Residues of Amitraz in Honey and Beeswax: Ensuring Safety in Beekeeping

Introduction:

Honey bees play a crucial role in global pollination, contributing significantly to agricultural yields and biodiversity. [1] However, the beekeeping industry faces a persistent threat from *Varroa destructor* mites, which can devastate bee colonies.

Amitraz, a widely used acaricide, is effective in controlling varroa mites, and monitoring its residue levels in hive products like honey and beeswax ensures safety for both bees and humans. [2] [3]

Residue Dynamics of Amitraz:

Amitraz breaks down into various metabolites. DMPF and DMF are major initial breakdown products which can be detected in honey and beeswax. [4] The degradation rate of amitraz is influenced by environmental conditions and the formulation used in treatments. [6]

Research shows that amitraz residues in honey are typically low due to its rapid degradation. [5,6] This fast degradation minimizes the risk of long-term accumulation in hive products, making amitraz a safer option for beekeeping compared to other acaricides (Tau-fluvalinate, thymol). [6]

Its shorter half-life minimizes the risk of chronic exposure and negative effects on bee health and hive product safety. This feature is particularly important for maintaining consumer confidence in honey and other hive products.

Amitraz Residue Levels in Honey and Beeswax:

Studies indicate that amitraz residues in honey, when using authorized veterinary treatments, remain below the maximum residue limits (MRLs) set by the authorities, ensuring both honey bee health and consumer safety. [7, 8]

A study investigating amitraz and its metabolites in honey and beeswax revealed that amitraz residues degrade rapidly in hive environments, ensuring minimal long-term presence. Amitraz (Apivar[®]) stands out among acaricides due to its instability in beeswax, where it degrades almost completely within one day, leaving DMF as the primary breakdown product [5](Corta et al., 2001). The study found significantly lower amitraz residues in honey compared to beeswax, underscoring its minimal impact on honey safety.

This is a notable advantage over some pyrethroids like tau-fluvalinate and flumethrin, which are more prone to absorption and retention in beeswax. [9-11] Wallner (1995) reported that flumethrin is 5–10 times more lipophilic than tau-fluvalinate. [12] This lipophilicity difference is evident in acaricides with low affinities for beeswax, such as coumaphos and tau-fluvalinate, which were detectable at only 1 mg/kg concentrations in honey. In contrast, acaricides with

very high affinities for beeswax, like flumethrin, were not detected in honey even at beeswax concentrations of 400 mg/kg (Wallner 1995; Persano et al. 2003). [12,13]

Moreover, environmental factors such as temperature and humidity play a crucial role in the degradation process of amitraz. Higher temperatures and humidity levels accelerate the breakdown of amitraz into less harmful metabolites. [14] The rapid degradation of amitraz significantly reduces the risk of long-term accumulation in hive products, offering a clear advantage over other acaricides that tend to persist longer in the hive environment. This quick breakdown also helps minimize the development of resistance in mite populations, making amitraz a more sustainable choice for long-term pest control. [11]

A study conducted by Pettis et al. (2013) evaluated amitraz residue transfer into honey. The study involved treating hives with different doses of Apivar[®] (Amitraz based product), including up to 10 times the recommended dosage. Results of this protocol (Figure 1) indicated **no detectable amitraz residues** (Table 1) in honey, and the metabolite levels were far below the established MRLs. [15]



Figure 1: One Apivar[®] strip being applied to a honey bee colony in Beltsville, MD USA, recommended dose (1X) is 2 strips per 6–10 frames of bees. Each Apivar[®] strip contains 3.3% amitraz in a slow-release matrix and was left in place according to label requirements for 42 days. Boxes of 1X, 5X and 10X represent approximate placement of 2, 10, or 20 Apivar[®] strips per respective treatment.

