**Appendix A to the main manuscript:**

**Sulfoxaflor and nutritional deficiency synergistically reduce survival and fecundity in bumblebees**

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Supplementary Information

**S1: References for the residue dataset.**

The following studies are owned by Dow AgroSciences (now Corteva Agrisciences) and subject to data protection. Therefore, original study reports were not available to the authors. However, key information on the experimental design and descriptive statistics were made publicly available in the background document of EFSA (2019), and EPA (2019). Our assessment is solely based on the open-access information contained in these regulatory decisions.

Regulatory decisions

EFSA, 2019. Peer review of the pesticide risk assessment for the active substance sulfoxaflor in light of confirmatory data submitted. EFSA J. 17. https://doi.org/10.2903/j.efsa.2019.5633

EPA, 2019. Decision memorandum supporting the registration decision for new uses of the active ingredient sulfoxaflor on alfalfa, cacao, citrus, corn, cotton, cucurbits, grains, pineapple, sorghum, soybeans, strawberries and tree plantations and amendments to labels. Epa 1–30. URL: ﻿https://www.regulations.gov/document?D=EPA-HQ-OPP-2010-0889-0570 (last access in May/2020)

Studies summarised in EFSA (2019):

Appeltauer, A.; 2017; Determination of residues of sulfoxaflor in nectar and pollen of apple after one application of GF-2626 in a semi-field residue study with honeybees (*Apis mellifera* L.) in Central and Southern Europe 2016; Eurofins Agroscience Services EcoChem GmbH/Eurofins Agroscience Services Ecotox GmbH, Niefern-Öschelbronn, Germany; lab study no. S16-00603; DAS study no. 160356; 05 May 2017; full report unpublished; study sponsored by Dow Agrosciences Ltd., Oxfordshire, OX14 4RN, UK; summary of results published in background documents of EFSA, 2019; Peer review of the pesticide risk assessment for the active substance sulfoxaflor in light of confirmatory data submitted; EFSA Journal 2019;17(3):5633; DOI: 10.2903/j.efsa.2019.5633.

Appeltauer A.; 2017; Determination of residues of sulfoxaflor in nectar and pollen of strawberry plants after one application of GF-2626 in a semi-field residue study with bumblebees (*Bombus terrestris* L.) in Central and Southern Europe 2016; Eurofins Agroscience Services EcoChem GmbH/Eurofins Agroscience Services Ecotox GmbH; lab study no. S16-00602-L1; DAS study no. 160355; study finalization date: 05 May 2017; full report unpublished; study sponsored by Dow Agrosciences Ltd., Oxfordshire, OX14 4RN, UK; summary of results published in background documents of EFSA, 2019; Peer review of the pesticide risk assessment for the active substance sulfoxaflor in light of confirmatory data submitted; EFSA Journal 2019;17(3):5633; DOI: 10.2903/j.efsa.2019.5633.

Appeltauer, A.; 2017; Determination of residues of sulfoxaflor in nectar and pollen of pumpkin after one application of GF-2626 in a semi-field residue study with honeybees (*Apis mellifera* L.) in Central and Southern Europe 2016; Eurofins Agroscience Services EcoChem GmbH/Eurofins Agroscience Services Ecotox GmbH, Niefern-Öschelbronn, Germany;lab study no.S 16-00596; DAS study no. 160354; 05 May 2017; full report unpublished; study sponsored by Dow Agrosciences Ltd., Oxfordshire, OX14 4RN, UK; summary of results published in background documents of EFSA, 2019; Peer review of the pesticide risk assessment for the active substance sulfoxaflor in light of confirmatory data submitted; EFSA Journal 2019;17(3):5633; DOI: 10.2903/j.efsa.2019.5633.

Appeltauer, A.; 2017; Determination of residues of sulfoxaflor in nectar and pollen of winter oilseed rape after one application of GF-2372 in a semi-field residue study with honeybees (*Apis mellifera* L.) in Germany 2016; Eurofins Agroscience Services EcoChem GmbH/Eurofins Agroscience Services Ecotox GmbH, Niefern-Öschelbronn, Germany; lab study no. S16-00604; DAS study no. 160357; 06 May 2017; full report unpublished; study sponsored by Dow Agrosciences Ltd., Oxfordshire, OX14 4RN, UK; summary of results published in background documents of EFSA, 2019; Peer review of the pesticide risk assessment for the active substance sulfoxaflor in light of confirmatory data submitted; EFSA Journal 2019;17(3):5633; DOI: 10.2903/j.efsa.2019.5633.

