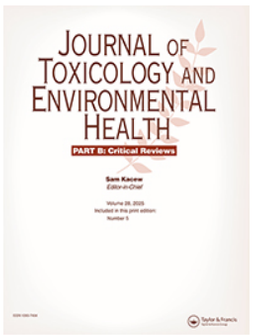

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Acute and repeated exposure toxicity of the insecticide sulfoxaflor on hymenopteran pollinators; sulfoxaflor environmental science review part III

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ABSTRACT

To support regulatory risk assessment, standardized laboratory tests of toxicity to representative species including honeybees (*Apis mellifera L.)*, orchard bees (*Osmia spp.*), and bumblebees (*Bombus spp.*) provide the benchmark toxicity values for use in preliminary Tier 1 assessments and more detailed and realistic higher-tier assessments. In this analysis, we summarize the results of studies of toxicity of SFX to pollinators conducted by the registrant as well as results published in the literature. The geometric mean of 48-hr adult acute oral LD_{50} values for SFX for honeybees was 0.0740 µg SFX bee^{-1} ($n = 5$). Toxicity values for technical grade SFX (SFX-T) and formulated products were not significantly different. The geometric mean 48 hr adult acute contact LD_{50} values for SFX-T and several formulated products were 0.432 ($n = 2$) and 0.202 ($n = 3$) µg SFX bee^{-1}, respectively. Exposures sprayed foliage was not significant after the spray had dried did not cause significant toxicity. Transformation products were not significantly toxic to adult or larval honeybees or other representative bee species. Results showed that, to complete the risk assessment, higher-tier studies were required. Differences in results between standard test methods and the nonstandard methods used in published work affect the outcome of the risk assessment. An understanding of these differences reconciled the differences in the reported findings.

Introduction

This is the third paper in a series of environmental science reviews for the systemic insecticide, sulfoxaflor (SFX) (Kramer et al. 2025). SFX is formulated for spray application to control sap-feeding insects. For a general summary of its chemical, physical and environmental properties (see Solomon et al. 2025b). In this review, laboratory studies on the toxicity of SFX to representative hymenopteran pollinator species, including honeybees (*Apis mellifera L.*), bumblebees (*Bombus* spp.), and several solitary bees (e.g., blue orchard bee, *Osmia bicornis*) are considered and evaluated. Results of studies conducted using standard guidelines, as well as information from the open literature were included in a weight-of-evidence (QWoE) assessment of potential adverse effects.

In this assessment, the available studies were evaluated for quality and relevance using a standard rating system in which each study was ranked on a scale of 1–4 and the mean value of the ratings (±2SE) was determined to obtain an overall measure of the quality and relevance of the data in each section of the assessment (Solomon and Stephenson 2017). The results were then graphed (Figures 1–5). In most cases, studies that used validated, standard methods and met Good Laboratory Practice (GLP) standards ranked higher in quality. Use of standard methods designed for use in risk assessment also ranked higher in relevance. The details of the rating process and the individual ratings for each study are included in the supplementary information (SI). For reviews of the methodology used to evaluate studies, see (Farruggia et al. 2022) and (Solomon and Stephenson 2017).

In this review, the risk assessment is limited to a Tier 1 oral calculation, which is sufficient to show the need for higher tier studies. An assessment of risks

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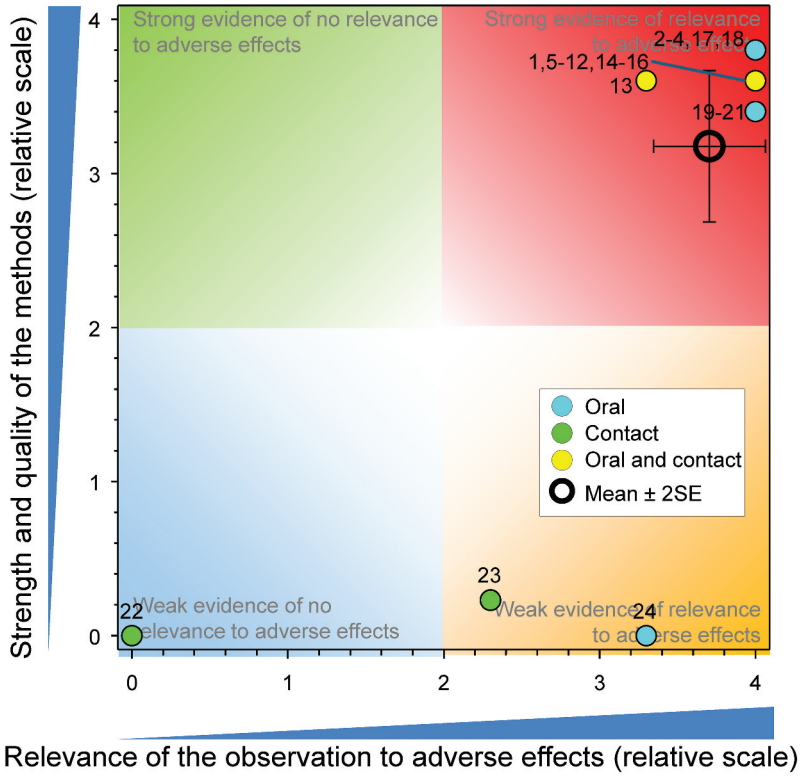

Figure 1. QWoE ratings for honeybee adult acute oral and contact toxicity studies. Some data points overlap ($N = 24$). See this graphic in the SI for identification of individual studies and the scoring sheets.

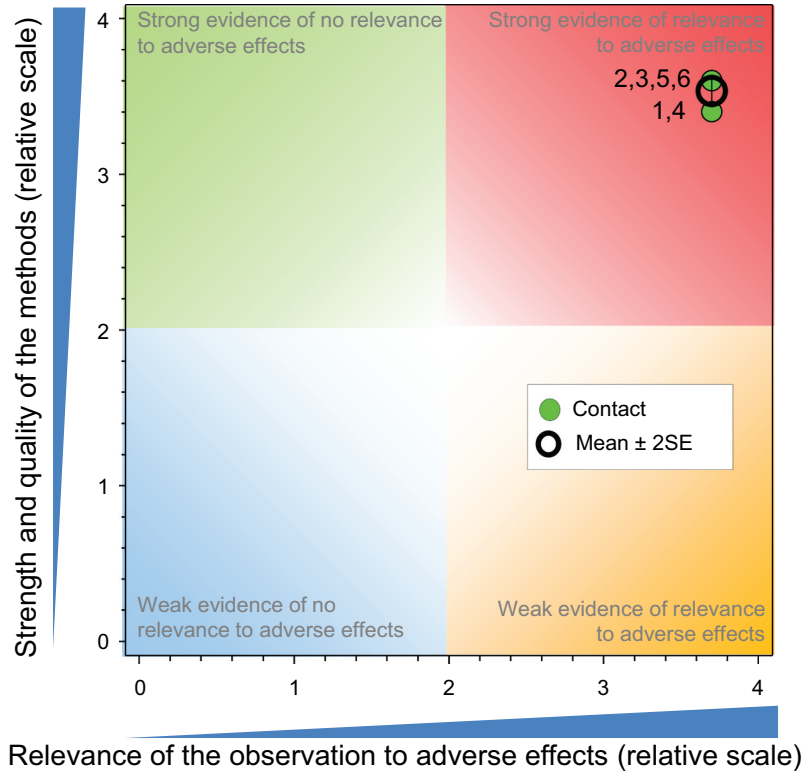

Figure 2. QWoE ratings for honeybee adult aged foliar contact studies toxicity studies. Some data points overlap ($N = 6$). See this graphic in the SI for identification of individual studies and the scoring sheets.

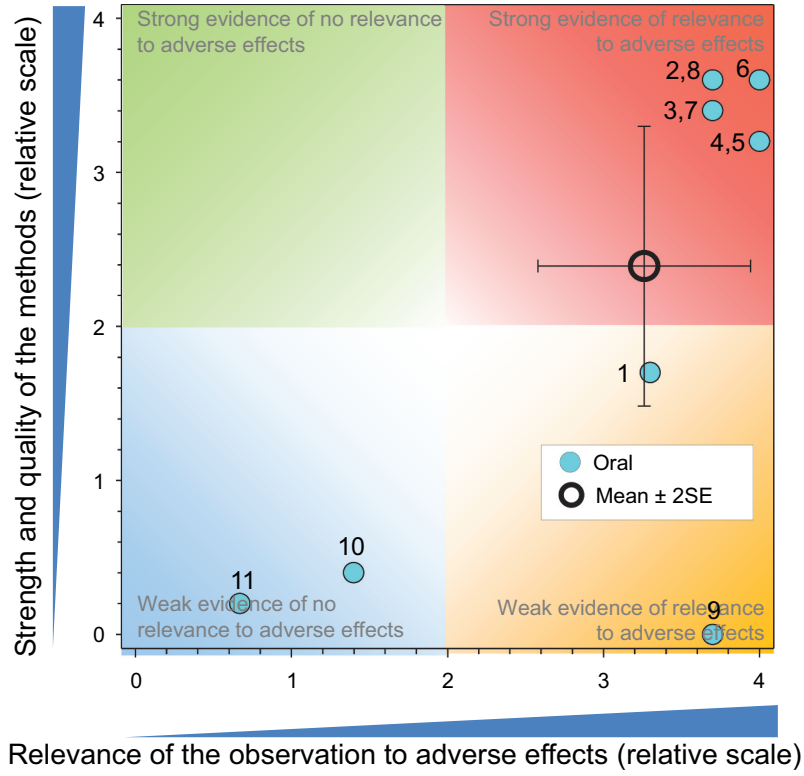

Figure 3. QWoE ratings for honeybee adult repeated dose and chronic studies with SFX. Some data points overlap ($N = 11$). See this graphic in the SI for identification of individual studies and the scoring sheets.

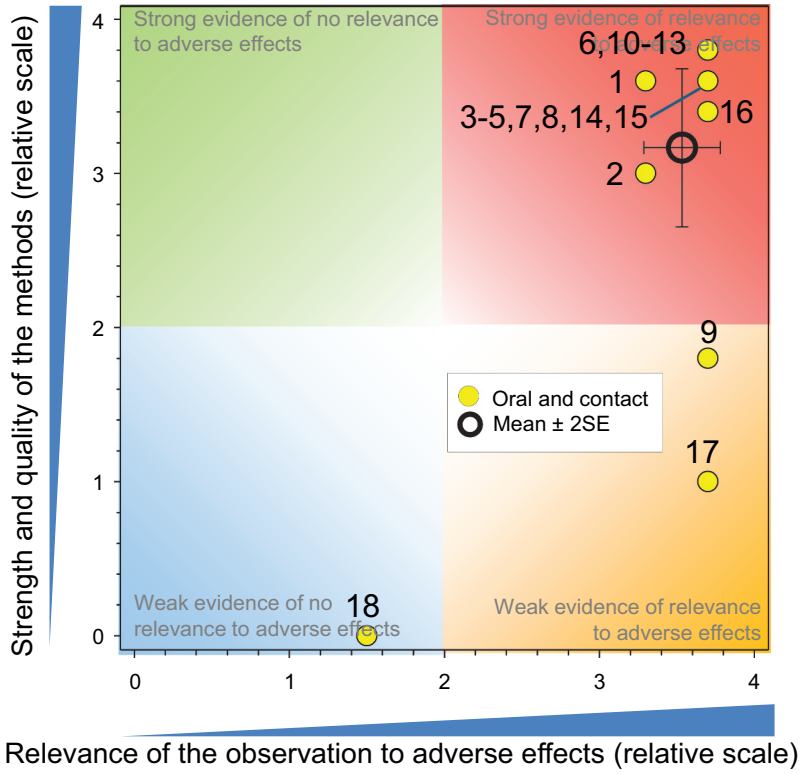

Figure 4. QWoE ratings for honeybee larval toxicity studies. Some data points overlap ($N = 18$). See this graphic in the SI for identification of individual studies and the scoring sheets.

