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## **Impacts of neonicotinoids on biodiversity: a critical review**

Laure Mamy, Stéphane Pesce, Wilfried Sanchez, Stéphanie Aviron, Carole Bedos, Philippe Berny, Colette Bertrand, Stéphane Betoulle, Sandrine Charles, Arnaud Chaumot, et al.

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# 1 **Impacts of neonicotinoids on biodiversity: a critical review**

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9

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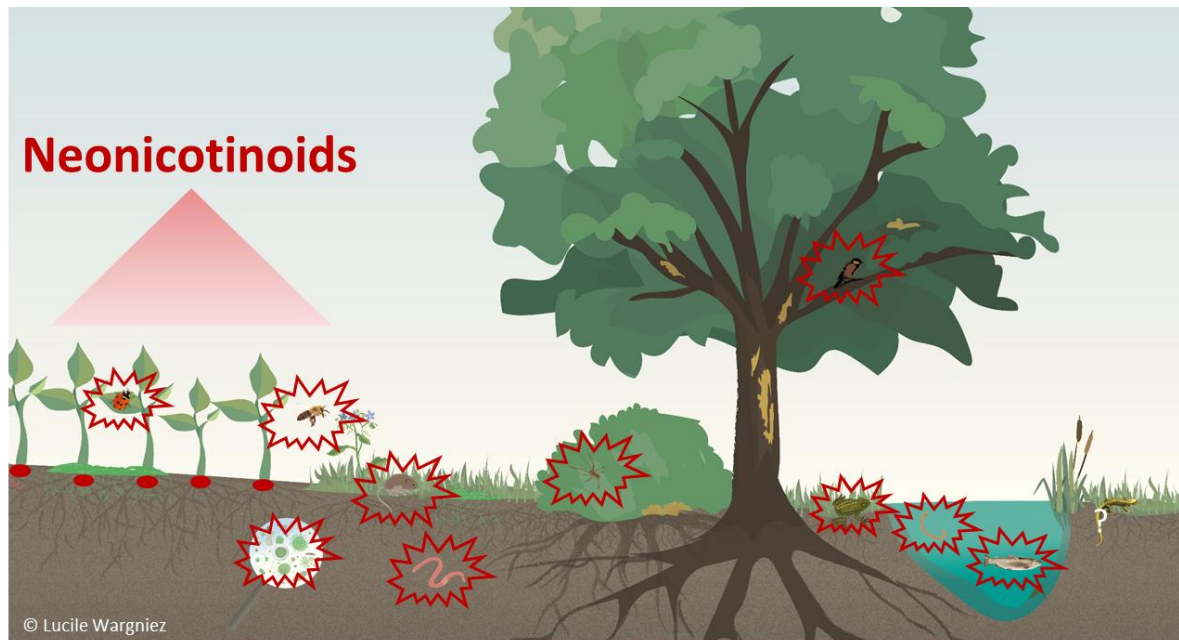
## 44 **Abstract**

45 Neonicotinoids are the most widely used class of insecticides in the world but they have raised numerous concerns  
46 regarding their effects on biodiversity. Thus, the objective of this work was to do a critical review of the  
47 contamination of the environment (soil, water, air, biota) by neonicotinoids (acetamiprid, clothianidin,  
48 imidacloprid, thiacloprid, thiamethoxam) and of their impacts on terrestrial and aquatic biodiversity.  
49 Neonicotinoids are very frequently detected in soils and in freshwater, and they are also found in the air. They  
50 have only been recently monitored in coastal and marine environments, but some studies already reported the  
51 presence of imidacloprid and thiamethoxam in transitional or semi-enclosed ecosystems (lagoons, bays and  
52 estuaries). The contamination of the environment leads to the exposure and to the contamination of non-target  
53 organisms, and to negative effects on biodiversity. Direct impacts of neonicotinoids are mainly reported on  
54 terrestrial invertebrates (e.g., pollinators, natural enemies, earthworms) and vertebrates (e.g., birds), and on aquatic  
55 invertebrates (e.g., arthropods). Impacts on aquatic vertebrate populations and communities, as well as on  
56 microorganisms, are less documented. In addition to their toxicity to directly exposed organisms, neonicotinoid  
57 induce indirect effects via trophic cascades as demonstrated in several species (terrestrial and aquatic

58 invertebrates). However, more data are needed to reach firmer conclusions and to get a clearer picture of such  
59 indirect effects. Finally, we identified specific knowledge gaps that need to be filled to better understand the effects  
60 of neonicotinoids on terrestrial, freshwater and marine organisms, as well as on ecosystem services associated with  
61 these biotas.

62

## 63 Graphical abstract



64

65

66 **Keywords** Pesticides · Plant protection products · Ecotoxicity · Ecotoxicology · Agrosystems · Collective  
67 scientific assessment

68

## 69 Introduction

70 Neonicotinoids are systemic insecticides (i.e., they diffuse throughout the treated plants to protect them from pests)  
71 that act on the central nervous system of insects by targeting nicotinic acetylcholine receptors (nAChRs) in the  
72 brain (Simon-Delso et al. 2015; Thompson et al. 2020). They are the world's fastest-growing and currently the  
73 most widely used class of insecticides against a broad spectrum of sucking and chewing insects (plant hoppers,  
74 thrips, micro-lepidopteras), and they are also involved in veterinary medicine (e.g., against fleas in pets) and in  
75 biocidal products such as those used for the treatment of livestock buildings or in pest baits for domestic use  
76 (Klingelhöfer et al. 2022; Thompson et al. 2020). In agriculture, neonicotinoids are mainly applied through seed

77 treatments, but they are also employed as granular application, spraying or soil treatment (Simon-Delso et al. 2015;  
78 Thompson et al. 2020). The five most used active substances are acetamiprid, clothianidin, imidacloprid,  
79 thiacloprid and thiamethoxam (clothianidin is also the main transformation product of thiamethoxam). Among  
80 these substances, only acetamiprid is still approved in the European Union (EU Pesticides database 2023).  
81 Clothianidin and thiamethoxam were withdrawn in 2019, while imidacloprid and thiacloprid were withdrawn in  
82 2020 (European Commission 2023). However, for example in France, derogations have been granted in 2021 and  
83 2022 for the use of coated seeds treated with imidacloprid or thiamethoxam in the context of the infestation of beet  
84 crops by aphids (JORF 2021; JORF 2022). Consequently, because of their wide use all over the world, and because  
85 of the high persistence of clothianidin, imidacloprid and thiamethoxam (average half-life in soils is 121 days for  
86 thiamethoxam (PPDB 2023), 187 days for imidacloprid (PPDB 2023) and 545 days for clothianidin (PPDB 2023)  
87 which could reach 20 years (Thompson et al. 2020)), neonicotinoids are likely to be ubiquitous in the environment,  
88 and present a potential environmental health concern (Bonmatin et al. 2015; Goulson 2013; Humann-Guilleminot  
89 et al. 2019a; Morrissey et al. 2015).

90 Neonicotinoids were first presented as having key attributes such as systemic nature, versatility in  
91 application (especially as seed treatments), selective toxicity to arthropods, lower binding efficiencies to vertebrate  
92 compared to invertebrate receptors, and assumed lower impacts on non-target aquatic and terrestrial organisms  
93 (Simon-Delso et al. 2015; Thompson et al. 2020). Neonicotinoids should also theoretically not target organisms  
94 lacking nAChRs and thus nervous systems, such as protists, fungi, prokaryotes and plants (Simon-Delso et al.  
95 2015).

96 However, neonicotinoids appeared to have lethal and sublethal effects on non-target organisms, including  
97 pollinators, insect predators and vertebrates (especially birds) (Alsafran et al. 2022; Mineau and Kern 2023;  
98 Mineau and Palmer 2013; Simon-Deslo et al. 2015). Thus, for many years, the use of neonicotinoid-based products  
99 in agriculture has raised concerns in several countries, particularly because of their effects on pollinators  
100 (Demortain 2021; Suryanarayanan 2013), and EFSA (2018) concluded that most uses of neonicotinoid substances  
101 do represent a risk to wild bees and honeybees. In addition, as more than 80% of neonicotinoid seed treatments  
102 can remain in the soil (Alford and Krupke 2017; Sur and Stork 2003), soil invertebrates may be exposed to high  
103 doses of neonicotinoids, with recognized lethal and sublethal effects (Gunstone et al. 2021). Neonicotinoids also  
104 contaminate freshwater ecosystems worldwide and could impact aquatic invertebrates, over broad spatial scales  
105 (Cavallaro et al. 2019; Hallmann et al. 2014; Morrissey et al. 2015). Moreover, they were demonstrated to exert

106 negative effects on terrestrial and aquatic vertebrates (Gibbons et al. 2015; Thompson et al. 2020; Wood and  
107 Goulson 2017).

108 In this context, the objective of this work was to do a critical review of (1) the contamination of the  
109 environment (soil, water, air, biota) by neonicotinoids and (2) their impacts on terrestrial and aquatic biodiversity.  
110 Although the literature focused on the ecotoxicological effects of neonicotinoids is abundant, to the best of our  
111 knowledge, no review has been published on the overall impacts of these substances on the whole biodiversity.

112

## 113 **Bibliographic corpus**

114 The review of the literature on the impacts of neonicotinoids on biodiversity was performed under the framework  
115 of a French collective scientific assessment focused on the impacts of plant protection products (PPPs) on  
116 biodiversity and ecosystem services (Pesce et al. 2023). Collective scientific assessment seeks to inform public  
117 policy and to foster public debate by analyzing the literature, but it is neither a meta-analysis nor a systematic  
118 review (Pesce et al. 2021). Though not quantitative, this review gives a detailed and complete overview of the  
119 impacts of neonicotinoids on the whole biodiversity.

120 In this framework, the bibliographic corpus was adapted and constructed as follows: six queries (Q)  
121 focused on neonicotinoids (Q1), ecotoxicology (Q2), biodiversity (Q3), terrestrial ecosystems (Q4), freshwater  
122 ecosystems (Q5) and marine ecosystems (Q6) were defined with related keywords (Table SII). The literature  
123 search was conducted on the Web of Science<sup>TM</sup>, from 2000 to 2020.

124 The corpus of publications was then built by combining Q1 with Q2, Q3, Q4, Q5 or Q6. The combination  
125 of Q1\*Q2 yielded 7349 references; that of Q1\*Q3, 457 references; Q1\*Q4, 3309 references; Q1\*Q5, 841  
126 references; and Q1\*Q6, 252 references. After removing duplicates, the total number of references was 7697.

127 The time course of the 7697 references showed a strong increase in the number of publications related to  
128 the impacts of neonicotinoids from 2000 to 2020 (Fig. 1). Among the five neonicotinoids retained in this review,  
129 imidacloprid was the most studied one (4218 occurrences in titles and abstracts), well above thiamethoxam (1672),  
130 acetamiprid (1176), clothianidin (887) and thiacloprid (674) (Fig. 2). The bibliometric measurements also  
131 demonstrated that terrestrial invertebrates were the most studied organisms and especially honeybees (Fig. 3).  
132 Apart from terrestrial invertebrates, fish come at the thirty second place (Fig. 3). In the first 35 occurrences, there  
133 are no other taxonomic group.

134 The categorization of references was based on titles and abstracts. The selected corpus was then divided  
135 according to the expertise of the different authors who proceeded to in-depth analysis of each reference. The

136 literature search was focused on the most integrative and ecologically realistic studies as possible. The results of  
137 single-species tests were not systematically reviewed, and were only used if they provided explanatory elements  
138 for processes observed under realistic environmental conditions.

139 The corpus was finally manually completed by various documents, papers and books known to the authors and  
140 which were not present in the 7697 references, and over time until April 2023. At the end, a total of 308  
141 publications were retained and cited in this work.

142

## 143 **Terrestrial ecosystems**

### 144 **Contamination of soils, plants and air**

145 Neonicotinoids are found in all environments: soil, water (see section “Contamination of freshwater and marine  
146 environments” below), plants and air.

147

### 148 **Contamination of soils**

149 Soil contamination by neonicotinoids has been studied under various climates, soil types, and agricultural practices  
150 (Table 1). A large study conducted on 74 French cultivated soils showed that imidacloprid (limit of quantification  
151 LOQ = 1 µg/kg) was present in 91% of the soil samples (excluding seven organically grown soils, with no  
152 detectable traces) although only 15% of the sites had been planted with treated seeds the year of the monitoring  
153 (Bonmatin et al. 2005a). In addition, imidacloprid was detected in 100% of the soils which received treated seeds  
154 (corn, wheat or barley) during the sampling year, and in 97% of the soils which received the same treatment one  
155 or two years before the study. Concentrations were higher in the soils which had been treated consecutively during  
156 two years before the monitoring than in those that received treated seeds only one year before, indicating that  
157 imidacloprid accumulates in soils over time. Silva et al. (2019) found that imidacloprid was present in 7% of the  
158 examined European topsoil samples (LOQ = 10 µg/kg, one order of magnitude higher than the above study) with  
159 a maximum content of 60 µg/kg, while Pelosi et al. (2021) found imidacloprid in 90 % of French sampled soils  
160 (n=180, 26 % when considering concentrations >10 µg/kg, LOQ = 0.4 µg/kg) and concentrations reaching 160  
161 µg/kg (Table 1). Thiamethoxam was present in 20% of the French soils at low concentrations (maximum of 2  
162 µg/kg, LOQ = 0.4 µg/kg) (Pelosi et al. 2021). In Switzerland, imidacloprid (LOQ = 0.9 10<sup>-3</sup> µg/kg) was quantified  
163 in 94% of cultivated field soils (n=82) and in 71% of ecological focus area soils (annual, biennial and perennial  
164 herbaceous plant species; n=68) (Humann-Guillemot et al. 2019a). Clothianidin (LOQ = 1.6 10<sup>-3</sup> µg/kg) was

165 also frequently observed in the sampled soils (77% of cultivated fields and 46% of ecological focus areas);  
166 followed by thiacloprid (LOQ =  $1.6 \cdot 10^{-3}$   $\mu\text{g}/\text{kg}$ ; 28% and 13%), thiamethoxam (LOQ =  $1.9 \cdot 10^{-3}$   $\mu\text{g}/\text{kg}$ ; 27% and  
167 6%) and acetamiprid (LOQ =  $2.0 \cdot 10^{-3}$   $\mu\text{g}/\text{kg}$ ; 13% and 3%) (Humann-Guillemint et al. 2019a). Similarly, Riedo  
168 et al. (2021) repeatedly observed imidacloprid (59% of soils, maximum concentration of 24  $\mu\text{g}/\text{kg}$ , LOQ = 0.14  
169  $\mu\text{g}/\text{kg}$ ), clothianidin (55%, 57  $\mu\text{g}/\text{kg}$ , LOQ = 0.15  $\mu\text{g}/\text{kg}$ ), thiamethoxam (21%, 24  $\mu\text{g}/\text{kg}$ , LOQ = 0.15  $\mu\text{g}/\text{kg}$ ) and  
170 thiacloprid (10%, 14  $\mu\text{g}/\text{kg}$ , LOQ = 0.073  $\mu\text{g}/\text{kg}$ ) in various Swiss agricultural soils (Table 1). The highest  
171 concentration of imidacloprid in Switzerland was measured by Chiaia-Hernandez et al. (2017) and was found to  
172 be 138  $\mu\text{g}/\text{kg}$  (LOQ = 3  $\mu\text{g}/\text{kg}$ ) (Table 1). Recently, Froger et al. (2023) monitored 111 PPP residues (48 fungicides,  
173 36 herbicides, 25 insecticides and/or acaricides, and two safeners) in 47 soils sampled across France under various  
174 land uses (arable lands, vineyards, orchards, forests, grasslands, brownfields). The most frequently quantified  
175 neonicotinoid was clothianidin (17% of the soil samples, maximum concentration of 2.7  $\mu\text{g}/\text{kg}$ , LOQ = 0.5  $\mu\text{g}/\text{kg}$ )  
176 followed by imidacloprid (9%, 13.8  $\mu\text{g}/\text{kg}$ , LOQ = 2  $\mu\text{g}/\text{kg}$ ), thiacloprid (6%, 0.26  $\mu\text{g}/\text{kg}$ , LOQ = 0.05  $\mu\text{g}/\text{kg}$ ) and  
177 acetamiprid (2%, 0.48  $\mu\text{g}/\text{kg}$ , LOQ = 0.01  $\mu\text{g}/\text{kg}$ ) (Table 1). Thiamethoxam was not quantified (LOQ = 0.5  $\mu\text{g}/\text{kg}$ ).  
178 In English arable soils, where neonicotinoids have been used as seed treatments, the concentrations of clothianidin  
179 ranged from  $< 0.02$  to 13.6  $\mu\text{g}/\text{kg}$  (LOQ = 0.02  $\mu\text{g}/\text{kg}$ ), that of imidacloprid from  $< 0.09$  to 10.7  $\mu\text{g}/\text{kg}$  (LOQ = 0.09  
180  $\mu\text{g}/\text{kg}$ ) and that of thiamethoxam from  $< 0.02$  to 1.5  $\mu\text{g}/\text{kg}$  (LOQ = 0.02  $\mu\text{g}/\text{kg}$ ) (Jones et al. 2014). Overall, most  
181 of the reviewed works focusing on the presence of neonicotinoids in soils is centered on imidacloprid, while the  
182 other substances are much less targeted. The environmental conditions, crops, agricultural practices, analytical  
183 methods and sampling time and strategies may explain the differences observed between the reviewed studies but,  
184 in general, they show the ubiquitous contamination of soils by neonicotinoids (Bonmatin et al. 2015; Froger et al.  
185 2023).

