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Effects of a Chronic Dietary Exposure of the Honeybee *Apis mellifera* (Hymenoptera: Apidae) to Imidacloprid

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Abstract. Previous studies have shown that imidacloprid and insecticidally active imidacloprid plant metabolites are rapidly metabolized by honeybees. Accordingly, no striking differences were expected between the acute and the chronic dietary toxicity of imidacloprid to honeybees. More recently, however, an unexpectedly high chronic dietary toxicity to honeybees was reported for imidacloprid and imidacloprid plant metabolites, and a novel pharmacologic mechanism unrelated to the parent toxophor was postulated. In an extensive literature survey, no further evidence was found for the reported high difference between the acute and the chronic dietary toxicity of imidacloprid and its plant metabolites to honeybees. The majority of data indicated a dietary no observed lethal-effect concentration >0.04 and 0.02 mg/L 50% sucrose solution, respectively, for an acute and a chronic dietary exposure of honeybees to either imidacloprid or its plant metabolites. Findings of chronic feeding studies with those plant metabolites where the toxophor had already been cleaved did not support the hypotheses of a novel pharmacologic mechanism unrelated to the parent toxophor. No increased treatment-related mortality or behavioral abnormalities were recorded in four independent research facilities during a 10-day dietary exposure of honeybees of different ages to sucrose solutions spiked with the respective metabolites at 0.0001, 0.001, and 0.010 mg/L 50% sucrose solution.

Neonicotinoid insecticides such as imidacloprid (1-[(6-chloro-3-pyridinyl)methyl]-N-nitro-2-imidazolidinimine) act agonistically on the insect nicotinic acetylcholine receptor (nAChR). The nAChR has also been identified as the molecular target site in the honeybee *Apis mellifera* (Nauen *et al.* 2001). Nauen *et al.* (2001) demonstrated that the binding of imidacloprid and its insecticidally active metabolites to the nAChR was fully reversible. Furthermore, imidacloprid and its insecticidally active metabolites are rapidly metabolized in the honeybee (Suchail 2001; Suchail *et al.* 2003). Accordingly, no striking differences were expected between the acute and the chronic toxicity of this compound to honeybees. However, Suchail *et al.* (2001) reported an unexpectedly high chronic dietary toxicity of imidacloprid to honeybees with an approximative median lethal concentration (LC_{50}) of 0.0001 mg/L 50% sucrose solution. A comparably high dietary toxicity was reported in the same article for six imidacloprid plant metabolites (5-hydroxy-imidacloprid, olefine-imidacloprid, 4,5-dihydroxy-imidacloprid, desnitro-imidacloprid, urea metabolite; and 6-chloro-nicotinic acid [6-CNA], of which some had already lost the toxophor (Fig. 1). Suchail *et al.* (2001) related the similarity of the observed dietary toxicity between metabolites with and without the toxophor to the chloropyridine structure, which all of these plant metabolites had in common. Data from binding and electrophysiologic studies (Nauen *et al.* 2001), however, are not in line with this hypothesis.

The objective of the present study was to corroborate the results presented by Suchail *et al.* (2001) concerning the chronic dietary toxicity of imidacloprid and its plant metabolites to honeybees. To achieve this objective, two approaches were followed. First, a survey of all available data (including unpublished data) was performed to examine whether other researchers had found a comparably high chronic dietary toxicity of either the parent or its plant metabolites to honeybees. Second, dietary toxicity tests were performed at four different research institutes with the urea metabolite (urea NTN) and the 6-CNA following closely the testing method given in Suchail *et al.* (2001). These two metabolites were chosen because the toxophor (=N-nitril-increment) is no longer present in these metabolites, and Suchail *et al.* (2001) had postulated a pharmacologic mechanism related to the chloropyridine structure of the molecules. Any chloropyridine-related toxicity should be most clearly expressed in these two metabolites because interference with the primary toxophor can be excluded for these two plant metabolites.