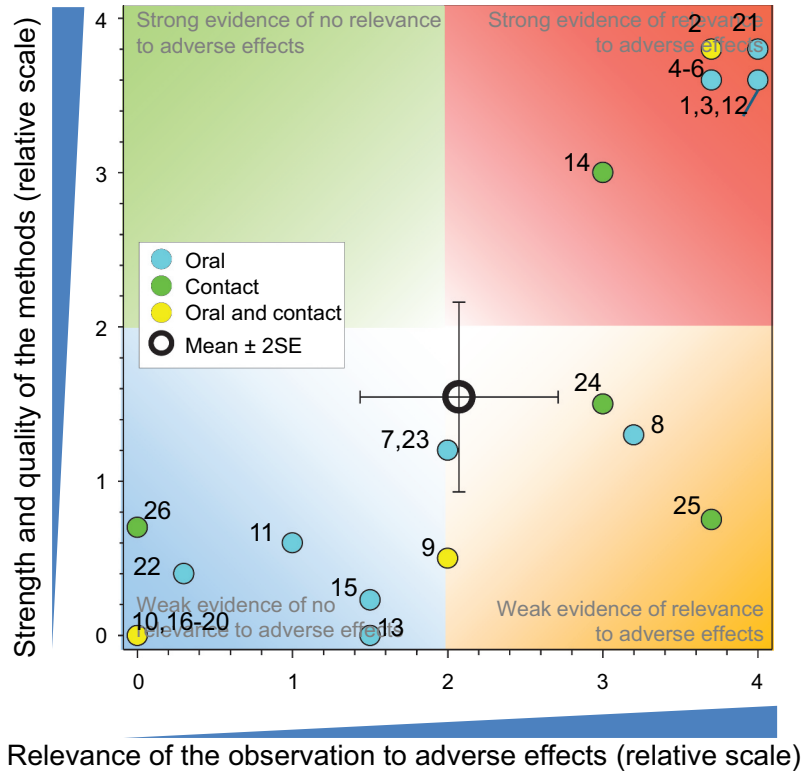

Figure 5. QWoE ratings for toxicity studies with sulfoxaflor in other species of bees. Some data points overlap ($N = 26$). See this graphic in the SI for identification of individual studies and the scoring sheets.

posed by SFX and its formulated products to pollinators based on field study results is presented in the fifth paper in the series (Purdy et al. 2025b). Discussion of some aspects of the standard test methods and the nonstandard methods used in published work is included in this review since these methods affect the outcome of the studies. An understanding of differences in methodology helps to reconcile the differences in the reported findings.

Review of laboratory studies of acute and delayed effects of SFX on bees

Determining the potential for harm to hymenopteran pollinators is a central component of conducting risk-benefit analyses for pesticides. Given the diversity of species, habitats, levels of social organization, and life stages that are present in agricultural environments, the approach has been to test surrogate species that can be reliably managed under laboratory conditions. Results of these studies, together with physical, chemical, and environmental properties can then be used to prescribe additional tests to answer specific questions about possible risks that might occur under current conditions of use. Several regulatory agencies, including the USEPA use a tiered approach to complete a pesticide risk assessment. The review of the initial set of laboratory tests generally takes place during Tier-1 of this risk assessment process. Tier-1 assessments are designed to be simple and conservative with the purpose of identifying those scenarios posing negligible risk with high confidence and those scenarios requiring further risk analysis. Additional semi-field and field tests may be conducted, where necessary, to provide more realistic estimates of risk. Semi-field and field studies are referred to as Tier-2 and Tier-3 studies, respectively (USEPA 2014). At each stage in the review, the results of the studies are compared to benchmark criteria to ascertain the need for higher tier more precise studies and analyses.

The higher-tier risk assessment outcome is still conservative but is more detailed and realistic. The study designs of higher-tier studies include more parameters such as colony-level effects, but they are still done to represent worst-case exposure scenarios for the approved uses of the pesticide. A widely held

misconception is that current regulatory risk assessments are based on the acute toxicities of pesticides (Mundy-Heisz, Prosser, and Raine 2022). However, as illustrated by the sulfoxaflor registration decision (USEPA 2019), regulatory decisions are based on the highest tier, most realistic studies needed to reach a risk assessment conclusion (USEPA 2019). The assessment considers any mandated mitigation measures. The risk assessment decision includes information from studies provided by registrants for regulatory purposes and results of studies published in the peer-reviewed literature, provided they meet basic criteria of quality, but these criteria are more flexible than those applied to regulatory studies. The use of standard test methods or method validation is not required, and published studies are exempt from compliance with Good Laboratory Practice (GLP) regulations (USEPA 2016). Furthermore, since many published studies are not designed to support risk assessment, they may not report key parameters such as the median lethal dose (LD_{50}) or the No Observed Effect Level (NOEL). The quality and relevance ratings presented in this review and in the supporting information (SI) include examples of published studies that were considered but were not usable for risk assessment.

Standardized test procedures are established for Tier-1 testing of effects of crop protection chemicals on various life stages of bees in the laboratory (OECD (2024); USEPA 2016). The Tier-1 studies are commonly conducted using easily available and manageable species, such as the western honeybee (*A. mellifera*), the eastern honeybee, which is also called the Asiatic honeybee or Asian honeybee (*A. cerana*), and various species of bumble bees (*Bombus* spp.), which are social bees. Tier 1 testing protocols are also under development and are sufficiently defined to be used for several solitary species, such as the mason bee (*O. bicornis*), and alfalfa leaf cutting bee (*Megachile rotundata*) (EFSA 2023; USEPA 2016). In this report, "honeybee" refers to *A. mellifera* except where specifically indicated.

In acute oral toxicity laboratory tests, honeybees are isolated from the regulatory processes of the colony and deprived of food prior to the test. When the test material is made available, it is consumed immediately unless the test material has a strong antifeedant effect (OECD 2017). Under these conditions, ingested food is not held in the crop for later consumption or sharing with other bees. The results are thus worst case for honeybees because there is no dilution arising from mixing and sharing of nectar, often from non-agricultural sources, as occurs in the colony nor is there degradation and dissipation prior to consumption as occurs following hive storage. Food deprivation also occurs prior to the first dose in repeated dose larval toxicity studies (OECD (2013, 2021).

Studies done with honeybees are considered to be protective of most other bee species, despite differences in individual characteristics (EFSA 2023; Farruggia et al. 2022). Furthermore, honeybees have higher nectar consumption rates to support thermoregulation of the hive, and this requirement increases when they are in smaller groups or isolated as in the laboratory test conditions(Free and Spencer-Booth 1958). This supports the use of laboratory tests with honeybees as a surrogate for most bee species in the Tier 1 screening level assessment. The influence of the individual sensitivity, life history, behavior, and physiology of additional bee species can then be evaluated in higher tier studies.

The body mass of honeybees varies greatly with gut contents (Allen 1959; Haydak 1934). Therefore, the toxic effects benchmarks, e.g., the no adverse effect dose level (NOAEL) for these bees are not typically normalized to body mass (OECD 2017). Other Apis bees are treated similarly (Corteva Agriscience 2012a), but endpoints for non-Apis bees are sometimes normalized to body mass (Medrzycki et al. 2013), generally assuming a standardized average body mass for the species (Pamminger 2021).

Results of adult acute oral and contact toxicity tests

The results from high quality GLP acute oral and contact toxicity tests on honeybees (*A. mellifera*) conducted with technical grade SFX (SFX-T) and several formulated products by the registrant are listed in Table 1 for products containing only SFX and Table 2 for multiple active ingredient products. Results of studies of acute oral toxicity for the significant transformation products SFX-sulfone, SFA-sulfoximine, SFX-urea and SFX-ethanol and the

Table 1. Acute oral toxicity of SFX and its transformation products to adult honeybees.

Test substance (%by wt.)	$LD_{50,}$ µg bee^{-1} (95% CL)[a]	Reference
SFX-T (95.6)	48 hr: 0.146 (0.098, 0.195)	Corteva Agriscience (2007b) (USEPA)
SFX-T (99.7)	48 hr: 0.0604 (0.0360, 0.102)	Corteva Agriscience (2022c)
SFX-A (100)	48 hr: 0.180 (0.162, 0.202)	
SFX-B (99)	48 hr: 0.0584 (0.0515, 0.0663)	
SFX 120 SC GF-2626 (12.0)	48 hr: 0.065 (0.052, 0.081) LOED 0.049 NOED 0.023	Corteva Agriscience (2010d)
SFX 240 SC GF-2032 (22.0)	48 hr: 0.0515[b] (0.0431, 0.0617) LOED 0.0498 NOED 0.0254	Corteva Agriscience (2009e)
SFX 500 WG GF-2372 (49.9)	48 hr: 0.075 (0.064, 0.093) LOED 0.055 NOED 0.064	Corteva Agriscience (2010b)
SFX-sulfone X11519540 (98)	48 hr: > 91.3 NOED ≥ 91.3	Corteva Agriscience (2010e)
SFX-sulfoximine X11579457 (97)	48 hr: 45.7 LOED 30.4 NOED 16.5	Corteva Agriscience (2010a)
SFX-urea X11719474 (99.9)	48 hr: > 100 LOED 99.0 NOED 82.4	Corteva Agriscience (2009f)
SFX-ethanol X11721061 (99)	48 hr: > 103.5 LOED n.r. NOED 103.5	Corteva Agriscience (2010f)

a) The endpoints are calculated from nominal concentration and measured food consumption and reported as the concentration of SFX, enantiomer, or transformation product as applicable. NOED and LOED values for mortality or other endpoints are included if they were reported.
b) Value selected by US EPA for risk assessment (USEPA 2019). Abbreviation: n.r. = not reported.

diastereomers SFX-A and SFX-B are also included in Table 1. The structures and other information for these substances are provided in (Solomon et al. 2025b). Results of acute contact studies are presented in Tables 2–4. The contact route of exposure is not considered to be relevant for the transformation products as they are not formed prior to contact.

