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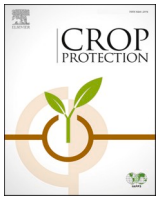

Short communication

Cyclobutrifluram (TYMIRIUM® technology): Low risks of a soil applied nematicide and fungicide to non-target soil invertebrates and bees

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ABSTRACT

Cyclobutrifluram (TYMIRIUM® technology) is a seed- and soil-applied nematicide and fungicide which protects the plant root mass. Cyclobutrifluram acts by inhibiting mitochondrial complex II electron transport and succinate dehydrogenase inhibition (SDHI). Concerns over the potential adverse effects on non-target species were addressed by assessing whether recommended field application rates of cyclobutrifluram would result in adverse impacts on soil invertebrates or honeybees. Studies conducted under laboratory conditions with the active ingredient and two formulations provided No Observed Effect Concentrations for earthworm (*Eisenia andreii*) reproduction of 71–171 mg a.i./kg dry soil with no effects on soil mite (*Hypoaspis aculifier)* reproduction. There were no effects on honeybee (*Apis mellifera*) adults or larvae following chronic exposure to doses up to 400 and 160 mg/kg diet respectively. Using Brazil as a target market (soybean seed treatment and in-furrow application in fruiting vegetables), our laboratory studies indicate that the risk to two species of soil invertebrates and honeybees of the use of cyclobutrifluram either in-furrow or as a seed treatment was orders of magnitude below any levels of concern.

1. Introduction

TYMIRIUM® technology (cyclobutrifluram) is a newly developed seed- and soil-applied IRAC group N-3 soil nematicide (IRAC Group N-3: Mitochondrial complex II electron transport inhibitors. Succinate-coenzyme Q reductase) (IRAC, 2023) and fungicide (FRAC Class 7 Complex II succinate dehydrogenase inhibitor (SDHI)) (FRAC, 2022). In combination with no-till and conservation tillage practices, use of cyclobutrifluram to protect the root mass from both nematodes, e.g. *Meloidogyne incognita*, and fungi, such as *Fusarium,* can support soil health (Atwood et al., 2022; Davies and Evans, 2006). Delayed germination of seed in cooler soils increases seed exposure to pests and pathogens (Atwood et al., 2022) Conventional tillage reduces populations of soil borne pathogens (Abawi and Widmer, 2000) and nematodes (Lenz and Eisenbeis, 2000) but has adverse effects on soil health, and therefore agricultural sustainability ((Congreves et al., 2015). Practices which can be used alongside no-till and conservation tillage, such as seed and soil treatments to control pathogens and parasitic nematodes, therefore have the potential to aid the farmer in supporting soil health (Atwood et al., 2022). However, due to the conserved sequence of succinate dehydrogenase, there have been concerns raised over the potential adverse effects of SDHI fungicides on non-target species, such as earthworms and honeybees (Bénit et al., 2019; Ernst et al., 2022; He et al., 2021). SDHIs are not limited to synthetic chemistry, they also occur in plants, insects, bacteria, and fungi (Becker et al., 2017). For example, 3-nitropropanoic acid is an effective SDHI produced in significant amounts in plants and beetles as well as fungi and significantly reduced the larval growth of the pest *Spodoptera littoralis* (Novoselov et al., 2015). Adverse impacts on soil organisms have potential consequences on soil health, e.g., through reductions in organic matter breakdown. In addition to soil organisms, adverse effects on honeybees (exposed through pollen, nectar or seed-dust (IBAMA, 2017) may indicate impacts on pollination services.

In the present work, we focus on the potential risks of cyclobutrifluram applied as a seed treatment in soybean or as a soil application in fruiting vegetables to non-target soil invertebrates

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(earthworms, and soil mites) and bees. We selected Brazil as the target market based on the use profile and interest in development of risk assessments for both bees and soil organisms in the region (Tincani et al., 2022). Our hypothesis was that the recommended application rates of cyclobutrifluram pose a low risk to non-target soil invertebrates and bees. First, we determined, under controlled laboratory conditions, the effects of cyclobutrifluram on the survival and reproduction of soil invertebrates (earthworms *Eisenia andrei* and the soil mite *Hypoaspis aculifier*) and on the survival of adult and larval honeybees (*Apis mellifera*). The active ingredient and two different formulations (VANIVA®, an SC formulation intended for in-furrow soil applications, and VICTRATO®, an FS formulation for seed treatment) were used in these assessments. We then used approaches proposed for use in, or adopted in, Brazil to assess whether the use of cyclobutrifluram as a seed treatment or in-furrow application posed any risk to soil invertebrates (Tincani et al., 2023) or to honeybees foraging on pollen and nectar from the treated crop or wild bees foraging in the off-crop area where seed-dust may be deposited (IBAMA, 2017).