Studies summarised in US-EPA (2019):

Belshay, TI; 2017; magnitude of residues of sulfoxaflor in nectar, pollen, and whole plants following foliar application of GF-2372 to Alfalfa; unpublished study performed by Smithers Viscient, Snow Camp, North Carolina; Laboratory project ID: 14050.4117; study sponsored by Dow AgroSciences LLC, Indianapolis, Indiana.

Louque, R. 2017; magnitude of residues of sulfoxaflor in nectar, pollen, and whole plants following foliar application of GF-2372 to canola; unpublished study performed by Smithers Viscient, Snow Camp, North Carolina; laboratory project ID: 14050.4118; study sponsored by Dow AgroSciences, Indianapolis, Indiana.

Bonetti, S. 2016; evaluate sulfoxaflor residues with nectar at different application periods. Unpublished study performed by Eurofins Agroscience Services, Inc., Lancaster, Pennsylvania; laboratory project ID: S15-00896; study sponsored by Dow AgroSciences, LLC, Indianapolis, Indiana.

Louque, R. 2017; magnitude of residues of sulfoxaflor in nectar, pollen, and whole flowers following foliar application of GF-2032 to peach trees; unpublished study performed by Smithers Viscient, Snow Camp, North Carolina; laboratory project ID: 14050.4121; study sponsored by Dow AgroSciences, Indianapolis, Indiana.

Louque, R. 2017; magnitude of residues of sulfoxaflor in nectar, pollen, and whole plants following foliar application of GF-2032 to pumpkin; unpublished study performed by Smithers Viscient, Snow Camp, North Carolina; laboratory project ID: 14050.4113; study sponsored by Dow AgroSciences, Indianapolis, Indiana.

Belshay, T. 2017; magnitude of residues of sulfoxaflor in nectar, pollen, and whole plants following foliar application of GF-2032 to strawberries; unpublished study performed by Smithers Viscient, Snow Camp, Florida; laboratory project ID: 14050.4116; study sponsored by Dow AgroSciences LLC, Indianapolis, Indiana.

Howerton, H. and Gilson, L. 2017; residues of sulfoxaflor in sunflower nectar and pollen after foliar application with GF-2372; unpublished study performed by SynTech Research Laboratory Services, LLC, 17745 S. Metcalf Avenue, Stilwell, Kansas 66085; laboratory project ID: 014SRUS15C116; study sponsored by Dow AgroSciences, Indianapolis, Indiana.

- All study summaries published as supporting materials to US-EPA (2019) were retrieved from the following link:

https://www.regulations.gov/docketBrowser?rpp=25&so=DESC&sb=postedDate&po=0&dct=SR%2BO&D=EPA-HQ-OPP-2010-0889

- All study summaries published as background documents to EFSA (2019) were retrieved from the “Sulfoxaflor Addendum: Confirmatory Information Volume 3 - Annex B.9 Co-Rapporteur Member State: Czech Republic (last updated on December, 2018)”. The Addendum, along with other background documents, was accessed at the following link:

https://open.efsa.europa.eu/study-inventory/EFSA-Q-2018-00709

**S2 Extended methods**

**2.1 the experimental design: a graphical representation**

![Diagram

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Figure 2.1-1: the treatment groups (a) and timeline of the experimental design (b) used across experiments. The decaying exposure is represented by a step-graph, similar to Figure 1 of the main manuscript. The exposure graph is only included for illustrative purposes, and is not to scale.

**2.2 design of time-variable sulfoxaflor exposure regimes**

Data on sulfoxaflor residues in nectar and pollen included in the addendum of a recent EU regulatory report (EFSA, 2019) were collated into a single dataset. Each study was initially screened on the basis of quality criteria (e.g., sampling methodology, design etc.) and the relevance of spraying regimes to currently authorised uses. Trials with at least four consecutive measurements of sulfoxaflor in nectar were used to model pesticide decay, by fitting a Single First-Order (SFO) kinetic model to residue data of individual trials. The SFO model is a two-parameter exponential equation assuming time of pesticide decay to be constant throughout the experiment, independently of the initial concentration (FOCUS, 2014). Once all models were fitted to the data, we assessed the goodness-of-fit by visual inspection of plots and residuals and assessment of statistical indicators, such as the χ2 error and the r2 (Appendix A, Table 2.3-1). Our analyses and model selection procedure was consistent with the EU peer-review of sulfoxaflor (EFSA, 2019).

