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## Synthetic varroacides in honey bee colonies: A comprehensive monitoring program across the European Union

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## ABSTRACT

Managing *Varroa destructor* in honey bee colonies remains a constant challenge for beekeepers, requiring a balance between maintaining mite levels low whilst minimizing the negative impacts of miticide treatments on bee health. Synthetic varroacides such as coumaphos, tau-fluvalinate, and amitraz are widely used due to their convenience, but they can have negative impacts on the colony and persist in hive materials, with residues detectable long after application. To investigate the presence and dynamics of these synthetic varroacides, the INSIGNIA-EU initiative conducted a large-scale monitoring program, covering 312 bee hive sites across the European Union. The study employed the APIStrip—a novel, non-invasive passive sampler based on TENAX® sorbent—which, when placed inside the hive, passively adsorbs chemical residues from the internal hive environment. This approach has demonstrated its effectiveness eliminating the need to sample bees, wax, honey, or pollen, while still providing representative contamination data from a single, standardized analytical matrix. This study reports results from APIStrip

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analyses deployed across all EU countries for residues of amitraz, tau-fluvalinate, and coumaphos, using a harmonized and validated analytical protocol. Additionally, thymol, regarded as an environmentally friendly alternative, was also included in the evaluation as a reference. Sampling was carried out over nine consecutive two-week periods from May to August 2023, ensuring synchronized data collection and enabling direct comparability of results across sites and time points. The study found these miticides to be pervasive across most EU regions, appearing in more than 85% of samples and greatly outnumbering detections of the natural alternative, thymol. In most cases, notable miticide residue concentrations persisted throughout the entire sampling period.

## 1. Introduction

The varroa mite (*Varroa destructor*) is a major threat to honey bee colonies worldwide. It parasitizes both the brood and the adult bees, weakening the growth of the larvae and the bee immune system, and transmitting harmful viruses.

Many methods exist to chemically control varroa, but treatments must be applied carefully to avoid harming bees and contaminating hive products. Their effectiveness varies with beekeeping practices, climate, and season<sup>1</sup>.

Authorized chemical treatments are often insufficient on their own, and mite resistance has emerged in many cases (Lipiński, 2007; Grindrod and Martin, 2021; Mitton et al., 2022; Bahreini et al., 2025), complicating control efforts. Among them, the so-called “soft” acaricides, mainly organic acids (formic, oxalic, lactic), essential oils (e.g., thymol), and especially long-used synthetic “hard” acaricides (Brodschneider et al., 2023; Bubnić et al., 2021) (tau-fluvalinate, coumaphos, and amitraz) have proven to be effective, but their success relies on correct application and local resistance dynamics. These miticides are usually highly toxic to varroa, but much less toxic to honey bees, a consequence of their mode of action and, in some cases, their high lipophilicity. However, the long-term adverse effects of these chemicals on bee health are well documented (Rondeau, 2022; Encerrado-Manriquez et al., 2024), and they are one of several stressors contributing to ongoing bee declines<sup>10</sup>. Moreover, numerous pesticide-poisoning incidents have been reported (Thompson and Thorbahn, 2009; Calatayud-Vernich et al., 2019), often under co-exposure conditions involving complex mixtures that include both in-hive varroacides and agrochemical residues from surrounding foraging landscapes. This “cocktail effect” can generate synergistic or additive toxicities, amplifying sublethal and lethal impacts and ultimately compromising not only individual bees but also overall colony viability (Johnson et al., 2009; Benito-Murcia et al., 2022).

As expected, varroacides are typically detected at substantially higher concentrations<sup>15</sup> than agrochemical residues originating outside the hive, because they are applied directly inside the beehives. Nonetheless, this situation is complex as combs may be reused for many years, and a common beekeeping practice is to recycle old wax combs to produce new wax foundation sheets on which bees build their combs (Marti et al., 2022; Bischoff et al., 2023). Amitraz, coumaphos, and tau-fluvalinate are highly lipophilic—especially the latter two—and their residues persist in newly manufactured wax foundation sheets because they are not degraded or removed during wax-recycling (Kast et al., 2019; Wilmart et al., 2016). Therefore, residual varroacides may be released back into the hive during subsequent honey bee seasons many years after their original application through passive diffusion and physical contact with contaminated wax. Additionally, bees can act as vectors, transferring residues between hive compartments during in-hive activities<sup>20</sup>.

While the use of “soft” and more eco-friendly miticides (e.g. oxalic acid) clearly offers important advantages, particularly their minimal absorption into beeswax, these also present challenges related to application management and generally lower toxicity against varroa mites<sup>21</sup>. These factors mean that “hard” synthetic varroacides remain widely used across many EU regions<sup>6</sup>.

Trace-level assessment of varroacides is intrinsically difficult. Differences in diffusion due to proximity to the application site or from wax comb and the hive-wide transfer of contaminated wax particulates on bee bodies create strong spatial heterogeneity, so fine-scale analyses of conventional hive matrices often return sparse or highly variable data (Rumkee et al., 2017; Murcia Morales et al., 2020). Consequently, the introduction of passive samplers such as the APIStrip has become an important analytical advancement, enabling more reliable and representative assessments of chemical evaluation (Murcia-Morales et al., 2023, 2021a, 2021b, 2020).