Treatment	2,4-dimethylaniline ($\mu g k g^{-1}$) (LOD = 50 $\mu g k g^{-1}$)			N-2,4-dimethylphenyl formamide (µg kg ⁻¹) (LOD = 4 µg kg ⁻¹)			Amitraz (μg kg ⁻¹) (LOD = 4 μg kg ⁻¹)		
	Min	Max	Average	Min	Max	Average	Min	Max	Average
Wax									
Control	N.D.			N.D.			N.D.		
IX		N	I.D.	196	460	336.67 ± 76.7	N.D.		
5X	N.D.	158	67.25 ± 40.00	1,020	2,510	1,520.00 ± 344.75	N.D.		
10X	N.D.	177	59.00 ± 59.00	2,830	6,160	4,763.33 ± 998.04	N.D.		
Honey									
Control	N.D.			N.D.			N.D.		
IX	N.D.			N.D.			N.D.		
5X	N.D.		N.D.	22	9.00 ± 5.48	N.D.			
10X	N.D.		N.D.	60.5	24.73 ± 18.31	N.D.			
Standard (At start)	88.4	120	99.7 ± 10.17	134	137	135.67±0.88		N.D	
Standard (At the end)		N	I.D.	80	87.2	84.23 ± 2.17		N.D	

Table 1: The residue levels of amitraz and its metabolites in honey and beeswax sampleswhen colonies were treated with Apivar[®] strips for 42 days and honey and wax sampled at28 days post-treatment.

Comparison with Other Acaricides:

Pyrethroids, such as flumethrin and tau-fluvalinate, show higher persistence in hive products, posing greater risks to bee health and hive product safety. [3] Organophosphates, like coumaphos, also have higher residue levels in beeswax and honey, leading to increased potential for adverse effects on bees and consumers.

In contrast, amitraz has a superior residue profile (Figure 2). Its rapid degradation and lower residue levels make it a safer choice for managing Varroa mites without compromising hive product safety. [16-18]

The lower residue levels of amitraz in honey and beeswax also reduce sub-lethal effects on bees, such as behavioral changes and compromised immune systems, which are more common with higher residue levels of other acaricides like flumethrin and tau-fluvalinate (Apistan®). [8]



Figure 2: Comparison of residue trends in conventional sampling (primary axis, lines) *vs* APIStrip¹ sampling (secondary axis, histogram) across various matrices (bee bread, honey and bee brood) and treatments (control, Check-mite[®], Amicel[®], Apivar[®] and Apistan[®]). [19]

¹ The APIStrip (Adsorb-Pesticide-Inhive Strips) is a passive sampling technique developed to evaluate the exposure of honey bee colonies to residues of pesticides, acaricides, and other contaminants.

The impact of acaricide residues has demonstrated significant effects on bee health and colony productivity. [21] Sub-lethal exposure to chemicals during critical developmental stages can cause developmental abnormalities and weakened immune systems in bees. [22] However, these effects are less pronounced with amitraz compared to other acaricides like pyrethroids. [23]

Research on acaricides and their residues in Spanish commercial beeswax has revealed chronic exposure to acaricides such as flumethrin and tau-fluvalinate can severely impact bee health. [24, 28] These chemicals persist longer in beeswax, leading to prolonged exposure and increased risk of adverse effects, [29] including reduced brood survival, impaired queen health, and increased bee mortality. [28] The presence of multiple pesticide residues in beeswax can have synergistic effects, potentially exacerbating the adverse impacts on bee health. [23, 26] In contrast, amitraz degrades rapidly, presenting a lower risk of adverse effects. [5] This quick degradation reduces the likelihood of synergistic interactions between multiple pesticide residues in beeswax, thereby minimizing the risk to bee colonies. [24]

Research by Orantes-Bermejo et al. (2010) highlighted that residues from treatments like coumaphos and fluvalinate in beeswax can negatively affect brood survival and bee health (Figure 3). Conversely, amitraz, with its favorable residue profile, offers a safer alternative for long-term Varroa control. [24]

A study on flumethrin residue levels in honey from Chinese apiaries using high-performance liquid chromatography found higher persistence and accumulation of residues compared to amitraz, further emphasizing amitraz's relative safety in terms of residue presence. [20]

Selecting acaricides with favorable residue profiles is crucial to minimizing their impact on bee health and ensuring the sustainability of beekeeping practices. Effective treatment and enhanced bee health can be achieved by opting for acaricides like amitraz, which present lower risks due to their rapid degradation. [24, 29]

