Brussels, Paris, 20 May 2025

Ms. Sandra Gallina, Director General, DG Health and Food Safety
European Commission
1049 Brussels
Belgium

Subject: Suspension of the EU-approval of acetamiprid, following recent scientific publications on developmental neurotoxicity of acetamiprid (art.69 from reg.(EC)1107/2009 and art.15 from reg.(UE) 528/2012)

Dear Mrs. Gallina,

In June 2023, PAN Europe sent a letter to DG Sante, asking to take action to better protect our children and the unborn, against the neurotoxicity of neonicotinoid insecticides, in the light of new scientific evidence. The letter has contributed to a mandate sent to EFSA, to take into account scientific publications contained in the letter. In May 2024, the EFSA published a Statement on the toxicological properties of acetamiprid[1], which led to a reduction in a series of MRLs for acetamiprid. We commend the fact that your services moved forward with the reduction of MRLs despite the opposition of a series of Member States.

PAN Europe, together with Générations Futures, welcomes this improvement in the protection of citizens' health, as well as the inevitable reduction in the use of acetamiprid, which will better protect the environment, and in particular pollinators.

PAN Europe and Générations Futures nevertheless regret that the EFSA statement regarding major findings on developmental neurotoxicity (DNT) did not lead to a ban on the use of acetamiprid in the EU. The recent regulatory decision contributed to a better protection of consumers but the Commission decided to keep this substance on the European market, ignoring the precautionary principle and thereby posing a risk to children's health.

Additional new scientific evidence tends to confirm the data assessed by EFSA, that acetamiprid harms the brain development of children exposed *in utero*, and poses an unacceptable risk for bees.

1. **Major inconsistencies between the implementation of the pesticide (reg.(UE)1107/2009) and the biocide (reg.(UE) 528/2012) regulations**

Acetamiprid was authorised under the pesticide regulation (UE) 1107/2009 in 2018 until 2033. We emphasize that this 15-year authorisation should never have occurred, as

[1] EFSA (European Food Safety Authority), (2024). Statement on the toxicological properties and maximum residue levels of acetamiprid and its metabolites. *EFSA Journal*, 22(5), e8759. https://doi.org/10.2903/j.efsa.2024.8759

acetamiprid must be considered a candidate for substitution under this piece of legislation, due to its persistence and toxicity.
Under the biocide regulation, acetamiprid is indeed considered as a very persistent (vP) substance in water and thus as a candidate for substitution. Its authorisation was therefore granted for only 7 years (from 2020 to 2027)[2].

The differences in the persistence assessment between the pesticide and biocide regulations is explained in the biocide dossier[3]: two countries (Germany and Belgium) noted that the model used in the pesticide dossier was not suitable and did not align with the recommendations of the PBT guidance document updated in 2017. The German and Belgian approach was subsequently validated by ECHA. This change in model leads to a significantly different result, with the half-life increasing from 27 days to 79.7 days!

We ask you to review the conclusions on persistence in the pesticide dossier and to consider acetamiprid as a very persistent substance in the aquatic environment and ultimately as a candidate for substitution. We also request that you consider that the 7-year authorisation period as a pesticide has already been exceeded.

2. Acetamiprid approval needs to be immediately withdrawn for pesticide uses

a. Maladministration

Instead of moving forward with a withdrawal of the approval of acetamiprid, the minutes of the December 2024 Scopaff meeting (phytopharmaceuticals, legislation) indicate that the Commission had sent a letter '*to the approval holder with a request to provide a testing plan for those properties. That plan, together with the associated deadlines for conducting the studies, will be evaluated by EFSA and the rapporteur Member State. The final list of studies that should be provided will be sent to the approval holder.*' It is puzzling to observe that in the case of scientific evidence of developmental neurotoxicity , the Commission gives the possibility to the applicant to decide itself which study it would run, instead of demanding specific data to be produced under a specific protocol. On the one hand, it is not surprising that the Commission has no clear idea on what studies to ask, as they have repeatedly refused to define mandatory data requirements to applicants in order to identify pesticides that lead to developmental neurotoxicity. On the other hand, EFSA has recommended since 2013 to conduct an *in vivo* test according to OECD 426[4]. However, this test was, to our knowledge, never performed nor required for acetamiprid.