As a result of this assessment, we validated six models, each representing a different degradation kinetic specific to real use conditions of sulfoxaflor. Each of these kinetics was considered a potential candidate for designing realistic time-variable exposure regimes. However, we aimed to explore the widest possible range of exposure conditions. Therefore, we selected the best- and worst-case exposure scenarios across these models, based on the resulting pesticide intake, using the following three-step process. First, we calculated predicted sulfoxaflor concentrations at daily intervals after pesticide application by using model-specific rate constants (k) and estimates of initial concentrations (C0) in Equation 2.2-1 (appendix A). In this calculation, exposure was conservatively assumed to start one day after application, to reflect the implementation of standard pollinator protection measures, such as the application of insecticides after sunset, when exposure is less likely to occur. Additionally, exposure was assumed to end when estimates of sulfoxaflor levels fell below the limit of detection (LOD) of 0.003 mg a.i./kg of the active ingredient.

Second, we calculated the pesticide intake of each model by multiplying the predicted concentrations described above by the daily food consumption. In this calculation, we assumed that a worker processes (i.e., eats and stores) 0.51 g contaminated nectar per day. This value corresponds to the daily average nectar consumption collected in a previous pilot, where the amount of food processed by bees in microcolonies was recorded in the same set-up used in this experiment (see Appendix A, 3.2).

Third, once models were ranked on the basis of their maximum daily and cumulative pesticide intake for the exposure period, we identified pumpkin and strawberry use scenarios to represent worst- and best- case exposure conditions respectively.

**Equation 2.2-1.** The Single First-Order kinetic function. C0: concentration of chemical at time t=0; k: kinetic rate constant.

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2.3 Analysis of degradation kinetics

Table 2.3-1. The summary and assessment of degradation kinetics of sulfoxaflor in nectar: re-analysis of data published within the addendum of EFSA (2019a). Models selected to represent worst- and best-case exposure conditions were highlighted in bold.

|  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Trial ID | Application rate (stage) | Crop | Location | K | C0 | DT50 | DT90 | 𝝌2 | r2 | Data points | Inclusion | Estimated sulfoxaflor intake  (µg a.s./bee) | |
| Cumulative | Max. daily |
| 603-01 | 1 \* 48 g a.s./ha  (flowering) | Apple | Germany | 0.8854 | 0.8854 | 0.783 | 2.6 | 3.49 | 0.9986 | 3 | N | *N.A.* | *N.A.* |
| 603-02 | 1.328 | 0.6558 | 0.522 | 1.73 | 22.8 | 0.9491 | 4 | N | *N.A.* | *N.A.* |
| 603-03 | France | 0.4349 | 0.2483 | 1.59 | 5.29 | 8.13 | 0.9887 | 4 | Y | 0.23 | 0.08 |
| 603-04 | NA | | | | | | 2 | N | *N.A.* | *N.A.* |
| 602-01 | 1 \* 24 g a.s./ha  (flowering) | Strawberry | Germany | 0.3709 | 0.05032 | 1.87 | 6.21 | 30.1 | 0.7565 | 4 | N | *N.A.* | *N.A.* |
| **602-02** | **1.226** | **0.5482** | **0.566** | **1.88** | **6.35** | **0.9985** | **4** | **Y** | **0.12** | **0.08** |
| 602-03 | France | 1.954 | 3.564 | 0.355 | 1.18 | 1.95 | 0.9999 | 4 | Y | 0.3 | 0.26 |
| 602-04 | 0.1304 | 0.7622 | 5.32 | 17.7 | 54.9 | 0.1764 | 4 | N | *N.A.* | *N.A.* |
| 596-01 | 1 \* 48 g a.s./ha  (flowering) | Pumpkin | Germany | 0.3104 | 0.01642 | 2.23 | 7.42 | 5.02 | 0.9875 | 3 | N | *N.A.* | *N.A.* |
| 596-02 | 0.5186 | 1.315 | 1.34 | 4.44 | 7.68 | 0.9919 | 4 | Y | 0.98 | 0.4 |
| 596-03 | France | 0.3314 | 0.06634 | 2.09 | 6.95 | 41.7 | 0.5198 | 4 | N | *N.A.* | *N.A.* |
| **596-04** | **2.358** | **14.37** | **0.294** | **0.977** | **9.43** | **0.9965** | **4** | **Y** | **0.77** | **0.69** |
| 604-01 | 1 \* 24 g a.s./ha  (flowering) | Oilseed rape | Germany | 0.9803 | 0.224 | 0.707 | 2.35 | 10.1 | 0.9949 | 3 | N | *N.A.* | *N.A.* |
| 604-02 | NA | NA | NA | NA | NA | NA | 2 | N | *N.A.* | *N.A.* |
| 604-03 | 0.6505 | 0.248 | 1.07 | 3.54 | 2.46 | 0.9996 | 4 | Y | 0.14 | 0.07 |
| 604-04 | 0.5373 | 0.268 | 1.29 | 4.29 | 2.88 | 0.9995 | 3 | N | *N.A.* | *N.A.* |