186

## 187 **Contamination of plants**

188 Neonicotinoids enter plants through the roots and/or leaves, and are transported into various organs, including  
189 foliage, flowers, pollen and nectar (Bonmatin et al. 2015). They are frequently detected in cultivated plants, as  
190 well as in wild plants. Bonmatin et al. (2005b) measured imidacloprid concentrations in corn whose seeds have  
191 been treated with this insecticide and observed that 76% of stem and leaf samples at flowering contained more  
192 than 1  $\mu\text{g}/\text{kg}$  of the substance (LOQ = 0.1  $\mu\text{g}/\text{kg}$ ). They also quantified from 1 to 10  $\mu\text{g}/\text{kg}$  of imidacloprid in  
193 sunflower flower heads, with notable variations depending on crop stage and seed variety. In the 29 analyzed  
194 samples of sunflower pollens, only two contained traces of imidacloprid. In parallel, imidacloprid was detected in

195 untreated sunflower heads grown on soil treated in previous years (from 0.1 to 2 µg/kg). In sugar beet crop treated  
196 with 90 g/ha of imidacloprid as seed coating, the concentration of imidacloprid in leaves initially reached 12.4  
197 mg/kg (fresh weight), then decreased but remained above 1 mg/kg 80 days after sowing, and was below the limit  
198 of detection (LOD = 10 µg/kg) at harvest (Rouchaud et al. 1994). Humann-Guilleminot et al. (2019a) analyzed  
199 imidacloprid, clothianidin, thiamethoxam, thiacloprid and acetamiprid in plant samples taken from 79 cultivated  
200 fields (mainly from cereals and beetroots, but also from potatoes, rapeseed, maize, peas and flax) and 69 ecological  
201 focus areas over Switzerland. The neonicotinoids were detected in 97% of plant samples taken in cultivated fields,  
202 and in 93% of plant samples from ecological focus areas. The most frequently detected substance was imidacloprid  
203 (87% in cultivated fields and 84% in ecological focus areas), followed by thiacloprid (43% and 59%), clothianidin  
204 (39% and 12%), acetamiprid (34% and 45%) and thiamethoxam (19% and 7%).

205 Neonicotinoid residues were also detected in various wildflowers present in non-treated area surrounding  
206 crops grown from treated seeds, with residues in foliage ranging from 0.06 to 106 µg/kg (LOQ ranged from 0.06  
207 to 0.60 µg/kg) (Botias et al. 2015; Botias et al. 2016). The authors pointed that these residues may overlap with  
208 lethal toxicity levels for some insect species (e.g., *Aphis glycines*). In addition, the widespread contamination of  
209 wild plants in agricultural landscape likely increases the exposure duration of pollinators though it is often  
210 supposed to be restricted to the crop flowering time (Botias et al. 2015).

211 Finally, in guttation droplets, potentially consumed by non-target species, works conducted in various  
212 European countries showed neonicotinoid concentrations of hundreds of mg/L at the emergence of plant, but only  
213 of a few µg/L one month after its emergence (Bonmatin et al. 2015; Tapparo et al. 2011).

214

## 215 **Contamination of air**

216 Neonicotinoids may also reach the atmosphere. The measurement of their concentrations relies on active air  
217 sampling systems and by trapping compounds on a sorbent from which the compounds are extracted and analyzed.  
218 Most of the time, the measured concentrations represent the sum of the compounds present in the atmosphere in  
219 both particulate and gaseous forms. Désert et al. (2018) monitored PPP concentrations in ambient air samples  
220 collected from February 2012 to December 2017 at one rural and six urban sites in the French Provence-Alpes-  
221 Côte d'Azur region. Imidacloprid was quantified in four locations, with concentrations higher than 1 ng/m<sup>3</sup> (LOD  
222 = 0.081 ng/m<sup>3</sup>), but with a low frequency of quantification (1 to 2% depending on the site). As it was detected both  
223 in the rural and urban sampling sites, the authors suggested an atmospheric transport from agricultural areas to  
224 cities given the air mass retro-trajectories. In the French Phytatmo database (2023), which synthesizes the data

225 obtained by the French Approved Air Quality Monitoring Associations (AASQAs) from 2002 up to now, the  
226 average imidacloprid concentration, calculated from 18 quantifications, was equal to 0.39 ng/m<sup>3</sup>, with a maximum  
227 of 2.3 ng/m<sup>3</sup> (Table 1), which was higher than the range of concentrations reported by Coscollà and Yusà (2016)  
228 (from 0.012 to 0.014 ng/m<sup>3</sup>) or by Raina-Fulton (2015) (from 0.01 to 0.36 ng/m<sup>3</sup> in the particulate phase, LOD =  
229 0.0039 ng/m<sup>3</sup>) in Canada. The analysis of the Phytatmo database (2023) also showed that acetamiprid and  
230 thiamethoxam were only detected once, while thiacloprid was found at an average concentration of 0.17 ng/m<sup>3</sup> out  
231 of 17 quantifications, and at a maximum concentration of 0.47 ng/m<sup>3</sup>. In Canada, for the particulate phase, Raina-  
232 Fulton (2015) and Coscollà and Yusà (2016) reported acetamiprid concentrations of 0.006 ng/m<sup>3</sup> and 0.018 ng/m<sup>3</sup>,  
233 respectively, and Raina-Fulton (2015) observed clothianidin concentrations ranging from 0.01 to 0.09 ng/m<sup>3</sup>.

234

## 235 **Impacts on terrestrial biodiversity**

### 236 **Terrestrial heterotrophic microorganisms**

237 Most studies devoted to the effects of neonicotinoids on functional activities and biodiversity of terrestrial  
238 heterotrophic microorganisms concerned imidacloprid. Acetamiprid, clothianidin and thiamethoxam were scarcely  
239 addressed, while there was no data for thiacloprid.

240 In laboratory experiments, Cycoń and Piotrowska-Seget (2015a) evaluated the impact of imidacloprid on  
241 soil microbial activities in soils spiked at the agricultural dose and at ten times this dose (1 and 10 mg/kg,  
242 respectively). At the agricultural dose, imidacloprid decreased microbial respiration, total bacterial count, and  
243 dehydrogenase, phosphatase and urease activities after 14 days. However, these effects were transient and the  
244 measured microbial functions recovered after 56 days of exposure. At ten times the agricultural dose, imidacloprid  
245 decreased the microbial parameters but no recovery was observed after 56 days suggesting irremediable impacts  
246 on communities. Consistently, nitrate concentration decreased while ammonium concentration increased, in  
247 agreement with the high sensitivity of nitrifying and nitrogen-fixing bacteria to imidacloprid. Under the same  
248 experimental conditions, the effect of imidacloprid on the structure of ammonia-oxidizing archaea (AOA) and  
249 bacteria (AOB) communities was analyzed using Denaturing Gradient Gel Electrophoresis (DGGE) (Cycoń and  
250 Piotrowska-Seget, 2015b). At the agricultural dose, imidacloprid did not affect the  $\alpha$  diversity of the bacterial  
251 communities. However, at ten times the dose, imidacloprid decreased the  $\alpha$  diversity of the AOA community in a  
252 durable way, and temporarily that of the AOB community. In addition, at the highest dose, imidacloprid decreased  
253 nitrification and increased ammonification. To determine the role of the microbial community diversity in the fate  
254 and impact of imidacloprid and acetamiprid, Zhang et al. (2017) used soil microcosms cropped with *Brassica*

255 *chinensis* L. They showed that the diversity of the microbial community did not affect the amount of imidacloprid  
256 or acetamiprid remaining in the soil but, when microbial diversity decreased, the amount of insecticide exported  
257 from the soil to the plant increased. Finally, a study conducted on microbial strains isolated from soil and exposed  
258 to imidacloprid or thiamethoxam in Petri dishes showed that both neonicotinoids altered the functions of *Klebsellia*  
259 sp. strain 19, a phosphate-solubilizing rhizobacterium exhibiting Plant Growth Promoting Rhizobacteria (PGPR)  
260 properties (Ahemad and Khan 2011). Thus, these two insecticides could compromise the PGPR activity of  
261 microbial inoculant used to decrease crop dependence on chemically derived fertilizers.

262 In field conditions, soybean imidacloprid treated seeds decreased the number of *Rhizobia* by a factor of  
263 three, while the number of *Rhizobia* was not affected after foliar application (Sarnaik et al. 2006). In contrast,  
264 regardless of the mode of application, the insecticide had no effect on phosphate solubilizing bacteria (Sarnaik et  
265 al. 2006). Li et al. (2018) studied the impact of imidacloprid or clothianidin treated seeds on the wheat rhizosphere  
266 microbial communities over nine months. The analysis of 16S rRNA and ITS amplicons generated from soil-  
267 extracted DNA revealed changes in the  $\alpha$  and  $\beta$  diversities of bacterial and fungal communities during plant  
268 development, but did not reveal any change due to seed treatment with each of the two insecticides. Furthermore,  
269 under these conditions, no effect of imidacloprid or clothianidin on some biocontrol agents (*Bacillus*,  
270 *Pseudomonas*, *Streptomyces*...) was observed in the wheat rhizosphere.

271 Two studies examined the impact of thiamethoxam on the taxonomic and metabolic diversity of soil  
272 bacterial communities using a laboratory setting. In forest land soils spiked with different amounts of  
273 thiamethoxam, an altered composition of the community was observed (Yu et al. 2020): the relative abundance of  
274 *Gemmatimonadetes* and OD1 decreased when compared to the control while the relative abundance of *Chloroflexi*  
275 and *Nitrospirae* increased. On the other hand, the catabolic diversity of the microbial community in soils treated  
276 with the lowest dose (0.02 mg/kg) of thiamethoxam was higher than that of the control while it was lower at the  
277 highest doses (0.2 mg/kg and 2 mg/kg). Analyzing soil samples from experimental plots where thiamethoxam was  
278 applied in field conditions, Filimon et al. (2015) showed that the insecticide only slightly reduced the phosphatase  
279 activity but reduced the number of nitrifying bacteria by about 60%.

280 In general, studies concerning the effects of neonicotinoids on terrestrial heterotrophic microorganisms  
281 revealed contradictory results depending on whether they were conducted in the laboratory (often under unrealistic  
282 agricultural conditions), showing impacts on the structure and on different microbial activities, or in the field (in  
283 more realistic conditions), showing no or very little effect of these substances.

284

## 285 **Terrestrial invertebrates**

286 Neonicotinoids have negative impacts on terrestrial invertebrates (pollinators, natural enemies, earthworms...) in  
287 agricultural environments despite variable responses depending on the traits and groups considered, as summarized  
288 below.

289

### 290 ***Pollinators***

291 Neonicotinoids are likely to have greater effects on insect pollinators than other insecticides because they are  
292 systemic insecticides regularly found in pollen, nectar, and other vegetative parts of plants throughout their  
293 flowering period (Krupke et al. 2012; Krupke and Long 2015), leading to risks of pollinators exposure via the oral  
294 route as well as through contact for a longer period of time. In addition, during their application, neonicotinoids  
295 can also contaminate the surrounding environments (Krupke et al. 2012; Krupke and Long 2015). Comparative  
296 toxicity studies among the different categories of neonicotinoids are scarce, but Arena and Sgolastra (2014)  
297 provided some insights. They showed that nitro-substituted neonicotinoids (“N-nitroguanidines”; including  
298 imidacloprid, thiamethoxam or clothianidin) were generally more toxic to pollinators than cyano-substituted  
299 neonicotinoids (“N-cyanoamidines”; including acetamiprid or thiacloprid).

300 **Honeybees.** Exposure of honeybees (*Apis mellifera*) to neonicotinoids has been repeatedly demonstrated (e.g.,  
301 Bonmatin et al. 2015; Hladik et al. 2016; Mitchell et al. 2017; Zhang et al. 2023). In pollens sampled in 2002-2003  
302 before spring, summer, autumn and winter, in apiaries located in five French departments, imidacloprid and/or its  
303 6-chloronicotinic acid transformation product were detected in 69% of the 81 samples, and quantified in 13.5%  
304 and 34.6% of the samples, respectively (Chauzat et al., 2006). The frequency of detection did not vary much  
305 according to the sampling period. This study was then continued until the end of 2005 (Chauzat et al. 2011):  
306 imidacloprid was detected in 11.2% of the bees (average concentration of 1.2 µg/kg) and in 40.5% of the pollen  
307 samples (0.9 µg/kg), and 6-chloronicotinic acid was detected in 18.7% of the bees (1.0 µg/kg) and in 33% of the  
308 pollen (1.2 µg/kg). In different sites cultivated with a corn/rapeseed rotation whose seeds were treated with  
309 thiamethoxam (or not), residues of thiamethoxam and clothianidin in pollens were close to the LOQ (1 µg/kg) in  
310 both corn and oilseed rape (from 1 to 2 µg/kg), and the amounts in oilseed rape nectar were lower than 1 µg/kg  
311 (LOQ = 0.5 µg/kg) (no corn nectar was analyzed) (Pilling et al. 2013). Wiest et al. (2011) detected imidacloprid  
312 in 1% of pollen and 2% of honey but nothing in bees sampled from hives located in the French Pays de la Loire  
313 region. Thiamethoxam and clothianidin were not detected in any of these samples. The multiple potential exposure

314 pathways and the size of the pollinator activity zone make it challenging to fully identify and quantify the exposure  
315 of pollinators to neonicotinoids (van der Sluijs et al. 2013).

316 In parallel of the awareness raised by exposure data on the possible role of neonicotinoids in the massive  
317 decline of insects, honeybees have been the subject of extensive research focused on the toxicological effects of  
318 neonicotinoids. Particular concern resulted from studies focused on honeybee behavior which revealed  
319 neonicotinoid-induced impairment of memory and learning abilities (Tison et al. 2019; Willemsen and Hailey  
320 2001) because such impairment is likely to affect navigation parameters and the ability to return to the hive (Henry  
321 et al. 2012; Henry et al. 2014). With regard to interaction with other factors or stressors, neonicotinoids were found  
322 to increase the susceptibility of honeybees to pathogens (*Nosema*) (Grassl et al. 2018; Müller 2018; Pettis et al.  
323 2013; Uhl and Brühl 2019). Furthermore, the effects of neonicotinoids were demonstrated to increase with  
324 decrease in temperature: the ability of bees to return to the hive following exposure to thiamethoxam decreased at  
325 lower temperatures (< 28°C) (Henry et al. 2014; Monchanin et al. 2019). Finally, neonicotinoids can interact with  
326 other PPPs as observed for clothianidin and propiconazole (fungicide) which impact honeybee survival via  
327 synergistic effects (Sgolastra et al. 2017).

328 However, the issue of the effects of neonicotinoids on honeybees has been the subject of much  
329 controversy. In their large-scale monitoring study, Rolke et al. (2016) showed that honeybee colonies placed in  
330 clothianidin-treated oilseed rape crops exhibited developmental and reproduction performances similar to those of  
331 non-exposed colonies. Under the same crop treatment, clothianidin was not found to pose a risk to colonies in  
332 terms of health, development, and overwintering success of honeybee colonies (Belsky and Joshi 2020). This result  
333 was also found by Rundlöf et al. (2015) for clothianidin-rape seed treated seed in combination with non-systemic  
334 pyrethroid (beta-cyfluthrin) treatments. Conversely, Samson-Robert et al. (2017) observed an increased mortality  
335 of honeybee colonies located in environments dominated by clothianidin-treated grain corn. More recently, Schott  
336 et al. (2021) demonstrated lethal effects of clothianidin on honeybee larvae, but found short-term resilience of  
337 colonies to treatments, which may result from compensation mechanisms (increased brood size). As to adults, seed  
338 treatments with clothianidin, thiamethoxam or imidacloprid resulted in increased worker bees mortality, but effects  
339 on colony growth were not observed thereafter (Lin et al. 2021). Actually, the effects of neonicotinoids on colony  
340 size vary across study areas (Woodcock et al. 2017). Spatial features, such as landscape characteristics and  
341 especially landmarks density (landscape elements that are used as visual cues for the orientation of bees), as well  
342 as the bee experience in the studied area (e.g., homing experiments carried out with foragers familiar or not with

343 the release point), influence the performance of individuals and therefore of colonies, which in turn can either limit  
344 or exacerbate the neonicotinoid-induced effects (Henry et al. 2014).