[2] Commission implementing regulation (EU) 2018/1129 of 13 August 2018 approving acetamiprid as an existing active substance for use in biocidal products of product-type 18
https://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=CELEX:32018R1129
[3] Competent Authority Report - Acetamiprid PT18 - August 2018.
https://echa.europa.eu/documents/10162/fd927384-b921-8d32-5313-1f7e897a2d13
[4] EFSA PPR Panel (EFSA Panel on Plant Protection Products and their Residues), 2013. ScientificOpinion on the developmental neurotoxicity potential of acetamiprid and imidacloprid. EFSA Journal 2013;11(12):3471,47 pp. doi:10.2903/j.efsa.2013.3471 "Considering the available DNT studies for imidacloprid and acetamiprid, important uncertainties still remain and further in vivo studies following OECD test guideline (TG) 426 are required to robustly characterise a DNT potential and dose-response relationships, particularly for acetamiprid"

It is thus unacceptable that the Commission is allowing the pesticide industry to define the rules and the planning, as it has been the case in too many instances in the past[5].
We respectfully ask you to ensure that this maladministration is corrected immediately and that your services withdraw the letter sent to the applicant. We kindly ask you to ensure that DG Sante plays its role and imposes to the industry to run specific tests with clearly defined protocols, instead of asking the applicant what it wants to do.

b. Toxicity for human health - 16 new scientific studies pointing at human toxicity

The Commission approach to allow the applicant to decide by itself a plan and deadlines for the production of new scientific studies on acetamiprid, followed by a series of exchanges and negotiations with DG Sante, EFSA and Member States will lead to acetamiprid remaining on the market for at least 4-5 years. PAN Europe and Générations Futures consider that this is not in line with the law and the case law, and that DG Sante's approach is putting at risk the health of the unborn and that of young children. Indeed, the pesticide regulation (EC) 1107/2009, as well as rulings from the Court of Justice of the EU impose to the Commission to take action when sufficient scientific evidence points at a potential harm to human health, following exposure to a pesticide.

Since the sending of PAN Europe's above mentioned letter, we have identified no less than 16 new peer reviewed scientific studies showing adverse effects to human health (see in Annex 1 of this letter), in only two years.

Specifically on developmental neurotoxicity, we have identified 3 new in vivo studies, published after the last EFSA statement of 2024. These new studies contribute to a growing body of evidence highlighting a developmental neurotoxicity effect. In total, we have identified one regulatory study and at least 13 academic studies that demonstrate the impact of acetamiprid on brain development (see in Annex 2). Additionally, as mentioned in the EFSA statement of 2024, there is evidence that a metabolite of acetamiprid crosses the blood-brain barrier and is found in cerebrospinal fluid in children. None of these academic studies were taken into account by ECHA for the harmonised classification. Taken together and using a weight of evidence approach they should lead to a stricter classification of acetamiprid as reprotoxic of category 1B and thus result in a ban of the substance.

c. Toxicity to bees and birds - 23 new scientific studies pointing at bee toxicity and 1 new study on birds

Moreover, over the last years, an important amount of findings have been published in the scientific literature, with regards to the toxicity of acetamiprid to honey bees and wild bees. Just over the last 2 years, at least 23 publications were made available (see annex 3), most of them showing harm to bees at field-realistic dose exposure.

[5] See PAN Europe report Industry writing its own rules.

A new study confirms that, due to their small size, solitary bees are much more sensitive than the bees studied in regulatory tests[6].

Several studies highlight the risks of sublethal effects (impacts on behaviour[7], microbiome[8], immunity[9], learning, and memory[10]) resulting from exposure to acetamiprid. These effects may affect colony survival, according to some authors.

Two studies also indicate that exposure to acetamiprid makes bees more susceptible to the parasite Varroa destructor[11]. This increased sensitivity to Varroa, which contributes to the decline in bee populations, cannot be detected by regulatory studies.

Multiple studies report on another aspect entirely overlooked by the regulatory assessment and EFSA, which is the impact of acetamiprid exposure in combination with fungicides, particularly on the bee microbiome, making them more vulnerable to diseases[12]. This alteration in microbiome composition can have serious effects on honeybee health, which may manifest only long after exposure. According to the authors, these effects have so far been completely neglected in studies of pesticide side effects.