One limitation of this approach is that APIStrips employ TENAX® as the adsorbent, which only poorly retains highly polar miticides such as formic and oxalic acids, hindering their recovery and analysis. For other compounds, their evaluation through APIStrip extraction after a defined sampling period is analytically straightforward, particularly when applying advanced techniques such as GC-MS/MS and LC-MS/MS, as recently demonstrated by our team<sup>27</sup>.

This APIStrip based method not only eliminates the need for active sampling of apicultural matrices—live bees, wax, honey, or pollen, but also provides representative and reproducible contamination data from the hive environment using a single, standardized matrix. Its practicality and analytical robustness make it very well suited for large-scale monitoring studies.

This work forms part of the INSIGNIA-EU Action <https://www.insignia-bee.eu>, which established a pan-European citizen-science network of beekeepers to monitor environmental pollution using honey bee colonies. In total, 315 citizen-scientist beekeepers from all 27 EU Member States followed a standardized protocol based on the effective, non-invasive APIStrip passive sampler<sup>28</sup>. The results establish a robust baseline for real-world exposure of honey bee colonies to ‘hard’ synthetic varroacides and document their widespread occurrence, probably driven both by intensified use in response to treatment resistance and by the slow release of legacy residues from contaminated comb wax. These findings underscore the need to accelerate the transition to newer and lower-impact varroacide alternatives.

## 2. Experimental methods

### 2.1. "APIStrips" passive samplers

The preparation of the APIStrip samplers was performed according to the protocol described in a previous study<sup>23</sup>. Briefly, the APIStrip sampler consisted of a polystyrene rectangle ( $5 \times 10 \times 0.2$  cm) was coated with a TENAX® solution in dichloromethane (6 ml, 125 mg/ml), resulting in 0.75 g of sorbent per APIStrip. The upper section was left uncovered to facilitate handling, and the samplers were not handled with bare hands in order to avoid cross-contamination. The APIStrips were individually stored, wrapped in aluminum foil, placed in sealed bags. After shipment to the apiaries, they were subsequently inserted into the bee hives, where they

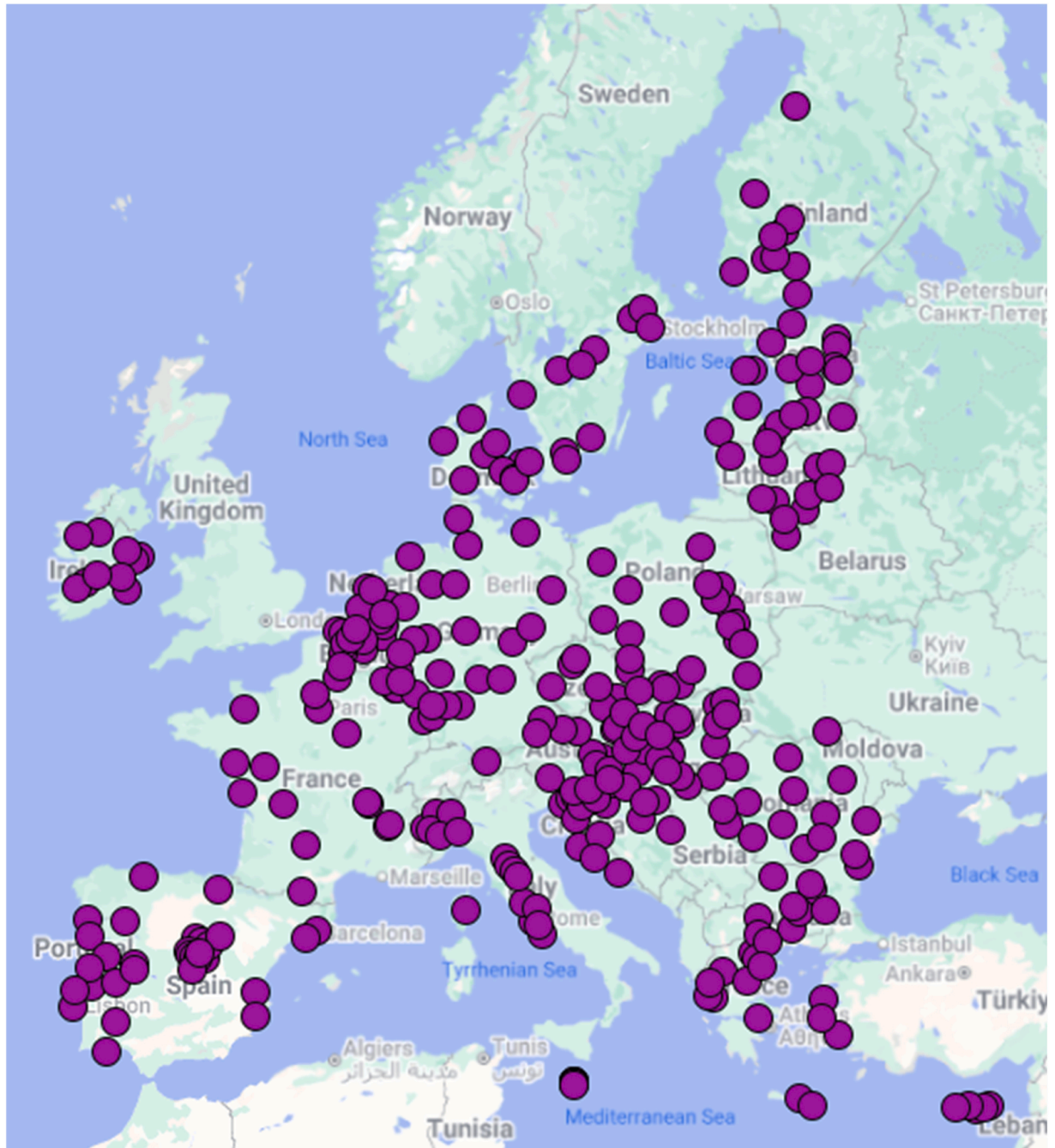


Fig. 1. Distribution of sampling points across the 27 EU member states.