Pesticide	Frequency	Maximum concentration	Minimum concentration	Mean concentration ^a				
	(%)	(ng∙g ⁻¹ beeswax)	(ng∙g ^{−1} beeswax)	(ng∙g ⁻¹ beeswax)				
Foundation beeswax (F)								
Coumaphos	100.0	17,370.7	25.0	9486.2 b				
Chlorfenvinphos	100.0	5284.8	433.9	1490.5 b				
Fluvalinate	100.0	3593.3	374.9	1085.3				
Acrinathrin	81.8	2584.9	96.3	414.8				
Flumethrin	81.8	170.1	48.0	90.5 b				
DMF (amitraz)	81.8	118.9	15.9	40.9 a				
Dichlofenthion	63.6	96.2	28.9	38.6				
Chlorpyrifos	54.5	327.2	19.4	69.7				
Malathion	27.3	189.7	67.5	39.8				
Azinphos-methyl	9.1	75.1	75.1	6.8				
Fenthion-sulfoxide	9.1	44.4	44.4	2.0				
Average total pesticide load ($ng \cdot g^{-1}$ beeswax): 12,765.0								

Figure 3: Summary of pesticide detections in foundation (F) beeswax. [25]

Regulatory Standards and Best Practices:

Regulatory bodies in Australia have established stringent maximum residue limits (MRLs) for amitraz in honey to safeguard consumer safety, with these limits being informed by thorough research and ongoing monitoring of amitraz residues in hive products. [30] Notably, Apivar stands as the sole amitraz product already registered in Australia that has successfully met all regulatory standards set forth by local authorities.

The European Commission has set MRLs for amitraz in honey at 0.02mg/kg, emphasizing its fast degradation and reduced persistence in hive products. [31, 32] Similar standards are applied in Australia, ensuring that amitraz residues remain within safe limits.

An Integrated Pest Management (IPM) strategy involving the rotation and diversification of acaricides, combined with mechanical interventions, is pivotal in reducing residues and preventing the development of Varroa resistance [33]. This proactive approach not only ensures efficient Varroa control but also safeguards hive product safety and enhances bee health.

Maximizing the benefits of Amitraz while minimizing residues involves beekeepers adhering to best practices. This entails administering chemical treatments during low honey flow periods, without the supers on, ensuring precise dosages, and consistently monitoring residue levels. [3] By following these practices, beekeepers can effectively utilize Amitraz, uphold hive product safety standards, and promote sustainable beekeeping practices.

Conclusion:

Amitraz stands out as the premier choice for Varroa control due to its rapid degradation and minimal residue levels, setting it apart from other acaricides like pyrethroids and organophosphates. Its exceptional residue profile not only safeguards bees but also guarantees the safety of hive products.

Embracing amitraz for Varroa control isn't just about beekeeping; it's about ensuring the longevity of the industry in Australia. Employing IPM strategies and vigilantly monitoring residue levels are essential steps toward sustainable beekeeping practices. These measures don't just protect bee populations; they instill confidence in consumers regarding hive product safety and support the enduring success of the beekeeping sector.

In summary, amitraz offers a solution to Varroa management, effectively controlling mites while minimizing residual impact. By adhering to regulatory guidelines and embracing best practices, beekeepers can safeguard bee health and the integrity of hive products, thus securing the future of their operations and the well-being of the environment.

References:

- Klein AM, Vaissière BE, Cane JH, Steffan-Dewenter I, Cunningham SA, Kremen C, Tscharntke T. Importance of pollinators in changing landscapes for world crops. Proc Biol Sci. 2007 Feb 7;274(1608):303-13. doi: 10.1098/rspb.2006.3721. PMID: 17164193; PMCID: PMC1702377.
- Rosenkranz P, Aumeier P, Ziegelmann B. Biology and control of Varroa destructor. J Invertebr Pathol. 2010 Jan;103 Suppl 1:S96-119. doi: 10.1016/j.jip.2009.07.016. Epub 2009 Nov 11. PMID: 19909970.
- 3. Bogdanov, S. (2006). Contaminants of bee products. Apidologie, 37(1), 1-18
- 4. Corta E, Bakkali A, Berrueta LA, Gallo B, Vicente F. Kinetics and mechanism of amitraz hydrolysis in aqueous media by HPLC and GC-MS. Talanta. 1999 Jan;48(1):189-99. doi: 10.1016/s0039-9140(98)00237-9. PMID: 18967458.
- Corta E, Bakkali A, Berrueta LA, Gallo B, Vicente F, Kilchenmann V, Bogdanov S. Study of acaricide stability in honey. Characterization of amitraz degradation products in honey and beeswax. J Agric Food Chem. 2001 Dec;49(12):5835-42. doi: 10.1021/jf010787s. PMID: 11743771.
- 6. Lodesani, M., Pellacani A., Bergomi S., Carpana E., Rabitti T., and Lasagni P. 1992. Residue determination for some products used against Varroa infestation in bees. Apidologie. 23: 257–272.
- Shimshoni JA, Sperling R, Massarwa M, Chen Y, Bommuraj V, Borisover M, Barel S. Pesticide distribution and depletion kinetic determination in honey and beeswax: Model for pesticide occurrence and distribution in beehive products. PLoS One. 2019 Feb 20;14(2):e0212631. doi: 10.1371/journal.pone.0212631. PMID: 30785931; PMCID: PMC6382162.
- Rix RR, Christopher Cutler G. Acute Exposure to Worst-Case Concentrations of Amitraz Does Not Affect Honey Bee Learning, Short-Term Memory, or Hemolymph Octopamine Levels. J Econ Entomol. 2017 Feb 1;110(1):127-132. doi: 10.1093/jee/tow250. PMID: 28028168.
- 9. Wallner K, Varroacides and their residues in bee products. Apidologie 30:235–248 (1999). 10.1051/apido:19990212.
- 10. Bonzini S., Tremolada P., Bernardinelli I., Colombo M., Vighi M. Predicting Pesticide Fate in the Hive (Part 1): Experimentally Determined τ-Fluvalinate Residues in Bees, Honey and Wax. Apidologie. 2011;42:378–390. doi: 10.1007/s13592-011-0011-2.
- 11. Dai P, Jack CJ, Mortensen AN, Ellis JD. Acute toxicity of five pesticides to Apis mellifera larvae reared in vitro. Pest Manag Sci. 2017 Nov;73(11):2282-2286. doi: 10.1002/ps.4608. Epub 2017 Jul 24. PMID: 28485079.
- 12. Wallner K. The use of varroacides and their influence on the quality of bee products. Am Bee J. 1995;135:817–821.
- 13. Persano Oddo L, Pulcini P, Morgia C, Marinelli E, Allegrini F, DePace VF, et al. Acaricide residues in wax: a research in Central Italy. XXXIII. Int Congr Apic, Ljubljana, Slovenia. Apimondia, paper number 232 (2003).
- 14. Martel A.C., Zeggane S., Aurières C., Drajnudel P., Faucon J.P., Aubert M.. Acaricide residues in honey and wax after treatment of honey bee colonies with Apivar[®] or Asuntol[®]50. Apidologie. 2007;38:534.