Material and Methods

Chemicals

Urea NTN and 6-CNA (Fig. 1) were purchased from Bayer AG, Leverkusen (Germany) and shipped to the test facilities within 4 days of receipt. Before shipping, purity was analytically determined and

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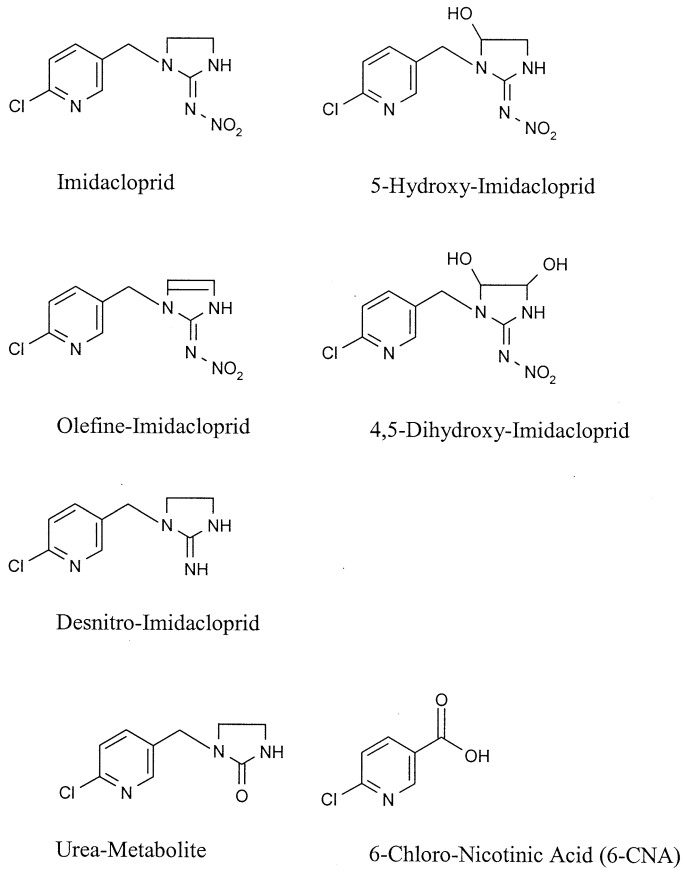

Fig. 1. Chemical structures of imidacloprid and plant metabolites referenced in this article

was found to be >95% for both the urea NTN and the 6-CNA metabolite.

Honeybees

Test bees were adult worker bees (*A. mellifera* L.) collected from a single colony owned and maintained by the respective test facility (for further details see Table 1). Two age cohorts were used for the feeding tests. Younger honeybees (hive bees) were obtained by either incubating combs with bee brood (Germany I) or sampling bees from the brood nest area (Germany II and III). Older honeybees (foragers) were sampled from the flight board or an exit bypass. One laboratory (United Kingdom I) used bees of widely varying ages (between 2 and 35 days) that were collected from the combs.

Preparation of Test Solutions

Hereafter, the term "solution" is used to include material that may be suspended or dispersed. Both metabolites, urea-NTN and 6-CNA, readily dissolved in 50% w/v aqueous sucrose up to the stock solution concentration to yield a clear, colorless solution. They remained in solution when further diluted in 50% w/v aqueous sucrose for test concentrations. Homogeneity of such solutions was checked visually during and immediately before use and solutions were stored at 4°C in the dark until required.