remained for  $14 \pm 2$  days. In each apiary, we installed two APIStrips simultaneously in two bee hives near the hive center. This procedure was repeated across nine consecutive coordinated sampling events at each apiary. The APIStrip collections from each country were centralized by a national coordinator, stored at  $4\text{ }^{\circ}\text{C}$  in a refrigerator, and shipped for analysis every three sampling rounds according to the INSIGNIA-EU standardized citizen-science protocol. Field blanks were also shipped to each beekeeper to assess potential contamination: these were treated, stored, and analyzed in the same way as the real samples, but they were not placed into bee hives. Additionally, blank APIStrips remained in the laboratory and were analyzed with each set of samples to assess in-house contamination. It should be noted that the Passive sampling, (PAS) sampling system based on TENAX® has limited efficiency in trapping highly polar pesticides, which may lead to an underestimation of their contribution.

## 2.2. Sample collection

Sampling was conducted in every EU country between 20 April and 24 August 2023 over nine consecutive two-week intervals, enabling synchronous data collection and direct comparability. The INSIGNIA\_EU action was developed as a citizen-science project; accordingly, one national coordinator in each of the 27 EU countries was responsible for centrally collecting their country's samples at each scheduled sampling time. All participants followed a strict protocol for installing the APIStrip devices, collecting samples, and transporting them to their national coordination point, with hives selected to be of broadly comparable colony strength (similar adult bee populations) (<https://www.insignia-bee.eu>). Every two weeks, the passive samplers were collected by the citizen science beekeepers and replaced with new ones, stored at  $4\text{ }^{\circ}\text{C}$ , wrapped in aluminum foil, and placed in labeled, sealable bags indicating the collection time and any relevant incidents. Every three sampling rounds, the national coordinators delivered their aggregated samples to the central laboratory. The sampling points are represented in Fig. 1.

## 2.3. Chemical analysis

After the sampling period, the APIStrips were shipped back to the analytical laboratory according to a specified protocol and chemical contaminants were desorbed following previously developed and optimized procedures (Murcia-Morales et al., 2023, 2021a). The extraction involved cutting each sampler into small pieces that were placed in 50-ml PTFE centrifuge tubes; then, surrogate standards (dichlorvos-D6, malathion-D10, parathion-D10, carbendazim-D3) were added, followed by 10 ml of acetonitrile. The samples were automatically shaken for 3.5 min and centrifuged for five minutes at 4000 rpm. Two aliquots of each sample (0.5 ml) were evaporated to dryness and reconstituted with 50  $\mu\text{l}$  of the injection solvent (acetonitrile for liquid chromatography -LC-, ethyl acetate for gas chromatography -GC-). Lindane-D6 and dimethoate-D6 were added as internal standards for GC and LC, respectively, as well as 200  $\mu\text{l}$  of ultrapure water to the LC vials. The analyses by GC-MS/MS were performed in an Agilent Intuvo 9000 GC system coupled to an Agilent 7010B GC MS/MS triple quadrupole, while an Agilent UPLC 1290 Series coupled to an Agilent 6490 Triple Quad LC/MS was employed for the LC-MS/MS analyses. The analytical methods have been previously described<sup>23</sup>. A detailed list of the contaminants included in the analytical scope, as well as the transitions and their limits of quantification, can be found in the [Supplementary Material](#) (Annex I).

The identification of the varroacide residues included in the present study was performed according to the guidelines established by the SANTE Document No. 11312/202127: Two fully overlapping transitions with a signal-to-noise ratio (S/N) higher than 3, whose ion ratio in the sample is within  $\pm 30\%$  from the average of the calibration standards, and a retention time in the sample within  $\pm 0.1$  min from that of the calibration standards. Recovery studies at 2 or 5 ng/APIStrip for all compounds were performed and described in a previous work<sup>27</sup>, and the recovery of more than 98% of compounds fell within the range 70–120%. The minimum matrix-matched standard calibration point that allowed the identification of each compound according to the criteria described above was set as the limit of quantification (LOQ). The LOQ for most compounds was determined to be 0.5 ng/APIStrip. Positive detections below the LOQ that were fully identified were assigned to a default limit of detection (LOD) value of 0.25 ng/APIStrip.

## 3. Results and discussion

During the sampling period, beekeepers acting as citizen scientists in the INSIGNIA-EU project received no specific instructions or restrictions regarding their use of varroacides. Consequently, the detected residues reflect routine, real-world treatments. The analysis spanned concentrations from 0.25 to 400 ng per APIStrip to characterize the kinetics of varroacide appearance both as a direct consequence of treatments and via gradual release from wax over time.