2. Material and methods

2.1. Toxicity assessment

All toxicity assessments were conducted to international guidelines published by the OECD (soil organisms and honeybees, references are supplied in the relevant sections below) and fulfilled the validity criteria in these guidelines. The active ingredient cyclobutrifluram (min 95% purity on dried basis) and two formulations (in-furrow formulation 450SC (38% a.i. w/w) and seed treatment formulation 500FS (42% a.i. w/w) were used. All studies were conducted to Good Laboratory Practice by contract research organisations. Soil organism studies were conducted by BioChem agrar (Gerichshain, Germany), honeybee studies with the active ingredient were conducted by Eurofins (Niefern-Oschelbronn, Germany) and with the formulations by BioChem agrar (Gerichshain Germany). The dose rates for soil invertebrates and honeybees were selected to identify a no observed effect dose, rate or concentration (NOED, NOER or NOEC respectively) and, if a lethal effect was observed, the dose (LD_{50}), rate (LR_{50}) or concentration (LC_{50}) resulting in 50% mortality. The maximum dose rate used was intended to be well in excess of the recommended application rate or residues in soil resulting from the use of the products (for details see section 2.2).

2.1.1. Soil organisms (earthworms and mites)

The rates used for soil organisms were 0, 16.3, 29.4, 52.9, 95.3, 171, 309, 556 and 1000 mg test item/kg soil dry wt. (mg a.i./kg dry wt. soil for studies using the active ingredient or mg formulation/kg soil dry wt. for studies using the formulations).

Chronic reproduction studies with earthworms of the species *Eisenia andrei* were conducted in artificial soil, as defined in the guideline, with a 5% peat content (OECD, 2016a). For each study, 4 replicates for each treatment rate and 8 replicates for the control (untreated) were used. There were 10 worms per replicate with assessments of mortality, behavioural effect (e.g. feeding activity), and biomass development of adults which were removed after 28 days, and of reproduction after 56 days. Reproduction was assessed by gradual heating of the containers in a water bath to 60°C so that juveniles moved to the surface. The temperature was maintained at 19.7–21.7 °C with an 16h light (590 lux): 8 h dark photoperiod. All aspects of the study were conducted in compliance with the OECD guideline (OECD, 2016a) as required for studies for regulatory approval. Control data were compared with the independent test item groups using ToxRat Professional 3.2.1 (2015) and details are supplied in Supplementary Information). The NOECs for mortality following exposure for 28 days to the active ingredient and the two formulations (450SC and 500FS) were identified by multiple sequentially-rejective Fisher Exact tests with Bonferroni-Holm correction (one sided greater, $\alpha = 0.05$). The NOECs for biomass change and numbers of juveniles were identified by William's *t*-test after testing for normality and homogeneity using Shaprio-Wilk's and Levine's tests respectively.

Reproduction studies with the soil mite *Hypoaspis aculeifer* (obtained from a synchronised culture with age difference less than 2 days) were conducted in artificial soil as defined in the guideline with a 5% peat content (OECD, 2016b). For each study, 4 replicates for each treatment and 8 replicates for the control (untreated) were used. There were 10 mites per replicate with assessments of adult mortality and reproduction after 14 days. The temperature was maintained at 19.5–21.1 °C with a 16h light (427–623 lux): 8 h dark photoperiod. All aspects of the study were conducted in compliance with the OECD guideline (OECD, 2016b) as required for studies for regulatory approval. After 14 days, adult and juvenile mites were extracted using MacFayden apparatus in which a temperature gradient was created between the soil and a collecting flask containing 70% ethanol by heating the soil from 25 to 45°C over period of 48 h. Control data were compared with the independent test item groups using ToxRat Professional 3.2.1 (2015) and details are supplied in Supplementary Information. The NOECs for mortality following exposure for 14 days to the active ingredient and the 500FS formulation (the 450SC formulation was not tested) were identified by multiple sequentially-rejective Fisher Exact tests with Bonferroni-Holm correction (one sided greater, $\alpha = 0.05$). The NOECs for biomass change and numbers of juveniles were identified by Dunnett's *t*-test after testing for normality and homogeneity using Shaprio-Wilk's and Levine's tests, respectively.