In the QWoE ratings the quality of the methods and the relevance of the findings to the risk assessment are rated on a scale from 1–4 for each study. The results of the QWoE ratings for the acute oral and contact studies cited in this section are presented graphically in Figure 1. The source documents are included in Section 3.1 of the SI.

The QWoE ratings for the aged foliar contact studies are presented in Figure 2. The figures include a symbol for the mean (±2SE) for the quality and relevance ratings. The key to the source of the data is provided in a similar figure in the SI, which also contains the summaries of the studies.

Lethality was the main effect at higher doses, with some behavioral effects at lower doses reported in the source documents. Effects after oral exposure were observed within 24–48 hr. A published acute oral toxicity study for SFX-T (Azpiazu et al. 2021) used older forager bees and was compromised by high mortality in the control group. The LD_{50} was only reported for 24 hr and was therefore excluded from calculation of the 48-hr geometric mean. In a better quality, GLP-compliant study of oral exposures to the two diastereomers (SFX-A and SFX-B) of SFX, the mixed diastereomers and the SFX-B diastereomer were approximately 3-fold more potent than SFX-A (Table 1) (Corteva Agriscience). This is attributed to differences in rates and extents of uptake after ingestion among the groups of bees in the study. The geometric mean of the 48-hr, acute, oral LD_{50} values for adults was calculated. The results from three GLP-compliant studies of formulated products containing only SFX (Table 1) were not

Table 2. Acute, oral, and contact toxicities of SFX formulated products containing a second active ingredient to adult honeybees.

Test substance and Active Ingredients (% a.i. by wt.)[a]	LD_{50} , µg product bee^{-1} (95% CL)[b]	Reference
SFX GF-2628 (SFX 9.61; lambda-cyhalothrin 14.4)	Oral 24 & 48 hr: 0.516 (0.452, 0.584)	Corteva Agriscience (2012b)
SFX GF-2628 (SFX 9.62; lambda-cyhalothrin 15.0)	Contact 24 hr: 0.733 (0.667, 1.032) 48 hr: 0.665 (0.429, 1.032) NOED 0.25 LOED 0.50	Corteva Agriscience (2011b)
SFX GF-2680 (SFX 20; Spinetoram 19.9)	Oral 24 hr: 0.63 (0.48, 0.82) 48 hr: 0.47 (0.36, 0.62) 72 hr: 0.45 (0.35, 0.58) Contact 48 hr: 0.21 (0.17, 0.26) 72 hr 0.16 (0.13, 0.20) 96 hr: 0.17 (0.14, 0.20)	Corteva Agriscience (2011a)
SFX GF-3052 SFX 30.0; spinetoram 10.0)	Oral 24 hr & 48 hr: 0.15 (0.14, 0.17)	Corteva Agriscience (2013b)
SFX GF-3971 (SFX 3.8; bifenthrin 11.4)	Contact 48 hr: 30 (26, 34) 72 hr: 0.28 (0.24, 0.33) Oral 24 &48 hr: 0.481 (0.374, 0.612) NOED 0.10 LOED 0.30	Corteva Agriscience (2022b)
SFX GF-3971 (SFX 3.8; bifenthrin 11.4)	Contact 24 & 48 hr: 0.772 (0.643, 0.989)	Corteva Agriscience (2022b)

a) Concentration is the measured percent by weight (g $100\,g^{-1}$).
b) Endpoint values are based on the measured weights and volumes dispensed and the amount of dose mixture consumed, with no chromatographic confirmation. NOED and LOED values for mortality or other endpoints are included if they were reported.

Table 3. Acute contact toxicity of SFX and formulated products containing only SFX to adult honeybees.

Test substance & concentration[a] (% measured)	Median lethal dose[b] LD_{50}, µg a.i. bee^{-1} (95% CL)	Reference
SFX-T (96.6)	48 hr: 0.539 (0.291, 0.932) 72 hr: 0.379 (0.303, 0.475) LOED 0.4 NOED 0.2	Corteva Agriscience (2007a)
SFX-T (99.7)	48 hr: 0.364 (0.312, 0.425) 72 hr: 0.358 (0.306, 0.418)	Corteva Agriscience (2022c)
SFX-A (100)	48 hr: 0.546 (0.448, 0.667) 72 hr: 0.519 (0.425, 0.634)	
SFX-B (99)	48 hr: 0.314 (0.257, 0.380)	
SFX 120 SC GF-2626 (12.0)	48 hr: 0.283 (0.222, 0.432) LOED 0.272 NOED 0.124	Corteva Agriscience (2010c)
SFX 240 SC GF-2032 (22.0)	48 hr: 0.130 (0.105, 0.161)[c] LOED 0.103 NOED 0.047	Corteva Agriscience (2009c)
SFX 500 WG GF-2372 (49.7)	48 hr: 0.224 (0.101, 0.301) LOED 0.08 NOED 0.032	Corteva Agriscience (2009d)

a) Concentration is the measured percent by mass (g 100^{-1} g).
b) Endpoint values were calculated from nominal dosing concentrations and measured food consumption per test chamber. They are listed for 48 hr observation time, with values for longer observation times if they were lower (more toxic). NOED and LOED values for lethality are included if they were reported. None of the reports provided endpoints in units of dietary concentration.
c) Value selected by USEPA for risk assessment (USEPA 2019).

Table 4. Acute toxicity from contact of adult honeybees to aged foliar residues.

Substance	Application rate (g a.i./ha)	Average % mortality[a] vs weathering time (hr)					Reference
		3	6	24	48	≥ 72	
SFX 240 SC	200	< 5	< 5	< 5	–	–	Corteva Agriscience (2008)
GF-2032							
SFX 500 WG	200	15	15	19	–	–	Corteva Agriscience (2009b)
GF-2372	100	7	8	19	–	–	

a) In the foliar residue exposure test, the results are from 24 hr exposure to foliage starting at listed weathering times after the liquid from a spray application had dried, typically 45 min. Therefore, the RT25 values were not calculated. In most cases, the tests were continued until no significant difference in mortality from control was seen.

significantly different by a T-test and thus were included in the geometric mean calculation, resulting in a value of 0.0740 µg a.i. bee^{-1} ($n = 5$, 95% confidence limits: 0.0528, 0.1006 calculated as the antilog of the $T_{0.05}$ confidence interval of the log-transformed values). The individual diastereomer values do not represent the actual active ingredient under field conditions and thus were excluded from the geometric mean calculation. For comparison to this geomean value, the USEPA chose the most conservative value of 0.0515 µg a.i. bee^{-1}, based on the formulated product SFX 240 SC as a point estimate (USEPA 2019). The two values are quite similar. Additional published papers are available but did not meet the standards of quality for use in risk assessment (See rating forms and Table S1 in the supplementary information).

The toxicity studies for the multiple active ingredient products in Table 2 were intended to measure the toxicity of the products themselves, not identify the individual contributions to the overall toxicity. Thus, these studies are not considered further but are provided here for completeness.

The formulated products had similar toxic potencies to the active ingredient by oral exposure. However, the formulated products were less toxic by contact exposure. The average contact LD_{50} from 2 studies with SFX-T was 0.452 µg a.i. bee^{-1}, whereas the mean from 3 single-active ingredient formulated products was 0.192 µg a.i. bee^{-1}. The difference is statistically significant ($p = 0.0439$, T-test, 2-sided, $n = 2$, 3), but this test might be unreliable due to the small sample sizes. Regardless, the geometric mean of the 48-hr contact LD_{50} values for SFX-T (excluding the diastereomers) and the formulated products (Table 3) were calculated separately and are 0.432 and 0.202 µg a.i. bee^{-1}, respectively ($n = 2,3$; 95% CI 0.280, 0.667, and 0.129, 0.317, respectively).

Results of toxicity tests with aged foliar residues of sulfoxaflor

The results from GLP compliant studies of the acute toxicity of aged foliar residues to adult honeybees are listed in Table 4. The tests involved foliage sprayed at the highest recommended application rate and then left for 3, 6, and 24 hr after application, at which point bees were exposed to the foliage for a 24-hr period. This scenario produces a worst-case representation of the bee crawling between flowers, such as a spike-type inflorescence or crawling on the surface of a larger flower.

Observations of mortality and sublethal impairment, including lethargy or immobility (lying on back) were recorded 4 hr and 24 hr after start of exposure and afterward, if necessary, until there was no difference in mortality from controls. The results show that the effects of SFX from contact with foliar spray deposits are minor and temporary with < 20% mortality when exposed to 2 hr aged residues. The results show that, while the residues remain in and on plant material, transfer of SFX onto bees during contact rapidly decreases after the spray on the foliage has dried, so that very little uptake occurs.

Four additional GLP-compliant studies involving aged foliar residues were conducted with products that contain SFX and other insecticides, but the results are not considered herein because the toxicity contribution from SFX was not

Table 5. Chronic dietary exposure effects for SFX, its formulations and its transformation products on adult honeybees.