Finally, one study demonstrates for the first time, according to its authors, the impact of acetamiprid on a species of solitary bee (Osmia bicornis) and the pollination services provided by these bees. These impacts can have very negative consequences, as these bees are “economically important pollinators for many crops”[13].

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[6] Catania R, Bernardes RC, Bonforte M, Ferreira LMN, Lima MAP, Teper D, Zappalà L, Mazzeo G. Susceptibility of solitary bees to agrochemicals highlights gaps in bee risk assessment. Environ Toxicol Pharmacol. 2025 Jan;113:104614. doi: 10.1016/j.etap.2024.104614. Epub 2024 Dec 18. PMID: 39706385.

[7] Shi J, Wang X, Luo Y. Honey bees prefer moderate sublethal concentrations of acetamiprid and experience increased mortality. Pestic Biochem Physiol. 2025 Mar;208:106320. doi: 10.1016/j.pestbp.2025.106320. Epub 2025 Feb 6. Erratum in: Pestic Biochem Physiol. 2025 Apr;209:106360. doi: 10.1016/j.pestbp.2025.106360. PMID: 40015911.

[8] Su Y, Shi J, Hu Y, Liu J, Wu X. Acetamiprid Exposure Disrupts Gut Microbiota in Adult and Larval Worker Honeybees (*Apis mellifera* L.). Insects. 2024 Nov 26;15(12):927. doi: 10.3390/insects15120927. PMID: 39769529; PMCID: PMC11678641.

[9] Erban T, Markovic M, Sopko B. Sublethal acetamiprid exposure induces immunity, suppresses pathways linked to juvenile hormone synthesis in queens and affects cycle-related signaling in emerging bees. Environ Pollut. 2024 May 15;349:123901. doi: 10.1016/j.envpol.2024.123901. Epub 2024 Mar 29. PMID: 38556147.

[10] Abuagla MIB, Iqbal J, Raweh HSA, Alqarni AS. Insight into Olfactory Learning, Memory, and Mortality of *Apis mellifera jemenitica* after Exposure to Acetamiprid Insecticide. Insects. 2024 Jun 25;15(7):473. doi: 10.3390/insects15070473. PMID: 39057206; PMCID: PMC11276894.

[11] -Kang Y, Wu T, Han B, Yang S, Wang X, Wang Q, Gao J, Dai P. Interaction of acetamiprid, Varroa destructor, and Nosema ceranae in honey bees. J Hazard Mater. 2024 Jun 5;471:134380. doi: 10.1016/j.jhazmat.2024.134380. Epub 2024 Apr 22. PMID: 38657514.
-Kang Y, Guo J, Wu T, Han B, Liu F, Chu Y, Wang Q, Gao J, Dai P. Insecticide and pathogens co-exposure induces histomorphology changes in midgut and energy metabolism disorders on Apis mellifera. Pestic Biochem Physiol. 2025 Jun;211:106414. doi: 10.1016/j.pestbp.2025.106414. Epub 2025 Apr 18. PMID: 40350227.

[12] Han W, Ye Z, Gu Y, Zhong Y, Gao J, Zhao S, Wang S. Gut microbiota composition and gene expression changes induced in the *Apis cerana* exposed to acetamiprid and difenoconazole at environmentally realistic concentrations alone or combined. Front Physiol. 2023 May 3;14:1174236. doi: 10.3389/fphys.2023.1174236. PMID: 37256066; PMCID: PMC10226273
Reiß F, Schuhmann A, Sohl L, Thamm M, Scheiner R, Noll M. Fungicides and insecticides can alter the microbial community on the cuticle of honey bees. Front Microbiol. 2023 Oct 30;14:1271498. doi: 10.3389/fmicb.2023.1271498. PMID: 37965543; PMCID: PMC10642971.

[13] O'Reilly AD, Stanley DA. Solitary bee behaviour and pollination service delivery is differentially impacted by neonicotinoid and pyrethroid insecticides. Sci Total Environ. 2023 Oct 10;894:164399. doi: 10.1016/j.scitotenv.2023.164399. Epub 2023 May 26. PMID: 37245806.