- 15. Chaimanee V., Johnson J., Pettis J.S. Determination of amitraz and its metabolites residue in honey and beeswax after Apivar[®] treatment in honey bee (Apis mellifera) colonies. J. Apicult. Res. 2022;61:213–218. doi: 10.1080/00218839.2021.1918943.
- 16. Calatayud-Vernich P, Calatayud F, Simó E, Suarez-Varela MM, Picó Y. Influence of pesticide use in fruit orchards during blooming on honeybee mortality in 4 experimental apiaries. Science of The Total Environment. 2016;541:33–41. doi: 10.1016/j.scitotenv.2015.08.131
- Mullin CA, Frazier M, Frazier JL, Ashcraft S, Simonds R, Vanengelsdorp D, Pettis JS. High levels of miticides and agrochemicals in North American apiaries: implications for honey bee health. PLoS One. 2010 Mar 19;5(3):e9754. doi: 10.1371/journal.pone.0009754. PMID: 20333298; PMCID: PMC2841636.
- Degrandi-Hoffman G, Chen Y, Watkins Dejong E, Chambers ML, Hidalgo G. Effects of Oral Exposure to Fungicides on Honey Bee Nutrition and Virus Levels. J Econ Entomol. 2015 Dec;108(6):2518-28. doi: 10.1093/jee/tov251. Epub 2015 Aug 28. PMID: 26318004.
- Alba Luna, María Murcia-Morales, María Dolores Hernando, Jozef J.M. Van der Steen, Amadeo R. Fernández-Alba, José Manuel Flores. Comparison of APIStrip passive sampling with conventional sample techniques for the control of acaricide residues in honey bee hives. Science of The Total Environment, 905, 2023, 167205, <u>https://doi.org/10.1016/j.scitotenv.2023.167205</u>
- 20. Yu LS, Liu F, Wu H, Tan HR, Ruan XC, Chen Y, Chao Z. Flumethrin residue levels in honey from apiaries of China by high-performance liquid chromatography. J Food Prot. 2015 Jan;78(1):151-6. doi: 10.4315/0362-028X.JFP-14-297. PMID: 25581190.
- 21. Sánchez-Bayo F, Goulson D, Pennacchio F, Nazzi F, Goka K, Desneux N. Are bee diseases linked to pesticides? A brief review. Environ Int. 2016 Apr-May;89-90:7-11. doi: 10.1016/j.envint.2016.01.009. Epub 2016 Jan 27. PMID: 26826357.
- 22. Gashout HA, Guzman-Novoa E, Goodwin PH, Correa-Benítez A. Impact of sublethal exposure to synthetic and natural acaricides on honey bee (Apis mellifera) memory and expression of genes related to memory. J Insect Physiol. 2020 Feb-Mar;121:104014. doi: 10.1016/j.jinsphys.2020.104014. Epub 2020 Jan 7. PMID: 31923391.
- Johnson RM, Dahlgren L, Siegfried BD, Ellis MD. Acaricide, fungicide and drug interactions in honey bees (Apis mellifera). PLoS One. 2013;8(1):e54092. doi: 10.1371/journal.pone.0054092. Epub 2013 Jan 29. PMID: 23382869; PMCID: PMC3558502.
- 24. Serra-Bonvehí J, Orantes-Bermejo J. Acaricides and their residues in Spanish commercial beeswax. Pest Manag Sci. 2010 Nov;66(11):1230-5. doi: 10.1002/ps.1999. PMID: 20661942.
- 25. Calatayud-Vernich P, Calatayud F, Simó E, Picó Y. Occurrence of pesticide residues in Spanish beeswax. Sci Total Environ. 2017 Dec 15;605-606:745-754. doi: 10.1016/j.scitotenv.2017.06.174. Epub 2017 Jul 2. PMID: 28679118.
- 26. O'Neal ST, Brewster CC, Bloomquist JR, Anderson TD. Amitraz and its metabolite modulate honey bee cardiac function and tolerance to viral infection. J Invertebr Pathol. 2017;149:119–26. Epub 2017/08/12. 10.1016/j.jip.2017.08.005 .

- 27. Boi M, Serra G, Colombo R, Lodesani M, Massi S, Costa C. A 10-year survey of acaricide residues in beeswax analysed in Italy. Pest Manag Sci. 2016 Jul;72(7):1366-72. doi: 10.1002/ps.4161. Epub 2015 Oct 27. PMID: 26423556.
- Weick J, Thorn RS. Effects of acute sublethal exposure to coumaphos or diazinon on acquisition and discrimination of odor stimuli in the honey bee (Hymenoptera: Apidae). J Econ Entomol. 2002 Apr;95(2):227-36. doi: 10.1603/0022-0493-95.2.227. PMID: 12019994.
- 29. Calatayud-Vernich P, Calatayud F, Simó E, Picó Y. Pesticide residues in honey bees, pollen and beeswax: Assessing beehive exposure. Environ Pollut. 2018 Oct; 241:106-114. doi: 10.1016/j.envpol.2018.05.062. Epub 2018 May 24. PMID: 29803024.
- 30. Australian Pesticides and Veterinary Medicines Authority (2022). Trade Advice Notice on amitraz in the product APIVAR 500 mg Bee Hive Strips for Honey Bees for use in bee hives.
- 31. https://food.ec.europa.eu/system/files/2018-%200/pesticides_mrl_guidelines_honey.pdf
- Pohorecka K, Kiljanek T, Antczak M, Skubida P, Semkiw P, Posyniak A. Amitraz Marker Residues in Honey from Honeybee Colonies Treated with Apiwarol. J Vet Res. 2018 Dec 10;62(3):297-301. doi: 10.2478/jvetres-2018-0043. PMID: 30584608; PMCID: PMC6295988.
- Vilarem C, Piou V, Vogelweith F, Vétillard A. *Varroa destructor* from the Laboratory to the Field: Control, Biocontrol and IPM Perspectives-A Review. Insects. 2021 Sep 7;12(9):800. doi: 10.3390/insects12090800. PMID: 34564240; PMCID: PMC8465918. Milani, N. The resistance of *Varroa jacobsoni* Oud. to acaricides. Apidologie. 1999. 30(2-3), 229-234.