Bee-driven mixing and compound volatility yield internal homogenization, making the APIStrip readout a highly representative, time-integrated measure of hive contamination versus other hive materials<sup>20</sup> while not being directly equivalent to concentrations in individual bees or specific hive compartments, but rather a global integrated indicator of presence. APIStrips were installed near the hive center, where certain strip-based varroacides would also be applied, whilst other varroa treatments may be more spatially heterogeneous, so the sampler's proximity to varroacide-impregnated strips or contaminated frames, together with compound-specific volatility probably influenced the measured concentrations. These proximity/volatility effects are expected to be most pronounced at higher concentrations. Considering this, and to simplify the results of the quantitative analysis and to avoid overly broad calibration curves, values exceeding 400 ng per APIStrip were not quantitatively evaluated and were instead assigned a generic value of  $> 400$  ng ([Supplementary Material](#), Annex II). Based on our experience, values of 100 ng/APIStrip or higher can be considered indicative of recent or immediate varroacide application, whereas values below 25 ng/APIStrip after the two-week sampling period are consistent with residual presence or desorption from the wax comb, provided no new treatment occurred (Luna et al., 2023; Murcia-Morales

et al., 2021b).

### 3.1. Varroacide detections

It is important to note that, although varroacide Regulations EU 37/2010 and EU 2019/6 are harmonized at the EU level to ensure that only substances evaluated for their efficacy and safety can be used, national authorizations for each commercial product (trade name, formulation, and conditions of use) remain the responsibility of each Member State. Owing to this dual authorization framework, validity periods, conditions of use, and restrictions can vary considerably from one Member State to another. In practice, amitraz and tau-fluvalinate are among the most widely authorized miticides, whereas coumaphos is authorized to a much lesser extent<sup>29</sup>. As a result, detection patterns across different regions can be strongly influenced by these national authorizations and product-specific dosing recommendations.

Of the 5522 APIStrips analyzed, 4663 had detections > 0.25 ng/APIStrip of synthetic varroacides, and over 45% exceeded 25 ng/APIStrip for at least one of them. Only in a small proportion of samples (15%) corresponding to 90 locations, were none of the varroacides (amitraz, coumaphos, tau-fluvalinate and thymol) found in any APIStrip analysed. It is important to emphasize the value of achieving these very low detection limits (ng), as they enable accurate assessment of the widespread occurrence of these chemicals, as even at sublethal levels, prolonged exposure may lead to adverse effects.

Notably, nearly 80% of the positive detections contained mixtures of at least two varroacides, and approximately 34% contained all three synthetic “hard” compounds evaluated (Table IA). These results align with both the common strategy of alternating varroacides with diverse modes of action and the pronounced release of residues from wax matrices, leading to carry over into later seasons. These combinations remained remarkably consistent across the nine sampling rounds (Table IA), indicating the persistence of these varroacides or mixtures over time. This pattern probably reflects cumulative treatments over successive seasons and/or the concurrent use of multiple products to address increasing resistance in varroa populations. In addition, desorption of residues from contaminated wax contributes substantially to the persistence of multiple compounds, potentially enhancing synergistic effects.

### 3.2. Detections of tau-fluvalinate

Tau-fluvalinate, with a LogP of 7.0–8.0, is the most lipophilic of the varroacides, conferring a strong affinity for beeswax and accounting for its remarkable persistence in the wax matrix. Although it was detected alone in only 10.07% of the analyzed samples, its co-occurrence with coumaphos and amitraz increased prevalence by a further 73.85%. As a result, whether detected alone or in mixtures, it was the compound with the highest number of detections (Table IB). The presence of a trifluoromethyl substituent on the aromatic ring of this compound (Fig. 2) is noteworthy, as it classifies the compound as a PFAS (per- and polyfluoroalkyl substances) pesticide prone to generating trifluoroacetic acid, a persistent “forever chemical” (Joerss et al., 2024).

It is well known that detoxification enzymes, such as cytochrome P450s, can metabolize tau-fluvalinate and mitigate its acute toxicity in honey bees (Mao et al., 2011; Johnson et al., 2006). However, regarding this negative impact, it is important to note that raising the reporting threshold from < 0.25 to > 25 ng/APIStrip, as noted above, reduces tau-fluvalinate positives from 73.85% to 6.46%. This suggests that most detections reflect residual contamination, often originating from desorption of previously treated wax. Overall, the total number of detections varied little across sampling rounds (< 10%). However, at concentrations ≥ 100 ng/APIStrip (indicative of actual in-hive application), the percentage decreased markedly to 3.7%, showing very few detections—about one-tenth of those observed when all concentrations were considered—and exhibited a clear decline from the initial to the final sampling rounds (Fig. 3). Notably, all detections at these high levels were confined to 27 locations. This trend reflects the EU-wide decline in

**Table IA**

Total detections per analyte/s across all APIStrips, with a breakdown across the nine sampling rounds (SR1–SR9).