3. Acetamiprid approval needs to be immediately withdrawn for biocidal uses

In addition to what is explained in point 2., another reason supports the ban of acetamiprid for its biocidal uses:

In both pesticide and biocide assessment, the same toxicity threshold (Acceptable Operator Exposure Level, AOEL) is used to assess the risk for human health. In the Competent Authority Report (CAR) of acetamiprid, ECHA used for human health assessment, an AEL of 0.025 mg/kg b.w/d as set by EFSA in its peer review of 2016[14].

It appears that when the new toxicity threshold set by EFSA in 2024 (AOEL of 0.005 mg/kg b.w/d) is applied, the risk for professional users is unacceptable, even when all recommended protective equipment (gloves, protective clothing, and masks) is used[15]. Additionally, the acute risk to children following a secondary exposure as a result of non-professional uses is no longer acceptable[16]. In other words, all uses of the reference product assessed in the dossier for European biocide authorisation lead to an unacceptable risk, which should immediately result in its ban in Europe of acetamiprid for its biocidal uses.

In conclusion, Générations Futures and PAN Europe consider that the available scientific evidence should lead to an immediate ban on acetamiprid in pesticides and biocides. We consider that the handling of the dossier by DG Sante is not in line with EU law. We therefore respectfully ask you to suspend the approval of this substance, in accordance with the pesticide and biocide regulations.

Kind regards,

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[14] EFSA (European Food Safety Authority), 2016. Conclusion on the peer review of the pesticide risk assessment of the active substance acetamiprid. *EFSA Journal* 2016; 14(11):4610, 26 pp. doi:10.2903/j.efsa.2016.4610
[15] Competent Authority Report - Acetamiprid PT18 - August 2018. pp 305/422
[16] Ibid, pp 309/422

Annex 1: New scientific evidence on negative effects of acetamiprid on human health

a. Developmental toxicity, including DNT

- Lee CLM, Brabander CJ, Nomura Y, Kanda Y, Yoshida S. Embryonic exposure to acetamiprid insecticide induces CD68-positive microglia and Purkinje cell arrangement abnormalities in the cerebellum of neonatal rats. Toxicol Appl Pharmacol. 2025 Feb;495:117215. doi: 10.1016/j.taap.2024.117215. Epub 2024 Dec 22. PMID: 39719252.

In this study, prenatal exposure to acetamiprid causes irregular neuronal arrangement, followed by Purkinje cell loss two weeks after birth in the developing cerebellum. According to the authors, these results suggest that prenatal acetamiprid exposure may have significant impacts on cerebellar development and function.

- Saito H, Furukawa Y, Sasaki T, Kitajima S, Kanno J, Tanemura K. Behavioral effects of adult male mice induced by low-level acetamiprid, imidacloprid, and nicotine exposure in early-life. Front Neurosci. 2023 Aug 16;17:1239808. doi: 10.3389/fnins.2023.1239808. PMID: 37662107; PMCID: PMC10469492.

In this study, significant effects have been observed for exposure to Acceptable Daily Intake of imidacloprid, acetamiprid and nicotine. Only 8 individuals were tested per condition. Significant differences in behaviour were observed between imidacloprid- and nicotine-treated groups. In the case of acetamiprid, comparable deviations were observed, but to a lesser extent. The low number of individuals did not allow to establish statistically significant differences but considering the fact that all neonicotinoids present the same mode of action, the same physicochemical properties, the similar trend observed in nearly all behavioural tests should be considered by risk assessors.

- Longoni V, Kandel Gambarte PC, Rueda L, Fuchs JS, Rovedatti MG, Wolansky MJ. Long-lasting developmental effects in rat offspring after maternal exposure to acetamiprid in the drinking water during gestation. Toxicol Sci. 2024 Feb 28;198(1):61-75. doi: 10.1093/toxsci/kfad122. PMID: 38011675

Results of this study showed no consistent findings indicating maternal, reproductive or developmental toxicity. However, the authors found acetamiprid effects on neurobehavioral responses, suggestive of a mild toxic action. According to the authors, this study showed a trend for developmental susceptibility at a dose so far considered subtoxic.