Testing Procedure

Before placing them into the test boxes, honeybees were acclimatized to the test conditions for at least 1 hour. Three to five batches of bees, in groups of 10 bees, were offered 0.1, 1.0, and 10.0 μg/L of the test substance in 50% w/v aqueous sucrose solution (same test concentrations as used by Suchail *et al.* 2001) with the dose being measured into two small, preweighed glass feeders within the cage using a variable volume pipette. Controls received 50% w/v aqueous sucrose solution. Each group of bees was offered 0.8 to 5 mL of a given concentration, which was provided in preweighed glass feeders within the cage. During the test period, the bees were kept in the dark (except during observations) in an incubator at 23°C to 28°C and 45% to 88% relative humidity. Mortality and sublethal effects were assessed at 24- to 48-hour intervals after the start of the test for up to 10 days. Sublethal effects were assessed according to predetermined categories: knocked down (i.e., alive but immobile), staggering (i.e., moving but poorly coordinated) and responsiveness (i.e., hyporesponsive or hyperresponsive). The glass test feeders containing any unconsumed portions of the doses were removed and weighed either daily or at regular intervals (every second or third day). Fresh test feed was supplied in preweighed glass feeders within the cages after each consumption measurement. The dose consumed was determined by comparing the weight of the dose remaining in the glass feeders with the weight of a known volume of the test solutions. Mortality data were analyzed for statistical differences between the treatment and the control groups using Fischer's Exact test (Germany I) and a one-sided Student *t* test (all other laboratories).

Table 1. Honeybee strains used in the chronic feeding tests and sampling and randomization procedure applied by the four testing facilities

Testing Facility	Bee Strain	Bee Sampling	Bee-Allocation Procedure
Germany I	*A. mellifera* L. var. *carnica* POLLM.	Raised from incubated comb pieces (young bees) or collected from flight board (old bees)	Test cages allocated to treatment by random list
Germany II	*A. mellifera* L. var. *carnica* POLLM.	Sampled from brood nest area (young bees) or collected from flight board (old bees)	No randomization procedure
Germany III	*A. mellifera* L. var. *carnica* POLLM.	Sampled from brood nest area (young bees) or collected from by-pass exit (old bees)	Bees impartially allocated to labeled test cages
United Kingdom I	*A. mellifera* L. (strain not specified)	Sampled from hive combs	No randomization procedure

Results

Literature Survey on the Chronic Dietary Toxicity of Imidacloprid and its Plant Metabolites to Honeybees

All accessible values (published or unpublished) on the chronic dietary toxicity of imidacloprid to honeybees were compiled. The studies and their results are listed in Tables 2 and 3. In the laboratory studies, corrected mortality figures $< 10\%$ were considered to indicate no observed lethal-effect concentrations (NOLEC). In the tunnel- and field-feeding studies those concentrations in which no increased mortality or significant antifeeding effects were noted were considered as NOLEC.

Laboratory feeding studies with imidacloprid indicated chronic NOLEC values between >10 and 48 μg/L. The dietary NOLEC value of 4 μg/L reported by Decourtye (1998) could not be confirmed by the same researcher (Decourtye *et al.* 2003) in a repeat study conducted with a higher number of replicates, i.e., a higher statistical power. Also in the reviewed tunnel- and field-feeding studies, dietary NOLEC values were much greater than the NOLEC of <0.1 μg/L reported by Suchail *et al.* (2001). The higher-tiered studies of Belzunces *et al.* (1999) and Kirchner (1999) may not be directly comparable with the laboratory-feeding study of Suchail *et al.* (2001) because there were uncontaminated food stores in the hives, which may have been preferably used by the bees. However, the test concentrations in these studies were 1,000 and 10,000-fold higher than the approximative LC_{50} value of Suchail *et al.* (2001), which should have resulted in substantial bee mortality even if only minute amounts of these sucrose solution were ingested during the studies. In the study by Schmuck *et al.* (2001), bees were exclusively fed with contaminated sunflower honey, and therefore a precise NOLEC >20 μg/kg can be concluded. The dietary toxicity values from all reviewed laboratory (NOLEC >10 μg/L) and field–semifield (NOLEC >20 μg/L) studies indicate a much lower dietary toxicity of imidacloprid to honeybees than suggested by the results of the laboratory tests by Suchail *et al.* (2001), where substantial mortality was found at 0.1 μg/L. Further to their feeding studies with imidacloprid, Decourtye and Pham-Delègue (2000) and Decourtye *et al.* (2003) also examined the chronic dietary toxicity of two insecticidally active plant metabolites of imidacloprid—the 5-hydroxy- and the olefin-metabolite—under laboratory conditions. Both metabolites still contain the primary toxophor of the parent molecule. Their findings on these two metabolites are listed in Tables 4 and 5. As for the parent compound, no increased honeybee mortality was recorded for the hydroxy- and the olefin-metabolite at the tested residue levels after chronic dietary exposure. Also Kirchner (personal communication, 2000) could not demonstrate mortality rates higher than control mortality for a dietary exposure of either 10 μg dihydroxy-imidacloprid/L sucrose solution or 10 μg olefin-metabolite/L sucrose solution fed during 10 days. As for the parent compound, these findings are in contradiction to the reported chronic dietary toxicity values of Suchail *et al.* (2001), who reported approximative LC_{50} values of 0.1 μg/L for all tested metabolites.

