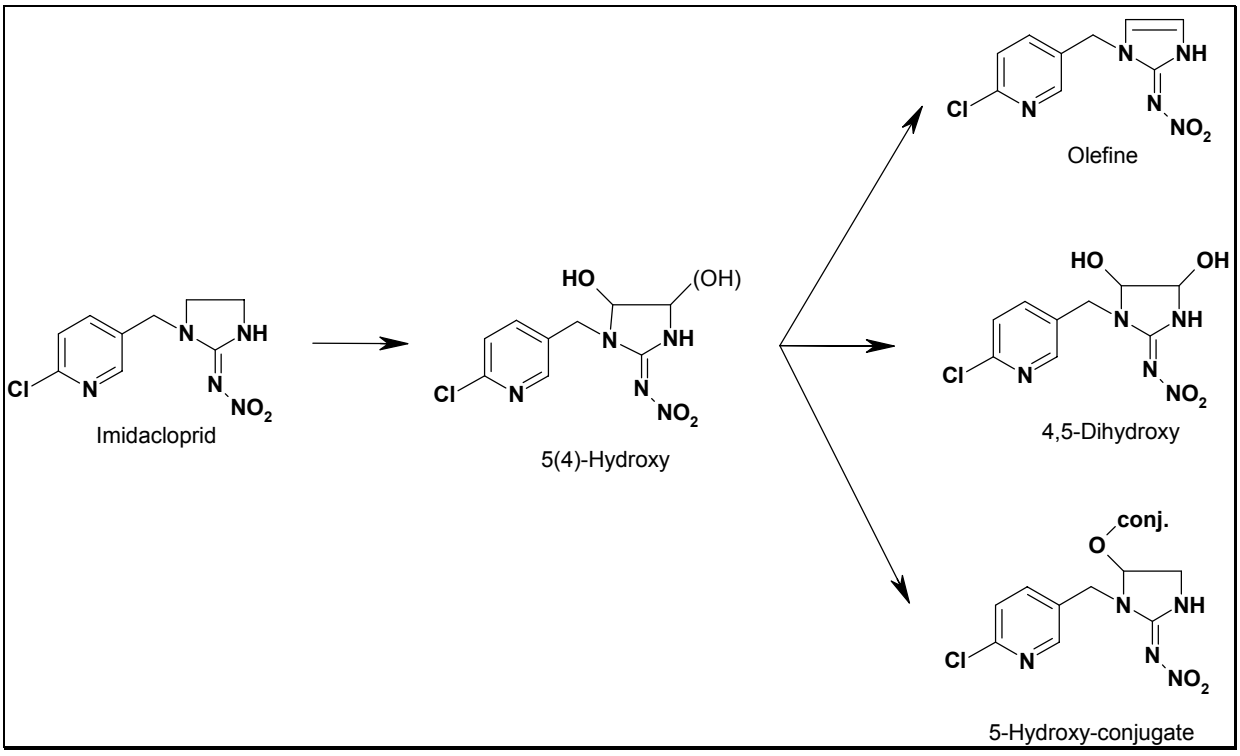

Figure 1. Metabolism of imidacloprid (I): ethylene-bridge hydroxylation of the imidazolidine ring and elimination of water.