Compound	Number of detections	% of detections	Sampling site	SR 1	SR 2	SR 3	SR 4	SR 5	SR 6	SR 7	SR 8	SR 9
Coumaphos	155	0.0281	53	15	11	26	26	16	16	20	15	10
Amitraz	199	0.036	49	16	23	24	22	16	19	24	30	25
Tau-fluvalinate	556	0.1007	100	59	60	47	50	59	66	67	74	74
Coumaphos + Amitraz	121	0.0219	32	13	15	20	18	12	11	14	10	8
Coumaphos + Tau	690	0.125	103	82	86	78	74	88	81	76	67	58
Amitraz + Tau-fluvalinate	935	0.1693	114	116	119	99	89	100	105	93	98	116
Coums + Amitraz + Tau	1893	0.3428	172	212	197	211	226	229	211	205	198	204
Zero detections	859	0.1556	90	83	90	90	95	84	97	108	115	97
Thymol (only)	114	0.0206	18	17	13	15	14	11	11	8	9	16
Total	5522	1	312	613	614	610	614	615	617	615	616	608
Thymol + Coumaphos	36	0.0952	11	2	2	7	6	3	4	3	4	5
Thymol + Amitraz	7	0.0185	6	1	0	0	1	1	0	1	2	1
Thymol + Tau-fluvalinate	30	0.0794	13	10	6	1	0	2	1	2	1	7
Thymol + Coum + Amitraz	12	0.0317	5	1	0	0	0	2	1	3	3	2
Thymol + Coum + Tau	54	0.1429	18	11	13	5	5	4	5	2	2	7
Thymol + Amitraz + Tau	34	0.0899	8	2	2	6	4	5	4	3	4	4
Thymol + Coum + Am + Tau	91	0.2407	23	12	10	7	12	7	9	12	8	14
Total Thymol	378	0.6984	57	56	46	41	42	35	35	34	33	56

**Table IB**

Total number of detections of each compound evaluated and numbers per sampling round (SR1-SR9).

Compound	Number of detections	% of detections	Sampling site	> 25 ng/ APIStrip	> 100 ng/ APIStrip	SR 1	SR 2	SR 3	SR 4	SR 5	SR 6	SR 7	SR 8	SR 9
Coumaphos Total	2859	51.77%	260	277	137	322	309	335	344	345	319	315	290	280
Amitraz Total	3148	57.01%	226	1053	486	357	354	354	355	357	346	336	336	353
Tau-fluvalinate Total	4074	73.78%	284	358	151	469	462	435	439	476	463	441	437	452
Total Thymol	378	6.23%	52	378	378	56	46	41	42	35	35	34	33	56

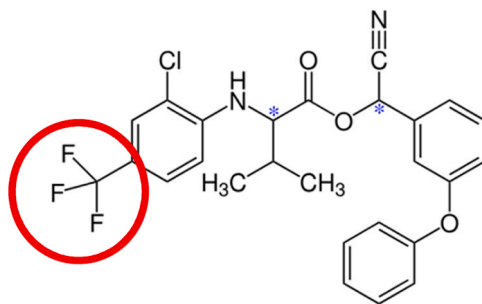


Fig. 2. Chemical structure of tau-fluvalinate (the trifluoromethyl group,  $-CF_3$ , is highlighted in the red circle).

tau-fluvalinate or coumaphos usage resulting from diminished effectiveness caused by mite resistance<sup>6</sup>.

### 3.3. Detections of coumaphos

With a LogP of approx. 4.1, coumaphos is lipophilic but markedly less than tau-fluvalinate. It therefore partitions less strongly into wax and desorbs more readily. Consequently, long-term wax-mediated release is expected to be lower; however, the typically higher application dose of coumaphos can partly offset this difference<sup>16</sup>. Although its acute contact toxicity to honey bees is relatively low, chronic exposure may exert cumulative, time-dependent effects. Its toxicity to honey bees is reportedly more than 10 times greater than that of tau-fluvalinate<sup>32</sup>. This finding is not fully explained by the acute toxicity values reported in the literature, but may be attributed to a phenomenon known as Time Reinforcement Toxicity (TRT), which results from prolonged exposure to low residue levels (Benito-Murcia et al., 2024; Xiao et al., 2025; Carnesecchi et al., 2019).

In addition, coumaphos poses significant occupational hazards for beekeepers, considering ECHA's Classification & Labelling (C&L) Inventory. These high-severity hazards necessitate stringent personal protective equipment (PPE) and risk-mitigation measures during handling and application, and they have contributed to the observed decline in coumaphos use. These factors help to explain why the number of coumaphos detections was some lower than that of tau-fluvalinate: overall 51.77% at  $< 0.25$  ng/APIStrip and declining to just 9.6% at  $> 25$  ng/APIStrip, indicating relatively infrequent application across the EU. Nevertheless, it was still detected at  $\geq 100$  ng/APIStrip at levels comparable to tau-fluvalinate (Table IB) and remained stable across all sampling rounds. However, these considered high-level detections were restricted to only 16 sampling sites (Fig. 4).

It is important to consider the co-occurrence of coumaphos and tau-fluvalinate, as combined exposure produces marked synergistic effects: both compounds compete for cytochrome P450 mediated detoxification in honey bees, reducing metabolic capacity and