In the reviewed feeding studies, reported average sucrose ingestion rates of honeybees were ≥ 23.5 μL/bee/d (Tables 2 through 5). These sucrose ingestion rates are at least twice as high than those reported by Suchail *et al.* (2001), who reported an average ingestion rate of only 12 μL/bee/d.

Chronic Dietary Toxicity of the Urea-NTN and the 6-CNA-Metabolite to Honeybees

During the feeding studies with the urea NTN and 6-CNA metabolites, control bees ingested sucrose volumes between 31.7 and 65.2 μL/bee/d (young bees; Table 6). These ingestion rates compare well with those reported in the surveyed literature (Tables 2 through 5) and are significantly higher than those reported by Suchail *et al.* (2001) under comparable environmental conditions.

Intake of the metabolite-containing solutions was not decreased up to the highest test concentration of 10.0 μg/L. No statistically increased mortality rates relative to the control were noted except in test run no. 1 by Germany II (Tables 7 and 8).

Tests starting with older workerbees (22 to 45 days old) do not appear to give reliable results as shown by the high mortality in the controls (20% to 44%). In the tests with older honeybees, mortality did not follow a dose–response relationship and fluctuated randomly between replicates (controls 10% to 60%) and dose groups (34% to 77%). This was also true in the test using bees of unspecified age (United Kingdom I). In the tests with bees of higher or unspecified age (United Kingdom I), individuals were occasionally classified as being

Table 2. Results of the literature survey on the chronic dietary toxicity of imidacloprid to honeybees: Laboratory Studies

Location/season	Age of Bees[a]/Strain	Feeding Duration (n = No. of Repetitions)	Syrup Ingestion Rate (μL/Bee/d)	Mortality rates (% Corrected for Control Mortality)	Reference
Bures, France/ March-April 1998	4 d (*A. mellifera ligustica*)	11 d (n = 2)	25–46 (50% sucrose, 33°C, dark)	4 μg/L (3) 8 μg/L (20) 40 μg/L (36) Control mort. (7) NOLEC: 4 μg/L	Decourtye (1998)
Bures, France/Nov-Feb, 1999–2000	4 d (*A. mellifera ligustica*)	11 d (n = 3)	c. 28.8–33.7 (50% sucrose)	1.5 μg/L (1) 3 μg/L (0) 6 μg/L (0) 12 μg/L (0) 24 μg/L (5) 48 μg/L (10) Control mort. (12) NOLEC: 24 μg/L	Decourtye *et al.* (2003) (repetition of 1998 study)
Bures, France/Jun-July, 2000	4 d (*A. mellifera ligustica*)	11 d (n = 3)	c. 28.8–33.7 (50% sucrose)	1.5 μg/L (5) 3 μg/L (5) 6 μg/L (2) 12 μg/L (4) 24 μg/L (5) 48 μg/L (6) 96 μg/L (15) Control mort (3) NOLEC: 48 μg/L	Decourtye *et al.* (2003) (repetition of 1998 study)
Avignon, France/ May-June, 1998	Not specified	15 d (n = 1)	Not specified (32°C)	No difference in mortality compared with control at 10 μg/L NOLEC: 10 μg/L[b]	Colin *et al.* (1998)
Konstanz, Germany/ May-June, 2000	4 d (*A. mellifera carnica*)	10–12 d	Not specified (30°C)	No difference in mortality compared with control at 10 μg/L NOLEC: 10 μg/L[b]	Kirchner (2000)
Avignon, France/ not specified	Age not specified (*Apis. mellifera* L.; strain not specified)		c. 12 (50% sucrose)	0.1 μg/L (ca. 35) 1 μg/L (ca. 65) 10 μg/L (ca. 70) Control mort (< 15) NOLEC: < 0.1 μg/L	Suchail *et al.* (2001)