2.1.2. Honeybees

For honeybees (*Apis mellifera* L.), adult acute contact and oral toxicity studies were conducted according to OECD guidelines (OECD, 1998a, 1998b) with the active ingredient and with both formulations. The active ingredient was dissolved in acetone and 4 replicates of 10 bees were dosed with 12.5, 25, 50, 100 or 200 μg a.i./bee in the contact study. For oral doses, the doses were prepared by further diluting the acetone solutions 1:10 in 50% w/v sucrose and the doses offered to 4 replicates of 10 bees were 4.8, 9.6, 19.2, 38.5 or 77 μg a.i./bee. For the formulations, dilutions were prepared in deionised water. For the formulation contact studies, doses contained 0.1% Triton-X100 to ensure spreading of the droplet and 3 replicates of 10 bees were dosed with of 62.5, 125, 250, 500 or 1000 μg formulation/bee. For oral doses, the water stock solutions were diluted 1:10 in 50% w/v sucrose and 3 replicates of 10 bees were dosed with of 62.5, 125, 250, 500 or 1000 μg formulation/bee. Control treatments for each test were prepared in the same way so that control groups of bees received the same solutions but excluding the active substance or formulation. Contact doses were applied in 1 μL/bee and oral doses offered as 200 μL/10 bees for 6 h (actual consumption was then measured). All bees were fed 50% w/v sucrose *ad libitum* with mortality recorded up to 48hrs after dosing.

No chronic exposure studies with formulation were conducted as prolonged exposure to the complete formulation via pollen and nectar was considered unlikely. An adult chronic 10-day oral toxicity study was conducted with the active ingredient according to OECD guideline 245 (OECD, 2017). The active ingredient was dissolved in acetone and diluted 1:20 in 50% w/v sucrose containing 0.1% Xanthan. Doses of 25, 50, 100, 200 or 400 mg a.i./kg sucrose were offered to 4 replicates of 10 bees *ad libitum* for 10 days. Two control groups were used, one offered 50% w/v sucrose and one provided 50% w/v sucrose containing 5% acetone and 0.1% Xanthan. Consumption and mortality were measured daily and the no observed effect dose after 10 days was determined by multiple Fisher's Exact test with Bonferroni-Holm correction (one sided greater, $\alpha = 0.05$).

A larval 22-day chronic toxicity study was conducted with the active ingredient according to OECD Guidance Document 239 (OECD, 2016c). The active ingredient was dissolved in acetone and then diluted in de-ionised water which was added 1:10 to larval diet to give a final acetone concentration in diet of 0.5%. Doses of 4.1, 10.2, 25.6, 64 or

160 mg a.i./kg diet were provided to 3 replicates of 16 larvae on days 3–6 (total 140 μL diet). Two control groups were used, one offered larval diet containing 10% de-ionised water and one provided larval diet containing 0.5% acetone and 9.5% de-ionised water. The treated larvae were allowed to pupate and emergence of adults was observed on day 22 (19 days after the first dosing). The no observed effect dose for adult emergence was determined by multiple sequentially rejective Fisher's Exact test with Bonferroni-Holm correction (one sided greater, $\alpha = 0.05$).

2.2. Exposure measures

Application rates used in exposure assessments were the highest global single application rate for in-furrow application of the 450SC formulation use in fruiting vegetables (250 g a.i./ha, the recommended single application rate for Brazil is slightly lower at 200 g a.i./ha) and 56 g a.i./ha for soybean seed treatment with the 500FS formulation.

2.2.1. Soil

Initial residues of cyclobutrifluram in soil after application of the formulations were estimated using a simple modelled approach (Tincani et al., 2022, 2023) assuming, as a worst case, 100% of the application rate incorporated uniformly throughout a 5 cm layer of the soil with a soil bulk density of 1.5 g dry wt/cm^3 (Zeri et al., 2018).