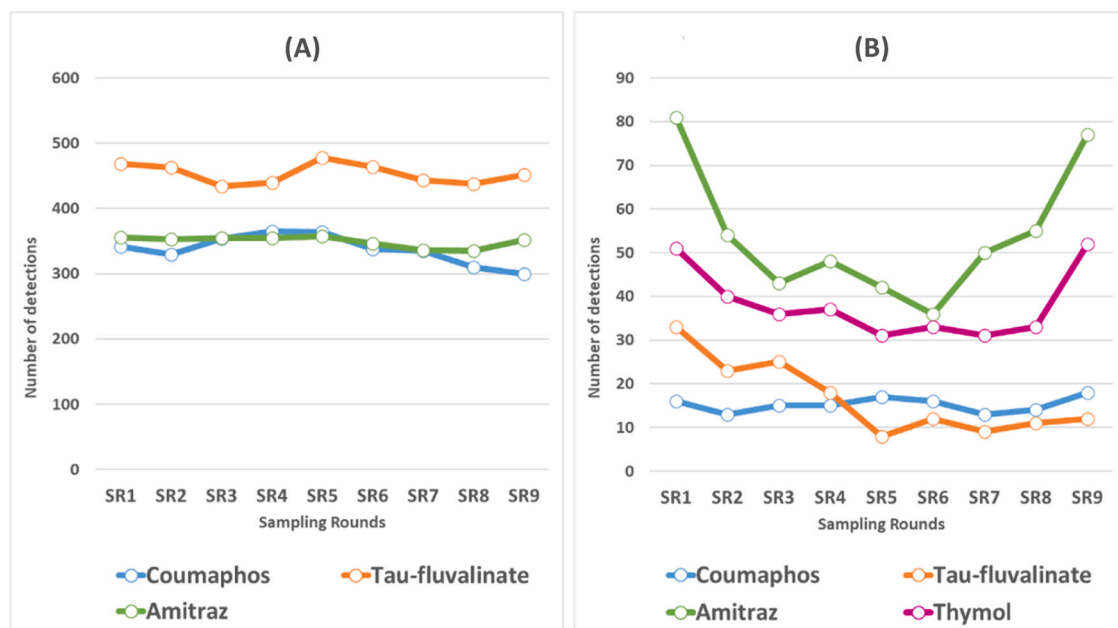


Fig. 3. Number of detections across the nine sampling rounds (A) considering all detections above 0.25 ng/APIStrip, and (B) above 100 ng/APIStrip.

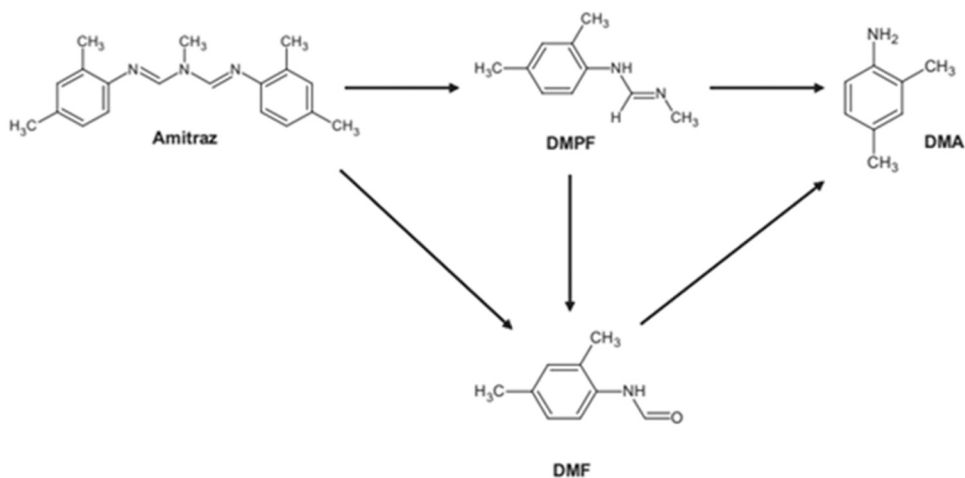


Fig. 4. Schematic representation of amitraz degradation.

increasing toxicity (Benito-Murcia et al., 2022; Xiao et al., 2025). Across the study, we recorded 690 co-detections of coumaphos together with tau-fluvalinate (>10% of all samples). When the reporting threshold was raised to > 25 ng/APIStrip, this fell to 57 (around 1% of samples), which, given nine sampling rounds at the same sites, correspond to fewer than 10 cases per sampling round (Table IA). No detections of this binary mixture at levels of 25 ng for both compounds were observed, clearly indicating that they were not applied concurrently during the study.

### 3.4. Detections of amitraz

The use of amitraz is particularly valuable because its mode of action differs notably from that of pyrethroids and organophosphates. This provides an effective alternative before varroa mites develop full resistance to pyrethroid or coumaphos-based treatments.

The analytical evaluation of amitraz is more complex, as it hydrolyzes rapidly into its main metabolites DMPF, DMF, and DMA (Fig. 4) and is therefore only detected through the presence of these degradation products. Obviously, the degradation products exhibit different physicochemical properties compared to the parent compound. While amitraz is highly lipophilic, DMPF and especially DMF are much less so; consequently, their retention in wax is much lower. Amitraz breaks down through multiple pathways to DMPF and DMF, and later to DMA. There is no fixed stoichiometric ratio between amitraz and its metabolites because degradation proceeds via multiple, branching pathways. The proportions depend on pH, temperature, and the matrix, with additional shifts from interconversion and losses. Because of these challenges, it is standard to use an operational “amitraz sum”: considering the principal transformation products observed under the given monitoring conditions.