Test substance (% by wt.)	10-day repeated dose oral toxicity study endpoint: Base[a]	Concentration in food[b] mg kg^{-1} (95% CL)	Daily dose[c] µg bee^{-1} day^{-1} (95% CL)	Reference
SFX-T (95.6)	n, m	LC_{50} n.r. LOAEC > 0.575 NOAEC 0.575 (NA[d], 0.143)	LD_{50} n.r. LD_{10} 0.008082 (NA, 0.0493) Mortality and food consumption LOAEL 0.01839 (USEPA)[c] NOAEL 0.01567, 0.01160 (EPA)[c]	Corteva Agriscience (2016a)
SFX-T (95.6)	n, m	LC_{50} > 0.433 LOAEC 0.433	LD_{50} > 0.01146 LD_{10} > 0.01146 Mortality: NOAEL 0.01146, 0.00998 (EPA)[c] Food consumption: LOAEL 0.010 (EPA)[c,e] NOAEL 0.0057, 0.00539 (EPA)[c]	Corteva Agriscience (2017b)
SFX 120 SC GF-2626 (11.5)	n	LC_{50} 1.54 (1.39, 1.71) LC_{20} 1.17 (1.01, 1.31) LC_{10} 1.02 (0.83, 1.15) NOEC 0.92	LD_{50} 0.027 (0.025, 0.029) LD_{20} 0.022 (0.018, 0.024) LD_{10} 0.019 (0.014, 0.021) NOEL 0.017	Corteva Agriscience (2023c)
SFX 500 WG GF-2372 (50.4)	m	LC_{50} 2.88 (2.65, 3.13) LC_{20} 2.33 (2.04, 2.54) LC_{10} 2.08 (1.75, 2.31) NOEC 1.59	LD_{50} 0.051 (0.047, 0.054)	Corteva Agriscience (2023b)
X11519540 SFX-sulfone (100)	m	LC_{50} 951.1 (246.7, 2255) LC_{20} 430.0 (13.6, 873.4) LC_{10} 254.2 (1.6, 590.4) Mortality: LOEC 124, NOEC 45.5 Body mass: LOEC 360, NOEC 124	LD_{50} 10.3 (5.53, 17.1) LD_{20} 5.86 (1.45, 9.10) LD_{10} 4.04 (0.53, 6.84) Mortality: LOEL 2.67, NOAEL 1.12 Body mass: LOEL 5.09, NOEL 2.67	Corteva Agriscience (2021b)
X11579457 SFX-sulfoximine (100)	n	LC_{50} > 3000 LC_{20} 742.4 (406.1, 1216) LC_{10} 242.8 (84.4, 437.0) Mortality: LOEC 111, NOEC 37 Body mass: LOEC n.r., NOEC ≥3000	LD_{50} > 38.3 LD_{20} 13.3 (8.06, 19.8) LD_{10} 5.40 (2.16, 8.74) Mortality: LOEL 2.75 NOAEL 0.97 Body mass: LOEL n.r. NOEL ≥ 38.3	Corteva Agriscience (2010a)
X11719474 SFX-urea (99.7)	n	LC_{50} 1469 (1261, 1711) Mortality: LOEC 3000, NOEC 1000 Body mass: LOEC 1000, NOEC 333	LD_{50} 23.3 (20.4, 26.8) Mortality: LOEL 39.3 NOEL 18.8 Body mass: LOEL 18.8 NOAEL 8.01	Corteva Agriscience (2021c)
X11721061 SFX-ethanol (99)	a	48 hr: >130 $LOEL_{emergence}$ 130 $LOEL_{body\ mass}$ 4.13 $NOEL_{body\ mass}$ < 4.13	LD_{50} 48 hr: > 20 $LOEL_{emergence}$ 20 $LOEL_{body\ mass}$ 0.636 $NOEL_{body\ mass}$ < 0.636	Corteva Agriscience (2010f)
X11721061 SFX-ethanol (82)	n	LC_{50} 1394 (1098, 1744) Mortality: LOEC 942, NOEC 314 Body mass: LOEC n.r., NOEC ≥ 2827	LD_{50} 24.3 (19.8, 29.8) Mortality: LOEL 18.4 NOAEL 7.50 Body mass: LOEL n.r., NOEL ≥ 43.8	Corteva Agriscience (2021a)

a) For studies with base "n" and "m," exposures were calculated with nominal and analytically measured values, respectively.
b) The dosing solution was fed daily for 10 days at each concentration. The endpoints reflect the actual consumption of food in each study but are reported as concentration of SFX or transformation product in the diet.
c) The endpoints reflect the nominal dosing concentrations with measured amounts of diet that was consumed. For SFX-T, the values based on measured concentration of SFX or transformation product. Food consumption endpoint values used in the EPA risk assessment are included for comparison (USEPA 2019).
d) Abbreviations: NA = not applicable or not possible to calculate; n.r. = not reported.
e) The LOEL of 0.01 µg a.i. bee^{-1} day^{-1} was selected for risk assessment.

Table 6. Acute toxicity of SFX-T (95.6%) to honeybee larvae.

Life stage & evaluation time (days)	Endpoint[a] Concentration in food mg a.i. kg^{-1} (95% CL)	Endpoint[a] Total dose µg a.i. bee^{-1} (95% CL)	Reference[b]
Larvae (day-7) mortality	LC_{50} 144[c] LOEC 22	LD_{50} 4.90 (2.77, 8.32) LOEL 0.74 NOEL < 0.74 Sublethal effects: None	Corteva Agriscience (2018)
Larvae (day-7) mortality	n.r.[d]	LD_{50} 2.65 (1.33, 5.27) (extrapolated) LD_{10} 0.22 (0.067, 0.740) NOEL 0.22[e] NOAEL > 0.415[e] Sublethal effects: None	Corteva Agriscience (2012d)
Pupae (day-18) mortality	n.r.	LD_{50} 0.22 (0.08, 0.35)[f] LOEL n.r. NOEL n.r.[e] Sublethal effects: None	
Larvae (day-7) mortality	n.r.	LD_{50} 11.4 (9.78–13.35) Sublethal effects: n.r.	Kim et al. (2022)

a) The results were based on the nominal concentrations. The dosing solution was fed once on day-4 and the total mortality was recorded on day-7, giving a 72 hr LD_{50} or on day-18.
b) The endpoints reflect the actual amounts of diet that was consumed.
c) USEPA reported 151 mg a.i. kg^{-1} based on measured concentration.
d) Abbreviation: n.r. = not reported.
e) USEPA assessment used a value of > 0.415 µg a.i. bee^{-1} as the NOAEL from the day-7 results from the repeated dose larval toxicity study (USEPA 2019).
f) High control mortality (50%) was reported after day-7, making this value less reliable.

distinguished from the other ingredients (Corteva Agriscience 2013c, 2013d, 2013e, 2020a).

Adult repeated dose (chronic) toxicity of sulfoxaflor

Results of tests with longer-term chronic dietary exposures of adult honeybees indicate that SFX is toxic to individual bees under laboratory conditions (Table 5), the results show no indication of additional toxic modes of action compared to the single-dose experiments. LD_{50} values could not be calculated for two studies with SFX-T (Corteva Agriscience 2016a, 2017b) because mortality was < 50% at the highest test doses (0.0157 and 0.0115 µg SFX bee^{-1} day^{-1}, respectively). For risk assessments, a conservative threshold NOAEL of 0.01 µg SFX bee^{-1} day^{-1} is consistent with the conclusions of the USEPA review (Table 5).

The geometric mean oral chronic LD_{50} for adult honeybees, based on assays of two formulated products, was 0.037 µg SFX bee^{-1} day^{-1}. The NOAELs, at which no lethality or behavioral effects were observed were 0.005 to 0.016 µg SFX bee^{-1} day^{-1} (Table 5). Additional endpoints and adjusted values for endpoints calculated by the USEPA, based on measured concentrations instead of nominal values, are included in Table 5 (USEPA 2019).

Toxicity of sulfoxaflor to larval honeybees

Studies of acute toxicity of insecticides to larval bees can be conducted using OECD guideline 237 (OECD 2013), which specifies a single dose on day 4 with post-treatment monitoring up to day-7, giving a 72-hr LD_{50}. This value represents the combined oral and topical intake since the larva is in contact with the liquid diet for much of its surface. Although a single day-exposure is less realistic than repeated dosing through the larval stage, these acute endpoints have value for comparison of toxicities among compounds (Farruggia et al. 2022). Results of acute toxicity tests on SFX with honeybee larvae are listed in Table 6 and scores for quality are shown in Figure 4.

In the repeated dose (chronic) larval test, the bees are raised from grafted larvae to emergence in artificial cells on an artificial diet and cells are not capped as would naturally be conducted by worker bees during pupation. In this way, cells, and the larvae they contain can be observed directly throughout the test. The bees are fed when grafted to get them established in the test cells day^{-1}, starved for a day and then the test material is added to the diet at a series of dose levels daily from day-3 to −6. The total consumption of the diet is recorded at the end of the larval stage. This

Table 7. Chronic toxicity of sulfoxaflor to honeybee larvae.

Life stage & evaluation time (days)	Endpoint: Base[a]	Endpoint: Concentration in food[b] mg kg^{-1} (95% CL)	Endpoint: Daily dose[c] µg bee^{-1} (95% CL)	Reference
SFX-T 95.6% w/w				
Larvae (day-7) mortality	NA	LC_{50} n.r.[d] (Avg. mortality ≤ 10% at highest dose)	LD_{50} n.r.	Corteva Agriscience (2017c)
Imago (day-22) adult emergence	n	EC_{50} 2.09 (1.83, 2.40) EC_{20} 1.30 (1.02, 1.51) EC_{10} 0.943 (0.659, 1.16) LOEC 1.30 NOEC 2.59	ED_{50} 0.322 (0.282, 0.370) ED_{20} 0.200 (0.157, 0.233) ED_{10} 0.145 (0.101, 0.179) LOEL 0.400 NOEL 0.200 Sublethal effects: None	
Larvae (day-8) mortality	m	No significant mortality vs control	Sublethal effects: None	Corteva Agriscience (2016b)
Imago (day-22) emergence	m	LC_{50} NA[d] LC_{10} 1.64 LOEC 2.59 NOEC 1.32	LD_{50} > 0.415 (mortality < 50%) LD_{10} 0.269 (NA − 0.366) LOEL 0.415 NOEL 0.212 Sublethal effects: None	
Larvae (day-7) mortality	n	n.r.	LD_{50} 0.247 (0.116, 0.526) (extrapolated) LD_{10} 0.085 (0.005, 1.49)	Corteva Agriscience (2012e)
Pupae (day-18) mortality	n	n.r.	LD_{50} 0.054 (0.001, 2.08)[e] LD_{10} 0.038 (0.001, 2.02) Sublethal effects: None	
Larvae (day-7)	n	n.r.	LD_{50} 11.4 (9.78, 13.35) Sublethal effects: reduced body mass	Kim et al. (2022)
Imago (day-22)	n	n.r.	LD_{50} 0.212 (0.180, 0.254) NOEL 0.0625 Sublethal effects: reduced body mass, deformed wings (not treatment-related).	
SFX 120 SC GF-2626 11.5%w/w				
Imago (day-22) Adult emergence	n	EC_{50} 3.04 (2.65, 3.47) EC_{20} 1.30 (1.16, 1.73) EC_{10} 0.891 (0.646, 1.12) LOEC 2.6 NOEC 0.500	ED_{50} 0.469 (0.409, 0.536) ED_{20} 0.224 (0.178, 0.266) ED_{10} 0.137 (0.099, 0.173) LOEL 0.154 NOEL 0.077 Sublethal effects: Malformed wings at ≥7.7 µg a.i. $larva^{-1}$ total dose	Corteva Agriscience (2023e)
SFX 240 SC GF-2032 21.6% w/w				
Imago (day-22) Adult emergence	m	EC_{50} 3.48 (2.76, 4.31) EC_{20} 1.07 (0.64, 0.149) EC_{10} 0.486 (0.229, 0.778) LOEC 1.20 NOEC 0.60	ED_{50} 0.54 (0.43, 0.61) ED_{20} 0.16 (0.10, 0.23) ED_{10} 0.08 (0.03, 0.12) LOEL 0.17 NOEL 0.08 Sublethal effects: Malformed wings at ≥0.17 µg a.i. $larva^{-1}$ total dose	Corteva Agriscience (2023f)
SFX 500 WG GF-2372 50.4% w/w				
Larvae (day-8)	NA	Avg. mortality ≤ 11% at highest dose		Corteva Agriscience (2023a)
Imago (day-22) Adult emergence	m	EC_{50} 2.592 (1.33, 20.08) EC_{20} n.r. EC_{10} n.r. LOEC 3.53 NOEC 1.12	ED_{50} 0.399 (0.103, 1.56) ED_{20} n.r. ED_{10} n.r. LOEL 0.544 NOEL 0.172 Sublethal effects: Malformed wings at ≥0.544 µg a.i. $larva^{-1}$ total dose	

(*Continued*)

Table 7. (Continued).