345 To go further into toxicity mechanisms and their consequences for bee colony survival, LaLone et al.  
346 (2017) built a network of six Adverse Outcome Pathways (AOPs) and used weight of evidence (WoE) evaluation  
347 to describe plausible causal relationships between neonicotinoid mechanisms of action (activation of nicotinic  
348 acetylcholine receptor as molecular initiating event and downstream molecular, cellular, or organism-level key  
349 events) and colony death, as adverse outcome of regulatory concern. However, WoE assessment identified  
350 uncertainty, and thereby need for further research, in some upstream-to-downstream key-event relationships (e.g.,  
351 between mitochondrial dysfunction and learning/memory, or between role change in the colony and further larval  
352 development).

353 **Wild bees.** Beside works on the emblematic species *Apis mellifera*, some studies have focused on wild bees. In  
354 ground nesting species (*Eucera pruinosa*), soil treatment with imidacloprid was found to affect reproduction  
355 (decreased number of nests and larvae) and pollen consumption whereas no effect was observed with  
356 thiamethoxam used as seed treatment (*Cucurbita pepo*) (Chan and Raine 2021). However, seed treatments may  
357 lead to soil contamination, even in fields adjacent to crops and in non-cropped borders, and affect native bee  
358 nesting and richness (Main et al. 2020; Rundlöf et al. 2015). In the field, exposure to various neonicotinoids and/or  
359 other PPPs have lethal and sublethal effects, as shown for the solitary bee *Osmia bicornis*: clothianidin or  
360 thiamethoxam, used in combination with other insecticides (beta-cyfluthrin) or fungicides (fludioxonil and  
361 metalaxyl-M) impaired the reproduction (Woodcock et al. 2017), as did the mixture of thiacloprid and prochloraz  
362 (fungicide) (Alkassab et al. 2020), while clothianidin and propiconazole (fungicide) induced mortality (Sgolastra  
363 et al. 2017). In a multistress context, the effects of neonicotinoids on wild bees can be exacerbated by food resource  
364 limitation (Stuligross and Williams, 2020). Indeed, the diversification of non-crop floral resources can provide  
365 complementary resources, counteracting the negative effects of neonicotinoids as shown on *O. bicornis*  
366 reproduction and larval development (Klaus et al. 2021). With regard to other physiological mechanisms  
367 underlying population-level responses under field conditions, the negative effects of neonicotinoids observed on  
368 *Osmia cornuta* reproduction (Stuligross and Williams 2020) or at population level (fitness, density; Sandrock et  
369 al. 2014) may have a male component (thiamethoxam-altered male fertility; Strobl et al. 2021a ) or not  
370 (clothianidin unaffected male survival, emergence and reproductive physiology; Strobl et al. 2021b). Using simple  
371 generalized and linear mixed models (GLMM), Stuligross and Williams (2021) demonstrated how past and current

372 exposure to neonicotinoids profoundly impact both individual reproduction and population growth rate of orchard  
373 blue bees (*Osmia lignaria*).

374 The impact of neonicotinoids on wild social bees of the *Melipona* group is very little studied. However,  
375 the meta-analysis of Botina et al. (2020) highlighted lethal effects on both larvae and adults, especially marked for  
376 imidacloprid.

377 In bumblebees, the effects of neonicotinoids were found to be expressed at the organism level (molecular,  
378 cellular, and physiological responses; lethal and sublethal effects) as well as at the population level (mortality,  
379 altered colony structure and turnover) (Camp and Lehmann 2021). Colonies of *Bombus terrestris* and *Bombus*  
380 *impatiens* exposed to acetamiprid, clothianidin or imidacloprid exhibited lower growth rates and decreased  
381 production of new queens (Camp et al. 2020; Rundlöf et al. 2015; Whitehorn et al. 2012). In addition, a suite of  
382 effects was observed, including increased mortality of new queens, delayed nest foundation (Wu-Smart and Spivak  
383 2018), acute and chronic effects on worker foraging activity (Gill and Raine 2014), reduced fecundity and brood  
384 production (imidacloprid; Laycock et al. 2012), disruption of their flight activity and endurance (imidacloprid;  
385 Kenna et al. 2019), and altered queen condition upon overwintering (thiamethoxam and clothianidin; Fauser et al.  
386 2017). Some works also showed that seed treatments affect *Bombus* spp. densities in adjacent fields and in non-  
387 cropped borders (Main et al. 2020; Rundlöf et al. 2015). With respect to interactions with other stressors, no  
388 synergistic nor additive effects could be detected between neonicotinoids (mixture of thiamethoxam and  
389 clothianidin) and the trypanosome parasite *Crithidia bombi* on post hibernation performances (queen survival and  
390 body mass) of *B. terrestris* (Fauser et al. 2017).

391 With a multi-species dynamic Bayesian occupancy model, Woodcock et al. (2016) highlighted the high  
392 impact of neonicotinoid seed treatments as use in oilseed rape on the extinction of 62 species of wild bee  
393 populations. Their model was spatially and temporally explicit and related population persistence to exposure over  
394 a wide time period of 18 years. This paper identifies the need of developing national scale management strategies  
395 to support wild bee populations persistence over the long-term.

396 **Butterflies.** The impacts of neonicotinoids on lepidopterans are very little investigated, but the few studies  
397 addressing this issue underline a critical role of the timing and mode of exposure. In the monarch butterfly (*Danaus*  
398 *plexippus*), exposure of young adults to realistic doses of imidacloprid did not affect oocyte production, but  
399 significantly decreased insect longevity, with likely consequences for population development, migration, and  
400 overwintering (James 2019). On the contrary, under exposure to clothianidin-treated plants in the larval stage,  
401 there was no significant effect on parameters characterizing monarch migration (flight orientation, movement

402 speed; Wilcox et al. 2021). Using a linear mixed effect random slope model, Gilburn et al. (2015) demonstrated  
403 that the populations of 15 butterfly species commonly occurring at farmland sites in England declined due to the  
404 use of neonicotinoids.

405 **Overview of the effects of neonicotinoids on pollinators.** In 2018, EFSA (2018) confirmed that the use of  
406 neonicotinoids causes a risk to wild bees and honeybees. Although results appeared sometimes contradictory,  
407 many studies highlighted negative effects of neonicotinoids on pollinators. The contradictions occasionally  
408 observed can be explained by several methodological biases (Walters 2016): (1) laboratory experiments consider  
409 exposure conditions (in particular doses and durations) to neonicotinoids that are not really representative of those  
410 observed in natura in relation to agricultural practices; (2) most of the studies focus on honeybees or bumblebees,  
411 whereas susceptibility to insecticides varies greatly among the different groups of pollinators (Lundin et al. 2015;  
412 Rundlöf et al. 2015); (3) studies are most often focused on one type of neonicotinoid which makes generalization  
413 difficult. Furthermore, there is a need to combine laboratory and field approaches, and to address the effects of  
414 neonicotinoids at the sub-individual and individual levels, as well as the consequences for colonies and populations  
415 (see LaLone et al. 2017). For example, Henry et al. (2015) showed that the mortality in honeybee colonies near  
416 neonicotinoid (thiamethoxam and imidacloprid)-treated oilseed rape fields was higher than in colonies surrounded  
417 by less treated fields. However, this effect was not observable at the colony level during and after the flowering  
418 period of oilseed rape, because the impact of this loss was buffered by the colonies' demographic regulation  
419 response. While very few models exist that are devoted to the effects of neonicotinoids at the bee colony/population  
420 levels, this research area appears promising given the difficulty of actually detecting unintended effects of  
421 neonicotinoids in the field using conventional risk assessment methods (Lundin et al. 2015). In particular, Henry  
422 et al. (2017) advocated the potentialities of mechanistic models in a multiple stressor context. Since then, the  
423 honeybee colony model (BEEHAVE, Becher et al. 2014) has been extended to the colony development of  
424 bumblebees in a realistic landscape (Becher et al. 2018), and to translate results from standard laboratory studies  
425 to relevant parameters and processes for simulating bee colony dynamics (Preuss et al. 2022). On a regulatory  
426 point of view, significant efforts have been undertaken at the EU level to improve risk assessment of the effects of  
427 neonicotinoid on bees with, among others, the development of the ApisRAM population model (Adriaanse et al.  
428 2023; EFSA PPR Panel 2015; EFSA Scientific Committee et al. 2021).

429

430

431

432 **Natural enemies**

433 Overall, neonicotinoids have negative impacts on natural enemies such as predators (mites, ladybugs) and  
434 parasitoids, especially in field crops (Douglas and Tooker 2016). By disrupting prey-predatory and host-parasitoid  
435 interactions, neonicotinoid-treated seeds also alter arthropod communities as a whole (Chen et al. 2016; Disque et  
436 al. 2019; Dubey et al., 2020).

437 **Ants.** In *Tetramorium caespitum*, increased mortality and disruption of locomotion without loss of hunting  
438 behavior was observed after exposure to imidacloprid (Penn and Dale 2017). In other ant species (*Pogonomyrmex*  
439 *occidentalis*, *Lasius niger*, *Lasius flavus*), imidacloprid was also found to alter socio-behavioral traits (e.g.,  
440 foraging, nest building, competition behavior) at environmentally relevant concentrations under experimental  
441 exposure (Sappington 2018; Thiel and Kohler 2016).

442 **Bugs.** Prey consumption was reduced in predatory bugs (*Pentatomidae*) feeding on herbivorous preys previously  
443 exposed to imidacloprid-treated plants, even when prey density increased (lack of a type II functional response)  
444 (Resende-Silva et al. 2019). Studies with *Orius insidiosus* concluded that imidacloprid was moderately to highly  
445 toxic when applied as seed treatment, while foliar toxicity showed conflicting results (Naranjo 2001). In *Podisus*  
446 *nigrispinus* predatory bugs, sublethal effects of thiamethoxam treatments resulted in longer larval development,  
447 decreased adult body weight and delayed oviposition (Torres et al. 2003). Imidacloprid may also alter the predatory  
448 behavior of spined soldier bugs (*Podisus maculiventris*), with negative consequences in terms of weight gain  
449 (Resende-Silva et al. 2019). However, some of these effects were only seen at certain treatment doses (> 0.5  
450 mg/plant) (Torres et al. 2003), and were sometimes transient (Pekar and Kocourek 2004).

451 **Carabids.** When fed with slugs contaminated with thiamethoxam, *Chlaenius tricolor* carabid beetles displayed  
452 altered mobility twitching and mild motor difficulties, up to partial to extensive paralysis (Douglas et al. 2015).

453 **Forficulidae.** As dominant earwig species in temperate orchards, *Forficula auricularia* is the most studied  
454 forficulidae species in the laboratory. Shaw and Wallis (2010) demonstrated impaired mobility and movement  
455 coordination in 70 % of earwigs exposed to thiacloprid, and that more than 80 % of them died after 10 days  
456 exposure. Thiacloprid was also shown to reduce larval growth and to decrease adult foraging behavior (Fountain  
457 and Harris 2015). Acetamiprid significantly decreased the predation behavior of adult males by 28 % but not of  
458 females nor nymphs when applied in apple orchards at the agricultural rate (Malagnoux et al. 2015).

459 **Lacewings.** Survival of the green lacewings *Chrysoperla carnea* reduced when adults feed on imidacloprid-treated  
460 plants (Rogers et al. 2007). In addition, imidacloprid was found to disrupt the mobility of individuals (appearance

461 of tremors; Rogers et al. 2007). It has to be underlined that, upon multigeneration exposure, this species was able  
462 to develop strong resistance to acetamiprid (Mansoor and Shad 2020).

463 **Ladybugs.** Ladybugs are impacted by neonicotinoids via prey ingestion, especially at early larval stage in  
464 *Coleomegilla maculata* feeding on cereal aphids exposed to thiamethoxam (Bredeson et al. 2015). Thiamethoxam  
465 reduces the mobility of ladybugs (the time to turn around when placed on their backs increases with the  
466 concentration of ingested insecticide) but not the number of eggs, while a negative correlation between the increase  
467 in the concentration of the insecticide and the number of developing eggs has been shown (Bredeson and Lundgren  
468 2018). Wang et al. (2018a) evaluated the toxicity of thiamethoxam to *Harmonia axyridis*, a predator of the *Myzus*  
469 *persicae* aphid, and its effect in term of functional response, by three exposure routes: direct contact of *H. axyridis*  
470 with thiamethoxam residues; cabbage leaves infested with *M. persicae* treated systematically with thiamethoxam  
471 which exposed *H. axyridis* to the insecticide indirectly (referred as systemic application, mimicking direct soil  
472 drench or seed treatments); and cabbage leaves infested with *M. persicae* treated with thiamethoxam by leaf-dip  
473 which exposed *H. axyridis* to thiamethoxam residues on both cabbage leaves and thiamethoxam-treated *M.*  
474 *persicae* (referred as leaf dip treatment, mimicking foliar spray application). Predation was negatively affected  
475 under the three conditions, but particularly when ladybugs were exposed following leaf dipping. For all exposure  
476 routes, *H. axyridis* rapidly recovered predatory ability, however, sublethal effects of thiamethoxam may reduce  
477 the population growth of *H. axyridis* and, therefore, impair the biological control of *M. persicae*, especially after  
478 leaf or contact exposure.

479 **Parasitoid hymenoptera.** Acetamiprid was demonstrated to cause significant reductions in the abundances of  
480 various groups of parasitoids (Aphelinidae, Braconidae, Encyrtidae, Eulophidae, Eupelmidae, Ichneumonidae,  
481 Mymaridae, Platygasteridae, Proctotrupidae, Pteromalidae, Scelionidae, Trichogrammatidae) (Khans and  
482 Alhewairini 2019), and these losses were generally accompanied by an increase in pest infestation levels (Saito et  
483 al. 2008). In various parasitoid species, systemic applications of imidacloprid were often minimally detrimental,  
484 whereas foliar applications could be highly toxic (Naranjo 2001).

485 **Predatory mites.** In the presence of neonicotinoids (acetamiprid, clothianidin, imidacloprid, thiacloprid or  
486 thiamethoxam), disruption of mite behavior (*Panonychus ulmi*, *Tetranychus urticae*), without loss of abundance,  
487 resulted in loss of biological control activity (Beers et al. 2005). Predatory mites (Phytoseiidae) are affected by  
488 acetamiprid, but studies have shown that they can develop resistance (Fountain and Medd 2015) which led to a  
489 growing interest in their use in sustainable agriculture (Duso et al. 2014; Fountain and Medd 2015).

490 **Spiders.** For several spider families (Araneidae, Lycosidae), contact exposure to neonicotinoids (acetamiprid,  
491 imidacloprid) appeared to be the most toxic pathway (compared to consumption of treated prey) inducing lethal  
492 and sublethal effects such as disruption of web construction (Pekar 2012). Furthermore, neonicotinoids  
493 (acetamiprid, thiacloprid) were demonstrated to affect the richness of spider communities (Rosas-Ramos et al.  
494 2020).

495

### 496 ***Detritivorous arthropods***

497 In a three-year field experiment, Pearsons and Tooker (2021) showed that seed treatments (corn, soybean) with  
498 neonicotinoids (clothianidin, imidacloprid) reduced saprophagous arthropod (millipede, springtails, oribatid mites)  
499 density and activity (litter decomposition) by more than 10%.

500

### 501 ***Earthworms***

502 Earthworms are likely to be exposed to neonicotinoids in soils. For example, in a French arable landscape, Pelosi  
503 et al. (2021) observed residues of imidacloprid in 79% of the sampled earthworms (*Allolobophora chlorotica*,  
504 n=155; maximum concentration of 777 µg/kg; 43 % of the earthworms contained imidacloprid concentrations  
505 >100 µg/kg, LOQ = 0.4 µg/kg), while thiacloprid was found in 34% of the earthworms (maximum concentration  
506 of 42.1 µg/kg, LOQ = 0.1 µg/kg).

507 Neonicotinoids (e.g., acetamiprid, clothianidin, imidacloprid, thiamethoxam) have negative effects on  
508 several endpoints of various earthworm species (e.g., *Eisenia fetida*, *Lumbricus terrestris*, *Aporrectodea*  
509 *caliginosa*), from sub-individual to community levels: tissue integrity, physiological activity, behavior, growth,  
510 reproduction, and survival (Dittbrenner et al. 2010; Dittbrenner et al. 2011a; Dittbrenner et al. 2011b; Qi et al.  
511 2018; Tu et al. 2011; Wang et al. 2015). They are also known to be toxic to compost worms (*E. fetida*) in laboratory  
512 conditions: they affect reproduction, cellulase activity and tissues, among others (Wang et al. 2015).