2.2.2. Seed dust

Untreated soybean seeds (variety S09–C3X) obtained from commercial facilities (cleaned, graded, and certified at source) were treated with the 500FS formulation at the rate of 100 mL formulation/100 Kg seed in a Hege 11 batch treater to ensure uniform coverage. The treated seeds were allowed to dry at room temperature before replicate batches of 100g seed were subjected to Heubach analysis to assess the amount of dust released (Thompson et al., 2023). The amount of dust liberated from the seed was determined by weighing the filter paper from the Heubach unit before and after analysis. This Heubach dust analysis was performed on five replicate batches of treated seed and five replicate batches of untreated seed, and the results expressed as g dust/50g seed.

2.2.3. Residues in pollen and nectar

Predicted residues of cyclobutrifluram in pollen and nectar after seed and soil treatments were generated using the US EPA BeeRex model (USEPA, 2014).

2.3. Risk assessment

2.3.1. Soil

There are two main approaches to assessing the risk to soil invertebrates from the two application scenarios (Tincani et al., 2023). The first uses the Risk Quotient approach (predicted residues in soil/-NOEC), with a Level of Concern (LOC) of 1 indicating an acceptable risk for values below 1 (AEA, 2009; PMRA, 2000). The other utilises the Toxicity Exposure Relationship (TER, effect/predicted residues in soil (EFSA, 2017) with a TER greater than 5 indicating acceptable risk. Both these approaches were used and the NOECs from the toxicity studies with the formulations were used in the risk assessments.

2.3.2. Seed dust

The honeybee contact LD_{50} for the 500FS formulation was used in the risk assessment in a hazard quotient (HQ) (dust deposition rate (g a.i./ha)/LD_{50}). In the absence of data for other bee species, an additional safety factor of 10 was applied to extrapolate to other bee species (IBAMA, 2017).

2.3.3. Residues in pollen and nectar

Uptake and transport of cyclobutrifluram within the plant following use as a soil or seed treatment is likely to result in transfer of residues of the active ingredient, rather than the complete formulation, to pollen and nectar. Similarly, contact exposure of honeybees with residues in pollen and nectar is likely to be negligible. Therefore, the honeybee adult acute oral and adult and larval chronic oral toxicity data for the active ingredient were used to generate Risk quotients (RQ) for the nectar and pollen following the soil treatment and seed treatment uses using BeeRex (USEPA, 2014). This approach assumes all uses are on highly bee attractive crops and honeybees satisfy all their needs only from the treated crop.

3. Results and discussion

3.1. Toxicity assessment

The results of the toxicity assessments are shown below and in Figs. 1 and 2 with further details available in the Supplementary Information.

3.1.1. Soil organisms

Four weeks after the start of the exposure, there was no significant increase in mortality of adult *E. andrei* in any of the treatments ($p > 0.05$). After exposure to the active ingredient cyclobutrifluram, all *E andrei* survived except one in each of the 29.4, 309 and 556 mg a.i./kg dry wt. soil treatments, one in the 1000 mg 450 SC formulation/kg dry wt. soil treatment and one in the 309 mg 500 FS formulation/kg dry wt. soil treatment. There were also no significant differences in change of biomass of adult *E. andrei* (Fig. 1) after exposure to the active ingredient cyclobutrifluram (Fig. 1D, $p > 0.05$, d.f. 31), the 450SC or the 500 FS formulations (Fig. 1E, $p > 0.05$, d.f. 31; Fig. 1F, $p > 0.05$, d.f. 31 respectively) in all treatment rates up to and including the maximum rate of 1000 mg test item/kg dry wt. soil. However, there were effects on the number of juvenile *E. andrei* (Fig. 1) at 309 mg test item/kg dry wt. soil and greater for both the active ingredient cyclobutrifluram (Fig. 1A, $p < 0.05$, d.f.31) and the 500FS formulation (Fig. 1C), and at 556 mg formulation/kg dry wt. soil and above for the 450SC formulation (Fig. 1B, $p < 0.05$, d.f. 31) (for more details see Supplementary Information). Thus, the NOECs for *E. andrei* were defined as 171 mg a.i./kg dry wt. soil for the active ingredient, 71 mg a.i./kg dry wt. soil for the 500FS formulation (42% a.i. w/w) and 117 mg a.i./kg soil for the 450SC formulation (38% a.i. w/w).