**S2 Extended methods**

**2.4 Data on allocation and rearing conditions.**

A picture containing schematic

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Figure 2.4-1 Figure 1 The allocation of bees to treatments by bodyweight (A, B) and colony of origin (C, D) in the worst- (A, C) and best-case (B, D) exposure experiments

A close up of a map

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Figure 2.4-2 The average temperature (A) and humidity (B) and C.I. recorded in the laboratory over the course of the experiments plotted against jittered data.

**2.5 analytical methods for the determination of sulfoxaflor presence and concentration in treatment samples.**

Table 2.5-1 The instrument setup (ULPC/HSS T3 UPLC column). Flow rate: 0.4 mL/min; volume injected: 10 µL; column temperature: 40°C; sample temperature: 10°C; run time: 5 min. Mobile phase A: water + 0.2% formic acid, mobile phase B: acetonitrile + 0.01% formic acid.

|  |  |  |
| --- | --- | --- |
| Time (min) | % A | %B |
| 0.00 | 90 | 10 |
| 0.50 | 0 | 100 |
| 2.50 | 0 | 100 |
| 4.00 | 90 | 10 |
| 5.00 | 90 | 10 |

Table 2.5-2 The MS Instrument set up (Acquity QDa, Waters)

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Compound | m/z quantifier | Cone quantifier | m/z qualifier | Cone qualifier |
| Sulfoxaflor | 278 | 13 | 174 | 20 |
| Atrazine-d5 | 221 | 8 |  |  |

Table 2.5-3 The Sulfoxaflor linearity. RA: ratio between the areas of analyte and internal standard (atrazine d-5). See also Figure S1

|  |  |
| --- | --- |
| **Concentration (ng/mL)** | **RA** |
| 5 | 0.007 |
| 10 | 0.131 |
| 12.5 | 0.179 |
| 15 | 0.227 |
| 20 | 0.345 |
| **Slope:** | 44.9 |
| **Intercept:** | 4.51 |
| **r²:** | 0.998 |

A screenshot of a person

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Figure 2.5-1. Sulfoxaflor calibration curve