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b. Neurotoxicity

- Shamsi, M., Soodi, M., Shahbazi, S. *et al*. Effect of Acetamiprid on spatial memory and hippocampal glutamatergic system. *Environ Sci Pollut Res* **28**, 27933–27941 (2021). https://doi.org/10.1007/s11356-020-12314-6

- Hirai A, Toda C, Yohannes YB, Collins N, Tamba M, Nomiyama K, Eguchi A, Hoshi N, Hirano T, Nakayama SMM, Ishizuka M, Ikenaka Y. Role of brain monoamines in acetamiprid-induced anxiety-like behavior. Toxicology. 2024 Jun;505:153839. doi: 10.1016/j.tox.2024.153839. Epub 2024 May 21. PMID: 38782113.

- Phogat A, Singh J, Malik V, Kumar V. Neuroprotective potential of berberine against acetamiprid induced toxicity in rats: Implication of oxidative stress, mitochondrial alterations, and structural changes in brain regions. J Biochem Mol Toxicol. 2023 Oct;37(10):e23434. doi: 10.1002/jbt.23434. Epub 2023 Jun 23. PMID: 37350525.

c. Other toxicity endpoints

- Pan D, Lin M, Mu C, Yu C, Ye B, Liang J, Sheng Y, Huang D, Liu S, Zeng X, Jennifer Tan HJ, Chongsuvivatwong V, Qiu X. Maternal exposure to neonicotinoid insecticides and fetal growth restriction: A nested case-control study in the guangxi Zhuang birth cohort. Chemosphere. 2023 Sep;336:139217. doi: 10.1016/j.chemosphere.2023.139217. Epub 2023 Jun 17. PMID: 37336441

- Phogat A, Singh J, Sheoran R, Hasanpuri A, Chaudhary A, Bhardwaj S, Antil S, Kumar V, Prakash C, Malik V. Berberine Attenuates Acetamiprid Exposure-Induced Mitochondrial Dysfunction and Apoptosis in Rats via Regulating the Antioxidant Defense System. J Xenobiot. 2024 Aug 7;14(3):1079-1092. doi: 10.3390/jox14030061. PMID: 39189176; PMCID: PMC11348026.

- Abdelrahman RE, Hassan MS, Morgan AM, Ibrahim MA, Hassanen EI. Acetamiprid induces cardiotoxicity in rats by dysregulating α7 nAChR and its downstream targets: The ameliorative role of resveratrol. Food Chem Toxicol. 2024 Sep;191:114892. doi: 10.1016/j.fct.2024.114892. Epub 2024 Jul 25. PMID: 39067744.

- Abdelrahman RE, Hassan MS, Ibrahim MA, Morgan AM. Mechanistic insights into acetamiprid-induced genotoxicity on the myocardium and potential ameliorative role of resveratrol. Environ Toxicol Pharmacol. 2024 Sep;110:104526. doi: 10.1016/j.etap.2024.104526. Epub 2024 Aug 5. PMID: 39111560.

- Benchikh I, Ziani K, Benalia A, Djebbar AA, Argoub H, Khaled MB. Thirty-day oral exposure to acetamiprid induces biochemical and histological alterations in rat pancreas: protective effects of carnosine supplementation. Toxicol Mech Methods. 2024 Dec 3:1-11. doi: 10.1080/15376516.2024.2435350. Epub ahead of print. PMID: 39627014.

- Su Q, Luo J, Zhou Y, Liu M, Zeng S, Li Y, Gao J. Sex steroid hormones mediate the association between neonicotinoids and obesity among children and adolescents. Ecotoxicol Environ Saf. 2025 Jan 1;289:117708. doi: 10.1016/j.ecoenv.2025.117708. Epub 2025 Jan 10. PMID: 39793286.

- Guo LC, Zhu P, Gui C, Deng J, Gao Y, Long C, Zhang H, Lv Z, Yu S. Disrupting effects of neonicotinoids and their interaction with metals on thyroid hormone, an evidence of children in a rural area, South China. Ecotoxicol Environ Saf. 2025 Jan 15;290:117788. doi: 10.1016/j.ecoenv.2025.117788. Epub 2025 Jan 23. PMID: 39854865.