Life stage & evaluation time (days)	Endpoint: Base[a]	Endpoint: Concentration in food[b] mg kg^{-1} (95% CL)	Endpoint: Daily dose[c] µg bee^{-1} (95% CL)	Reference
X11519540 100% w/w, SFX-sulfone				
Larvae (day-8)	No significant mortality vs control			Corteva Agriscience (2021f) Repeated[f], see Corteva Agriscience (2022d)
Imago (day-22) Adult emergence	n	EC_{50} > 130 LOEC 8.32 NOEC 3.33	ED_{50} > 20 LOEL 1.28 NOEL 0.513 Sublethal effects: NOEL body mass ≥ 20	
X11519540 100% w/w, SFX-sulfone				
Larvae (day-8)	No significant mortality vs control			
Imago (day-22) Adult emergence	n	EC_{50} > 130 NOEC 130	ED_{50} > 20 NOEL 20	Corteva Agriscience (2022d)
X11579457 100% w/w, SFX-sulfoximine				
Larvae (day-8)	n	Larval mortality was 15–31% on day-8 vs 7.3% in pooled controls		Corteva Agriscience (2021e) Repeated[f], see Corteva Agriscience (2022e)
Imago (day-22)	n	Adult emergence EC_{50} > 130 LOEC 20.8 NOEC 8.32 Sublethal effects: NOEC body mass ≥ 130	Adult emergence ED_{50} > 20 LOEL 3.20 NOEL 1.28 Sublethal effects: NOEL body mass ≥ 20	
X11579457 100% w/w, SFX-sulfoximine				
Larvae (day-8)	No significant mortality vs control			
Imago (day-22)	n	EC_{50} > 130 $NOEC_{emergence}$ 130	ED_{50} > 20 $NOEL_{emergence}$ 20	Corteva Agriscience (2022e)
X11719474 99.7% w/w, SFX-urea				
Larvae (day-8)	No significant mortality vs control			Corteva Agriscience (2021g) Repeated[f], see Corteva Agriscience (2022f)
Imago (day-22)	n	Adult emergence EC_{50} > 130 LOEC n.r. NOEC 130 Sublethal effects: NOEC body mass ≥ 130	Adult emergence ED_{50} > 20 LOEL n.r. NOEL > 20 Sublethal effects: NOEL body mass ≥ 20	
X11719474 99.5% w/w, SFX-urea				
Larvae (day-8)	No significant mortality vs control			
Imago (day-22)	n	EC_{50} > 130 $NOEC_{emergence}$ 130	ED_{50} > 20 $NOEL_{emergence}$ 20	Corteva Agriscience (2022f)
X11721061 82% w/w SFX-ethanol				
Larvae (day-8)	n	Mortality was consistently above control mortality, but there was no dose-response relationship		
Imago (day-22)	n	EC_{50} > 130 $LOEC_{emergence}$ 130 $NOEC_{emergence}$ 50 $LOEC_{body\ mass}$ 4.13	ED_{50} > 20 $LOEL_{emergence}$ 20 $NOEL_{emergence}$ 8.01 $LOEL_{body\ mass}$ 0.636	Corteva Agriscience (2021h) Repeated[f], see Corteva Agriscience (2022g)
X11721061 82% w/w SFX-ethanol				
Larvae (day-8)	No significant mortality vs control			Corteva Agriscience (2022g)
Imago (day-22)	n	Adult emergence EC_{50} > 130 LOEC n.r. NOEC 130 Sublethal effects: None	Adult emergence ED_{50} > 20 LOEL n.r. NOEL 20 Sublethal effects: None	

a) For studies with base "n" and "m," exposures were calculated as nominal or measured values respectively.
b) The dosing solution was fed daily on day-4–7 at each concentration. The endpoints reflect the actual consumption of food in each study but are reported as concentration of SFX or transformation product in the diet.
c) The endpoints reflect the actual amounts of diet that was consumed for the entire exposure interval (development period). For SFX-T, the values based on measured concentration of SFX or transformation product.
d) Abbreviations: NA = not applicable or not possible to calculate; n.r. = not reported.
e) The reported LC_{50} was excluded from averages due to high control morality after Day -7.
f) Four studies with the transformation products were repeated to follow the new OECD guideline. The results did not change.

feeding of larvae also results in a combination of oral and contact exposure until the larvae stop eating (day-5). Survival of the larvae on an artificial diet without being tended by nurse bees has been a problem in these studies (Schmehl et al. 2018), and the latest version of the guidance document for chronic studies has been updated to address this issue (OECD 2021). At the beginning of pre-pupation, i.e., seven days of age, dead larvae are removed and the cells with live larvae are cleaned to remove feces and unconsumed food. Measurement endpoints include ECx and EDx values at various development stages, particularly EC_{50}, ED_{50}, EC_{10} and ED_{10} for mortality on day-7 and −22, and percent emergence by day-22. These results should be adjusted for control mortality.

The repeated dose (chronic) results for technical grade active ingredient, formulations, and transformation products are presented in Table 7. The repeated dose is a chronic exposure scenario because the four daily doses represent the entire development phase when larvae might be exposed. All transformation products of SFX were essentially nontoxic to the brood stages, although several resulted in reduced body mass at greater doses (Table 7).

Results of toxicity tests with sulfoxaflor in other species of bees

The available laboratory acute toxicities of SFX and its formulated products for species other than *A. mellifera* are listed in Table 8 and the quality of the studies is shown in Figure 5. The acute oral LD_{50} for *A. cerana indica* (0.0835 µg a.i. bee^{-1}) was similar to the geometric mean of 0.074 µg a.i. bee^{-1} reported for *A. mellifera*. Acute, oral toxicities of SFX-T and the formulated SFX products to bumblebees were between 0.027 and 0.71 µg a.i. bee^{-1}, showing no strong formulation-related increase in toxicity, but, in some cases, a possible reduction in oral toxicity in the presence of the formulants (Table 8). Acute contact toxicity results in Table 8 show that, as found for honeybees, SFX is less toxic by contact than by oral exposure for other bee species. In addition, bumblebees are much less susceptible to SFX than are honeybees when exposed via contact exposure (USEPA 2019).

As expected, the transformation products of SFX were all essentially nontoxic to bumblebees (Corteva Agriscience 2020b, 2020c, 2020d, 2020e). Because they are formed within the plant or soil, and there is no significant contact of adult honeybees with excreta, the contact route of exposure from foliar or other surfaces is not considered to be significant for transformation products (Solomon et al. 2025a). Exposure of soil-dwelling pollinators to these substances is also considered to be insignificant.

Discussion

QWoE risk assessment

The quality and relevance ratings summarized in Figures 1–5 provide an overview of the available laboratory toxicity studies that support the QWoE risk assessment of SFX. Some of the studies ranked high in quality and relevance, as expected for laboratory studies, which are designed to intentionally produce effects to support dose-response determinations and provide measured apical toxicity endpoints. Therefore, the endpoint values are well supported. The QWoE risk assessment indicates that more detailed studies are required to define the risk of SFX to honeybees, in line with the Tier-1 hazard assessment, which is described in Section 3.3.

Relationship between doses and daily consumption of sugar

A significant observation from the laboratory studies of chronic, oral toxicity to adult bees is that the consumption rates of the dosing syrup, 50 w/w % sucrose in water, which is highly appetitive, are higher than natural food consumption rates. Continuous, *ad libitum* feeding of honeybees in the laboratory over a period of ten consecutive days, as in the two SFX-T studies cited in Table 5, resulted in dietary intakes of 14.4 and 22.2 mg sugar bee^{-1} day^{-1}. For comparison, the values in the two studies were approximately 1.7-fold the median rate of consumption of 13.3 mg sugar bee^{-1} day^{-1} (44.4 mg of 30% nectar bee^{-1} day^{-1}) for an active forager (Rodney and Kramer 2019), which can be considered realistic for active adult

Table 8. Acute oral and contact toxicity of SFX and formulations of SFX to additional species of bees.

Test substance and concentration[a] (% measured)	Median lethal dose LD_{50} , µg a.i. bee^{-1} (95% CL)		Reference
	Oral	Contact	
B. terrestris Adult			
SFX-T[c] (98)	48 hr: 0.0835 (0.0687, 0.0982)	n.r.[b]	Azpiazu et al. (2021)
SFX-T ("pure")	Worker 48 hr: 0.126 (0.12, 0.13) Male 48 hr: 0.08 (0.066, 0.095) Queen 96 hr: 0.452 (0.369, 0.535)	Worker 48 hr: 6.23 (4.67, 7.98) Male 72 hr: 0.6 (0.399, 0.801) Queen 96 hr: 37.51 (28.78, 46.25)	Linguadoca et al. (2021)
SFX-T ("pure")	n.r.	48 hr SFX-T: 14.9 (13.2, 16.6) SFX-T + Azoxystrobin: 31.2 (29.3, 33.3).	Rohesalu (2021)
SFX 240 SC GF-2032 (21.6)	48 hr: 0.027 (0.016, 0.041)	48 hr: 7.57 (n.r.) 72 hr: 7.55 (2.99, 21.01)	(Corteva Agriscience 2009a)
SFX 120 SC GF-2626 (12)	48 hr: 0.15 (n.r.)	48 hr: 187.1 (n.r.) 72 hr: 26.1 (n.r.) 96 hr: 23.3 (n.r.)	Corteva Agriscience (2017a)
SFX 120 SC GF-2626 (11.16)	48 hr: 0.71 (0.56, 1.01)	n.r.	Cabezas and Farinos (2022)
A. cerana indica Adult			
SFX -T (98)	n.r.	24 hr: 0.126 (0.111,0.140)	Corteva Agriscience (2012a)
SFX 240 SC GF-2032 (22.2)	n.r.	48 hr: 0.0835 (0.0733, 0.0939)	Corteva Agriscience (2013a)
O. bicornis Adult			
SFX-T[c] (98)	48 hr 0.00906 (0.00680, 0.0112) 72 hr: 0.00719 (0.00412, 0.00973) 96 hr: 0.00590 (0.00197, 0.00928)	n.r.	Azpiazu et al. (2021)
SFX-T ("pure")	Male 48 hr: 0.01 (0.007–0.013) Female 48 hr: 0.013 (0.007, 0.018)	Male 96 hr: 0.029 (0.018, 0.041) Female 96 hr:0.051 (0.032, 0.069)	(Linguadoca et al. 2021)
O. excavata (Larvae)			
SFX-T[c]	48 hr 1.46 (1.18–1.87) no significant effects on larval weight gain	n.r.	Song et al. (2021)

a) Concentration is the measured percent by weight (g 100 g^{-1}).
b) Abbreviation: n.r. = not reported.
c) These findings need confirmation in view of the deficiencies in design and reporting of the cited work (See SI for details).

foragers and conservative for other bees. This difference demonstrates that the standard test methods for honeybees are conducted at the maximum potential feeding rates.