513

### 514 ***Nematodes***

515 Compared to arthropods, nematodes tend to be less sensitive to neonicotinoids (Kudelska et al. 2017; Neury-  
516 Ormanni et al. 2019; Bradford et al. 2020). In entomopathogenic species (*Steinernema glaseri*, *Steinernema*  
517 *carpocapsae*, *Steinernema feltiae*, *Heterorhabditis bacteriophora*, *Heterorhabditis megidis*), a positive effect of  
518 imidacloprid was observed at low dose on reproduction (Koppenhöfer et al. 2003).

519

520 **Terrestrial vertebrates**

521 ***Birds (excluding raptors)***

522 Numerous studies demonstrated that bird decline in agroecosystems is related to the use of neonicotinoids (Ertl et  
523 al. 2018; Lennon et al. 2019; Li et al. 2020; Mineau and Palmer 2013; Mineau and Kern 2023).

524 In agricultural areas and other environments across Europe and North America, the analyses of  
525 neonicotinoid residues in various biological components (eggs, feathers, livers, plasmas) of several avian trophic  
526 groups such as nectarivores, granivores, insectivores and carnivores showed ubiquitous exposure of birds  
527 (gamebirds, house sparrows, hummingbirds, songbirds...) (Bishop et al. 2020; Bro et al. 2016; Fuentes et al. 2023;  
528 Humann-Guillemot et al. 2019b; Humann-Guillemot et al. 2021; Lennon et al. 2020a; Lennon et al. 2020b;  
529 Poisson et al. 2021; Prouteau 2021; Roy et al. 2020). The prevalence of exposure greatly varies from one study to  
530 another and among species, but, even if some studies detected neonicotinoids only in a few individuals (e.g.,  
531 Graves et al. 2022), the vast majority of works underlined pervasive exposure of numerous species and pointed  
532 out high frequencies of detection.

533 Granivorous birds are directly exposed to neonicotinoids following the consumption of neonicotinoid  
534 treated seeds (Lopez-Antia et al. 2016; Prosser and Hart 2005; Roy et al. 2019). For example, Lennon et al. (2020b)  
535 demonstrated that the detection of clothianidin in the plasma of several farmland bird species increased from 11%  
536 before sowing to 51% after sowing. In French cereal dominated landscape, where neonicotinoid treated seeds were  
537 widely used, the eggs or livers of grey partridge (*Perdix perdix*) and of some *Columba* species were found to be  
538 contaminated by neonicotinoids (Bro et al. 2016; Millot et al. 2017). In Ontario fields (Canada), the analysis of  
539 carcasses of wild turkey (*Meleagris gallopavo silvestris*), which consumes neonicotinoid-coated seeds, showed  
540 detectable levels of clothianidin and/or thiamethoxam in 22.5% of individuals (detection of both substances in 5%)  
541 (MacDonald et al. 2018). These studies underlined that the crop sowing periods are the most at risk (especially in  
542 autumn compared to early spring, Millot et al. 2017) for bird exposure through neonicotinoid treated seeds, because  
543 it also corresponds to a period of low food availability and of migration stopover for some species. Along  
544 agricultural gradients in Minnesota (USA), at least one neonicotinoid among the seven compounds screened  
545 (acetamiprid, clothianidin, dinotefuran, imidacloprid, nitenpyram, thiacloprid, thiamethoxam) was detected in 93  
546 % and 80 % of fecal pellets of sharp-tailed grouse (*Tympanuchus phasianellus*) and greater prairie-chickens (*T.*  
547 *cupido*), respectively, and in 90 % and 76 % of their livers, respectively (Roy and Chen 2023). Imidacloprid and  
548 clothianidin were the most detected substances. To document the exposure of wild bird communities, Anderson et  
549 al. (2023) analyzed seven neonicotinoids (acetamiprid, clothianidin, dinotefuran, imidacloprid, nitenpyram,

550 thiacloprid, thiamethoxam) in plasma samples from 55 species across 17 avian families, in four counties in Texas  
551 (USA). Imidacloprid was detected in 36 % of samples (n=294), and two birds contained imidacloprid, acetamiprid  
552 and thiacloprid. Clothianidin, and thiamethoxam were not detected but their LOD (0.3 µg/L, 0.05 µg/L,  
553 respectively) were higher than that of imidacloprid (0.005 µg/L). Temporal variations have been evidenced, with  
554 lower frequencies of detection in summer and winter than in spring and fall which correspond to the usual planting  
555 days for the most common crops across the state. Some species showed higher prevalence of exposure such as the  
556 American robin (*Turdus migratorius*) and the red-winged blackbird (*Agelaius phoeniceus*). Importantly, the study  
557 evidenced a chronic or repeated exposure of wildlife since six birds out of seven re-sampled over time exhibited  
558 at least one detection of neonicotinoid, and three exhibited multiple exposure at different time points (Anderson et  
559 al. 2023). In Europe, several measurements of neonicotinoid residues in bird carcasses (livers or gizzards) revealed  
560 very large numbers of accidental direct bird poisonings (passerine, *Columba* and game species) following the  
561 ingestion of neonicotinoid-treated seeds, especially with imidacloprid (Berny et al. 1999; Bro et al. 2010;  
562 Buchweitz et al. 2019; Millot et al. 2017; Mineau and Kern 2023; Mineau and Palmer 2013). Despite biases in the  
563 detection of carcasses in the field survey (de Snoo et al. 1999; Vyas 1999), a significant number of birds have been  
564 categorically identified as victims of acute and lethal poisoning induced by neonicotinoids used in seed treatments.  
565 Nevertheless, these lightning mortality events would likely not be the primary cause of the significant decline of  
566 some bird species (gray partridge) in agricultural environments, but they are undeniably an aggravating factor  
567 (Millot et al. 2017). This is all the more since many other direct sublethal (physiological and behavioral) and  
568 indirect effects of neonicotinoids have been demonstrated, for many more species than just granivores (Gibbons  
569 et al. 2015; Wood and Goulson 2017). Improved seeding techniques can limit the risk of direct poisoning by  
570 ensuring that treated seeds are effectively buried so that the proportion of seeds on the surface after planting is low  
571 (McGee et al. 2018). However, the effectiveness of these methods depends on planting techniques and on seed  
572 type and are not generalizable to all coated seed situations (McGee et al. 2018). Coatings have been suggested to  
573 induce an aversion which limits ingestion to a few coated seeds, representing only a small fraction of the  
574 neonicotinoid LD50 (Lethal Dose causing the death of 50% of exposed organisms) (Avery et al. 1994), but these  
575 results have been shown to depend on the experimental context, including the availability of alternative food  
576 resources or the state of food stress (Millot et al. 2017; Mineau and Kern 2023; Mineau and Palmer 2013).  
577 Furthermore, the repellent effect results from the induction of a physiological disorder following initial ingestions  
578 of treated seeds, involving that significant sublethal effects can occur well before ingestion of a lethal dose (Lopez-  
579 Antia et al. 2014; Lopez-Antia et al. 2015; Mineau 2017). It has to be underlined that some passerine species,

580 especially *Fringillidae*, can de-husk seeds which lowers their direct exposure by ingestion (Prosser and Hart 2005).  
581 Other contexts of neonicotinoid poisoning of passerines (American goldfinches *Spinus tristis*) have also been  
582 identified in public spaces in California (Rogers et al. 2019): the mortality of birds was due to the ingestion of  
583 natural elm seeds remaining on the ground which were contaminated with imidacloprid during the drench  
584 application.

585         While neonicotinoids were initially thought to be less harmful to birds than insects due to their lower  
586 affinity for vertebrate nicotinic receptors, mounting evidence now challenges this view and birds appear to be more  
587 sensitive to neonicotinoids than other vertebrates (Mineau and Kern 2023; Mineau and Palmer 2013). The acute  
588 toxicity of neonicotinoids was reported to be underestimated by a factor of ten for some wild bird species compared  
589 to the one determined on model species of mallard or bobwhite quail (*Colinus virginianus*) (Mineau and Kern  
590 2023; Mineau and Palmer 2013). Chronic toxicity is poorly taken into account, as well as sublethal effects which  
591 are scarcely investigated.

592         Several reviews of the individual and sub-individual effects of neonicotinoids on birds have been  
593 published (Gibbons et al. 2015; Moreau et al. 2022; Pisa et al. 2015; Wood and Goulson 2017). The literature  
594 shows that imidacloprid induces weight loss or reduces energy reserves (fat mass) in the white-crowned sparrow  
595 (*Zonotrichia leucophrys*) (Eng et al. 2017; 2019). In hummingbirds (*Selasphorus rufus*), the consumption of  
596 imidacloprid in flower nectar induces underactivity and decreased energy expenditure (-25%), with no other effect  
597 detected on feeding activity or immune response (Bishop et al. 2018; English et al. 2021). On the contrary, some  
598 studies showed an impact of imidacloprid on the immune status of adult (Lopez-Antia et al. 2013) and juvenile  
599 (Lopez-Antia et al. 2015) red-legged partridges (*Alectoris rufa*). These contrasting results could be explained by  
600 interspecific variability and various exposure conditions (dose x species x biomarkers x duration) (English et al.  
601 2021; Gibbons et al. 2015; Lopez-Antia et al. 2015). Behavioral alterations were also observed (Eng et al. 2019),  
602 and disruption of flight and/or navigation efficiency emerged as a sensitive and relevant endpoint of imidacloprid  
603 exposure and sublethal effect on the white-crowned sparrow (Eng et al. 2017). These effects have been associated  
604 with loss of energy reserves. Thus, even if transient under the tested conditions, these sublethal effects can likely  
605 lead to impaired migration success of white-crowned sparrows using agricultural environments as staging areas  
606 (Eng et al. 2017; 2019). Furthermore, reductions in feeding and activity most often resulting in weight loss and  
607 risk to survival have been demonstrated in migratory birds exposed to sublethal doses of imidacloprid (Eng et al.  
608 2017; 2019). Finally, exposure to sublethal dose of acetamiprid has been associated to reduced sperm density in  
609 the house sparrow (*Passer domesticus*) (Humann-Guillemint et al. 2019c).

610 In controlled experiments on red-legged partridges (*Alectoris rufa*) fed with control seeds or seeds treated  
611 with imidacloprid at 20%, 100% or 200% of the recommended dose, analyses in livers showed an increase in the  
612 accumulation of imidacloprid with exposure time, and mortality of 50% of the females within five days even at  
613 agricultural or lower doses (Lopez-Antia et al. 2013; Lopez-Antia et al. 2015). Moreover, breeding investment was  
614 lowered with reduced clutch size, eggs size and fertilization rate, and chick survival was diminished when birds  
615 were exposed to imidacloprid.

616 Sabin and Mora (2022) performed an ecological risk assessment to evaluate the potential effects of  
617 neonicotinoids (acetamiprid, clothianidin, imidacloprid, thiamethoxam) on populations of the northern bobwhite  
618 (*C. virginianus*) in the South Texas Plains Ecoregion (USA). The assessment of the exposure of both juveniles and  
619 adults showed levels which can induce adverse effects on growth, reproduction success, and long-term survival.

620 The analysis of the literature thus demonstrated that neonicotinoids are one of the factors responsible of  
621 the decline in the abundance and diversity of birds. Depending on the bird species and their diet, this impact results  
622 mainly either from a direct effect (e.g., ingestion of treated seeds), or from an indirect effect (e.g., reduction in  
623 food resources following the decline of prey). Such indirect effects are addressed hereafter in the “Food webs”  
624 section.

625

## 626 ***Raptors***

627 Several works showed the presence of neonicotinoids in raptors. Imidacloprid was detected in the blood of  
628 Eurasian eagle owl (*Bubo bubo*) in Spain (Taliensky-Chamudis et al. 2017), imidacloprid and thiacloprid in the  
629 blood of honey buzzards (*Pernis apivorus*) in Finland (Byholm et al. 2018), and acetamiprid, clothianidin,  
630 thiacloprid, and thiamethoxam in the feathers of barn owls (*Tyto alba*) in Switzerland (Humann-Guilleminot et al.  
631 2021). The detection frequencies were contrasted: 3% of the analyzed samples were positive in the Eurasian eagle  
632 owl, whereas in the insectivorous honey buzzard, imidacloprid and thiacloprid were detected in 40 and 70% of the  
633 samples, respectively. In the barn owl, more than 80% of the individuals were positive, notably for thiacloprid, the  
634 frequent detection in chicks suggesting a trophic exposure. The feeding specialization of the barn owl on insects  
635 would not be sufficient to explain the high detection frequency of neonicotinoids. In northern Germany, Badry et  
636 al. (2021) investigated the impregnation of the livers of three raptor species (red kite *Milvus milvus*, common  
637 buzzard *Buteo buteo*, Montagu’s harrier *Circus pygargus*; n=186). Among the neonicotinoids, only thiacloprid  
638 was detected in two red kites. Recently, no neonicotinoid was detected in the blood of chicks of the same three

639 raptor species in Germany (Badry et al. 2022). No study examining the toxicity of neonicotinoids on raptors has  
640 been identified.

641

### 642 ***Mammals (excluding chiropterans)***

643 One of the largest mammalian studies conducted to date resulted in the simultaneous analysis of 480 substances  
644 in muscle of 42 wild boars (*Sus scrofa*), 79 roe deer (*Capreolus capreolus*) and 15 deer (*Cervus elaphus*) in Poland  
645 (Kaczynski et al. 2021). The five neonicotinoids were among the most frequently detected compounds  
646 (imidacloprid and thiacloprid showing mean concentrations in the top five values). They were detected in 100%  
647 of the wild boar samples, while acetamiprid was detected in three deer, and thiacloprid and clothianidin were  
648 detected in two deer. Acetamiprid, clothianidin and thiacloprid were detected in 13 roe deer (16.5%). The mean  
649 residue concentrations ranged from 0.6 µg/kg (thiamethoxam) to 4.3 µg/kg (imidacloprid) in the liver. In France,  
650 multi-residues analyses targeting 140 PPPs (67 withdrawn and 73 currently used PPPs) and transformation  
651 products were performed in hair samples of small omnivorous rodents (wood mouse *Apodemus sylvaticus*) and  
652 insectivorous shrews (greater white-toothed shrew *Crocidura russula*) sampled in arable landscapes (Fritsch et al.  
653 2022). Again, acetamiprid, imidacloprid and thiacloprid were among the most frequently detected substances  
654 (more than 80% of individuals) and/or quantified at high concentrations (up to 70.7 µg/kg) (Fritsch et al. 2022).  
655 The ubiquity of exposure to neonicotinoids was demonstrated as residues were detected in all animals regardless  
656 of the type of habitat (hedgerows, cereal crops, grasslands) or of the agricultural practices (conventional or organic  
657 farming) (Fritsch et al. 2022). Assessing the exposure of wild raccoons (*Procyon lotor*) captured in Hokkaido  
658 (Japan) to neonicotinoids (acetamiprid, imidacloprid, clothianidin, dinotefuran, thiacloprid, thiamethoxam, and  
659 desmethyl-acetamiprid), Shinya et al. (2022) showed that either one of the six screened neonicotinoids or one  
660 transformation product was detected in the urine of 90% of the raccoons. Neonicotinoids were also found in the  
661 hair of red fox (*Vulpes vulpes*) in Italia; acetamiprid, clothianidin, and imidacloprid being detected in 100% of the  
662 analyzed individuals (n=11), and thiacloprid in 91% of them (Picone et al. 2023).

663         The toxicity of neonicotinoids to mammals have been reviewed by Tomizawa (2004) and Gibbons et al.  
664 (2015), showing the potential for various deleterious effects on growth, development and reproduction as well as  
665 other sub-lethal effects such as genotoxic and cytotoxic effects, immunotoxicity, neuro-behavioral disorders and  
666 changes in behaviors related to anxiety and fear, impairments of the thyroid and retina, and reduced movement.  
667 The study of the effects of imidacloprid (112 and 225 mg/kg, daily gavage for 60 days, which is above realistic  
668 environmental exposure concentrations) on rat reproduction, a mammal model organism, showed a decrease in

669 sperm vitality and number, a reduction in sex organ mass, and a decrease in the production of sex hormones FSH  
670 and LH in males (Nafaji et al. 2010; Tetsatsi et al. 2019). A significant impact of imidacloprid on the rat body  
671 weight was also reported but no published evidence of reproductive disorders in relation to neonicotinoid exposure  
672 in wild mammals was found. However, most of the research on mammals have been performed on rats or mice,  
673 and under laboratory conditions, hampering the assessment of direct toxicity to wild mammals which may exhibit  
674 different sensitivity and may be exposed to other chemical or biological stressors. As for birds, Gibbons et al.  
675 (2015) emphasized that neonicotinoids can also impact terrestrial mammals via indirect effects which are reviewed  
676 in the “Food webs” section.