[a] At study initiation.
[b] Highest test concentration.
Mort = Mortality.
NOLEC = No observed lethal-effect concentration.

knocked down or stumbling, but in these tests the same number of affected specimens were recorded in the control as in the treatment groups. It appears that in tests with bees of higher or unspecified age, it is difficult to distinguish between natural mortality at the end of the life cycle of the honeybee and mortality from compound-related lethal effects. Accordingly, these tests do not provide reasonable results on potential treatment-related effects. In contrast, younger bees remained vital during the entire test period, and tests with these bees ended in reliable results as shown by reasonably low mortality rates in the controls (≤10%).

Chronic Dietary Toxicity Tests—Artifact Analysis

In Germany II, an increased mortality relative to the controls was recorded for both metabolites in test run no. 1. However, no clear dose–response relations were recorded in these tests. For example, between the three replicates of the highest dose group with the urea metabolite, mortalities of 90%, 80%, and 20% were recorded for ingested doses of 2.3, 4.1, and 4.3 ng metabolite/bee, respectively. A more detailed analysis of the testing procedure followed for this test run revealed that honeybees had not been randomly allocated to the treatment groups. Rather, bees sampled first from the storage container were placed in the control boxes, and bees sampled next were allocated to the lowest treatment level of the urea-metabolite, etc. Arranging the recorded mortality rates in order of loading the test boxes with bees yields the results shown in Table 9.

This obvious correlation between loading sequence and increasing mortality rates indicates a sampling artefact (the bees sampled latest were the least vital) rather than a treatment-related effect. Because of these experimental uncertainties and the fact that the honeybees from this apiary had revealed no

Table 3. Results of the literature survey on the chronic toxicity of imidacloprid to honeybees: Tunnel and field feeding studies

Location/Season	Initial No. of Bees/Strain	Exposure Duration	Syrup-Ingestion Rate (μL/Bee/d)	Mortality Rates (% Corrected for Control Mortality)	Reference
Avignon, France/April 1998	5,000 (*A. mellifera* Buckfast)	6 d[a] Individually marked bees	Not specified (50% sucrose)	No difference in mortality compared with control reported for 100 μg/L; decreased foraging at 1,000 μg/L NOLEC: 100 μg/L[b]	Belzunces *et al.* (1999)
Konstanz, Germany/ May-June 1999	5,000 (*A. mellifera carnica*)	4–10 d Individually marked bees	Not specified (50% sucrose, 21°C, semidark)	No difference in mortality compared with control reported for 10, 20, 50 and 100 μg/L; decreased foraging at 50 and 100 μg/L NOLEC: 20 μg/L[b]	Kirchner (1999)
Euskirchen, Germany/ June-July 1999	500 (*A. mellifera carnica*)	39 d	c. 25[c] (90% sucrose)	No difference in mortality compared with control reported for 2, 5, 10, and 20 μg/kg NOLEC: > 20 μg/kg[d]	Schmuck *et al.* (2001)

[a] Belzunces et al. (1999) erroneously reported an exposure duration of 10 days; this figure was corrected in Guez (2001).
[b] No increased mortality reported for higher concentration, but this was perhaps mainly due to the observed strong antifeedant effect.
[c] Related to the initial number of bees (no relevant increase in bee number before day 32).
[d] Highest test concentration.