The OECD acute and chronic larval toxicity studies include an interval with no food provided prior to dosing (OECD (2013), 2021). The larval assays are more conservative than the adult tests based on the amount of sugar consumed (see section 5.2.3 for additional consideration). Survival of bee larvae depends on a sequence of three diets that contain royal jelly and increasing amounts of an aqueous solution of glucose and fructose as well as other ingredients. The OECD guideline 237 and guidance document 239 do not specify the amount of these sugars in the royal jelly, but a reported median value is 11% total sugars by mass (Schmidt and Buchmann 1985), and the royal jelly is 50% of the diet used in the study (Asencot and Lensky 1988). The specified concentrations of glucose and fructose in the aqueous solution are 30% on day-3 and 36% on days 4–6. The volumes fed are 20, 20, 40, and 50 µL on days 3–6, respectively, and densities are approximately 1.1 (OECD 2021). Based on these values, the total amounts of sugar fed, expressed as 30% nectar equivalents, are 15.0,

17.2, 34.5, and 43.1 mg larva^{-1}, with a total of 109.8 mg, if all the diet is consumed. This is only 61% of the 180 mg larva^{-1} referred to in the honeybee risk assessment guideline (USEPA 2016). Therefore, this guideline results in a significant but intended overestimation of the potential exposure of larvae to residues in nectar both in amount and duration of sustained maximal exposure. The deficit in caloric content of the artificial larval diet might also provide an explanation for rates of failed adult emergence in the control treatment. Up to 30% failure is considered "acceptable" by the guidance, which is often observed in studies following the OECD 239 test guidance document. Thus, results of studies conducted under these guidelines will significantly overestimate the potency of insecticides on a per bee basis. The risk assessment must be conservative, but the quantification and possible reduction of the overestimation should be considered when conducting more realistic risk assessments.

Factors affecting toxic potency

Consideration of factors affecting exposure or toxicity, including test conditions and details of physiology, behavior, and life history is fundamentally important for assessment of hazard and risks (Farruggia et al. 2022; Pamminger 2021). In the time since the USEPA guideline for assessments of risks were updated (USEPA 2016), scientific knowledge of honeybee biology, physiology, behavior, and lifestyle has fundamentally changed, including our understanding of food distribution within a colony, and food consumption rates vs age of the bee (Purdy 2024; Rodney and Kramer 2019). However, important details for honeybees are still needed and the life histories of other important species of bees are only now being described (Cane 2024). Here, we consider some significant influencing factors and trends for assessment of risks posed by SFX.

Oral exposures involve feeding test material in sugar solution to starved bees, which results in almost immediate uptake (Blatt and Roces 2002). Due to faster uptake of the oral dose, acute oral LD_{50} values expressed as a.i. adult bee^{-1} were significantly less (more potent) for dietary exposures than those from topical assays (T-test, one-sided, p =0.005) (Tables 1 and 2), as expected from previous work (Farruggia et al. 2022).

In some tests, the contact route of exposure was also slower-acting than oral dosing, with additional mortality observed at 72 hr or even 96 hr (Table 3). Toxic potencies of the diastereomers exhibited a small, but statistically significant difference in the contact LD_{50}. However, toxic potency calculated from the contact LD_{50} values of the diastereomers (Table 3) using the sum of Toxicity Units (Gennings et al. 2005) was 0.088 µg a.i. bee^{-1}. This is within experimental variability of the value of 0.060 µg a.i. bee^{-1} reported for SFX-T in the same study (Corteva Agriscience 2022c). Regardless, the diastereomers are so rapidly interconverted under physiological conditions that the difference in toxicity is biologically insignificant (Solomon et al. 2025b).

The contact route of exposure must also be considered for the activities of honeybees inside the hive. When consuming nectar or touching surfaces bearing residues, contact exposure via mouth parts and appendages is possible, however, results of studies with radiotracers have demonstrated that this pathway is insignificant (Nixon and Ribbands 1952). Furthermore, the contribution from this pathway would be included in the endpoint for oral toxicity (Purdy et al. 2025a).

Information on behavioral ecology of pollinators augments the selectivity of SFX, and other products used to control sap feeding insects. Although these crop pests consume the residues after they dry on the plant, pollinating insects that crawl over the plant to get to the flowers have minimal contact with foliage and flying pollinators have even less contact. Honeybees seldom land on plant parts other than floral tissue and they prefer newly opened, warm, dry flowers (Lawson and Rands 2019; Nicolson et al. 2013). This behavioral preference reduces the potential for contact exposure to the dried residue on flowers or inflorescences that were open during spray application and remain attractive to bees. The potential for oral exposure is limited to the residues in nectar and pollen that

arise due to systemic distribution of residues within plants. For additional discussion of potential routes of exposure, see (Purdy et al. 2025a)

Adult bees - acute exposure and Tier 1 risk assessment

As expected from its intended use to control insect pests, SFX is classified by the USEPA as "highly toxic to honeybees" (Farruggia et al. 2022). Using the EPA Tier 1 formula(Thompson and Pamminger 2019), and the geometric mean acute oral LD_{50} of 0.0740 µg a.i. bee^{-1} for honeybees the Hazard Quotient (HQ) = 39.1. This is consistent with the assessment by the USEPA (USEPA 2019). Since this is above the criterion of 0.4, the results do not refute the presumption of risk. Higher tier studies are therefore needed to complete the SFX risk assessment. These higher-tier studies are reviewed in (Purdy et al. 2025a, 2025b; Solomon et al. 2025a).

For SFX, such tests included a series of foliar contact toxicity studies with aged foliar residues from application of the formulated products (Table 4). The oral route of exposure is discussed based on a series of crop residue studies conducted to measure the concentrations of SFX and its transformation products in bee-relevant matrices such as pollen and nectar in a companion paper (Solomon et al. 2025a).

The results from colony-level field studies are described in a companion paper in the series on SFX (Purdy et al. 2025b). This paper includes calculation of risk quotients based on comparison of the measured concentrations to the LOAECs in Tables 1 and 2. Based on the results of these tests, a refined risk assessment was conducted using a new model of the distribution of residues within the colony, the *A. mellifera* agent-based hazard and risk assessment model (AMAHRA) in a companion paper (Purdy et al. 2025a).

For formulations containing SFX alone, the aged foliar contact toxicity studies showed that the potency of SFX residues on foliage declines rapidly after application, even at double the maximum recommended label rate (Table 4). In these studies, honeybees are unnaturally confined and forced to crawl in or on a pile of chopped, treated foliage, which they would not normally do, since they hover and fly directly to the flowers (Willmer 2011b). This exposure scenario greatly exaggerates potential exposure because pollinator bees spend only brief intervals, resting on foliage or manipulating flowers. For example, the average total time per day outside the hive for honeybee foragers was 2.08 ± 7.49 hr and much of that time was spent in flight (Rodney and Kramer 2019). Therefore, it is likely that, under real-world conditions, no effects occur after the spray has dried (~45 minutes) (Corteva Agriscience 2013d). The results of aged residue foliar contact studies are reported as the RT_{25}, which is the time for the rate of mortality of exposed bees in a 48-hr interval to drop to 25%. The RT_{25} for formulations containing only SFX was < 45 min (Corteva Agriscience 2008, 2009b). Some behavioral abnormalities, mainly a lack of coordination and lethargy, were seen initially, but not in bees that survived for longer intervals of time. The sublethal effects, observed at this stage in the tiered risk assessment framework, are considered in more detail, during higher-tier studies. In summary, the effects from the contact route of exposure were minor and temporary at the highest application rate on the product use label (101 g ai ha^{-1}) or lower, in or near the treated crop.

Although the results of the aged foliar residue contact test demonstrate that there is no significant harm to pollinators after the spray has dried, there is little documentation of effects of wet foliage on foraging activity. However, because of interference with landing and take-off, and the energetic cost of drying, it is likely that pollinators are averse to landing on wet foliage, even for brief rests. In addition, the dislodgeable portion of the residues for insecticide residue that is on the surface and most susceptible to transfer onto bees, is also most likely to be washed off in rainfall.

Adult bees - repeated dose (chronic) exposure

The symptoms of intoxication in repeated-dose studies were like those in acute studies except that a decrease in body mass was observed in some studies over time. The toxicity of SFX did not increase with multiple days of feeding, and there was no indication of long-term accumulation of SFX by bees.

The interpretation of toxicity study results requires consideration of the consequences of the study design choices in the guidelines. The current guideline for chronic toxicity to adult honeybees is OECD guideline 245 (OECD 2017) specifies the use of ≤2-day old

bees. This includes imagoes (newly emerged adult bees) less than 1-day old that are not physiologically mature. These bees are fed by nurse bees and therefore not as exposed to pesticides under natural conditions. However, they are still maturing and have lower expression of detoxification enzymes than older hive bees (Maiwald et al. 2023). While they are not representative of older hive bees, they are expected to be more sensitive, making the results conservative by an undetermined factor. Furthermore, testing groups of bees with a wider than necessary range of physiological maturity introduces variability that can be reduced by using a method for producing cohorts of worker honeybees with uniform (within 12 hr) age. This indicates that the SFX adult bee toxicity results may be conservative compared to older hive bees.

Acute and repeated dose toxicity to brood

A detailed understanding of larval feeding and ontogeny in the hive is essential for the design, conduct, and interpretation of honeybee toxicity studies. Recent advancements in this field of research have made it possible to rear honeybee larvae under controlled laboratory conditions (OECD 2021). Some key aspects are summarized here. Larvae of honeybees are entirely dependent on adult nurse bees for nutrition. Their diet is so completely digestible that honeybee larvae have an incomplete alimentary canal until the last instar, which is called the prepupal stage because it includes a metamorphosis into the pupa (page 49 in Winston 1987). This protection from predation and secure food supply likely contributed to the evolutionary success of the social lifestyle (Purdy et al. 2025b).

The prepupal stage is also called the defecation stage. Many larvae have stopped eating at this stage, although it is the final day of dosing in the standard method (OECD 2021). Transcuticular uptake continues, but is incomplete, and it is not possible to quantify the amounts of toxin eliminated in feces that are removed by nurse bees in the hive or left behind when the prepupa is transferred to a new cell for pupation in the standard assay method. A qualitative assessment of unconsumed food to be conducted at the end of the larval stage is recommended. Nonetheless, the overall proportion of dosed material taken up by the larvae is expected to be similar within an experiment and included in the reported results unless the test materials affect consumption of food by the larvae.