To avoid overestimation, it is essential to identify the predominant degradation products before interpreting detection counts. Although DMPF is the primary hydrolysis product and it may appear most abundant immediately after treatment, under bee hive degradation kinetics DMF becomes by far the dominant product, with DMPF at lower levels and DMA practically negligible in all cases (Pohorecka et al., 2018; Lozano et al., 2019). This effect is particularly pronounced with APIStrips, as the higher volatility of DMF facilitates its transfer to the sampling surface. Accordingly, DMF served as the reference for amitraz detection; DMPF was included in the sum of detections only when DMF was absent. DMA was found in just two samples across the entire monitoring period. In general, a slight quantitative underestimation may occur; however, given the thresholds established in this study, it does not affect the interpretation of the results. A total of 3148 detections were recorded, representing 57% of all samples. When applying a threshold of > 25 ng/APIStrip, this percentage decreased to 19%, still considerably higher than the values observed for coumaphos and tau-fluvalinate at this concentration level (Fig. 3 A). The difference becomes even more pronounced at concentrations above 100 ng/APIStrip, where the detected levels were, on average, close to five times higher than those of the other two miticides (Fig. 3 B). These elevated values are also accompanied by substantial variability throughout the sampling period, as consequence of more intense numbers of applications. Similarly, the number of locations with these high values reaches 87, as shown in Fig. 5, higher than with the other “hard” varroacides.

### 3.5. Detections of thymol

In this study, we selected thymol as the reference compound to enable comparability across application levels and between ‘hard’ (synthetic) and ‘soft’ (organic) miticides, given thymol’s widespread use and consistently high efficacy (Jack and Ellis, 2021; O’Connell et al., 2025). Thymol is relatively nonpolar and therefore partitions into beeswax; however, its appreciable volatility leads to rapid evaporation, particularly at temperatures above 30 °C and under well-ventilated hive conditions. As a result, thymol concentrations may decline more rapidly than those of other compounds; however, the required dosing levels and intervals often lead to elevated peak

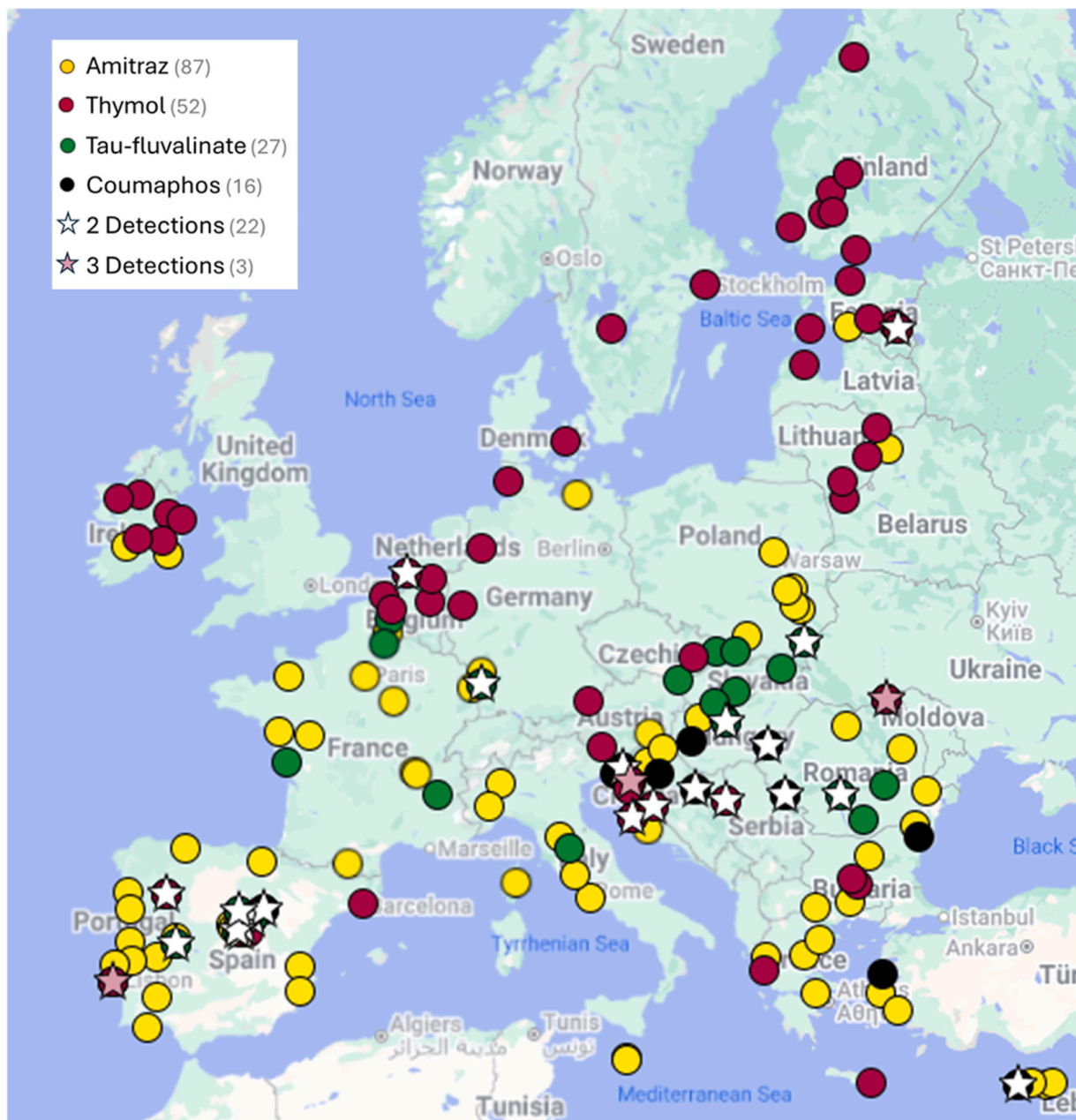


Fig. 5. Distribution of sampling locations where concentrations exceeded 100 ng per APIStrip for amitraz, tau-fluvalinate, coumaphos, and thymol.

concentrations<sup>40</sup>.