Table 4. Results of the literature survey on the chronic toxicity of imidacloprid plant metabolites to honeybees: Hydroxy-imidacloprid

Location/Season	Age of Bees[a]/ Strain	Exposure Duration (n = No. Repetitions)	Syrup-Ingestion Rate (μL/Bee/d)	Mortality Rates (% Corrected for Control Mortality)	Reference
Bures, France/ Nov-Feb	4 d (*A. mellifera ligustica*)	11 d (n = 3)	c. 23.5–32.6[b] (50% sucrose)	7.5 μg/L (0) 15 μg/L (0) 30 μg/L (3) 60 μg/L (0) 120 μg/L (11) 240 μg/L (29) Control mort (17) NOLEC: 60 μg/L	Decourtye *et al.* (2003)
Avignon, France/ not specified	Not specified (*A. mellifera* L.; strain not specified)	10 d (n = 3)	c. 12 (50% sucrose)	0.1 μg/L (ca. 25) 1 μg/L (ca. 50) 10 μg/L (ca. 55) Control mort (< 15) NOLEC: < 0.1 μg/L	Suchail *et al.* (2001)

[a] At study initiation.
[b] Taken from Decourtye and Pham-Delegue (2000).

higher sensitivity to imidacloprid than did bees from the other laboratories (acute toxicity data are reported in Nauen *et al.* 2001), one repeat feeding test was conducted at this laboratory. For this repeat test (run no. 2), 6-CNA was chosen because in test run no. 1 this metabolite had shown a higher dietary toxicity than the urea metabolite. In the repeat test, a computer-generated random list was used for allocating test bees to the different treatment groups, and bees were sampled closer to the broodnest to get younger stages. In this repeat test, no increased mortality was recorded at any treatment level (Table 8).

Discussion

A review of chronic dietary toxicity data of imidacloprid to honeybees from various publications revealed NOLEC values ≥0.01 mg/L sucrose solution (Colin *et al.* 1998; Belzunces *et al.* 1999; Kirchner 1999, 2000; Schmuck *et al.* 2001; Decourtye *et al.* 2003). This chronic NOLEC value is not very different from the acute dietary NOLEC value of 0.046 mg/kg as reported by Schmuck *et al.* (2001). Such a similarity between acute and chronic values is to be anticipated given the rapid

Table 5. Results of the literature survey on the chronic toxicity of imidacloprid plant metabolites to honey bees: Olefin-imidacloprid

Location/ Season	Age of bees[a]/ Strain	Exposure Duration (n = No. of Repetitions)	Syrup-Ingestion Rate (μL/Bee/d)	Mortality Rates (% Corrected for Control Mortality)	Reference
Bures, France/ Nov-Feb	4 d, (*A. mellifera ligustica*)	11 d (n = 3)	c. 27.6–33.3 (50% sucrose)	1.4 μg/L (4) 2.8 μg/L (12) 5.6 μg/L (0) 11.2 μg/L (24) 22.5 μg/L (6) 45.0 μg/L (0) Control mort (8–11) NOLEC: ND	Decourtye and Pham-Delegue (2000)
Avignon, France/Not Specified	Not specified (*A. mellifera* L.; strain not specified)	10 d (n = 3)	c. 12 (50% sucrose)	0.1 μg/L (ca. 50) 1 μg/L (ca. 50) 10 μg/L (ca. 55) Control mort: (< 15) NOLEC: < 0.1 μg/L	Suchail *et al.* (2001)

[a] At study initiation.
ND = not determined due to inconsistent mortality pattern.