**2.6 statistical analyses: model selection and results**

Table 2.6-1 Model selection table showing candidate models for each analysis. Models in bold are the ones within the 95% confidence set.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **A) Sucrose consumption - microcolony was included as a random effect** | | **AICc** | **ΔAICc** | **weight** |
| Worst- case exposure | treatment \* day | -3174.1 | 10.46 | 0.005 |
| **treatment + day** | -3184.5 | 0.00 | 0.995 |
| treatment | -3160.6 | 23.90 | 0.00 |
| day | -3159.9 | 24.59 | 0.00 |
| null | -3136.5 | 48.09 | 0.00 |
| Best-case exposure | **treatment \* day** | -6051.3 | 0.00 | 1 |
| treatment + day | -6001.1 | 50.18 | 0.00 |
| treatment | -5911.3 | 139.98 | 0.00 |
| day | -5943.3 | 108.01 | 0.00 |
| null | -5954.9 | 196.36 | 0.00 |
|  | | | | |
| **A1) Syrup consumption - microcolony was included as a random effect** | | **AICc** | **ΔAICc** | **weight** |
| Worst-case exposure | **treatment \* day** | -270.6 | 0 | 1 |
| treatment + day | -248.4 | 22.23 | 0 |
| treatment | -198.4 | 72.20 | 0 |
| day | -50.6 | 220.01 | 0 |
| null | -0.6 | 269.96 | 0 |
| Best-case exposure | **treatment \* day** | 259.7 | 0 | 1 |
| treatment + day | 394.8 | 135.09 | 0 |
| treatment | 536.3 | 276.52 | 0 |
| day | 586.3 | 326.54 | 0 |
| null | 726.0 | 466.21 | 0 |
|  |  |  |  |  |
| **C) egg production – colony as a random effect** | | **AICc** | **ΔAICc** | **weight** |
| Worst- case exposure | **treatment** | 599.7 | 0.00 | 1 |
| null | 621.0 | 21.26 | 0 |
| Best-case exposure | **treatment** | 1148.5 | 0.00 | 1 |
| null | 1242.3 | 93.77 | 0.00 |
|  |  |  |  |  |
| **C1) larval production – colony as a random effect** | | **AICc** | **ΔAICc** | **weight** |
| Worst- case exposure | **treatment** | 715.6 | 0.00 | 1 |
| null | 806.4 | 90.83 | 0.00 |
| Best-case exposure | **treatment** | 1088.3 | 0.00 | 1 |
| null | 1135.4 | 47.17 | 0.00 |
|  |  |  |  |  |
| **C2) egg production (interaction) - colony as a random effect** | | **AICc** | **ΔAICc** | **weight** |
| Best-case exposure | **pesticide exposure + sugar deficit + pesticide exposure:sugar deficit** | 1148.5 | 0.00 | 0.999 |
| pesticide exposure + sugar deficit | 1161.9 | 13.39 | 0.01 |
| pesticide exposure | 1232.9 | 84.41 | 0.00 |
| sugar deficit | 1175.6 | 27.08 | 0.00 |
| null | 1242.3 | 93.77 | 0.00 |
|  |  |  |  |  |
| **C3) larval production (interaction) - colony as a random effect** | | **AICc** | **ΔAICc** | **weight** |
| Best-case exposure | **pesticide exposure + sugar deficit + pesticide exposure:sugar deficit** | 1088.3 | 0.20 | 0.474 |
| **pesticide exposure + sugar deficit** | 1088.0 | 0.00 | 0.52 |
| pesticide exposure | 1123.8 | 35.78 | 0.00 |
| sugar deficit | 1105.0 | 16.96 | 0.00 |
| null | 1135.4 | 47.8 | 0/00 |
|  |  |  |  |  |
| **D) ovary development (binomial process)** | | **AICc** | **ΔAICc** | **weight** |
| Worst- case exposure | **treatment \* size** | 206.0 | 3.92 | 0.063 |
| **treatment + size** | 205.3 | 3.24 | 0.088 |
| **treatment** | 203.3 | 1.22 | 0.242 |
| **size** | 204.1 | 2.02 | 0.162 |
| **null** | 202.1 | 0.00 | 0.445 |
| Best-case exposure | **treatment \* size** | 369.8 | 2.51 | 0.157 |
| **treatment + size** | 367.3 | 0.00 | 0.552 |
| **treatment** | 370.7 | 3.39 | 0.101 |
| **size** | 369.8 | 2.43 | 0.164 |
| null | 373.4 | 6.11 | 0.026 |
|  |  |  |  |  |
| **D1) mean terminal oocyte length** | | **AICc** | **ΔAICc** | **weight** |
| Worst- case exposure | treatment \* size | 491.7 | 4.92 | 0.037 |
| **treatment + size** | 489.6 | 2.92 | 0.101 |
| **treatment** | 488.0 | 1.25 | 0.234 |
| **size** | 488.4 | 1.65 | 0.191 |
| **null** | 486.7 | 0.00 | 0.436 |
| Best-case exposure | treatment \* size | 1085.9 | 10.90 | 0.004 |
| **treatment + size** | 1080.2 | 5.26 | 0.064 |
| treatment | 1086.4 | 11.38 | 0.003 |
| **size** | 1075.0 | 0.00 | 0.893 |
| null | 1081.4 | 6.42 | 0.036 |
|  |  |  |  |  |
| **E) exposure concentration distribution** | | **AIC** | **ΔAIC** | **weight** |
| lognormal | | 153 | 0 | 1 |
| log-logistic | | 273 | 120 | 0 |
| gamma | | 217 | 63.6 | 0 |
| Weibull | | 176 | 22.1 | 0 |
| normal | | 763 | 610 | 0 |