- Arafa SS, Elnoury HA, Badr El-Din S, Sakr MA, Hendawi FF, Masoud RAE, Barghash SS, Elbehairy DS, Hemeda AA, Farrag IM, Abdelrahman DS, Elsadek AM, Ghanem SK, AboShabaan HS, Atwa AM, Nour El Din M, Radwan AF, Al-Zahrani M, Alhomodi AF, Abdulfattah AM, Abdelkader A. Acetamiprid-induced pulmonary toxicity via oxidative stress, epithelial-mesenchymal transition, apoptosis, and extracellular matrix accumulation in human lung epithelial cells and fibroblasts: Protective role of heat-killed Lactobacilli. Food Chem Toxicol. 2025 Apr;198:115322. doi: 10.1016/j.fct.2025.115322. Epub 2025 Feb 15. PMID: 39961414.

- Hassanzadeh R, Joursaraei GA, Hejazian LB, Feazi F, Najafzadehvarzi H. Evaluation of the protective effect of melatonin on oocyte, embryo and ovarian tissue parameters in female mice exposed to acetamiprid. JBRA Assist Reprod. 2023 Sep 12;27(3):407-413. doi: 10.5935/1518-0557.20220068. PMID: 37257062; PMCID: PMC10712808.

- Wang R, Yang X, Wang T, Kou R, Liu P, Huang Y, Chen C. Synergistic effects on oxidative stress, apoptosis and necrosis resulting from combined toxicity of three commonly used pesticides on HepG2 cells. Ecotoxicol Environ Saf. 2023 Sep 15;263:115237. doi: 10.1016/j.ecoenv.2023.115237. Epub 2023 Jul 12. PMID: 37451096.

Annex 2 – synthesis of available data (old and new data) on DNT effects (non-exhaustive list)

4 In vitro studies

Kimura-Kuroda J, Komuta Y, Kuroda Y, Hayashi M, Kawano H (2012) Nicotine-Like Effects of the Neonicotinoid Insecticides Acetamiprid and Imidacloprid on Cerebellar Neurons from Neonatal Rats. PLOS ONE 7(2): e32432. https://doi.org/10.1371/journal.pone.0032432

Loser, D., Hinojosa, M.G., Blum, J. *et al.* Functional alterations by a subgroup of neonicotinoid pesticides in human dopaminergic neurons. *Arch Toxicol* **95**, 2081–2107 (2021). https://doi.org/10.1007/s00204-021-03031-1

Christen V, Rusconi M, Crettaz P, Fent K. Developmental neurotoxicity of different pesticides in PC-12 cells in vitro. Toxicol Appl Pharmacol. 2017 Jun 15;325:25-36. doi: 10.1016/j.taap.2017.03.027. Epub 2017 Apr 3. PMID: 28385489.

Lee, J., Escher, B.I., Scholz, S. *et al.* Inhibition of neurite outgrowth and enhanced effects compared to baseline toxicity in SH-SY5Y cells. *Arch Toxicol* 96, 1039–1053 (2022). https://doi.org/10.1007/s00204-022-03237-x

3 studies with the zebrafish model

Hussain, A.; Audira, G.; Malhotra, N.; Uapipatanakul, B.; Chen, J.-R.; Lai, Y.-H.; Huang, J.-C.; Chen, K.H.-C.; Lai, H.-T.; Hsiao, C.-D. Multiple Screening of Pesticides Toxicity in Zebrafish and Daphnia Based on Locomotor Activity Alterations. *Biomolecules* **2020**, *10*, 1224. https://doi.org/10.3390/biom10091224

Ma X, Li H, Xiong J, Mehler WT, You J. Developmental Toxicity of a Neonicotinoid Insecticide, Acetamiprid to Zebrafish Embryos. J Agric Food Chem. 2019 Mar 6;67(9):2429-2436. doi: 10.1021/acs.jafc.8b05373. Epub 2019 Feb 20. PMID: 30735371.

Von Hellfeld R, Ovcharova V, Bevan S, Lazaridi MA, Bauch C, Walker P, Hougaard Bennekou S, Forsby A, Braunbeck T. Zebrafish embryo neonicotinoid developmental neurotoxicity in the FET test and behavioral assays. ALTEX. 2022;39(3):367–387. doi: 10.14573/altex.2111021. Epub 2022 Feb 23. PMID: 35229877.