Fourteen days after the start of exposure, there was no significant increase in mortality of adult *H. aculeifer* in any of the treatments ($p > 0.05$). After exposure to the active ingredient cyclobutrifluram, all adult *H aculeifer* survived except one in each of the 16.3 and 1000 mg a.i./kg dry wt. soil treatments and two in each of the 29.4, 309 and 556 mg a.i./kg dry wt. soil treatments. After exposure to the 500 FS formulation, all adult *H aculeifer* survived except four in the control, three in the 16.3 mg formulation/kg dry wt. soil, two in each of the 29.4, 171, 556 and 1000 mg formulation/kg dry wt. soil and one in the 95.3 mg formulation/kg dry wt. soil treatment. There were also no significant differences in the number of juveniles produced after exposure to the active ingredient (Fig. 2A, $p > 0.05$, d.f. 31) or the 500FS formulation (Fig. 2B, $p > 0.05$, d.f. 31) at all treatment rates up to and including 1000 mg test item/kg dry wt. soil. Thus, the NOECs for *H. aculeifer* were 1000 mg a.i./kg dry wt. soil for the active ingredient and 420 mg a.i./kg dry soil wt. for the 500FS formulation (42% a.i. w/w).

3.1.2. Bees

There were no mortalities in any of the control groups in the honeybee acute studies. The only mortality in the active ingredient cyclobutrifluram treated groups was 2.5% mortality (1 of 40 bees) observed in a single oral group (8.97 μg a.i./bee). Therefore, the acute contact and oral LD_{50} were >200 and > 72 μg a.i./bee respectively; the oral LD_{50} was based on the actual dose consumed. There were no mortalities observed at any dose in the acute contact studies with either formulation nor in the acute oral study with the 450FS formulation. In the acute oral toxicity with the 500 FS formulation, 3.3% mortality (1 of 30 bees) of the

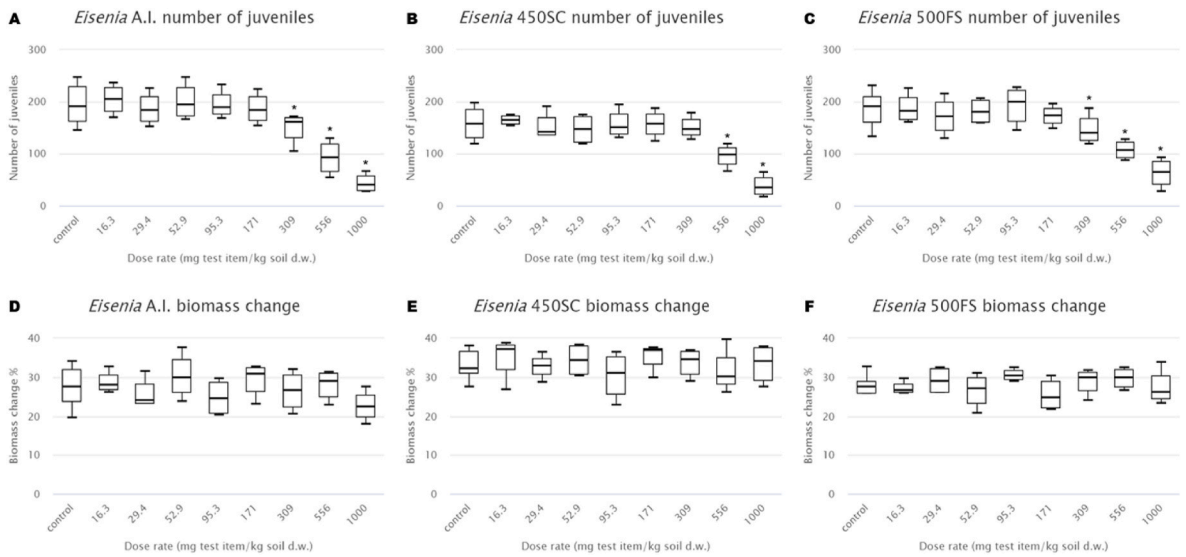

Fig. 1. Effects of exposure to cyclobutrifluram (test item refers to the active ingredient, 450SC or 500FS formulation) on number of juveniles (A–C) and biomass change (D–F) of *Eisenia andrei*. *Statistically significant compared to control (William's *t*-test $p < 0.05$, one sided smaller).