Table 6. Sucrose (50% w/v)-ingestion rates of control honeybees in the repeat chronic feeding tests compared with rates reported by Suchail *et al.* (2001)

Test Facility	Average Temperature (°C)	Young Bees (μL/Bee/d)[a]	Old Bees (μL/Bee/d)[a]	Bees of Unspecified Age (μL/Bee/d)
Germany I	24–28	37.0–57.9	66.1–99.2	
Germany II (test run I)[b]	25–27	38.8–48.2	45.3–52.1	
Germany II (test run II)	23–25	31.7–61.5	Not tested	
Germany III	24–26	55.7–65.2	68.5–84.2	
United Kingdom I	24–26			79.2–119.6
Suchail *et al.* (2001)	25			12

[a] Corrected for mortalities during the feeding period: 1 mL sucrose solution = 1.2 g.
[b] Classified as invalid because of (1) lack of dose–response relation, (2) lack of randomization procedure, and (3) irreproducibility of results by same laboratory (see Table 8, test run II).

metabolism of imidacloprid and its insecticidally active plant metabolites in the honeybee (Suchail 2001; Suchail *et al.* 2003). Accordingly, the very high chronic dietary toxicity of imidacloprid reported by Suchail *et al.* (2001) could not be confirmed based on a survey of literature information from published and unpublished sources.

In addition to the literature search, no-choice chronic feeding tests were conducted with urea NTN and 6-CNA (Fig. 1) at four different independent laboratories to test the hypotheses of Suchail *et al.* (2001) of a secondary toxicity mechanism of imidacloprid and its metabolites, which is related to the chloropyridine part of the molecule. Three of the four laboratories found NOLEC values >10 μg/L for both metabolites. One laboratory reported a chronic dietary toxicity comparable with that found by Suchail *et al.* (2001). A more detailed analysis of this test showed that it was not conducted according to standard experimental practices (e.g., randomization procedure). After amending the experimental deficiencies, a repeat test at the same laboratory resulted in no mortality, and data were consistent with those of the other three independent research institutes.

An analysis of all chronic feeding studies (n = 9) reported in this article highlights two experimental factors that could cause false-positive results in chronic feeding studies with honeybees when following the test protocol of Suchail *et al.* (2001):

1. Age of tested bees: Foraging honeybees sampled from the flight board do not qualify for chronic dietary toxicity tests because of their limited life span. In all tests with forager bees (22 to 42 days old), high control mortalities were observed (>20%) at the end of the 10-day feeding period.
2. Randomization procedure for allocating bees to treatment groups: As shown for various experiments, nonrandomization can strongly bias the test results. This could be demonstrated in the first test run of Germany II where mortality rates were strongly correlated with the sequence of the test-box loading.

Table 7. Dose-related mortality of honeybees during the repeat chronic feeding tests compared with values reported by Suchail *et al.* (2001): Urea-metabolite

	Young Honeybees (1–17 d)		Old Honeybees (22–45 d)		Honeybees of Unspecified Age	
Test Facility	Dose (ng a.i./ Bee/d)	Mortality (%)	Dose (ng a.i./ Bee/d)	Mortality (%)	Dose (ng a.i./ Bee/d)	Mortality (%)
Germany I	0	0	0	20[a]		
	0.004	8	0.003	34		
	0.045	6	0.029	20		
	0.432	0	0.288	16		
Germany II (test run I)[b]	0	10	0	30		
	0.005	37	0.004	60		
	0.039	3	0.048	50		
	0.357	63	0.478	60		
Germany III	0	4	0	44		
	0.008	10	0.008	26		
	0.073	8	0.071	36		
	0.727	12	0.730	36		
United Kingdom I					0	44
					0.007	34
					0.066	46
					0.640	50
Suchail *et al.* (2001)					0	<15
					0.001	c.50
					0.012	c.60
					0.120	c.75

[a] Test had to be prematurely terminated on day 4 because of increased control mortality.
[b] Classified as invalid because of (1) lack of dose-response relation, (2) lack of randomization procedure, and (3) irreproducibility of results by same laboratory (see Table 8, test run II).