Because queen-larvae are fed a diet (royal jelly) with a controlled composition that is entirely produced in food glands of nurse bees, queen larvae are isolated to a considerable extent from exposure to food borne natural toxins and pesticides (Purdy 2015). Worker and drone larvae receive brood food (worker jelly) that is similarly produced for the first 3 days of their lives but then start to receive food with a greater proportion of sugars. At this stage, more direct exposure to residues in the nectar and pollen collected by foragers can occur, but the amount is moderated by the mixing and dilution that take place as the larvae are fed by the nurse bees. A portion of the sugars and some pollen originates in crops of nurse bees, but the amount is variable and has not been quantified, because it is not known how much continues to come from the food glands (Purdy et al. 2025a). Although development time varies with conditions and genetics, worker and drone larvae are typically fed the high-sugar diet from day-4 of age until the prepupation stage. For current guideline risk assessment, sugar consumed by older larvae is assumed to be entirely from the nurse bee crop (USEPA 2019), and the pesticide is assumed to be undiluted from concentrations in nectar that arrive in the hive. Dilution of the pesticide in the pollen fed to larvae from the nurse bee crop is also ignored in the risk assessment to ensure that the results are conservative and thus protective (USEPA 2014).

Addition of pollen to diets of older worker and drone larvae in real world conditions was initially thought to be incidental due to the presence of pollen in the nurse bee crop (Simpson 1955). Later work showed that, although it is incidental and of insignificant nutritional value, pollen exposes larvae to natural toxins and induces or upregulates the metabolic enzymes needed to transform and detoxify these materials (Mao, Schuler, and Berenbaum 2015). This contributes to the evolutionary advantage of social brood care. Although not reported in the literature, it is likely that the natural toxins in the nectar fed from the nurse bee crop contributes to the induction of enzymes just as do the toxins in pollen. Some

pesticides, including SFX are known to upregulate expression of detoxification enzymes (Mao, Schuler, and Berenbaum 2015; Shi et al. 2020). The induced enzymes increase detoxification of pesticides (Gong and Diao 2017).

Results of acute toxicity tests on SFX with honeybee larvae are listed in Table 6. The lowest LD_{50} of SFX-T for toxicity to larvae was 4.9 µg SFX bee^{-1}, which is obviously greater than the corresponding value of 0.0740 µg SFX bee^{-1} day^{-1} described previously for adult bees, indicating larvae are much less susceptible to SFX after a single dose. The reported mortality from repeated dose studies was insignificant or well below 50% at the end of the larval stage (day-7). This shows that dividing the dose into daily aliquots reduces the susceptibility at day-7 compared to the single dose results in Table 6, which demonstrates the importance of a more realistic dosing pattern and a longer observation time.

The LD_{50} for SFX-T at emergence in repeated-dose studies with larvae, ranged from 0.212 to 0.312 µg SFX bee^{-1} development $stage^{-1}$ (Table 7). The units are expressed per development stage to denote the total dose over the 4 days in the larval development stage and not the daily individual doses. For comparison, the repeated dose LD_{50} values for larvae determined using formulated products containing SFX range from 0.40 to 0.54 µg SFX bee^{-1} development $stage^{-1}$ (Table 7).

The frequency of observed effects in several repeated-dose studies was less than 50% and thus too small to enable calculation of the ED_{50} values (Table 7). Results of most studies revealed no effects on morphology or rate of development, but several repeated-dose studies involving either SFX alone or SFX in formulated products on larvae reported greater rates of missing or malformed wings at emergence when bees were exposed to ≥0.06 µg SFX bee^{-1} development $stage^{-1}$ (Table 7). This effect was not observed in the control groups. There is one report that developmental effects including deformed antennae, abdomen, and/or wings are more likely to occur when the plates used to rear the larvae are horizontal than when they are kept in a more natural vertical orientation during incubation (Kim et al. 2021). The deformities reported in the laboratory studies are not observed in more realistic field studies of brood development and are therefore attributed to the study conditions (Purdy et al. 2025b). However, the absence of the effect in the control groups of the study remains unexplained but is unique to this study and may also have been an aspect of the study conditions.

The body mass per bee at the end of the larval stage and in the imago were measured in several studies (Table 7). At the end of the larval stage, a reduction in body mass relative to the unexposed control was observed for exposures ≥2.5 µg SFX bee^{-1}, but there was little mortality until the pupal stage (Kim et al. 2022). The reduction in body mass at the end of the larval stage or at emergence was also not observed consistently among studies. Based on these considerations, the NOEL for SFX exposure to honeybee larvae was 0.2 µg SFX bee^{-1}, i.e., the lowest of the relevant values in Table 7.

For comparison, the chronic LD_{50} values determined using formulated products containing SFX ranged from 0.40 to 0.54 µg SFX bee^{-1} (Table 7). The formulated products were significantly less toxic to larvae than SFX-T in chronic toxicity assays (T-test, one sided equal variance: $p = .0078$), and the adult chronic LD_{50} of 0.00539 µg SFX bee^{-1} is much less than the chronic larval toxicity values, which were 0.247 (0.201, 0.302) and 0.466 (0.0408, 0.532) µg SFX bee^{-1} for SFX-T and the formulated products in Table 7, respectively. This is reasonable since tests involving larvae include combined oral and trans-cuticular routes of exposure and should show trends in toxicity similar to what was observed in the adult oral versus contact toxicity tests.

These data are consistent with other reports that show that larvae are less sensitive to SFX and other substances than adult bees.

Additional published studies of effects of SFX on A. mellifera

There are multiple reports of adverse effects on honeybees and other pollinators from exposure to sulfoxaflor observed in laboratory studies at

purported field relevant or sublethal doses (Chakrabarti et al. 2020; Li et al. 2021; Shi et al. 2020). These studies used nonstandard methods that have not been validated or corroborated (see details in SI). For example, Chakrabarti et al. (2020) used generic indicators of stress but did not attempt to validate the methods and used nonstandard terminology. The method involved a single, direct overspray with two post-exposure observation intervals. The design of that study had critical weaknesses in the experimental design including lack of a positive control; no calibration of exposure per bee to a field application rate, no dose-response series; use of imago bees that have not yet completed their development and would not be exposed to the spray under real-world conditions; and lack of comparison to standard acute toxicity test methods. The reported results do not provide useable standard endpoints such as the NOEL and do not explain the biological relevance of the effects, such as a 30% increase in apoptosis, to apical endpoints. The findings are not supported by the data due to multiple unexplained significant differences among treatments.

Some of the published reports cited the standard test guidelines but deviated significantly from those guidelines. For example, in their adult acute toxicity test, Li et al. (2021) used newly emerged bees instead of older bees of foraging age, omitted a positive control treatment, did not use a logarithmic dose series, and did not report the sucrose concentration in the exposure matrix. The latter might affect the amount of dosing solution that the bees consumed. Their chronic toxicity test also lacked a positive control. No explanation of the impact of these design choices on the outcome was provided. The adult enzyme activity test showed significant differences but failed to explain the toxicological relevance of these differences (Li et al. 2021). For example, quercetin, a common natural pollen constituent, could be included as a reference compound in the study to give a measure of the significance of the observed changes in enzyme activity for bee health versus an established benchmark.

Similarly, Ibrahim et al. (2023) omitted positive controls and did not report NOEL or NOAEL values. Without these endpoints for comparison to exposure, the significance of the conclusions could not be evaluated, and the results could not be used in risk assessment. In addition, the quality of this work is reduced by citing unreliable literature, such as the work by Chakrabarti et al. (2020) described above, and by including several statements that are not logically sound: e.g., the authors conclude that having fewer types of detoxification enzymes than other insects makes honeybees more "susceptible to pesticide exposure." The number of enzymes is not related to exposure, nor does it matter how many enzymes they have when those they have meet their needs. Furthermore, this statement ignores the importance of the social lifestyle of honeybees in colony-level protection against food-borne toxins (Purdy 2015). More generally, studies conducted to identify molecular biology endpoints in bees can provide insights into new toxicological outcomes of exposure, but no examples of such studies were found.

Wherever possible, the results of published literature were included in this assessment (Tables 1–5). The available published studies were rated using the same protocol that was used for all other studies, and the ratings are included in the SI. The SI includes a summary table of studies not used for risk assessment with supporting rationales. Several of the cited authors concluded that their work required a revision of the risk assessment process (Sgolastra et al. 2019), but no support was found for this. No published studies were found that would support changing the risk assessment profile of SFX.

Toxicity of SFX to other species of bees

Honeybees and other insect pollinator species have an effective metabolic detoxification capability that has evolved to protect them against the diverse toxins produced by plants as a protection against herbivores (Nauen et al. 2022; Purdy 2024; Willmer 2011a). Those same generic pathways for detoxification can transform pesticides, including SFX. The results of the chronic studies as well as published literature show that SFX is metabolized relatively rapidly by bees (Liao 2016; Zhu et al. 2020).

In a published study on three bee species, SFX was found to have greater toxic potency to *O. bicornis* than to *A. mellifera* and *B. terrestris*

was least sensitive when expressed as dose bee^{-1} (Tables 1 and 8). When body mass was taken into consideration, *B. terrestris* was more sensitive than *A. mellifera*, but *O. bicornis* was still the most sensitive (Azpiazu et al. 2021). However, the honeybee data in this study are unusable due to the use of foraging aged bees instead of hive bees and the high mortality in the controls. This study also attempted to characterize interactions of SFX with a fungicide, fluxapyroxad, but the use of the formulated product makes interpretation of the results ambiguous due to possible effects of the formulants, which would not occur in the real world. In conclusion, the findings of this study should be confirmed in view of these weaknesses (Azpiazu et al. 2021) (See SI for details).

Another published acute toxicity study reported that male bumblebees are more sensitive than queens based on dose of a.i. bee^{-1} (Linguadoca et al. 2021), but this should be confirmed, since the study was considered unusable for risk assessment for the following reasons. The study was not GLP compliant, and although OECD guidelines were cited, the study did not follow a standard guideline method or include a method validation. The claim of synergy is not supported due to confounding factors of unexplained experimental variability and the interaction of the well-known effect of the attractiveness of more concentrated sugar and the antifeedant effect of SFX. The actual doses transferred in stored nectar and pollen to nestmates are not known (see SI).

There is a high quality, 10-day repeated dose study with adult bumblebees (Corteva Agriscience 2022a) although the chronic LD_{50} is not used by the USEPA in risk assessment, the results included chronic oral LD_{50} values (95% C.L.) of 0.29 (0.27, 0.32) µg a.i. bee^{-1} day^{-1} or 3.44 (3.02, 3.83) mg a.i. kg^{-1} diet based on measured concentrations and actual food consumption. The NOELs based on lethality were 0.37 µg a.i. bee^{-1} and 2.4 mg a.i. kg^{-1} diet, respectively. The corresponding NOELs for reduction in body mass were 0.38 µg a.i. bee^{-1} and 2.4 mg a.i. kg^{-1}. When compared to other available data, the results show that *A. mellifera* is neither the most nor least sensitive bee species and that the range of sensitivity within a genus can be as great as the difference among genera.