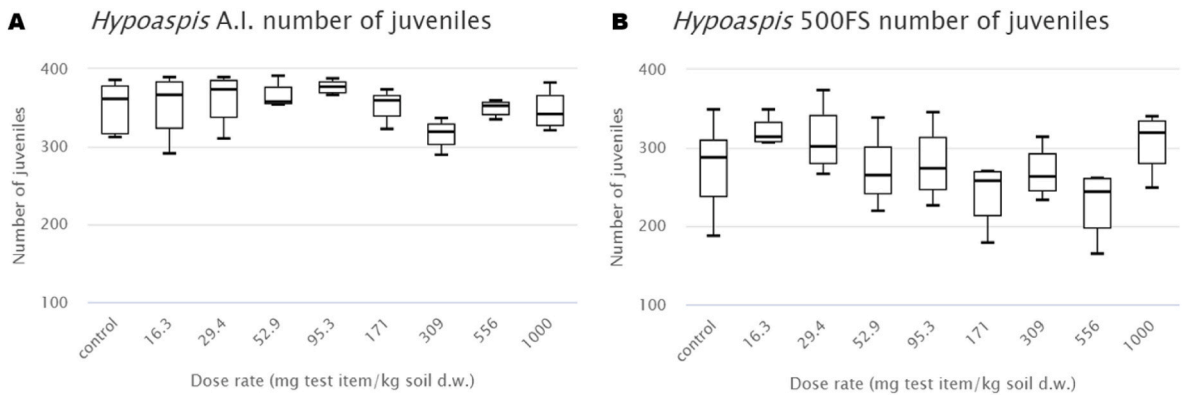

Fig. 2. Effects of exposure to cyclobutrifluram (A) active ingredient, and (B) 500FS formulation on number of juveniles produced by *Hypoaspis aculeifer*.

dosed bees was recorded at the highest dose (1000 μg formulation/bee). Therefore, the acute contact and oral LD_{50} of the 450SC and 500FS formulations were all >1000 μg formulation/bee (>380 μg a.i./bee contact and oral and >420 μg a.i./bee contact and oral for the 450SC and 500FS formulations, respectively).

In the adult chronic (10 days continuous feeding) study, mortality in the solvent control was 15% (within the OECD control validity criteria) and there was no significantly increased mortality at any dose ($p > 0.05$), with mortality ranging from 5 to 20% in a non-dose related manner. Therefore, the adult chronic NOED for cyclobutrifluram was identified as 6.11 μg a.i./bee/day (the highest dose tested).

Control emergence in the 22-day larval study (from 3-day old larva to emergence) was 70.8% in the control containing 0.5% acetone (within the OECD guidance validity criteria) and ranged, in a non-dose related manner, from 50% to 72.9% in the cyclobutrifluram-treated larvae. There was no significant difference in mortality from control in any treatment ($p > 0.05$). Therefore, the NOEC for larval toxicity for cyclobutrifluram was 160 mg a.i./kg diet (NOED 24.6 μg a.i./larva) (the highest dose tested).

3.2. Exposure

3.2.1. Soil

Initial residues of cyclobutrifluram in soil after application were estimated using a simple modelled approach based on a 5 cm layer of the soil (Tincani et al., 2022, 2023). For the 250 g a.i./ha application in-furrow (450SC formulation), this resulted in predicted residues of 0.334 mg a.i./kg soil and for the 56 g a.i./ha seed treatment (500FS formulation) predicted residues of 0.075 mg a.i./kg soil.

3.2.2. Seed dust

The levels of dust liberated during Heubach analysis of the 500FS treated soybean seed was 8 ± 6 μg dust/50g seed (mean ± SD). Based on a maximum drilling rate for soybean in Brazil of 70 kg seeds/ha (Thompson et al., 2023), this equated to 11.2 mg dust liberated per ha of treated seed drilled. The exposure of bees following deposition of liberated dust onto off-crop areas was evaluated using the worst-case assumption that the dust contained 100% cyclobutrifluram and, in the absence of soybean dust drift data, the default worst-case dust drift scenario that 17% of the dust is deposited off-crop (Thompson et al., 2023). Thus, based on these default assumptions, 1.9 mg a.i./ha could be considered as a worst-case estimate of deposition of cyclobutrifluram onto adjacent off-crop plants during drilling of treated soybean seed.