677

### 678 ***Chiropterans***

679 The exposure of wild bats to clothianidin, imidacloprid and thiamethoxam was demonstrated through the detection  
680 of the three substances in the hair of big brown bats (*Eptesicus fuscus*) sampled in Missouri (USA) (Hooper et al.  
681 2022). Imidacloprid showed the highest frequency of detection and was found in all samples (Hooper et al. 2022).  
682 In Turkey, in a large screening targeting 322 PPPs and organic contaminants in adult bat carcasses of *Pipistrellus*  
683 *pipistrellus* and *Myotis myotis*, 87 compounds were detected but they didn't include neonicotinoids (Kuzukiran et  
684 al. 2021). Habitat preferences of these bats (urban and forest species) may limit their exposure to neonicotinoids.  
685 Several studies mention a risk of exposure of chiropterans to neonicotinoids by the trophic route, based on the  
686 monitoring of chiropteran activities and dosages in their prey present in the foraging sites (Stahlschmidt and Brühl  
687 2012; Stahlschmidt et al. 2017).

688 In rare experimental studies, Hsiao et al. (2016) and Wu et al. (2020) reported the neurotoxic effects of  
689 imidacloprid (at 20 mg/kg/day) on the echolocation ability of insectivorous bats (*Hipposideros terasensis*).  
690 Memory loss in bats has been associated with apoptosis lesions in certain areas of the hippocampus (Hsiao et al.  
691 2016). Another study supports these behavioral data and suggests that altered echolocation movements likely  
692 affects bat movement and hunting activities (Wu-Smart and Spivak 2018). In addition, neonicotinoid use appears  
693 to be associated with an increased frequency of white-nose syndrome, caused in chiropterans by a fungal infection,  
694 in both the USA and Europe (Bayat et al. 2014; Oliveira et al. 2021). Upon awakening, bats experience a massive  
695 inflammatory response phase with destruction of part of the immune tissue before reconstruction making them  
696 particularly vulnerable to infection (Mineau and Callaghan 2018). Neonicotinoids can thus come as an aggravating  
697 factor during this critical period. In their review, Mineau and Callaghan (2018) concluded that there is sufficient  
698 evidence to support the assert that bats are being negatively affected by neonicotinoids, directly through functional

699 impairment, and indirectly through reduction in insect abundance (trophic cascades are detailed in the “Food webs”  
700 section): the levels of neonicotinoid residues in the environment are high enough to put bats at risk of motor  
701 impairment and death. Knowledge remains currently too incomplete to be able to thoroughly characterize the  
702 impacts of neonicotinoids on chiropterans.

703

#### 704 ***Reptiles***

705 Neonicotinoids (imidacloprid and thiamethoxam) have been detected in several Mongolian racerunner (*Eremias*  
706 *argus*) organs and tissues (blood, brain, heart, lungs, stomach, intestine, liver, kidney, skin, fat, and gonads),  
707 showing different internal distributions and post-exposure temporal variations depending on the substance  
708 considered. However, the limited number of individuals which were analyzed prevents any attempt at  
709 generalization (Wang et al. 2018b; Wang et al. 2019).

710         The exposure of *E. argus* to thiamethoxam and imidacloprid under controlled conditions led to variations  
711 in thyroid, stress or sex hormone levels, endocrine gland damage, or changes in expression of genes involved in  
712 endocrine functions (Wang et al. 2019; Wang et al. 2020). Yang et al. (2020) also reported the endocrine disrupting  
713 effect of imidacloprid to *E. argus* with decreased levels of testosterone and estradiol in plasma. Further research  
714 is required to better characterize the impacts of neonicotinoids on reptiles.

715

#### 716 ***Amphibians***

717 Amphibians are one of the biological groups most affected by the collapse of biodiversity on a planetary scale, in  
718 particular because of the use of PPPs (Hayes et al. 2010). However, the number of studies of the effects of  
719 neonicotinoids on the terrestrial stages of amphibians is low. Comparing dermal exposure of *Hyla gratiosa* and  
720 *Hyla cinerea* to imidacloprid via direct exposure of the frog present on the soil at the time of insecticide spraying,  
721 and via indirect exposure following soil contact after application, van Meter et al. (2015) showed that cumulative  
722 concentrations and bioconcentration factors were significantly higher for the direct exposure. In the Pampa region  
723 of Argentina, imidacloprid was detected in the terrestrial *Leptodactylus latinasus* frog living in close association  
724 with row crops (soybean, corn, wheat) (Brodeur et al. 2022).

725         Thompson et al. (2022) used both aquatic mesocosms, and terrestrial locomotor and behavior trials to  
726 study the effects of sublethal exposure of the wood frog (*Rana sylvatica* or *Lithobates sylvaticus*) to imidacloprid.  
727 The results showed a decrease in larval survival to metamorphosis under imidacloprid exposure in interaction with  
728 shorter hydroperiod. However, the effect of imidacloprid depends on the frog stage: terrestrial locomotor

729 performances were improved following aquatic exposure of the larvae, while an important loss in these  
730 performances was observed after terrestrial exposure to imidacloprid. In addition, high effects on population sex  
731 structure and sexual development were observed: a skewed juvenile sex ratio was evidenced in imidacloprid  
732 treatments with about 10% fewer males than in controls, and 15.7% of individuals exposed to imidacloprid could  
733 not be assigned to either sex (ambiguous reproductive organ morphology) (Thompson et al. 2022). A great deal of  
734 research remains to be done.

735

## 736 **Aquatic ecosystems**

### 737 **Contamination of freshwater and marine environments**

#### 738 **Freshwater environment**

739 Neonicotinoids used in agricultural fields can enter surface waters (from rivers to lakes) through spray drift, dust  
740 from coated seeds, runoff, subsurface flow (for example, subsurface tile drainage), input of treated leaves, and/or  
741 plant decomposition in water (Alford and Krupke 2019; Stehle et al. 2018; Wang et al. 2023). The primary routes  
742 of transfer are direct contamination due to spray drift or to dust abrasion of coated seeds, and re-distribution from  
743 surface runoff or subsurface drainage (Schaafsma et al. 2019; Wettstein et al. 2016). Neonicotinoids are stable in  
744 water, and because of their high mobility, they are mainly transported in the dissolved phase (Bonmatin et al. 2015;  
745 Morrissey et al. 2015; PPDB 2023).

746         After neonicotinoid applications, the delivery ratio to surface water was estimated to be less than 2% for  
747 thiamethoxam and clothianidin together, and 0.48% for imidacloprid (Frame et al. 2021; Wettstein et al. 2016).  
748 The detection rates in surface water are higher after seed treatment than after spraying (Wettstein et al. 2016). In  
749 North America, clothianidin was found before, during and after planting (i.e., in 98% of the samples), while the  
750 detection of thiamethoxam mainly occurred in the post-plant season (54% of the samples), and that of imidacloprid  
751 during the planting season (48% of the samples) (Evelsizer and Skopec 2018). Clothianidin is both a PPP and a  
752 transformation product of thiamethoxam which could explain its higher frequency of detection (Wang et al. 2023).

753         Neonicotinoids have been quantified in various types of surface waters including wetlands, ditches, ponds  
754 and rivers (Table 1). Acetamiprid, imidacloprid, and thiamethoxam are the most frequently detected substances  
755 (Pietrzak et al. 2019). Overall, maximum concentrations of neonicotinoids in surface waters were found to be 9.14  
756 µg/L for imidacloprid, 6.90 µg/L for thiamethoxam, 4.00 µg/L for acetamiprid, 3.50 µg/L for clothianidin, and  
757 1.37 µg/L for thiacloprid (Alford and Krupke 2019; de Araújo et al. 2022; Criquet et al. 2017; Evelsizer and Skopec

2018; Kuechle et al. 2019; Nélieu et al. 2021; Pietrzak et al. 2019; Schaafma et al. 2019; Wang et al. 2023) (Table 1). Most of these reported maximum concentrations exceed the ecological thresholds for neonicotinoid water concentrations (0.2 µg/L for short-term acute exposure and 0.035 µg/L for long-term chronic exposure) which were defined to avoid lasting effects on aquatic invertebrate communities (Morrissey et al. 2015). A recent review provided a meta-analysis of neonicotinoid concentrations in water, based on more than 40 papers published in ten countries (Wang et al. 2023). It reported mean concentrations of 0.222 µg/L (n=1056) for clothianidin, 0.120 µg/L (n=879) for imidacloprid, 0.059 µg/L (n=863) for thiamethoxam, 0.023 µg/L (n=428) for acetamiprid, and 0.011 µg/L (n=295) for thiacloprid.

Some mitigation measures could consist in improving the application material to prevent dust during planting of treated seeds, and to improve water interception of surface and subsurface flow thanks to buffer zones such as wetlands. For example, in constructed wetlands, removal of neonicotinoids due to direct accumulation in macrophytes and to enhanced biodegradation was estimated to range from 10 to 100% in 28 days (Liu et al. 2021; Main et al. 2017).

771

## 772 **Marine environment**

Neonicotinoids have only been recently monitored in coastal and marine environments. Consequently, data are just available for imidacloprid and thiamethoxam, which are generally searched for using passive integrative POCIS samplers or directly in water. In mainland France, these substances were not found in the Channel/North Sea coast (Menet-Nedelec et al. 2018). On the contrary, on the other two maritime facades (Bay of Biscay and Mediterranean), imidacloprid and thiamethoxam were quantified quite frequently (with maximum frequencies of detection of 20%) in the coastal waters of the Arcachon Basin (maximum of 0.14 µg/L and 0.0039 µg/L for imidacloprid and thiamethoxam, respectively, in spot samples) (Auby et al. 2011; Tapie and Budzinski 2018) (Table 1), in transitional waters of the Gironde estuary (maximum imidacloprid concentration of 0.0053 µg/L with integrative sampling) (Levesque et al. 2018), in Marennes-Oléron bay (maximum of 0.0238 µg/L and 0.0004 µg/L for imidacloprid and thiamethoxam, respectively, with integrative sampling) (Pepin et al. 2017), and in Mediterranean lagoons (maximum of 0.028 µg/L and 0.0025 µg/L, for imidacloprid and thiamethoxam, respectively with integrative sampling) (Munaron et al. 2020; Munaron et al. 2023). Imidacloprid has also been detected in the Charente estuary and in the Loire estuary since 2006 (GIP Loire Bretagne 2013). According to ecotoxicological data collected in the OBSLAG (Observatory of the Mediterranean Lagoons) study, only imidacloprid would cause a chronic risk for the biota of lagoon ecosystems (exceeding its chronic marine predicted

788 no effect concentration PNEC in several lagoons since the beginning of the monitoring in 2017) (Munaron et al.  
789 2022). This risk can be extended to the Arcachon basin and Marennes-Oléron bay given the reported data. No  
790 neonicotinoid was found in French marine sediments and no reference from the French overseas territories  
791 mentions their research in the water of the marine environment.

792 Only scarce information is available evidencing the contamination of marine waters worldwide. In the  
793 Queensland region of Australia, streams flowing into the marine waters of the Great Barrier Reef were found to  
794 be contaminated with imidacloprid at levels ranging from 0.0005 to 1.3 µg/L (Warne et al. 2022). The  
795 contamination concerned observation sites located in downstream sectors near the mouths of large rivers (Warne  
796 et al. 2022). This pattern appeared similar in the Bohai Sea (China), where Naumann et al. (2022) observed the  
797 seasonal variation in neonicotinoid concentrations in rivers and marine water. In their study, the detection  
798 frequency of acetamiprid was 100% in both river (n=72) and marine (n=81) waters in summer and fall. Despite  
799 dilution in the coastal environment, the risk quotient associated with the contamination levels were reported as  
800 high risk for marine organisms regarding imidacloprid, thiamethoxam and acetamiprid (Naumann et al. 2022).  
801 Due to their slow degradation rates in the environment and binding properties to particulate organic matter (PPDB  
802 2023), neonicotinoids are likely to accumulate in sediments: Chen et al. (2022) reported contamination of marine  
803 sediments in East China Sea, due to the Yangtze River inputs, several tenths of kilometer from the river mouth.  
804 The mean concentration of total neonicotinoids was 11.9 µg/kg (dry weight). The authors concluded that marine  
805 sediments were a major sink for neonicotinoids, highly used in continental China as PPPs (Chen et al. 2022).

806

## 807 **Impacts on aquatic biodiversity**

### 808 **Aquatic microorganisms**

809 Few studies have been published on the effects of neonicotinoids on aquatic microorganisms. They suggest that  
810 imidacloprid does not affect the activity and respiration of aquatic microbial decomposers (Kreutzweiser et al.  
811 2007; Kreutzweiser et al. 2008). With the exception of the study of Neury-Ormanni et al. (2020a), who observed  
812 that an exposure of the freshwater diatoms *Planothidium lanceolatum* and *Gomphonema gracile* to 5 µg/L  
813 imidacloprid resulted in indirect effects via competition and predation, effects of neonicotinoids on different  
814 microalgae (e.g., *Desmodesmus subspicatus*; Malev et al., 2012) and cyanobacteria (e.g., *Synechocystis* sp.; Li et  
815 al., 2010) were only observed at very high concentrations (i.e., several mg/L), irrelevant to environmental  
816 contamination levels. Using a quantitative structure activity-toxicity modeling approach, Gökçe and Saçan (2019)  
817 also predicted an absence of effects of acetamiprid on microalgae exposed to up to 100 mg/L. Neonicotinoids are

818 therefore unlikely to be toxic to aquatic microbes, including primary producers, except under extreme events of  
819 contamination.

820

## 821 **Aquatic invertebrates**

822 Works focused on the effects of neonicotinoids on aquatic invertebrates are increasingly investigated (as compared  
823 to other insecticide classes, such as carbamates and organophosphates) due to the relative recentness of their use  
824 (first homologations date back from the 1990s), and to the risk specifically posed to aquatic invertebrates because  
825 of the levels of water contamination reported (see above). Morrissey et al. (2015) highlighted strong evidence that  
826 water-borne neonicotinoid exposure is frequent, long-term and at concentrations which commonly exceed several  
827 existing water quality guidelines. In addition, several monitoring studies of watercourses in either agricultural or  
828 urban landscapes demonstrated a significant contamination of freshwater amphipods (*Gammarus pulex*) by  
829 neonicotinoids (e.g., Shahid et al. 2018a; Švara et al. 2021).

830 Despite awareness of these contamination levels, works devoted to the effects of neonicotinoids on  
831 aquatic invertebrate biodiversity are still limited. A first review published in 2015 noted the weak level of  
832 knowledge available on the effect of neonicotinoids on the invertebrate fauna of freshwater and marine  
833 environments (Pisa et al. 2015). Since then, various field case studies have provided data and  
834 documented/predicted effects of neonicotinoids on aquatic invertebrate communities. For example, in Canadian  
835 wetlands near treated rapeseed crops, a correlation was established between neonicotinoids (acetamiprid,  
836 clothianidin, imidacloprid, thiamethoxam) transfer during rainfall events and changes in emergent insect (Diptera)  
837 diversity (Cavallaro et al. 2019). Through an experimental rice mesocosm study, imidacloprid was found to  
838 significantly reduce populations of various insects (dragonfly, bug, beetle) (Kobashi et al. 2017). A drastic decline  
839 in zooplankton biomass in Japanese brackish lakes also coincided with the introduction of neonicotinoids  
840 (clothianidin, imidacloprid, thiamethoxam) in rice agriculture since the 1990s, followed by collapse of predator  
841 fish populations (Yamamuro et al. 2019). In the Netherlands, where imidacloprid residues in water are particularly  
842 high, correlations between these residues and decline in arthropod taxa such as mayflies, odonates, diptera, and  
843 some crustaceans were revealed on a national scale (van Dijk et al. 2013). This was also observed in a study  
844 adopting a PAF (Potentially Affected Fraction) approach, but with much lower proportions of species potentially  
845 affected by neonicotinoids taking into account the co-occurrence of other PPPs in the studied environments (Vijver  
846 and van den Brink 2014).

847 Comparing recorded or predicted concentrations of neonicotinoids in the aquatic environment to  
848 ecotoxicity thresholds has raised some concerns for the potential effects of these insecticides in freshwater  
849 environments. The review by Sánchez-Bayo et al. (2016) reported widespread effects of neonicotinoids on aquatic  
850 species in the USA, and the major risk for aquatic invertebrates was reaffirmed in 2017 (Wood and Goulson 2017).  
851 More recently, a study based on an agricultural region located in an ecologically important wetland (Nebraska's  
852 Rainwater Basin, USA), showed negative correlations between neonicotinoid concentrations and  
853 macroinvertebrate biomass (which represents potential resources for various migratory birds) despite  
854 concentrations below the acute toxicity risk thresholds proposed by the USEPA (Schepker et al. 2020).