Table 2.6-2 Parameter estimates, standard errors and 95% confidence limits derived in each statistical model. Significant parameters highlighted in bold.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Larval production -**  **worst-case exposure** | **Parameter Estimate** | **Standard Error** | **Lower 95% CI** | **Upper 95% CI** |
| *Conditional model:* | | | | |
| (Intercept) Sucrose 50% | 3.18 | 0.09 | 3.00 | 3.36 |
| Sucrose 15% | **-0.51** | **0.05** | **-0.61** | **-0.40** |
| *Zero inflation model:* | | | | |
| (Intercept) Sucrose 50% | -3.69 | 1.01 | -5.67 | -1.70 |
| Sucrose 15% | 1.15 | 1.18 | -1.16 | 3.46 |
|  |  |  |  |  |
| **Larval production -**  **best-case exposure** | **Parameter Estimate** | **Standard Error** | **Lower 95% CI** | **Upper 95% CI** |
| *Conditional model:* | | | | |
| (Intercept) Sucrose 50% | 2.73 | 0.07 | 2.60 | 2.86 |
| Sucrose 15% | **-0.37** | **0.08** | **-0.52** | **-0.21** |
| Sulfoxaflor + sucrose 50% | **-0.32** | **0.07** | **-0.45** | **-0.18** |
| Sulfoxaflor + sucrose 15% | **-0.43** | **0.11** | **-0.64** | **-0.22** |
| *Zero inflation model:* | | | | |
| (Intercept) Sucrose 50% | -1.27 | 0.38 | -2.01 | -0.53 |
| Sucrose 15% | **1.17** | **0.49** | **0.22** | **2.13** |
| Sulfoxaflor + sucrose 50% | 0.35 | 0.51 | -0.65 | 1.35 |
| Sulfoxaflor + sucrose 15% | **2.17** | **0.52** | **1.15** | **3.19** |
|  |  |  |  |  |
| **Larval production –**  **best-case exposure (interaction)** | **Parameter Estimate** | **Standard Error** | **Lower 95% CI** | **Upper 95% CI** |
| *Conditional model:* | | | | |
| Intercept | **2.72** | **0.07** | **2.58** | **2.85** |
| Pesticide exposure | **-0.28** | **0.07** | **-0.43** | **-0.14** |
| Sugar deficit | **-0.32** | **0.08** | **-0.49** | **-0.16** |
| Pesticide exposure:sugar deficit | 0.12 | 0.16 | -0.19 | 0.43 |
| *Zero inflation model:* | | | | |
| Intercept | **-1.37** | **0.37** | **-2.10** | **-0.65** |
| Pesticide exposure | 0.54 | 0.47 | -0.38 | 1.45 |
| Sugar deficit | **1.34** | **0.45** | **0.46** | **2.23** |
| Pesticide exposure:sugar deficit | 0.30 | 0.58 | -0.83 | 1.44 |
|  |  |  |  |  |
| **Egg production -**  **worst-case exposure** | **Parameter Estimate** | **Standard Error** | **Lower 95% CI** | **Upper 95% CI** |
| *Conditional model:* | | | | |
| (Intercept) Sucrose 50% | 2.58 | 0.21 | 2.17 | 2.99 |
| Sucrose 15% | **-0.41** | **0.09** | **-0.58** | **-0.23** |
| *Zero inflation model:* | | | | |
| (Intercept) Sucrose 50% | -0.49 | 0.36 | -1.20 | 0.23 |
| Sucrose 15% | **0.92** | **0.46** | **0.01** | **1.83** |
|  |  |  |  |  |
| **Egg production -**  **best-case exposure** | **Parameter Estimate** | **Standard Error** | **Lower 95% CI** | **Upper 95% CI** |
| *Conditional model:* | | | | |
| (Intercept) Sucrose 50% | 2.82 | 0.07 | 2.67 | 2.96 |
| Sucrose 15% | **-0.28** | **0.08** | **-0.43** | **-0.13** |
| *Zero inflation model:* | | | | |
| (Intercept) Sucrose 50% | -0.75 | 0.37 | -1.47 | -0.03 |
| Sucrose 15% | 0.85 | 0.46 | -0.06 | 1.76 |
| Sulfoxaflor + sucrose 50% | -0.19 | 0.49 | -1.14 | 0.76 |
| Sulfoxaflor + sucrose 15% | **0.99** | **0.48** | **0.05** | **1.92** |
|  |  |  |  |  |
| **Egg production - best-case exposure (interaction)** | **Parameter Estimate** | **Standard Error** | **Lower 95% CI** | **Upper 95% CI** |
| *Conditional model:* | | | | |
| Intercept | **2.82** | **0.07** | **2.67** | **2.96** |
| Pesticide exposure | -0.10 | 0.07 | -0.23 | 0.03 |
| Sugar deficit | **-0.28** | **0.08** | **-0.43** | **-0.13** |
| Pesticide exposure:sugar deficit | **-0.54** | **0.13** | **-0.80** | **-0.28** |
| *Zero inflation model:* | | | | |
| Intercept | **-0.75** | **0.37** | **-1.47** | **-0.03** |