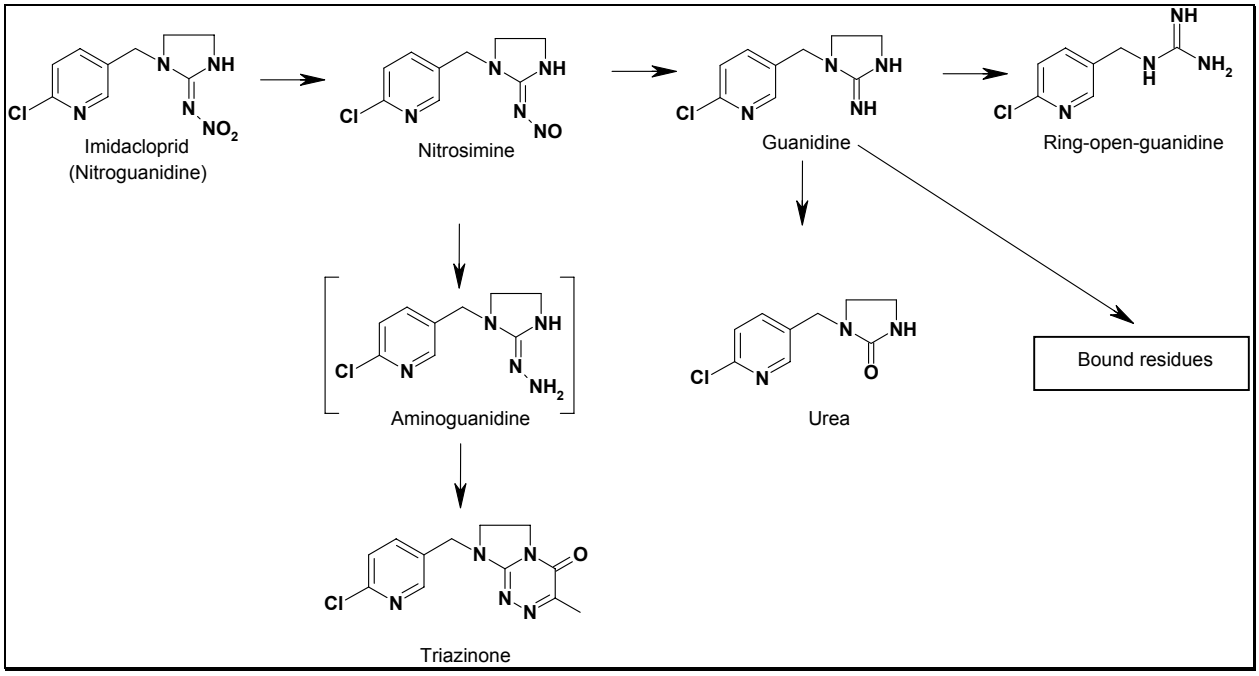

Figure 2. Metabolism of imidacloprid (II): nitro-group reduction to nitrosimine and further loss of NO to form guanidine.

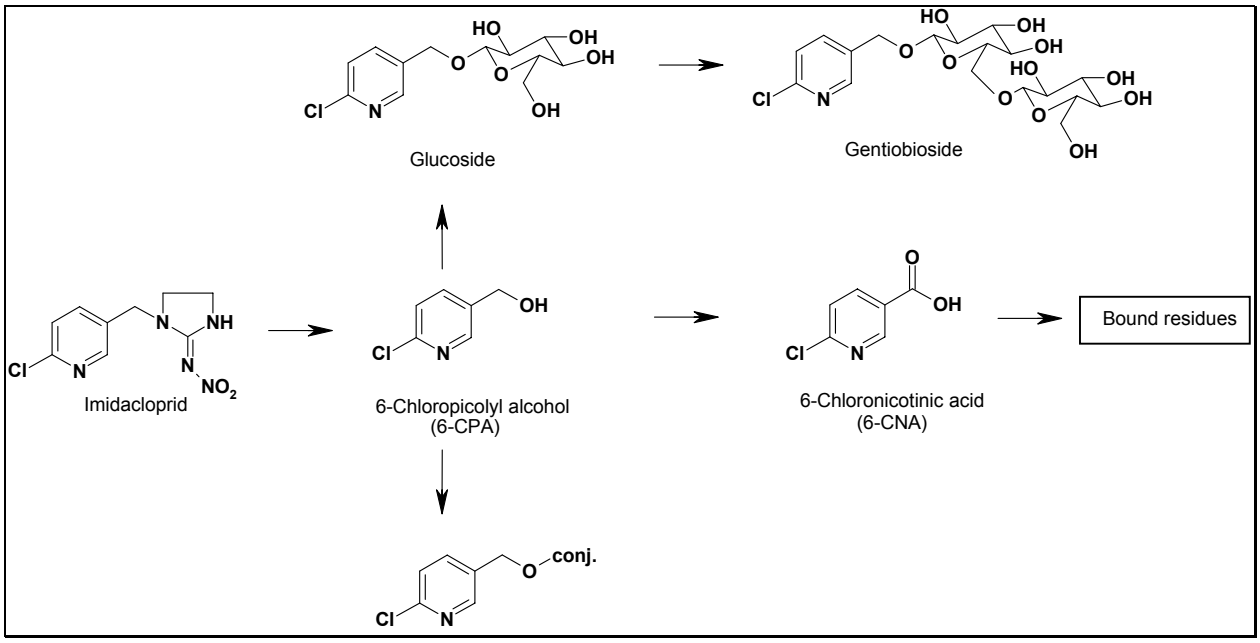

Figure 3. Metabolism of imidacloprid (III): Oxidative cleavage of the methylene bridge to form 6-chloropicolyl alcohol and subsequent oxidation to 6-chloronicotinic acid.