855 Long-term ecological impact of neonicotinoids is a particularly salient issue for aquatic invertebrates. The  
856 chronic risk mainly results from the ability of neonicotinoids to reach aquatic environments (high solubility in  
857 water) and to persist there when they are adsorbed on particles (Armbrust and Peeler 2002). However, this risk is  
858 poorly assessed because most often based on toxicity tests on *Daphnia*, an organism more tolerant than insects and  
859 other arthropods to neonicotinoids (Beketov and Liess 2008; Wood and Goulson 2017). Neonicotinoids can have  
860 chronic effects on abundance and community structure of freshwater arthropods and other macroinvertebrates at  
861 doses in the  $\mu\text{g/L}$  range and below (Beketov and Liess 2008; Kattwinkel et al. 2016). After cessation of treatments,  
862 the onset of delayed effects was also demonstrated in situ (limnocorrals) for much lower concentrations of  
863 imidacloprid and clothianidin ( $< 0.05 \mu\text{g/L}$ ) resulting in a significant advancement of the emergence date of  
864 chironomids and zygopteran odonates (Cavallaro et al. 2018; Williams and Sweetman 2019). From a functional  
865 point of view, the desynchronization of phenology of these organisms could have important consequences on  
866 ecosystems, especially in terms of biomass input to the terrestrial environment (trophic resource for terrestrial  
867 predators such as birds). Lethal and sublethal effects of thiacloprid have been demonstrated in various aquatic  
868 invertebrates, several days after exposure, for moderate acute toxicity concentrations (Beketov and Liess 2008).  
869 Neury-Ormanni et al. (2020b) documented altered feeding behavior in chironomids exposed to environmental  
870 doses of imidacloprid. The insecticide induced changes in motility, feeding selectivity, and browsing ability. The  
871 reduced abundance and altered emergent aquatic insect assemblages in wetlands exposed to neonicotinoids could  
872 explain the reduction in densities of insectivorous birds in such environments (Cavallaro et al. 2019).

873 Investigating the idea of long-term impact of neonicotinoids beyond the lifespan of exposed individuals,  
874 recent works with the model amphipod crustacean, *G. pulex*, suggested the development of tolerance towards  
875 clothianidin within populations from watercourses in agricultural landscapes (Becker and Liess 2017; Becker et  
876 al. 2020; Shahid et al. 2018b). According to the authors, in these populations, the evolution of resistance by natural

877 selection could be facilitated by factors acting at the population and/or community levels: distance from non-  
878 tolerant populations, which would favor selection locally by limiting gene flow and the influx of non-adapted  
879 genes into populations (Hoffmann and Willi 2008), and low community diversity which would intensify intra-  
880 specific competition in gammarids. Nevertheless, the shift in sensitivity of this non-target species to the  
881 neonicotinoid appeared very moderate (less than three-fold change in LC50 for example) in comparison to the  
882 genetic resistance reported for other neurotoxic insecticides (pyrethroids and organophosphates) in the amphipod  
883 *Hyalella azteca* (Gamble et al. 2023; Weston et al. 2013). In addition, an inverse pattern with increased sensitivities  
884 of long-term exposed *G. pulex* populations towards imidacloprid was found in non-agricultural context presenting  
885 complex mixture of organic contaminants (Švara et al. 2021). Overall, these results demonstrate the unsuspected  
886 importance of evolutionary adaptive processes underway in natural populations unintentionally exposed to  
887 neonicotinoids, and the urgency to develop assessment tools specifically focused on long-term effects (Oziolor et  
888 al. 2016). Such processes should be anticipated, at least in insects and probably in other arthropods, from the  
889 current knowledge on the selective evolution of resistance to neonicotinoids in pests, based either on target-site  
890 mutation or on metabolic resistance (Bass et al. 2015).

891         Although environmentally less realistic than field approaches, experimental studies performed in  
892 mesocosms and in the laboratory (e.g., common garden), offer the statistical power required to test patterns  
893 observed in natura (Barmantlo et al. 2021), as well as interactions with other environmental factors susceptible to  
894 alleviate or aggravate the effects of neonicotinoids, such as PPP mixtures (Sanchez-Bayo and Goka 2012; Rico et  
895 al. 2018; Sol Dourdin et al. 2023), temperature/climate (Mohr et al. 2012; Sumon et al. 2018; Rico et al. 2018),  
896 nutrients/fertilizers (Barmantlo et al. 2019; Chara-Serna et al. 2019), vegetation disturbance (Cavallaro et al. 2019),  
897 and indirect effects between species representative of different functional groups in the community (e.g., such as  
898 predator-prey relationships; Miles et al. 2017). In this regard, Alexander et al. (2013) used artificial streams to  
899 examine the impact of mixing three insecticides expected to act additively, i.e., imidacloprid (which acts on the  
900 acetylcholine receptor) and two organophosphates which act on the acetylcholine esterase enzyme, chlorpyrifos  
901 and dimethoate, and under oligotrophic vs mesotrophic (nitrate input), along a Toxic Unit (TU) gradient  
902 established for concentrations consistent with environmental data. The study showed a significant interaction  
903 between insecticides and nutrients on macroinvertebrate communities, with notably, under mesotrophic condition  
904 and low insecticides pressure, an increase in the total abundance and species richness of ephemeropteran,  
905 plecopteran and trichopteran insects. At higher insecticides pressure, the overall density of these groups and the  
906 entire community was the most reduced in mesotrophic streams. In contrast, for other species groups such as

907 chironomids, detritus feeders, and the odonate predator *Gomphus* spp., no significant interaction between  
908 insecticides and nitrate was detected. In oligotrophic environments, increasing PPP doses decreased predation  
909 intensity, which in turn affected abundance patterns while, in mesotrophic environments, a bottom-up effect of  
910 nutrients on the periphyton explained the variation in macroinvertebrates abundance and richness. Such cause-  
911 and-effect relationships were also analyzed with Structural Equation Modeling (SEM) approaches which describe  
912 effect pathways among different variables of interest (Miller et al. 2020; Schmidt et al. 2022). At low doses, the  
913 toxicity of PPPs appeared hidden by nutrients because of increased compensatory consumption, expression of  
914 adaptive plasticity at the intraspecific level, or differential responsiveness across taxa, processes which are not  
915 captured by traditional community study methods (taxonomic determination and records of relative abundances).  
916 Interactions between nutrients and PPP can thus result in a redirection of energy within food webs towards non-  
917 productive pathways (Davis et al. 2010) or in a shift in communities towards more tolerant groups (Vinebrooke et  
918 al. 2004). This type of interactions was also studied in terms of convergence/divergence of invertebrate community  
919 structure in open artificial ditches (naturally assembled communities), by combining NPK elements with  
920 thiacloprid (Barmantlo et al. 2019). Following thiacloprid treatments designed to maintain concentrations for one  
921 month (two spikes separated by two weeks), no effect of treatments, other than an increase in total abundance after  
922 four months due to nutrient input, was found in terms of taxon richness, overall abundance, or within-treatment  
923 community divergence/convergence through time ( $\beta$  dispersion). However, significant changes were observed in  
924 community composition under the effect of thiacloprid, nutrients and combination thereof. This effect persisted  
925 several months after the disappearance of thiacloprid from the medium. The main compositional changes were a  
926 reduction in the abundance of insects and large predators, and an increase in multivoltine species. Some results,  
927 such as the particularly strong increase in *Helophorus* beetles under nutrients and thiacloprid, may reflect a PPP-  
928 induced rippling effect on the community amplified by nutrient supply. This study shows that thiacloprid, in  
929 addition to its short-term toxicity, induces indirect longer-term ecological effects.

930 Overall, the corpus analyzed pointed to a marked impact of neonicotinoids on aquatic arthropods at low  
931 doses, as demonstrated once again in a recent study which reports the decline in emerging aquatic insects during  
932 a three-month semi-field experiment considering environmentally realistic contamination scenarios of thiacloprid  
933 (Barmantlo et al. 2021). However, more studies remain to be performed to determine the relationship between the  
934 impacts of neonicotinoids and fitness of organisms, in relation to the ecological functions to which they contribute,  
935 as well as on the relationship between the impacts of neonicotinoids on the nervous system and the behavior of  
936 aquatic invertebrates.

937 **Aquatic vertebrates**

938 ***Amphibian larvae and tadpoles***

939 The sensitivity of amphibian species to neonicotinoids through water contamination has been rarely studied. Green  
940 frog (*Rana clamitans*) tadpoles were found to be relatively insensitive to imidacloprid with mortality observed  
941 after 96h of exposure to high concentrations only (150 mg/L) (Puglis and Boone 2011). This lack of sensitivity is  
942 likely due to differences in the vertebrate nicotinic acetylcholine receptor relative to their invertebrate homologs  
943 (Li et al. 2016). On the contrary, spotted marsh frog tadpoles (*Limnodynastes tasmaniensis*) suffered high mortality  
944 rates (up to 17%) when they were exposed to imidacloprid concentrations as low as 0.50 µg/L (Sievers et al. 2018).  
945 This exposure level reduced swimming speed and distance, and escape responses which then made the tadpoles  
946 more susceptible to predation, while increasing erratic swimming (Sievers et al. 2018). The toxicity of imidacloprid  
947 has also been demonstrated in the tadpoles of *Leptodactylus luctator* and *Physalaemus cuvieri* (Samojeden et al.  
948 2022). The consequences of exposure to environmental concentrations (3-300 µg/L) led to a decrease in size, to  
949 morphological malformations (for the two species), and to changes in tadpole swimming activity (only for *L.*  
950 *luctator*).

951 In the current literature, there is limited evidence of the effects of neonicotinoids on amphibians under  
952 chronic exposure to aquatic environmental concentrations. However, neurotoxic responses can be observed.  
953 Campbell et al. (2022; 2023) demonstrated the ability of imidacloprid to cross the blood-brain barrier and to  
954 concentrate over 300-fold in the brain of juvenile northern leopard frogs (*Rana pipiens*) with some consequences  
955 on foraging behavior (e.g., a decrease in reaction times to a food stimulus by 1.5 to 3.2 times for organisms exposed  
956 to concentrations up to 10 µg/L). At concentrations ranging from 0.1 to 10 µg/L and over a 21 day exposure period,  
957 bioaccumulation of imidacloprid in frog brains is accompanied by a decreased reactivity in individuals subjected  
958 to feeding stimuli. Beyond the active substance, the transformation product imidacloprid-olefin was detected in  
959 the brains of amphibians at much lower concentrations, which does not mean that this compound cannot be  
960 responsible for any toxic action. Surprisingly, exposure of leopard frogs to imidacloprid led to increased growth  
961 primarily affecting body length (Campbell et al. 2022). Recent research has further demonstrated that wood frogs  
962 (*R. sylvatica* or *L. sylvaticus*) exposed to imidacloprid (10 or 100 µg/L) at the tadpole stage were less likely to  
963 escape simulated predator attacks in the laboratory, suggesting that exposure to this insecticide may negatively  
964 impact tadpole perception and cognitive function (Lee-Jenkins and Robinson 2018; Sweeney et al. 2021).  
965 However, at a lower concentration of 0.1 µg/L, imidacloprid did not induce any modulation of acetylcholinesterase  
966 activity in bullfrog (*Lithobates catesbeiana*) tadpoles after three weeks of exposure (Rios et al. 2017). For other

967 less studied neonicotinoids as clothianidin, frog tadpoles are among the least sensitive species in case of  
968 laboratory exposure at sublethal concentrations (Miles et al. 2017). The tadpoles are tolerant to clothianidin,  
969 confirming the low toxicity of neonicotinoids in vertebrates (Miles et al. 2017). As stated in the section focused  
970 on the impacts of neonicotinoids on amphibians during their terrestrial life, numerous research remain to be done  
971 to characterize their impacts on amphibians in aquatic media.

972

### 973 ***Fish***

974 In general, neonicotinoids exhibit low acute toxicity to fish. The 96h LC50 of clothianidin ranges from 93.6 mg/L  
975 for sheepshead minnow (*Cyprinodon variegatus*) to 117 mg/L for bluegill sunfish (*Lepomis macrochirus*)  
976 (Anderson et al. 2015). A similar trend is observed for imidacloprid, with 96h LC50 ranging from 211 mg/L for  
977 rainbow trout (*Oncorhynchus mykiss*) to 280 mg/L for common carp (*Cyprinus carpio*) (Anderson et al. 2015).  
978 Two formulations of thiamethoxam have 96h LC50 above 100 mg/L (Anderson et al. 2015). These results indicate  
979 that fish are insensitive to neonicotinoids, probably because of the properties of the vertebrate nicotinic  
980 acetylcholine receptor (Li et al. 2016).

981         Nevertheless, the available data indicate that exposure of aquatic vertebrates to sublethal concentrations  
982 of neonicotinoids results in pro-oxidative responses from which genotoxic perturbations arise. A short 48h  
983 exposure of the freshwater cichlid fish (*Australoheros facetus*) to imidacloprid concentrations of 100 and 1000  
984 µg/L affected the integrity of fish erythrocyte DNA (COMET assay and micro-nuclei test) (Iturburu et al. 2018).  
985 Under short-term exposure to a much lower concentration of thiamethoxam (3.75 µg/L), the siluriform catfish  
986 (*Rhamdia quelen*) showed activity inhibition of two liver enzymes, adenylate kinase and pyruvate kinase, as early  
987 as 24h of exposure (Baldissera et al. 2018). These inhibitions were associated with a decrease in ATP levels in the  
988 liver. The energetic deregulation appeared to persist after the fish were no longer contaminated (Baldissera et al.  
989 2018). Beyond these non-specific effects, neonicotinoids can act on the nervous function of non-target organisms,  
990 given their mode of action (binding to nicotinic acetylcholine receptors at neuromuscular junctions leading to  
991 insect paralysis) (Kimura-Kuroda et al. 2012). Imidacloprid was found to be neurotoxic to adult rainbow trout (*O.*  
992 *mykiss*) exposed for 21 days to high concentrations (10 and 20 mg/L) (Topal et al. 2017). This neurotoxicity  
993 resulted in inhibition of acetylcholinesterase activity, oxidative stress, and a concomitant increase in DNA damage  
994 in the fish brains (Topal et al. 2017).

995         Neurotoxicity of neonicotinoids may also impact the behavior of fish. A laboratory test developed to  
996 investigate two key responses of fish anti-predator behaviors revealed that zebrafish (*Danio rerio*) larvae exposed

997 for 24 hours to acetamiprid exhibited increased fear reflex and faster habituation compared to unexposed larvae  
998 (Faria et al. 2020). The concentrations tested in this study were considered to be realistic (0.04 and 0.40  $\mu\text{g/L}$ ) in  
999 relation to measured concentrations of acetamiprid in surface water (0.008 to 44  $\mu\text{g/L}$ ) (Faria et al. 2020). The  
1000 modulations of fish larvae anti-predator behavior observed in the laboratory raise questions about the  
1001 environmental reality of such effects and about their hypothetical consequences in terms of survival capacity in  
1002 the environment. Könemann et al. (2021) observed that zebrafish larvae were able to avoid imidacloprid  
1003 contamination, but did not react to other neonicotinoids such as thiacloprid. In addition, the experimental ablation  
1004 of olfaction abolished aversive responses of individuals, indicating that fish may sense insecticides. In this species,  
1005 the assessment of neural activity in 289 different brain regions revealed a particular modulation of hypothalamic  
1006 areas involved in the fish stress response, indicating that the observed behavioral patterns are close to those  
1007 observed for other stress responses (Könemann et al. 2021). Juvenile medaka (*Oryzias latipes*), exposed to  
1008 imidacloprid under rice cultivation field conditions, were consecutively infected by a *Trichodina* parasite  
1009 (Sánchez-Bayo and Goka, 2005). Such pathology was linked to the chemical stress induced by imidacloprid. If  
1010 toxicity of imidacloprid to vertebrates was extensively studied, the toxicity related to imidacloprid transformation  
1011 products (5-hydroxy-imidacloprid, imidacloprid-urea and 6-chloronicotinic acid) was not taken into account until  
1012 now, despite their presence in various tissues as observed, for example, in muscle, gonads, brain and gills in  
1013 Goldfish (*Carassius auratus*) (Xu et al. 2023).