A study with larvae of *O. excavata* reported a 48-hr LD_{50} of 1.46 µg a.i. bee^{-1} (Song et al. 2021) (Table 8), which is slightly less than the 72-hr value of 4.9 µg a.i. bee^{-1} for honeybees (Table 6). The LD_{90} was 19.54 (95% CL 12.07, 37.59). The results are included for comparison although the study lacked a positive control, and the dose levels were not verified (see SI).

There have been multiple studies of potential sublethal effects on non-Apis bees, particularly bumblebees. Studies of *B. terrestris* by Siviter et al (Siviter et al. 2018, 2019; Siviter, Brown, and Leadbeater 2018; Siviter, Folly, et al. 2020; Siviter, Horner, et al. 2020) reported reproductive effects, but these were unusable due to multiple significant deficiencies. The EFSA review found that the first of these papers was not representative of normal healthy colonies and was unusable in risk assessment due to the lack of a dose-response series in the study design and multiple other concerns (EFSA 2020). In addition, the study used an unrealistic sustained exposure at relatively high dose levels over 2 weeks despite the known exponential decline in concentration found in pollen and nectar (Solomon et al. 2025a; USEPA 2019). The other 2018 paper was a review article of published papers on multiple pesticides including SFX that lacked criteria or thresholds for effects or a dose-response relationship (Siviter et al. 2018). The 2019 paper reported no adverse impacts on bee olfactory conditioning or working memory from acute exposure to SFX-T (Siviter et al. 2019). The first 2020 paper reported a study of potential effects of SFX-T on *B. terrestris* larval growth. The design of this study did not include a logarithmic series of doses to enable a dose-response calculation. The results were compromised by excessive mortality in the control group. No quantitative endpoints, e.g., LC_{50}, NOEL, were reported(Siviter, Folly, et al. 2020), therefore, the results were not usable in risk assessment. The second 2020 study reported potential effects of SFX-T on egg laying in *B. terrestris* (Siviter, Horner, et al. 2020). This study had similar deficiencies including excessive mortality in the controls, lack of a dose-response relationship, and no reported quantitative endpoints. (See SI for additional deficiencies). None of the papers reported the methods to ensure the stability, uniformity, and concentration of the dosing solutions. Viewed from a broader perspective, they illustrate the concern

Table 9. Summary of toxicity endpoints for SFX-T and formulated products from laboratory tests.

			Adult		Larvae	
Endpoint	Test type	Test substance	Value[a]	EPA[b]	Value[a]	EPA[b]
LD_{50}	Acute oral	SFX-T	0.074[c]	0.146	4.9	> 0.415[d]
LD_{50}	Acute oral	Formulated		0.05	n.r[e]	n.r.
LD_{50}	Acute contact	SFX-T	0.432	0.379 (72 hr)	n/a	n.r.
LD_{50}	Acute contact	Formulations	0.202	0.130, 0.224	n/a	n.r.
LD_{50}	Chronic oral	SFX-T	n.r.	n.r.	0.371	n.r.
LD_{50}	Chronic oral	Formulations	0.037	n.r.	n.r	n.r.
$NOAEL_{mortality}$	Chronic oral	SFX-T	0.01	0.0054	0.2	0.212

a) The values are the geometric means of currently available values in Tables 1–7 and are in µg a.i. bee^{-1} for acute studies and µg a.i. bee^{-1} day^{-1} for chronic studiesThe values are geometric means except for the acute oral larvae values, which was the lowest available point estimate (Confidence intervals are included in Section 2). The acute LD50's are for 48 hr except as noted.
b) USEPA values are from (USEPA 2019).
c) Geometric mean for SFX-T and formulations listed in Table 1 (see Section 4.1).
d) USEPA value from the day-8 assessment in a chronic study
e) n.r. = not reported

raised using nonstandard study designs outside the context of developing and validating new methodology. Additional reports of sublethal effects of SFX, which were similarly unusable are described in the SI.

Recent work on the chemical defense processes of pollinating insects has broadened knowledge of sublethal effects by elucidating the biochemical processes involved. Molecular methods and a fluorescence-based in-vitro screening method have been developed which extend the capacity to study the mode of action and sensitivity of multiple bee species to pesticides and to quantify pesticide interaction effects at the molecular level (Dermauw, Van Leeuwen, and Feyereisen 2020; Haas et al. 2022). The detoxification system in honeybees includes five protein superfamilies: cytochrome P450 monooxygenases (P450), carboxylesterases, glutathione S-transferases (GST), UDP-glycosyl transferases (UGT) and ATP-binding cassette (ABC) transporters, in 47 transcriptomes and their expression in honeybee life stages and tissues have been mapped (Maiwald et al. 2023, Nauen et al. 2022). In honeybees, the CYP9Q3 family of P450 enzymes can detoxify pesticides from five diverse chemical classes including pyrethroids, neonicotinoids, organophosphates, diamides, and butenolides and orthologs in other species are expected to have similarly broad substrate specificity (Haas et al. 2022). In addition to receptor binding site affinity, there are three components in the chemical defense system: pharmacokinetics, enzyme turnover rate, and enzyme expression (Manjon et al. 2018; Zaworra et al. 2019). Contrary to earlier work reporting that honeybees are deficient in detoxifying enzymes (Claudianos et al. 2006; Gong and Diao 2017), this work reveals a robust capacity to detoxify xenobiotics that is consistent with their co-evolution with flowering plants known to produce a diverse array of natural toxins (Purdy 2024).

Molecular biology provides a broad base for comparison, and prediction of the potential sensitivity of other insect species to pesticides, which has only recently begun to be exploited. A phylogenetic study of the variability of P450 detoxifying enzyme activity in a range of bee taxa revealed that the capability to metabolize certain insecticides has been extensively conserved through evolution and is present in all major bee families (Haas and Nauen 2021). This conclusion is corroborated by the greater susceptibility of some tribes in the genus *Megachile*, which have evolved with P450 enzymes in the CYP9DU family in place of those in the CYP9DM family found in most other bees (Hayward 2022). Obviously, this method has potential for screening species of bees and other hymenopteran pollinators that cannot easily be tested under laboratory conditions (Haas et al. 2022; Katsavou et al. 2022). Molecular phylogeny also provides a method for corroborating the use of honeybees as a surrogate in toxicity tests as well as a powerful and efficient means of identifying more

Table 10. Summary of measurement and assessment toxicity endpoints for the major transformation products of SFX-T.

Life stage	Endpoints (mg kg^{-1} diet)	Endpoints (µg a.i. bee^{-1})
SFX-sulfone		
Larvae (day-8)	No significant mortality vs control	
Imago (day-22)	Adult emergence EC_{50} > 130 NOEC 3.33	Adult emergence LD_{50} > 20 NOEL 0.513 Sublethal effects: NOEL body mass ≥ 20
SFX-sulfoximine		
Larvae (day-8)	Larval mortality was 15–31% on day-8 vs 7.3% in pooled controls	
Imago (day-22)	Adult emergence EC_{50} > 130 NOEC 8.32 Sublethal effects: NOEC body mass ≥ 130	Adult emergence LD_{50} > 20 NOEL 1.28 Sublethal effects: NOEL body mass ≥ 20
SFX-urea		
Larvae (day-8)	No significant mortality vs control	
Imago (day-22)	Adult emergence EC_{50} > 130 NOEC ≥130 Sublethal effects: NOEC body mass ≥ 130	Adult emergence LD_{50} > 20 NOEL > 20 Sublethal effects: NOEL body mass ≥ 20
SFX-ethanol		
Larvae (day-8)	No significant mortality vs control	
Imago (day-22)	Adult emergence EC_{50} > 130 NOEC 130 Sublethal effects: None	Adult emergence LD_{50} > 20 NOEL 20 Sublethal effects: None

susceptible species. The consensus of the literature is that *A. mellifera* is not more sensitive than other species but sufficiently sensitive to be used as a representative species (Haas et al. 2022; Thompson and Pamminger 2019). In addition to these laboratory studies, a series of field studies have been conducted with SFX or its formulated products with *Bombus* and *Osmia* sp. These studies are reviewed separately (Purdy et al. 2025a).

Conclusions

The toxicity endpoints from the laboratory tests with SFX-T and formulated products are summarized in Table 9. The values used by USEPA for risk assessment are included for comparison. The differences are mainly due to the use of geometric means in the present work except that the acute oral LD50 for larvae exposed to SFX-T was assigned a value of > 0.2 µg a.i. bee^{-1} by the USEPA instead of the reported 4.9 µg a.i. bee^{-1}. No explanation for the use of this value by USEPA was found. These values indicate that SFX is potentially harmful to honeybees, but the actual hazard or risk depends on the magnitude and duration of exposure. The contact exposure assay results for SFX-T discussed in Section 2.4 are also summarized in Table 9 and show that this compound is less potent via contact than by oral dosing. The major transformation products are nontoxic to bees at environmentally relevant concentrations. The toxicity data are summarized in Table 10.

In some tests, the contact route of exposure was slower acting, with additional mortality at 72 hr, but not significantly more potent than the oral exposure (Tables 1 and 2). The observed difference in acute toxicity between the diastereomers is not relevant for risk assessment because these compounds are rapidly interconverted in the environment and under physiological conditions (Solomon et al. 2025b). The repeated-dose oral LD_{50} at emergence of adults was 0.217 µg SFX/larva.

Several of the studies of bee larvae reported developmental malformations, and reduced body mass, but the NOAEL of 0.005 µg SFX $larva^{-1}$ for reduced

food consumption (USEPA 2019) is more protective than the endpoints for these effects. Malformations were not consistently observed in a study of vertical vs horizontal rearing trays (Kim et al. 2021). These effects were not observed in whole colony field studies (Purdy et al. 2025b) and were attributed to the laboratory study conditions. In general, the available studies did not support the finding of any mode of action other than the primary mode of action of SFX, which involves binding to a unique site on the nicotinic acetylcholine receptor (nAChR).

Pollinators like bees that have co-evolved with flowering plants are also insects that feed on plant materials, i.e., pollen. The need to avoid harm to pollinators when using pesticides is widely recognized, but it is often overlooked that the plants produce toxic secondary chemicals to protect themselves from herbivores, and they began the process of balancing their need for protection against the need to attract pollinators very early in the co-evolution that has given us the current biodiversity of insect pollinated plants (Chapter 4 in Purdy 2024; Willmer 2011a). Indeed, several classes of insecticides have been developed from the diverse range of natural products as lead compounds including those like SFX that act on the nicotinic acetylcholine receptor (nAChR).

The results and discussion presented herein indicate that higher tier testing is required to complete the risk assessment for SFX. They form a conservative but reliable set of benchmarks for use in higher tier refined risk assessment and emphasizes the importance of standardized, validated test methods with representative species. The refined risk assessments are presented in the companion papers (Purdy et al. 2025a, 2025b; Solomon et al. 2025a).

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Data availability statement

Additional data on the studies cited in the this paper are provided in the Supplemental Information. Access to copies of the Corteva reports cited in this paper can be requested via the web site listed in (Kramer and Solomon 2025).

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