7 In vivo studies on mammals

DNT regulatory study according to the guideline OPPTS 870.6300 - An oral developmental neurotoxicity study of acetamiprid in rats (2008)

Kagawa N, Nagao T. Neurodevelopmental toxicity in the mouse neocortex following prenatal exposure to acetamiprid. J Appl Toxicol. 2018 Dec;38(12):1521-1528. doi: 10.1002/jat.3692. Epub 2018 Jul 25. PMID: 30047162.
Sano K, Isobe T, Yang J, Win-Shwe TT, Yoshikane M, Nakayama SF, Kawashima T, Suzuki G, Hashimoto S, Nohara K, Tohyama C, Maekawa F. In utero and Lactational Exposure to Acetamiprid Induces Abnormalities in Socio-Sexual and Anxiety-Related Behaviors of Male Mice. Front Neurosci. 2016 Jun 3;10:228. doi: 10.3389/fnins.2016.00228. PMID: 27375407; PMCID: PMC4891355.

Nakayama A, Yoshida M, Kagawa N, Nagao T. The neonicotinoids acetamiprid and imidacloprid impair neurogenesis and alter the microglial profile in the hippocampal dentate gyrus of mouse neonates. J Appl Toxicol. 2019 Jun;39(6):877-887. doi: 10.1002/jat.3776. Epub 2019 Jan 29. PMID: 30693975.

Lee CLM, Brabander CJ, Nomura Y, Kanda Y, Yoshida S. Embryonic exposure to acetamiprid insecticide induces CD68-positive microglia and Purkinje cell arrangement abnormalities in the cerebellum of neonatal rats. Toxicol Appl Pharmacol. 2025 Feb;495:117215. doi: 10.1016/j.taap.2024.117215. Epub 2024 Dec 22. PMID: 39719252

Saito H, Furukawa Y, Sasaki T, Kitajima S, Kanno J, Tanemura K. Behavioral effects of adult male mice induced by low-level acetamiprid, imidacloprid, and nicotine exposure in early-life. Front Neurosci. 2023 Aug 16;17:1239808. doi: 10.3389/fnins.2023.1239808. PMID: 37662107; PMCID: PMC10469492.

Longoni V, Kandel Gambarte PC, Rueda L, Fuchs JS, Rovedatti MG, Wolansky MJ. Long-lasting developmental effects in rat offspring after maternal exposure to acetamiprid in the drinking water during gestation. Toxicol Sci. 2024 Feb 28;198(1):61-75. doi: 10.1093/toxsci/kfad122. PMID: 38011675

1 study pointing at the presence of acetamiprid metabolite in cerebrospinal fluid in children

Laubscher, B., Diezi, M., Renella, R. *et al*. Multiple neonicotinoids in children's cerebro-spinal fluid, plasma, and urine. *Environ Health* 21, 10 (2022). https://doi.org/10.1186/s12940-021-00821-z

Annex 3: New scientific evidence of acetamiprid's negative impact on bees made available since 2023

- Shi J, Wang X, Luo Y. Honey bees prefer moderate sublethal concentrations of acetamiprid and experience increased mortality. Pestic Biochem Physiol. 2025 Mar;208:106320. doi: 10.1016/j.pestbp.2025.106320. Epub 2025 Feb 6. Erratum in: Pestic Biochem Physiol. 2025 Apr;209:106360. doi: 10.1016/j.pestbp.2025.106360. PMID: 40015911.

- O'Reilly AD, Stanley DA. Solitary bee behaviour and pollination service delivery is differentially impacted by neonicotinoid and pyrethroid insecticides. Sci Total Environ. 2023 Oct 10;894:164399. doi: 10.1016/j.scitotenv.2023.164399. Epub 2023 May 26. PMID: 37245806.

- Lu Y, Gao J, Wu T, Han B, Qian B, Shi M, Yang S, Diao Q, Bu C, Dai P. Exposure of chlorothalonil and acetamiprid reduce the survival and cause multiple internal disturbances in *Apis mellifera* larvae reared *in vitro*. Front Physiol. 2023 Feb 13;14:1114403. doi: 10.3389/fphys.2023.1114403. PMID: 36860521; PMCID: PMC9968791.

- Han W, Ye Z, Gu Y, Zhong Y, Gao J, Zhao S, Wang S. Gut microbiota composition and gene expression changes induced in the *Apis cerana* exposed to acetamiprid and difenoconazole at environmentally realistic concentrations alone or combined. Front Physiol. 2023 May 3;14:1174236. doi: 10.3389/fphys.2023.1174236. PMID: 37256066; PMCID: PMC10226273.

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