| Pesticide exposure | -0.19 | 0.49 | -1.14 | 0.76 |
| Sugar deficit | 0.85 | 0.46 | -0.06 | 1.76 |
| Pesticide exposure:sugar deficit | 0.33 | 0.67 | -0.98 | 1.64 |
|  |  |  |  |  |
| **Syrup consumption-**  **worst-case exposure** | **Parameter Estimate** | **Standard Error** | **Lower 95% CI** | **Upper 95% CI** |
| (Intercept) Sucrose 50% | **0.63** | **0.03** | **0.57** | **0.69** |
| Sucrose 15% | **1.00** | **0.04** | **0.92** | **1.09** |
| day | **0.00** | **0.00** | **0.00** | **0.01** |
| Sucrose 15% : day | **0.02** | **0.00** | **0.01** | **0.02** |
|  |  |  |  |  |
| **Sucrose consumption-**  **worst-case exposure** | **Parameter Estimate** | **Standard Error** | **Lower 95% CI** | **Upper 95% CI** |
| (Intercept) Sucrose 50% | **0.31** | **0.01** | **0.29** | **0.32** |
| Sucrose 15% | **-0.06** | **0.01** | **-0.08** | **-0.04** |
| day | **0.00** | **0.00** | **0.00** | **0.00** |
|  |  |  |  |  |
| **Syrup consumption-**  **best-case exposure** | **Parameter Estimate** | **Standard Error** | **Lower 95% CI** | **Upper 95% CI** |
| (Intercept) Sucrose 50% | **0.63** | **0.05** | **0.54** | **0.72** |
| Sucrose 15% | **0.85** | **0.07** | **0.72** | **0.98** |
| Sulfoxaflor + sucrose 50% | -0.07 | 0.07 | -0.20 | 0.06 |
| Sulfoxaflor + sucrose 15% | **0.40** | **0.07** | **0.27** | **0.53** |
| day | 0.00 | 0.00 | -0.01 | 0.00 |
| Sucrose 15% : day | **0.02** | **0.00** | **0.02** | **0.03** |
| Sulfoxaflor + sucrose 50% : day | **0.01** | **0.00** | **0.00** | **0.02** |
| Sulfoxaflor + sucrose 15% : day | **0.0** | **0.00** | **0.04** | **0.06** |
|  |  |  |  |  |
| **Sucrose consumption-**  **best-case exposure** | **Parameter Estimate** | **Standard Error** | **Lower 95% CI** | **Upper 95% CI** |
| (Intercept) Sucrose 50% | 0.31 | 0.01 | 0.30 | 0.33 |
| Sucrose 15% | **-0.09** | **0.01** | **-0.12** | **-0.07** |
| Sulfoxaflor + sucrose 50% | **-0.03** | **0.01** | **-0.06** | **-0.01** |
| Sulfoxaflor + sucrose 15% | **-0.16** | **0.01** | **-0.19** | **-0.13** |
| day | **0.00** | **0.00** | **0.00** | **0.00** |
| Sucrose 15% : day | **0.00** | **0.00** | **0.00** | **0.01** |
| Sulfoxaflor + sucrose 50% : day | **0.00** | **0.00** | **0.00** | **0.01** |
| Sulfoxaflor + sucrose 15% : day | **0.01** | **0.00** | **0.01** | **0.01** |
|  |  |  |  |  |
| **Ovary development -**  **worst-case exposure** | **Parameter Estimate** | **Standard Error** | **Lower 95% CI** | **Upper 95% CI** |
| (Intercept) Sucrose 50% | 2.61 | 2.21 | -1.72 | 6.95 |
| Sucrose 15% | -0.57 | 2.32 | -5.12 | 3.98 |
| size | -0.08 | 0.45 | -0.96 | 0.80 |
| Sucrose 15% : size | 0.09 | 0.47 | -0.83 | 1.01 |
|  |  |  |  |  |
| **Ovary development -**  **best-case exposure** | **Parameter Estimate** | **Standard Error** | **Lower 95% CI** | **Upper 95% CI** |
| (Intercept) Sucrose 50% | -0.83 | 4.03 | -8.73 | 7.08 |
| Sucrose 15% | -2.03 | 4.19 | -10.25 | 6.20 |
| Sulfoxaflor + sucrose 50% | -0.96 | 3.98 | -8.77 | 6.85 |
| Sulfoxaflor + sucrose 15% | -2.54 | 5.38 | -13.09 | 8.02 |
| size | 0.69 | 0.84 | -0.95 | 2.33 |
| Sucrose 15% : size | 0.32 | 0.87 | -1.39 | 2.02 |
| Sulfoxaflor + sucrose 50% : size | 0.26 | 0.83 | -1.38 | 1.89 |
| Sulfoxaflor + sucrose 15% : size | 0.41 | 1.12 | -1.77 | 2.60 |
|  |  |  |  |  |
| **Ovary length -**  **worst-case exposure** | **Parameter Estimate** | **Standard Error** | **Lower 95% CI** | **Upper 95% CI** |
| (Intercept) Sucrose 50% | 2.42 | 0.34 | 1.76 | 3.08 |
| Sucrose 15% | -0.02 | 0.05 | -0.12 | 0.08 |
| size | 0.02 | 0.07 | -0.11 | 0.16 |
|  |  |  |  |  |
| **Ovary length -**  **best-case exposure** | **Parameter Estimate** | **Standard Error** | **Lower 95% CI** | **Upper 95% CI** |