1014 A few studies deal with the combined effects of neonicotinoids with other PPPs but sometimes with  
1015 experimental approaches that are more or less relevant in the context of ecological risk assessment. Thus, adult  
1016 zebrafish exposed by immersion during 24 hours to high concentrations of imidacloprid (13.75 mg/L) associated  
1017 with the organophosphate insecticide dichlorvos (7.5 mg/L) and the herbicide atrazine (1.5 mg/L) showed high  
1018 levels of lipid peroxidation, particularly in the liver, compared to fish exposed to the same active substances tested  
1019 in isolation (Shukla et al. 2017). Although this type of study is useful to test the hypothesis of expected synergistic  
1020 effects, it does not allow estimation of the actual environmental risk, particularly in view of the contamination of  
1021 surface waters reported by the authors (in the Ebro River in Spain: minimum concentration of imidacloprid of  
1022 0.0016  $\mu\text{g/L}$  and maximum concentration of 0.015  $\mu\text{g/L}$ ) (Shukla et al. 2017). It is therefore important to consider  
1023 such data with caution when assessing the ecotoxicity of neonicotinoids. Similarly, mixture of the order of mg/L  
1024 imidacloprid and organophosphate insecticide triazophos used to assess embryotoxicity to zebrafish early larvae  
1025 (blastula stage: 2h post-fertilization) exposed during 96h revealed a strong synergistic effect in terms of acute  
1026 toxicity (Wu et al. 2018). Although relevant in terms of mixture toxicity assessment, such high concentrations still

1027 lack environmental relevance. It is worth noting that, though concentrations were still high, synergistic effects  
1028 were also demonstrated on zebrafish larvae (72h post-hatching) for various combinations of imidacloprid with  
1029 atrazine, butachlor, chlorpyrifos or lambda-cyhalothrin (mixtures containing from two to five substances) (Wang  
1030 et al. 2017).

1031 No study has been devoted to the effects of neonicotinoid mixtures on aquatic vertebrates (Anderson et  
1032 al., 2015). In addition, there is a lack of ecosystem-scale studies (mesocosm approaches and/or field studies) to  
1033 investigate the effects of these insecticides. Work is also needed on sub-lethal or chronic effects to reflect  
1034 environmental concentration levels. Finally, most of the studies focus on imidacloprid, with very little attention  
1035 paid to the effects of other neonicotinoids.

1036

### 1037 *Aquatic birds*

1038 Aquatic birds include waterbirds, which live in freshwater environments, and seabirds, which feed on the resources  
1039 of seas and oceans.

1040 The exposure of seabirds to neonicotinoids (acetamiprid, clothianidin, imidacloprid, thiacloprid,  
1041 thiamethoxam) was characterized by analyzing residues in feathers sampled from the piscivorous Sandwich tern  
1042 (*Thalasseus sandvicensis*) and the mixotrophic Mediterranean gull (*Ichthyaetus melanocephalus*) in fledglings  
1043 from the Lagoon of Venice (Distefano et al. 2022). Neonicotinoids were detected in both species, and imidacloprid  
1044 and clothianidin were the most often quantified ones (100% in Mediterranean gulls and 58% in Sandwich terns,  
1045 and 100% in Mediterranean gulls and 61% in Sandwich terns, respectively). The detection of thiacloprid was lower  
1046 (<20% of samples in both species) (Distefano et al. 2022). On the contrary, no residue of neonicotinoids was found  
1047 in the liver or blood of white-tailed sea eagles (*Haliaeetus albicilla*) and ospreys (*Pandion haliaetus*) (Badry et al.  
1048 2021; Badry et al. 2022).

1049 For waterbirds, data are even more scarce. In some rice-growing regions, aquaponic practices involve  
1050 ducks for the control of weed and pest in rice fields (Mburia, 2016). In this very particular context, ducks may be  
1051 contaminated with neonicotinoid residues (Khidkhan et al., 2022).

1052 To date, no result on the direct effects of neonicotinoids on seabirds and waterbirds were available in the  
1053 literature. Thus, even if the toxicity of neonicotinoids to aquatic vertebrates is presumed to be limited, there are  
1054 still many areas of knowledge that need to be clarified and completed such as toxicity of transformation products,  
1055 and levels of impregnation of agricultural wetland-living organisms by native substances and their transformation  
1056 products (Frank and Tooker, 2020).

## 1057 **Food webs**

1058 Neonicotinoids can affect terrestrial and aquatic biodiversity by spreading through food webs, by the propagation  
1059 of adverse biological effects in food webs and disturbance of trophic interactions (e.g., reduced predation rate,  
1060 increased mortality of predators), and/or by reducing food resources (Alsafran et al. 2022). However, the number  
1061 of results which have been published in the literature remains limited.

1062

## 1063 **Terrestrial ecosystems**

1064 Focusing on insects, Tooker and Pearsons (2021) reviewed the mechanisms underlying the effects of insecticides  
1065 on food webs. They highlighted how neonicotinoids influence trophic interactions and food webs, and contribute  
1066 to insect declines. Neonicotinoids spread across trophic levels, primary and secondary consumers being exposed  
1067 through several routes (including dietary and trophic routes), and they may also bioaccumulate in some organisms  
1068 (Tooker and Pearsons 2021). Neonicotinoids distort food webs by significantly decreasing insect abundance and  
1069 diversity of both preys and consumers, as evidenced in various ecosystems (e.g., croplands, woodlands,  
1070 watercourses). Depopulated and less diversified insect communities lead to food scarcity for their predators,  
1071 thereby adversely impacting their local population dynamics. Importantly, food web disruption can occur even  
1072 when neonicotinoids do not bioaccumulate or biomagnify in food webs, depending on the sensitivity of the taxa  
1073 constituting the lower trophic levels (i.e., toxic effects on prey inducing adverse effects on higher levels via trophic  
1074 cascades) and/or the sensitivity of higher trophic levels (i.e., relatively low concentrations but high enough to  
1075 induce toxic effects on sensitive predators) (Tooker and Pearsons 2021).

1076 In terrestrial invertebrates, thiamethoxam has been reported to have no effect on the predation rates of  
1077 two predators, *Orius insidiosus* insidious flower bug and *Hippodamia convergens* ladybug, after consuming aphids  
1078 reared on thiamethoxam-treated plants (Esquivel et al. 2020). On the contrary, insidious flower bug survival, unlike  
1079 that of ladybugs, was reduced following aphid consumption. However, the reduction in bug survival was only  
1080 observed in the first few weeks after thiamethoxam application, and no reduction was noted one month after  
1081 treatment or beyond. In an urban context (Central Park, New York City, USA) where trees were treated with  
1082 imidacloprid against an alien beetle (*Anoplophora glabripennis*), unexpected outbreaks of a formerly innocuous  
1083 herbivore, *Tetranychus schoenei* (Tetranychidae), followed insecticide applications to elms (Szczepaniec et al.  
1084 2011). Changes in the structure of arthropod communities sampled in elm canopies after imidacloprid treatments  
1085 were evidenced, mainly related to an increase in the abundance of *T. schoenei*. Laboratory tests showed that

1086 exposure to imidacloprid through consumption of imidacloprid-treated elm foliage enhanced the fecundity of *T.*  
1087 *schoenei* by 40%: adult *T. schoenei* fed leaves from treated elms laid more eggs than when fed with leaves from  
1088 untreated elms (Szczepaniec et al. 2011). However, no effect of imidacloprid on *T. schoenei* fecundity was detected  
1089 when mites were directly sprayed with the insecticide. The longevity of mites was also not affected by exposure  
1090 to imidacloprid via food. Two model predators of spider mites, the Coccinellidae *Stethorus punctillum* (adult) and  
1091 the Chrysopidae *Chrysoperla rufilabris* (larva), showed significant decrease in feeding rates when offered mites  
1092 from imidacloprid-treated elms as preys. Moreover, the predators exhibited signs of intoxication (partial or  
1093 complete lack of response to touch, tremors, regurgitation, excessive grooming, and inability to right themselves  
1094 when placed on their back) and deleterious effects when exposed to imidacloprid by consuming prey from leaves  
1095 of treated trees such as impaired mobility and reduced longevity (about one-two days when mites fed from treated  
1096 trees versus 9-13 days when *T. schoenei* fed from untreated trees) (Szczepaniec et al. 2011). By stimulating  
1097 reproduction of mites while poisoning insect predators of spider mites which may reduce top-down regulation,  
1098 imidacloprid tree treatments finally led a non-target innocuous herbivore to reach a pest status (Szczepaniec et al.  
1099 2011). This study underlined how neonicotinoids may disrupt ecosystem functioning and impair ecological balance  
1100 that ultimately can favor pest outbreaks. Studying the effect of thiamethoxam on the spider mite (*Tetranychus*  
1101 *urticae*, considered as a pest in various agricultural systems) and its predator *Phytoseiulus persimilis*, Pozzebon et  
1102 al. (2011) showed that the neonicotinoid was toxic to both *T. urticae* and *P. persimilis*, but that the impact of  
1103 thiamethoxam varied according to the routes of exposure. The authors demonstrated that topical exposure led to  
1104 sublethal effects in predators and preys while residual and contaminated food exposures led to both lethal and  
1105 sublethal effects. In addition, toxicity increased when several exposure routes were involved. By limiting exposure  
1106 to thiamethoxam to ingestion of contaminated food only, the impact of the insecticide was more favorable to *P.*  
1107 *persimilis* than to its prey (Pozzebon et al. 2011).

1108           The propagation of sublethal effects of neonicotinoids via trophic interactions was evidenced in a three-  
1109 level food chain gathering wild strawberry (*Fragaria vesca*), wood cricket (*Nemobius sylvestris*) and nursery web  
1110 spider (*Pisaura mirabili*): strawberries were treated with imidacloprid at different doses and crickets were allowed  
1111 to feed on them (Uhl et al. 2015). In this tritrophic system, feeding, mass gain, thorax growth and mobility of wood  
1112 crickets was reduced, and herbivory and predation diminished at sublethal imidacloprid doses in the non-target  
1113 organisms (Uhl et al. 2015). The effects of thiamethoxam, applied as a soybean seed treatment, on interactions  
1114 between soybeans, non-target herbivorous mollusks (pests), and predatory insects was studied in the laboratory  
1115 and in the field (Douglas et al. 2015). In the laboratory, the slug *Deroceras reticulatum* was not affected by

1116 thiamethoxam, but predatory ground beetles (*Chlaenius tricolor*) which ate these slugs were affected or died in  
1117 over 60% of cases. In the field, thiamethoxam seed treatments decreased the activity and density of predatory  
1118 arthropods, thereby releasing slug predation and reducing soybean densities by 19% and yield by 5%. The analyses  
1119 of thiamethoxam residues revealed a transfer in food webs: they showed that insecticide concentrations decreased  
1120 throughout the food chain, but that levels in slugs collected in the field were still high enough to adversely affect  
1121 predatory insects. According to Douglas et al. (2015), this work on the trophic transfer of thiamethoxam challenges  
1122 the idea that seed treatments with neonicotinoids specifically target herbivore pests, and underscores the need to  
1123 consider predatory arthropods and soil organism communities in neonicotinoid risk assessment and management.

1124 If neonicotinoids can affect vertebrates through direct effects, as reviewed above, they can also affect  
1125 wildlife through a reduction in food resources (Gibbons et al. 2015). Further, the trophic transfer of neonicotinoids  
1126 has been recently evidenced, especially in birds. The presence of 54 residues of PPPs or transformation products  
1127 was investigated in the food bolus (insects) provided by the parents of the tree swallow (*Tachycineta bicolor*) to  
1128 their chicks, in 40 Canadian farms (Poisson et al. 2021). This multi-residue analysis included seven neonicotinoids  
1129 (acetamiprid, clothianidin, dinotefuran, imidacloprid, nitenpyram, thiacloprid, thiamethoxam). The results attested  
1130 to the ubiquitous trophic exposure, with nearly half of the food boluses showing contamination by at least one  
1131 substance, clothianidin being among the most frequently detected PPPs (9%). Mixtures of 2 to 16 PPPs, among  
1132 which five (clothianidin, dinotefuran, imidacloprid, thiacloprid, thiamethoxam) of the seven neonicotinoids, were  
1133 also detected in 21% of the food boluses (and 45% of the contaminated boluses). A study conducted in Switzerland  
1134 reported that at least one neonicotinoid was detected in 100% of food boluses collected from Alpine swift  
1135 (*Tachymarptis melba*) provisioning their nestlings, 75% of the food boluses exhibiting measurable concentrations  
1136 (Humann-Guillemot et al. 2021). Both acetamiprid and thiacloprid were found, and thiacloprid showed the  
1137 highest occurrence (up to 66.7%) and the highest concentrations (up to 0.6 µg/kg). Surveys on birds in the USA  
1138 and Europe revealed exposure/accumulation of neonicotinoids in all trophic groups such as nectarivores and  
1139 granivores, insectivores and predators including top-predators (raptors), and piscivores, strongly suggesting the  
1140 occurrence of trophic transfer in food webs (Badry et al. 2021; Bishop et al. 2020; Bro et al. 2016; Byholm et al.  
1141 2018; Distefano et al. 2022; Humann-Guillemot et al. 2021; Taliany-Chamudis et al. 2017). In 60 sites over a  
1142 wide cereal plain in France, the bioaccumulation of several neonicotinoids has been evidenced in both  
1143 granivorous/omnivorous rodents, and insectivorous shrews as well as in earthworms and carabid beetles, which  
1144 were their potential preys (Pelosi et al. 2021; Fritsch et al. 2022). Finally, residues in tissues have also been detected

1145 in terrestrial invertebrates and vertebrates, including wildlife species other than granivores (which can be exposed  
1146 directly via ingestion of treated seeds) as detailed in previous sections (e.g., chiropterans).

1147 Some studies highlighted the potential for neonicotinoids to negatively impact terrestrial insectivorous  
1148 vertebrate abundance and diversity through indirect effects related to the reduction in quantity and quality of food  
1149 resources. Such indirect effects have rarely been studied on vertebrates but Gibbons et al. (2015) showed that  
1150 systemic insecticides can induce effects on wildlife via trophic cascades: the reduction in food supply related to  
1151 the use of imidacloprid led to impairments in fish species.

1152 Long before major publications based on large-scale correlative analyses between PPP use and  
1153 population, Tennekes and Zillweger (2010) argued that neonicotinoid contamination of surface waters in Europe  
1154 was one of the factors responsible for the continental-scale decline in insect biomass, which in turn led to many of  
1155 the widespread declines in birds (golden oriole *Oriolus oriolus*, northern wheatear *Oenanthe oenanthe*, starling  
1156 *Sturnus vulgaris*...). This was studied by Hallmann et al. (2014) who observed that insectivorous bird populations  
1157 in the Netherlands declined in areas with surface water concentrations of imidacloprid higher than 0.02 µg/L. Spatial  
1158 differences in land-use changes related to agricultural intensification (urban area, natural area, cropped area,  
1159 fertilizers) have been considered but they did not alter the significance of the observed effects. In the USA, Li et  
1160 al. (2020) found that the increase in neonicotinoid use was related to reductions of 4% and 3% in grassland and  
1161 insectivorous bird biodiversity, respectively, over 2008-2014. Such a trend was also found for non-grassland and  
1162 non-insectivorous birds, with an average annual rate of reduction of 2%. Recently, Kraus et al. (2021) conducted  
1163 surveys in wetlands of cropland and grassland landscapes which allowed to characterize cross-ecosystem fluxes  
1164 of PPPs mediated by aquatic insect emergence, and discussed their implications for terrestrial insectivores. Aquatic  
1165 insects were estimated to transfer fluxes ranging from 2 to 180 µg of total insecticides per wetland per day to the  
1166 terrestrial ecosystem. Seven PPPs were detected in newly emerged insects, among which clothianidin and  
1167 imidacloprid, and biomass of emerging aquatic insects was reduced up to 73% in cropland wetlands. The authors  
1168 suggested that the availability of emerging adult aquatic insect prey for insectivores was reduced by insecticides,  
1169 and that accumulated insecticide could be responsible for insectivore exposure to insect-borne PPPs. Along the  
1170 observed gradient in PPP levels among the different wetlands, a decrease of 43% in insect emergence but an  
1171 increase of 50% in insect-mediated PPP flux with increasing insecticide concentrations were reported (from 3 to  
1172 577 ng of insecticide per gram of insect) (Kraus et al. 2021). In addition, the presence of these neonicotinoids also  
1173 led to a reduction in insect resources for consumer invertebrates (Kraus et al. 2021). Although bioaccumulation in  
1174 organisms and transfer in food webs have been demonstrated together with sublethal and lethal effects propagated

1175 along food chains, the major process involved in shaping the impact of neonicotinoids in food webs is considered  
1176 as being food web simplification (Tooker and Pearsons 2021). Such indirect effect of neonicotinoids affects both  
1177 prey and predator populations through trophic cascade mechanisms and feedbacks. The initial decrease in  
1178 resources when lower trophic levels are directly impacted by the use of the insecticides affect the dynamics of  
1179 consumer populations at higher trophic levels through food scarcity (bottom-up control). When consumers are  
1180 adversely impacted either directly (toxicity) or indirectly (lack of food supply), a subsequent decrease in predation  
1181 occurs, affecting the dynamics of prey populations (top-down control). Compensatory mechanisms for consumers  
1182 to overcome the decrease of one or a few food resources, such as switching to other food items, hardly occur when  
1183 the predator of concern are specialist species, and seemed currently hampered in the case of neonicotinoids because  
1184 of their widespread use (huge spatial extent worldwide, perennial and frequent use), the ubiquity of their  
1185 environmental contamination, their broad toxicity to non-target fauna, and time-cumulative toxicity (Tooker and  
1186 Pearsons 2021).