Together, these factors help explain why thymol on APIStrips exceeded 400 ng in 100% of detected cases. Thymol was detected in 344 samples (6% of the total), a clearly much lower proportion than for the other miticides evaluated in this study, nevertheless at much higher concentrations per APIStrip (Table IB). It is important to note that although thymol is a natural substance and generally recognized as safe, concentrations above 1.1–1.2 µg/g in honey may cause detectable off-flavours, potentially affecting its sensory quality. The variability of the number of detections across sampling rounds was similar to that observed for amitraz (Fig. 3), with increased use at the beginning and at the end of the sampling period. These high values, clustered at 52 locations primarily in northern regions (Fig. 5), may be attributed to temperature constraints affecting its use.

### 3.6. Detection of co-occurring miticides

Frequent co-occurrence of miticides is expected because colonies can receive multiple treatments over the study period and,

especially because contaminated beeswax is commonly reused during colony establishment and maintenance, leading to the recurrent release of residues into the hive environment. As a result, the majority of detections involved multiple compounds, with nearly 80% corresponding to multi-residue findings and only 20% to single-compound detections, considering the “hard” varroacides studied.

However, when restricting the analysis to co-occurrence cases where each compound exceeded a threshold of 100 ng/APIStrip, the percentage was negligible, indicating that simultaneous or very close application of these miticide mixtures was probably uncommon. Nonetheless, a limited number (11) of sampling sites exhibited simultaneously high concentrations of these compounds, as shown in Fig. 5.

The pattern of co-occurrence observed for thymol was similar. This compound was detected alone in 32% of the total thymol cases, while in nearly 70% of detections it appeared in combination with one or more of the other three miticides. Notably, a substantial portion of this 70% involved the simultaneous presence of all four compounds evaluated (Table IA). However, as with the previously discussed combinations, the number of cases where all chemicals were present at high concentrations was practically negligible. Only relevant is the case of three locations with all four compounds (amitraz, coumaphos, tau-fluvalinate and thymol) with values higher than 100 ng/APIStrip.

#### 4. Conclusions

One key finding of the study is that beekeepers in the EU continue to depend on synthetic miticides despite long-standing resistance, even as studies have linked the unprecedented honey bee colony losses in 2025 to viruses spread by *Varroa* mites due to the widespread failure of amitraz to control *Varroa*<sup>41</sup>.

Residues of coumaphos and tau-fluvalinate remain prevalent. Their high lipophilicity promotes partitioning into long-term retention by beeswax; when contaminated wax is recycled into foundation, this reservoir sustains a persistent background over multiple seasons.

Amitraz continues to be widely used; accordingly, its transformation products, particularly DMF and DMPF, are frequently detected, reflecting recurrent treatment patterns. Thymol shows a broadly similar application pattern but in fewer cases.

As a result of successive treatments and the slow release from wax, co-occurrence of miticides is common, implying that honey bees are often exposed to mixtures rather than single active ingredients. These observations reinforce the need within any integrated pest management framework to actively manage wax quality actively through traceability and purification/replacement in order to reduce residue burdens within the colony.

APIStrips, as standardized passive samplers, provided a major advantage for EU-wide monitoring of miticides: their simplicity and ease of handling enabled broad citizen-science participation in this study, yielding a robust, well-defined snapshot of varroacide status across the EU.

#### CRedit authorship contribution statement

**Maria Jesus Martinez-Bueno:** Writing – review & editing. **Valters Brusbardis:** Writing – review & editing, Resources, Conceptualization. **Maria Murcia-Morales:** Writing – review & editing, Methodology, Conceptualization. **Buddendorf Bas:** Writing – review & editing, Formal analysis, Conceptualization. **Van der Steen Jozef:** Writing – review & editing, Methodology, Funding acquisition, Conceptualization. **Robert Brodschneider:** Writing – review & editing, Methodology, Conceptualization. **M. Alice Pinto:** Resources, Conceptualization. **Dirk C. de Graaf:** Conceptualization. **Andreia Quaresma:** Conceptualization. **Kristina Gratzner:** Writing – review & editing. **José Luis Oller-Serrano:** Writing – review & editing, Conceptualization. **Norman Carreck:** Writing – review & editing, Conceptualization. **Marco Pietropaoli:** Resources, Conceptualization. **Ellen Danneels:** Conceptualization. **Flemming Vejsnæs:** Resources, Conceptualization. **Konstantinos M. Kasiotis:** Writing – review & editing, Conceptualization. **Fernández-Alba A.R.:** Writing – review & editing, Writing – original draft, Methodology, Conceptualization. **Ole Kilpinen:** Resources, Conceptualization. **Ivo Roessink:** Writing – review & editing, Methodology, Conceptualization. **Alison Gray:** Writing – review & editing, Conceptualization. **Evangelia Tzanetou:** Conceptualization. **Fani Hatjina:** Resources, Conceptualization. **José Antonio Martínez:** Writing – review & editing, Conceptualization.

#### Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

#### Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at [doi:10.1016/j.eti.2026.104861](https://doi.org/10.1016/j.eti.2026.104861).

## Data availability

Data will be made available on request.

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