In figure 1 the ethylene-bridge hydroxylation at the imidazolidine (dihydroimidazole) ring of the a.s. leads to the formation of the hydroxy-metabolite, which mainly undergoes a subsequent dehydration to form the olefine-compound. Secondly, as depicted in figure 2, a nitro-group reduction takes place to form the nitrosimine compound. In a further step, after loss of the NO-group the cyclic guanidine and urea metabolites are formed. Starting with nitrosimine a cyclisation with endogeneous pyruvic acid (created in respiration during glycolysis) to the triazinone metabolite via a supposed but not found aminoguanidine intermediate occurs. However, minute amounts of this compound were found only in potato leaves. Thirdly, imidacloprid is oxidized to 6-chloropicolyl alcohol and in turn to 6-chloronicotinic acid. In addition, alcohol conjugates with glucose and isomaltose to glucopyranoside and gentiobioside, respectively, are also formed (figure 3).

In the reproductive organs of seed-treated crop plants, only very low amounts of imidacloprid metabolites were detected. Based on the quantitative aspect the 5-hydroxy-, olefine-, dihydroxy-, urea- and 6-CNA-metabolite were most commonly found in these parts of seed-dressed crop plants.

It has to be mentioned that the findings of the metabolism and translocation experiments reflect worst-case scenarios and that under practical field conditions lower amounts of metabolites and a more rapid degradation are observed. This is on the one hand due to UV-photolysis and weather conditions being only of negligible importance in greenhouse trials and the low amount of soil in the plant boxes compared to the root mass causing exaggerated uptake of imidacloprid.

As far as bee-relevant matrices are concerned, a metabolism study in sunflower after seed-dressing with imidacloprid WS 70 was carried out (Stork, 1999; Schmuck *et al.*, 2001). The applied amount was 0.79 mg a.s./seed. During flowering, the nectar was collected after sampling the female florets from the inflorescence by tweezers and extracting the nectar using glass capillaries. Pollen samples were collected in plastic boxes fixed below the inflorescences before flowering. The total radioactive residue (TRR) in nectar and pollen was very low and amounted to 0.0019 and 0.0039 mg/kg, expressed as parent compound equivalents, respectively. In nectar 100% of the TRR consisted of imidacloprid. In pollen, the whole extractable radioactivity (85.8%, 0.0033 mg/kg) represented imidacloprid. The remaining radioactivity in pollen (= non-extractable residues) was not further analysed due to the very low absolute amount. As only imidacloprid was found in the metabolism study, this was considered as the only relevant residue in those sample materials and consequently, the residue definition was expressed as "parent only".

Field-residue trials with non-labelled imidacloprid on sunflower, corn and rape were additionally carried out to confirm the findings of the metabolism study under practical conditions (Schmuck, 1999). The samples of these studies were not only analyzed for imidacloprid but also for hydroxy- and olefine-metabolites, as these compounds also show biological activity against certain insect species (Nauen *et al.*, 1999; Nauen *et al.*, 2001). The results of all six field trials are compiled in table 3. Neither in pollen nor in nectar samples of corn, sunflower and summer rape any residues of imidacloprid and the two other metabolites have been determined above the limit of quantitation (LOQ of imidacloprid and hydroxy-imidacloprid = 0.005 mg/kg, LOQ of olefine metabolite = 0.01 mg/kg).

Conclusions

More than 15 studies with imidacloprid have been carried out concerning uptake, translocation and metabolism in various plant species mainly after foliar, soil or seed treatment. The uptake after soil or seed treatment is about 5% of the applied dose and the a.s. shows good acropetal mobility within the xylem and poor basipetal translocation within the phloem. Three principal metabolic pathways of imidacloprid in plants were identified showing a quick degradation of the a.s., especially after seed or soil application. The findings of the metabolism studies show a clear and consistent picture. It can be concluded that in nearly all crops the metabolic pathway of imidacloprid runs via the same three routes and results in qualitative and quantitative similar composition of the metabolic spectrum. All identified metabolites still contain the 6-chloropicolyl moiety of imidacloprid. Hence, the relevant residue to be analysed in field residue trials can be defined as the sum of imidacloprid and its metabolites containing the 6-chloropicolyl moiety, expressed as imidacloprid.

The nature of the residue in bee-relevant matrices of oilseeds (sunflower nectar and pollen) was determined to consist of the parent compound imidacloprid only. Field residue trials with imidacloprid after seed-dressing of sunflower, corn and rape revealed that no residues above the limit of quantitation of the residue analytical method were present in pollen and nectar.

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