1187

## 1188 **Aquatic ecosystems**

1189 Adverse effects of neonicotinoids can propagate through aquatic food webs via contaminated primary producers  
1190 (Lima-Fernandes et al. 2019). Lima-Fernandes et al. (2019) used imidacloprid-contaminated and uncontaminated  
1191 black alder tree (*Alnus glutinosa*) leaves to feed the stonefly shredder *Protonemura* sp., which were later given as  
1192 prey to *Isoperla* sp. They showed that survival, body length and biomass of the shredders as well as leaf  
1193 decomposition were 20% to 50% greater in the uncontaminated treatment in comparison to imidacloprid exposure.  
1194 The biomass and length of predators were 11% and 4.3% higher, respectively, when fed with uncontaminated prey  
1195 than when fed with imidacloprid exposed prey (Lima-Fernandes et al. 2019). Bioaccumulation of imidacloprid has  
1196 been evidenced in both *Desmognathus* salamanders (*D. monticola* and *D. fuscus*) and benthic macroinvertebrates  
1197 sampled from water streams adjacent to treated hemlock stands in the USA (Crayton et al. 2020), which represents  
1198 a potential source of exposure for consumers at higher trophic levels. If exposure via the trophic route was likely  
1199 for salamanders, several non-exclusive routes of exposure might be involved in the subsequent bioaccumulation,  
1200 including dermal and dietary uptake (Crayton et al. 2020).

1201 Hayasaka et al. (2012) showed that successive applications of imidacloprid and the phenylpyrazole  
1202 insecticide fipronil (also a systemic insecticide) in experimental rice fields resulted in reduced growth of medaka  
1203 fish, *Oryzias latipes*, adults and fry, most likely through reduced medaka prey abundance. Indeed, the  
1204 concentrations (approximately 1 to 50 µg/L) were too low to have a direct effect on fish. As indicated above, the

1205 decline of emerging insects from aquatic ecosystems towards riparian and surrounded terrestrial landscapes  
1206 strongly decrease the prey availability for numerous consumers, and overall minor energy transfer across  
1207 ecosystems (Kraus et al. 2021).

1208 In a Japanese lacustrine ecosystem, Yamamuro et al. (2019) demonstrated the existing relationship  
1209 between decline in fishery yields and neonicotinoids. The use of neonicotinoids on watersheds since 1993  
1210 coincided with an 83% decrease in average zooplankton biomass in spring, causing the smelt (*H. nipponensis*)  
1211 harvest to collapse from 240 to 22 tons. Young smelts consume zooplankton crustaceans, and their decreased  
1212 abundance was linked to the reduction of zooplankton biomass caused by the introduction of neonicotinoids. This  
1213 study demonstrates the indirect effects of neonicotinoids along an aquatic food web through cascading effects.

1214 Waterbirds living and feeding in lakes and ponds (ducks, waders, cormorants...) may depend on aquatic  
1215 invertebrates as their food source. Consequently, the depletion of this food source must necessarily affect them  
1216 (Sánchez-Bayo et al. 2016). Duckling abundance is thus related to aquatic macroinvertebrate abundance, which is  
1217 consistent with other studies, and collectively suggests that neonicotinoids contamination could influence duckling  
1218 abundance indirectly by impacting aquatic macroinvertebrate communities (Tyler 2022). The available data  
1219 indicate that the effects of neonicotinoids on aquatic bird life are indirect, as for other bird families, and are  
1220 associated with the direct toxic impacts of these contaminants on invertebrates (Sánchez-Bayo et al. 2016).

1221

## 1222 **Conclusion**

1223 Neonicotinoids, in particular imidacloprid, and to a lesser extent thiamethoxam and clothianidin, are very  
1224 frequently detected in soils and freshwaters, even several years after their use. In addition, the presence of  
1225 acetamiprid, imidacloprid, thiacloprid and thiamethoxam was observed in the air. Neonicotinoids have only been  
1226 recently monitored in coastal and marine environments (since 2010s), but many studies report the presence of  
1227 imidacloprid and thiamethoxam in different transitional ecosystems such as Mediterranean lagoons.

1228 This contamination of the environment leads to the exposure of non-target organisms and impacts  
1229 biodiversity. The ecotoxicological effects of neonicotinoids depend on the studied organisms, but this review  
1230 showed that these substances have particularly high direct and indirect impacts on terrestrial invertebrates and  
1231 vertebrates, and on aquatic invertebrates. The impacts on aquatic vertebrates are less documented.

1232 The effects of neonicotinoids on terrestrial heterotrophic microorganisms vary according to the  
1233 conditions: in field studies, these substances have little or no effect, while in the laboratory, impacts on the structure  
1234 and on different microbial activities were observed (however, the tested concentrations are sometimes unrealistic).

1235 Laboratory studies are not always environmentally relevant, but they are complementary to field approaches as  
1236 they can help to understand the effects at lower levels of biological organization (sub-individual, individual) that  
1237 have consequences on higher levels (populations, community) observed in the field. Although contradictory results  
1238 have been noted in the literature, neonicotinoids have negative effects (mortality, mobility disturbance) at the  
1239 individual level on pollinators (honeybees in particular). In addition, exposure to neonicotinoids increases the  
1240 susceptibility of honeybees to diseases and pests. Despite the importance of wild pollinators and their crucial role  
1241 in pollination, the number of studies focused on the impacts of neonicotinoids on this highly diverse group of  
1242 organisms is very limited. Furthermore, neonicotinoids have been shown to have effects on other terrestrial  
1243 invertebrates such as natural enemies, earthworms or nematodes. Neonicotinoids are also largely involved in the  
1244 decline of birds. Consumption of treated seeds is mainly responsible for neonicotinoid direct poisoning, but birds  
1245 could be exposed to these insecticides especially by trophic route after consumption of contaminated insects.  
1246 Neonicotinoids have negative effects on bats, amphibians, and on reptiles (though available data are still scarce  
1247 for this group). For aquatic invertebrates and vertebrates, the data on the effects of neonicotinoids remain limited.  
1248 The available results indicate correlations between neonicotinoid concentrations and declines in arthropod taxa.  
1249 Neonicotinoids seem to be not very toxic to aquatic vertebrates such as fish, but recent studies provide worrying  
1250 results for amphibians. However, the number of studies remains low and few studies focused on marine organisms.  
1251 In addition to their toxicity to directly exposed organisms, neonicotinoid-induced indirect effects via trophic  
1252 cascades have been demonstrated to affect some species (terrestrial and aquatic invertebrates) but data are still too  
1253 few to get a clear picture.

1254           This critical review highlighted numerous knowledge gaps. First, there was a lack of data regarding the  
1255 effects of neonicotinoids on primary producers (although the mode of action of neonicotinoids is unlikely to result  
1256 in effects; Anderson et al. 2015), aquatic heterotrophic microorganisms, wild pollinators, raptors, mammals,  
1257 reptiles, amphibians, aquatic vertebrates, and on organisms in the marine environment in general. In addition: (1)  
1258 the majority of studies focused on only one neonicotinoid making generalization difficult; (2) while imidacloprid  
1259 is the most commonly studied neonicotinoid, data are limited for the other substances; (3) most laboratory studies  
1260 do not reflect realistic and representative uses under in field application conditions; (4) very few studies consider  
1261 transformation products and mixtures with other PPPs; (5) the number of studies considering the impact of  
1262 neonicotinoids on high levels of biological organization (i.e., beyond individual and population) is low; (6) the  
1263 effects of neonicotinoids on maintenance of pest regulation and soil functions are hardly reported; (7) there is a  
1264 lack of time series to survey mid- or long-term effects as well as post-exposure effects; (8) there is a lack of data

1265 regarding the effects of neonicotinoids on ecosystem functioning and services, yet the few existing studies suggest  
1266 that they might significantly alter important provision and regulation ecosystem services (Pesce et al. 2023). More  
1267 research remains to be done to better characterize the impacts of neonicotinoids to protect biodiversity.

1268

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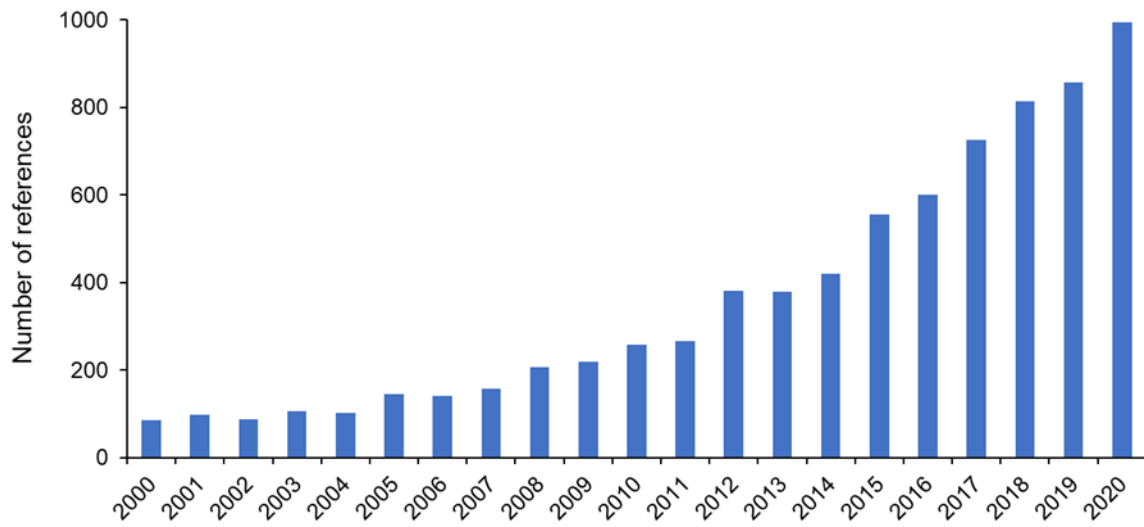
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2221 **Figures**

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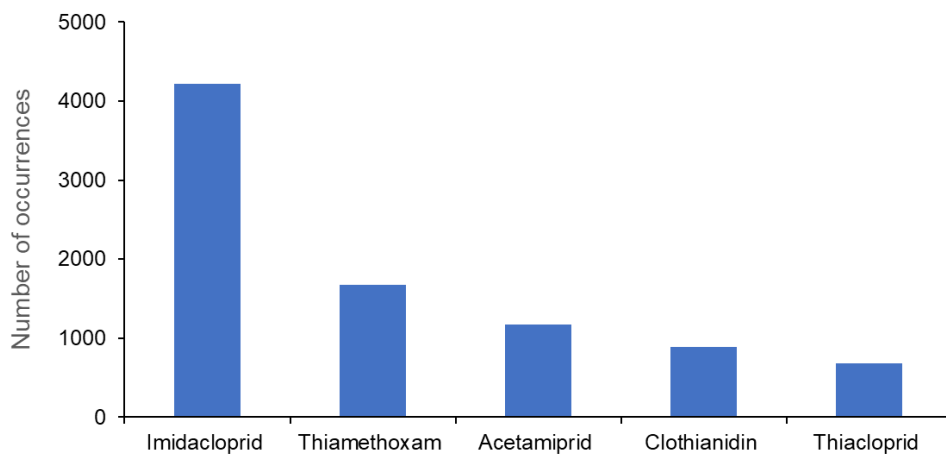


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2224 **Fig. 1** Time course of references focused on the impacts of neonicotinoids on biodiversity.

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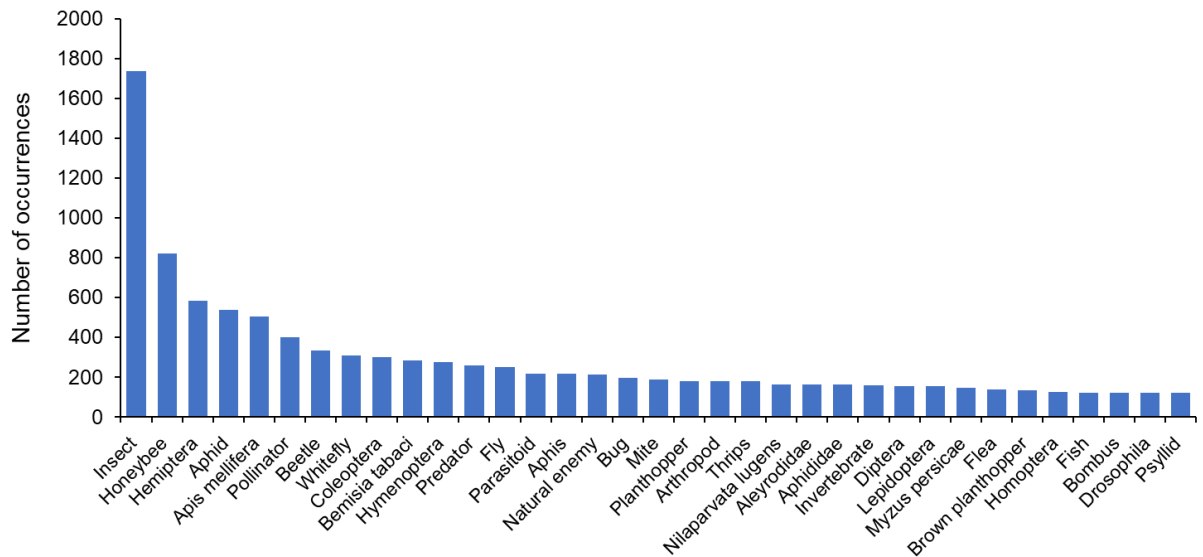
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2229 **Fig. 2** Occurrences of imidacloprid, thiamethoxam, acetamiprid, clothianidin and thiacloprid in title and abstract

2230 of the references constituting the bibliographic corpus on the impacts of neonicotinoids on biodiversity, from 2000

2231 to 2020.

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2234 **Fig. 3** Occurrences of the first 35 organisms studied in the bibliographic corpus on the impacts of neonicotinoids  
 2235 on biodiversity, from 2000 to 2020. Occurrences are counted from titles and abstracts. When occurring, alternative  
 2236 spellings were gathered into one category, for example “honeybee”, “honey bee”, “honeybees” and “honey bees”.

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2252 **Table**

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2254 **Table 1** Maximum concentration levels of acetamiprid, clothianidin, imidacloprid, thiacloprid and thiamethoxam in soil, air and water observed in France,

2255 Europe and in the world. nd: not determined, \*particulate phase.

Neonicotinoid	Geographic zone	Soil		Air		Water	
		Concentration (µg/kg)	Reference	Concentration (ng/m <sup>3</sup> )	Reference	Concentration (µg/L)	Reference
Acetamiprid	France	0.48	Froger et al. (2023)	0.26	Phytatmo database (2023)	nd	nd
	Europe	nd	nd	0.031 (Spain)	Coscollà and Yusà (2016)	4.00 (Spain, freshwater)	de Araújo et al. (2022)
	World	nd	nd	0.036* (Canada)	Raina-Fulton (2015)	2.86 (Turkey, freshwater)	de Araújo et al. (2022)
Clothianidin	France	2.7	Froger et al. (2023)	nd	nd	nd	nd
	Europe	57 (Switzerland)	Riedo et al. (2021)	nd	nd	nd	nd
	World	nd	nd	0.09* (Canada)	Raina-Fulton (2015)	3.50 (USA, drained wetlands)	Evelsizer and Skopec (2018)
Imidacloprid	France	160	Pelosi et al. (2021)	2.3	Phytatmo database (2023)	0.132 (USA, freshwater)	de Araújo et al. (2022)
	Europe	138 (Switzerland)	Chiaia-Hernandez et al. (2017)	0.014 (Spain)	Coscollà and Yusà (2016)	2.22 (peri-urban ponds)	Nélieu et al. (2021)
	World	nd	nd	0.36* (Canada)	Raina-Fulton (2015)	0.905 (agricultural/urban rivers)	Criquet et al. (2017)
Thiacloprid	France	1.4	Pelosi et al. (2021)	0.47	Phytatmo database (2023)	0.14 (marine waters)	Auby et al. (2011)
	Europe	14 (Switzerland)	Riedo et al. (2021)	nd	nd	0.342 (Spain, freshwater)	de Araújo et al. 2022
	World	nd	nd	nd	nd	9.14 (USA, freshwater)	Wang et al. (2023)
Thiamethoxam	France	2.0	Pelosi et al. (2021)	0.06	Phytatmo database (2023)	nd	nd
	Europe	24 (Switzerland)	Riedo et al. (2021)	nd	nd	0.159 (Portugal, freshwater)	de Araújo et al. (2022)
	World	nd	nd	nd	nd	1.37 (Australia, lagoon)	Wang et al. (2023)
						0.0039 (bay)	Tapie and Budzinski (2018)
						0.215 (Portugal, freshwater)	de Araújo et al. (2022)
						6.90 (USA, drained wetlands)	Evelsizer and Skopec (2018)
						3.82 (Canada, freshwater)	Wang et al. (2023)

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