3.2.3. Residues in pollen and nectar

Using the USEPA BeeRex model (USEPA, 2014) with average measured K_{oc} of cyclobutrifluram in Brazilian soils of 470 mL/g and Log K_{ow} of cyclobutrifluram of 3.2, the predicted residues in pollen for a soil treatment at 250 g a.i./ha were 0.17 mg/kg in pollen and nectar. For a seed treatment, the USEPA BeeRex model uses a worst-case default

assumption of 1 mg/kg in pollen and nectar, i.e., the residues are not related to the application rate.

3.3. Risk assessment

3.3.1. Soil

Although the toxicity of 450SC formulation to *H. aculifier* was not directly tested, based on the lack of effects at the highest rate tested of both cyclobutrifluram and the 500FS formulation, it was assumed for the purposes of the risk assessment that the NOEC for the 450SC formulation would be the same, i.e., of 1000 mg test item/kg dry soil wt. (380 mg a.i./kg dry soil wt.). The Risk Quotients and TERs for the identified formulation application scenarios are shown in Table 1. Both these methods of risk assessment concluded low risk to earthworms and soil mites from both formulation application scenarios (see Table 2).

3.3.2. Dust from treated seeds

All the HQ values were far lower than the level of concern indicating low risk for both bees (including honeybees) following any dust deposition during drilling of soybean seed treated with the 500FS formulation containing cyclobutrifluram.

3.3.3. Pollen and nectar

All scenarios (larvae and workers, soil and seed treatment uses) resulted in RQs well below the levels of concern (LOC) (Table 3). The LOC for chronic exposure is 1 and the RQs for larvae were 192- to 1450-fold below this value. For workers the highest values were at least 137- to 998-fold below the acute LOC of 0.4 and 21 to 152-fold below the chronic LOC of 1 (Table 3).

3.4. Conclusion

Bénit et al. (2019) highlighted potential effects of SDHI fungicides on non-target organisms based on the conservation of the target site (succinate dehydrogenase) across fungi, honeybees and earthworms. However, effects detected *in vitro* or predicted *in silico* do not necessarily reflect *in vivo* responses, e.g. due to metabolism or dose-response relationships. Reproduction in *E. andrei* was more sensitive to cyclobutrifluram than in the soil mite *H. aculifier*. However, earthworms exposed *in vivo* to cyclobutrifluram were up to orders of magnitude less sensitive (mortality, number of juvenile produced and biomass change) than to other fungicidal SDHIs (Ernst et al., 2022; He et al., 2021; Ji et al., 2023; Lewis et al., 2016). Toxicity of cyclobutrifluram to honeybees was also shown to be low, with 10-day adult and 22-day larval honeybee chronic studies showing no adverse effects even at the highest doses. Reproduction cannot currently be addressed for the honeybee under laboratory conditions and requires colony level studies (e.g. Thompson et al. (2014)). However, the 22-day honeybee larval study, which assesses effects from 3-day old larvae through to emergence, addresses potential concerns about effects of SDHIs on larval growth (Novoselov et al., 2015). The data for these soil invertebrates and honeybees also showed there are no major differences between the toxicity of cyclobutrifluram and the two formulations (450SC and 500FS), suggesting the toxicity of the formulations is driven by that of the active ingredient. However, in considering effects on non-target species after application of the products in the field, the utility of laboratory generated toxicity, i.e., hazard, data in isolation are limited.

Table 2
Comparison of the predicted maximum dust deposition rate off-crop for the 500FS formulation seed treatment use with the toxicity of the active ingredient to bees (based on honeybee contact LD_{50}/10 (IBAMA, 2017) with acceptable risk concluded when HQ < 50 for bees.

Endpoint	Endpoint value (contact LD_{50}/10)	Exposure (dust deposition in off-crop)	Hazard Quotient (HQ)
Non-Apis bees – (Honeybee contact LD_{50}/10)	>42 μg a.i./bee	0.0019 g a.i./ha	<0.000045