Table 8. Dose-related mortality of honeybees during the repeat chronic feeding tests compared with values reported by Suchail *et al.* (2001): 6-chloro-nicotinic acid

	Young Honeybees (1–17 d)		Old Honeybees (22–45 d)		Honeybees of Unspecified Age	
Test Facility (Testing Period)	Dose (ng a.i./ Bee/d)	Mortality (%)	Dose (ng a.i./ Bee/d)	Mortality (%)	Dose (ng a.i./ Bee/d)	Mortality (%)
Germany I	0	0	0	20[a]		
	0.005	2	0.003	8		
	0.046	4	0.033	10		
	0.468	0	0.273	6		
Germany II (test run I)[b]	0	10	0	30		
	0.004	67	0.003	77		
	0.038	77	0.040	70		
	0.388	97	0.404	73		
Germany II (test run II)	0	7	ND	ND		
	0.006	10				
	0.055	7				
	0.580	7				
Germany III	0	4	0	44		
	0.007	6	0.008	32		
	0.073	4	0.082	40		
	0.724	10	0.806	30		
United Kingdom I					0	54
					0.007	58
					0.065	58
					0.667	52
Suchail et al. (2001)					0	<15
					0.001	c.50
					0.012	c.55
					0.120	c.70

[a] Test had to be prematurely terminated on day 4 because of increased control mortality.
[b] Classified as invalid because of (1) lack of randomization procedure, and (2) irreproducibility of results by same laboratory (see test run II and artifact analysis in the text).
ND - not determined.

Table 9. Recorded mortality rates arranged in order of loading test boxes with bees

Loading Sequence	Test Variant	Mortality (%)
1	Control	10
2	0.1 μg/L urea-metabolite	37
3	1.0 μg/L urea-metabolite	3
4	10.0 μg/L urea-metabolite	63
5	0.1 μg/L 6-CNA-metabolite	67
6	1.0 μg/L 6-CNA-metabolite	77
7	10.0 μg/L 6-CNA-metabolite	97

Certainly, it cannot be concluded from the presented analysis which factor(s) might have influenced the test results from Suchail *et al.* (2001). However, the data reported by Suchail *et al.* (2001) have at least to be considered with care because of the following:

1. They are not consistent with unpublished and published data generated by various other researchers.
2. They could not be reproduced by four different independent research facilities, which all had a minimum testing experience of three years (Germany II had only 1 year of testing experience).
3. The sucrose ingestion rates reported by Suchail *et al.* (2001) were significantly lower than those reported by all other laboratories, which indicates that bees in the tests of Suchail *et al.* (2001) may have suffered from starvation stress.
4. The experimental methodology was not fully described in Suchail *et al.* (2001). Accordingly, no information on the age of bees and the randomization procedure applied in this test are available from the article.

Conclusion

The present study tested the findings of Suchail *et al.* (2001) of an unexpectedly high chronic dietary toxicity of imidacloprid and imidacloprid plant metabolites. Neither an intensive literature survey nor repeat tests with two metabolites at four different testing sites confirmed the results reported by Suchail *et al.* (2001). During the repeat tests, one test run showed similarly high mortalities, which could be related to an experimental artefact. Likewise, the hypotheses of low- and high-affinity binding sites in honeybees by Suchail *et al.* (2001) could not be supported by receptor binding and electrophysiologic studies (Nauen *et al.* 2001). Metabolites where the primary toxophor was no longer present neither revealed any displacement of hydrogen-3-labeled imidacloprid, nor did they induce any currents in honeybee neurons from the antennal lobes. Accordingly, the findings reported by Suchail *et al.* (2001) must be considered as scientifically unvalidated and must be considered with great care when they are used to evaluate a potential dietary risk posed by imidacloprid and its plant metabolites to honeybees.

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