Supplementary Information Text

**S3 Extended results**

**3.1 Effects of sulfoxaflor on bee fertility.**

Ovary development was not affected by sulfoxaflor exposure (Figure 1B, glm, best-case exposure: PE = -0.96, CI = -8.77 to 6.85), sugar deficit (Figure 1 A-B, glm, best-case exposure: PE = -2.02, CI = -10.25 to 6.20; worst-case exposure: PE = - 0.57, CI = -5.12 to 3.98) or their combination (Figure 1 B, glm, best-case exposure: PE = -2.54, CI = -13.09 to 8.01). Similarly, terminal oocyte length was not affected by pesticide exposure (Figure 1 B, glm, best-case exposure: PE = 3.2e-04, CI = -0.04 to 0.04), sugar deficit (Figure 1 A-B, glm, best-case exposure: PE = 1.4e-05, CI = -0.04 to 0.04; worst-case exposure: PE = - 0.02, CI = -0.12 to 0.08) or their combination (Figure 1 B, glm, best-case exposure: PE = 5.1e-03, CI = -0.05 to 0.065).

A screenshot of a cell phone

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Figure 3.1-1 The mean (± SE) terminal oocyte length per worker.

**3.2 pilot study determining food consumption in microcolonies across sugar concentrations**

Methods

The objective of this pilot was to quantify consumption of increasingly concentrated sucrose solutions by workers housed in microcolonies over a period of 14 days.

We used 6 queen-right colonies (*Bombus terrestris audax*, Agralan Ltd) of approximately 130 individuals with brood at various stages of development. Workers from these colonies were screened in a parallel experiment for the most prevalent gut parasites (*Apicystis bombi*, *Crithidia* spp., and *Nosema* spp.) through microscopic examination of faecal samples and no infections were detected.

216 medium-sized workers were allocated in groups of 4 to 54 microcolonies by weight and colony of origin. Microcolonies were allocated to 3 treatment groups, consisting of syrup diets with increasing sucrose concentration (i.e., 15%, 30% and 50% w/w). Workers for each microcolony were selected from the same queen-right colony. 15 workers died during the allocation and were replaced with individuals belonging to the same queen-right colony of origin. Microcolonies were set-up in custom-made acrylic boxes (width: 50 mm; length: 115 mm; height: 65mm) with a hole in one side to accommodate a horizontally positioned 5 ml syringe (BD emerald, Becton Dickinson, USA) with the tip removed to function as a syrup feeder. However, as the experiment started, it became apparent that syringe volume was inadequate. Therefore, 3 days after housing, syringes were replaced with 15 ml centrifuge tubes (Starstedt AG & Co. KG, DE) with a 2mm hole drilled at the end. A 35 mm diameter polystyrene petri dish was used as a pollen feeder in each box. Bees were given *ad libitum* supply of pollen balls (≈ 1.5 g) prepared by mixing fresh-ground pollen to distilled water in a 4:1 ratio. Over the course of the test, microcolonies were kept in darkness with temperature and humidity set at 26˚C and 60% respectively. Mortality was recorded daily and bees who died at this stage (N=2) were not replaced. Sucrose consumption was measured each day by weighing feeders to the nearest milligram (Scout® STX, Ohaus, CH). Daily consumption was adjusted for evaporation, which was measured as the mean weight change of 3 syrup feeders per sugar concentration kept in empty microcolony boxes under the same experimental conditions.

Results

The mean syrup consumption (Fig. 1) was quantified as 1.58 g (±SD=0.41), 0.9 g (±SD=0.22) and 0.51g (±SD=0.13) for the 15%, 30% and 50% sucrose groups respectively.

A close up of a map

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Figure 3.2-1 the daily (A) and overall (B) consumption of syrup. Error bars represent the standard error of the mean.