The use of a dose-response study design allowed the effects (LD50/LR50 or NOEC for reproduction) to be compared with potential exposure in standard laboratory surrogate species, e.g., the commercial use rates, and the margins of safety to be evaluated. Together, these data demonstrated no adverse effects on these soil invertebrates or honeybees from exposure to cyclobutrifluram when applied as a soil treatment or seed treatment with orders of magnitude margins of safety when applied at the commercial rates. To achieve residue levels in soil matching these effect levels would require application rates of products orders of magnitude above commercial rates. The use of no-observed effect concentrations, worst-case predicted soil residues and safety factors in the risk assessment with orders of magnitude between the resulting RQ/TER and LoC could be considered to contribute to addressing concerns about the relative sensitivity of these surrogate species compared with the multiple soil invertebrate species present in the field. The TER of 5 has been demonstrated to be protective of populations of earthworms in the field (Christl et al., 2016). Similarly, Ernst et al. (2022) demonstrated no risk to natural earthworm populations after the use of the SDHI fungicide bixafen (NOEC 100 mg/kg) under field conditions where, following commercial practices, soil residues were two orders of magnitude below the NOEC. The use of these approaches to risk assessment have been shown to be protective of effects at the field scale for soil organisms (Christl et al., 2016; Tincani et al., 2023) and honeybees (Thompson and Thorbahn, 2010).

In summary, comparing laboratory generated toxicity data with estimated exposure via soil, seed dust and pollen and nectar, showed that the risk to earthworms, soil mites and bees of the use of cyclobutrifluram either in-furrow or as a seed treatment was orders of magnitude below levels of concern. These lack of effects following use of cyclobutrifluram (TYMIRIUM® technology) for protection of the root mass from both nematodes and fungi such as *Fusarium* can also be considered to support soil health when used alongside no-till and conservation tillage practices.

CRediT authorship contribution statement

Helen Thompson: Writing – original draft, Formal analysis, Conceptualization. **F. Javier Peris-Felipo:** Writing – review & editing. **Natalia Peranginangin:** Writing – review & editing. **Mike Pocock:** Writing – review & editing. **Ana Lia Gayan-Quijano:** Visualization.

Table 1
Comparison of the predicted soil residue following 450SC formulation soil application and 500FS formulation seed treatment use with the reproduction no observed effect concentrations for *E. andrei* and *H. aculifier*. Risk Quotient (RQ, exposure/NOEC with acceptable risk concluded when RQ < 1 (AEA, 2009; PMRA, 2000). Toxicity Exposure Relationship (TER, NOEC/exposure with acceptable risk when TER>5 (EFSA, 2017),).

Species	450SC soil treatment 250 g a.i./ha				500FS seed treatment 56 g a.i./ha			
	NOEC (mg a.i./kg)	Predicted soil residue (mg a.i./kg)	RQ (LOC>1)	TER (LOC<5)	NOEC mg a.i./kg	Predicted soil residue mg a.i./kg	RQ (LOC>1)	TER (LOC<5)
E. andrei	117	0.334	0.00285	344	71	0.075	0.000105	947
H. aculiefer	380		0.000877	11401	420		0.000179	5573

Table 3
Risk quotients (RQ) following acute and chronic exposure of different honeybee castes to nectar and pollen from bee-attractive crops treated with cyclobutrifluram in-furrow or as a seed treatment.

Caste or task in hive	Soil treatment 450SC 250 g a.i./ha			Seed treatment 500FS 56 g a.i./ha		
	Total dose (μg a.i./bee)	Acute RQ (LOC = 0.4)	Chronic RQ (LOC = 1)	Total dose (μg a.i./bee)	Acute RQ (LOC = 0.4)	Chronic RQ (LOC = 1)
Larva	0.0212	–	0.00086	0.124	–	0.00502
Worker (cell cleaning and capping)	0.0114	<0.000159	0.00187	0.0667	<0.000667	0.010908
Worker (brood and queen tending, nurse bees)	0.0257	<0.000357	0.00421	0.150	<0.00150	0.0245
Worker (comb building, cleaning and food handling)	0.0106	<0.000147	0.00173	0.0617	<0.000617	0.0101
Worker (foraging for pollen)	0.00748	<0.000104	0.00122	0.0435	<0.000435	0.00713
Worker (foraging for nectar)	0.0502	<0.000697	0.00821	0.292	<0.00292	0.0478
Worker (maintenance of hive in winter)	0.00532	<0.0000739	0.000871	0.0310	<0.00031	0.00507

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: All authors are employed by Syngenta who manufacture and market cyclobutrifluram.

Data availability

Data are supplied in the supplemntary material

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.cropro.2024.106822.

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