Health & Ecological Risk Assessment

Realistic exposure of the fungicide bixafen in soil and its toxicity and risk to natural earthworm populations after multiyear use in cereal

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ABSTRACT
A comprehensive multiyear monitoring program was conducted to assess the exposure, effects, and long-term risk of the fungicide bixafen to earthworms in cereal fields. The realistic exposure of bixafen in soil was assessed at 10 representative field sites in Germany after a period of up to 8 years of use with five different products containing bixafen, followed by annual measurements from 2017 to 2019. The measured exposure concentrations were compared with modeled predicted environmental concentrations in soil (PECsoil) that are derived in the context of the European risk assessment of plant protection products. It was shown that the model assumptions, in particular the kinetic parameters describing the background accumulation, provided a conservative description of the observed residue data. This demonstrates that the exposure modeling tools are adequate for use in soil risk assessment. Laboratory and field ecotoxicological studies were performed to provide a comprehensive risk assessment on the long-term use of bixafen-based fungicides in cereals. While a laboratory reproduction study with the earthworm Eisenia fetida indicated a potential risk at the Tier 1 risk assessment for the end-use product Skyway XPro®, a 2.5-year field study showed no unacceptable long-term effects on natural earthworm populations. The exposure in this study exceeded the maximum recommended field rate of Skyway XPro® by a factor of 3 and the maximum measured bixafen concentrations from exposure monitoring study by a factor of 5.2. Hence, an acceptable long-term risk of bixafen-based cereal fungicides is concluded for earthworms. Integr Environ Assess Manag 2022;18:734–747. © 2021 Bayer AG. Integrated Environmental Assessment and Management published by Wiley Periodicals LLC on behalf of Society of Environmental Toxicology & Chemistry (SETAC).

KEYWORDS: Accumulation, Bixafen, Earthworms, Monitoring, Risk assessment

INTRODUCTION
Crop protection products are widely used in agriculture to control pests, weeds, and diseases (FAO and IPTS, 2017). An environmental risk assessment for pesticides is necessary to protect nontarget organisms and ecosystems from unacceptable side effects. Earthworms can play a major functional role in soil ecosystems by enhancing organic matter degradation and regulating water and nutrient cycling in soil (Lee, 1985). For this reason, an earthworm risk assessment is required to obtain the registration of a pesticide (e.g., EC, 1997). An acceptable risk is indicated at the Tier 1 level if the toxicity exposure ratio (TER), which is the no-observed effect concentration (NOEC) divided by the PECsoil, exceeds the value of 5 in Europe (EC, 2009). This safety factor of 5 is used to extrapolate from Tier 1 risk assessment based on E. fetida studies in artificial soil to natural earthworm populations in the more realistic field situation (Christl et al., 2016; EC, 2009). In case the Tier 1 TER indicates a potential concern (TER < 5), higher tier studies, such as earthworm field studies (International Standards Organisation, 1999; Kula et al., 2006), are recommended to assess whether the use of the pesticide under realistic conditions actually shows unacceptable long-term effects on earthworms (EC, 2009). An acceptable risk is shown if no unacceptable effects on earthworm populations are observed latest 1 year after application of the pesticide at the recommended field rate or above (EC, 2002).

Succinate dehydrogenase inhibitors (SDHIs), such as bixafen, are widely used as fungicides due to their good efficacy for Economic Co-operation and Development [OECD], 2004) are compared with the predicted environmental concentration in soil (PECsoil; EU Commission, 1995, 2000; FOCUS, 1997). An acceptable risk is indicated at the Tier 1 level if the toxicity exposure ratio (TER), which is the no-observed effect concentration (NOEC) divided by the PECsoil, exceeds the value of 5 in Europe (EC, 2009). This safety factor of 5 is used to extrapolate from Tier 1 risk assessment based on E. fetida studies in artificial soil to natural earthworm populations in the more realistic field situation (Christl et al., 2016; EC, 2009). In case the Tier 1 TER indicates a potential concern (TER < 5), higher tier studies, such as earthworm field studies (International Standards Organisation, 1999; Kula et al., 2006), are recommended to assess whether the use of the pesticide under realistic conditions actually shows unacceptable long-term effects on earthworms (EC, 2009). An acceptable risk is shown if no unacceptable effects on earthworm populations are observed latest 1 year after application of the pesticide at the recommended field rate or above (EC, 2002).

Succinate dehydrogenase inhibitors (SDHIs), such as bixafen, are widely used as fungicides due to their good efficacy
against phytopathogenic fungi (Avenot & Michailides, 2010). As for all approved pesticides, SDHIs are subjected to a thorough environmental risk assessment examination before being authorized for use in agriculture (e.g., EC, 2009). They are thoroughly investigated regarding their effects and risks on nontarget organisms, including earthworms (e.g., EFSA, 2008, 2012a, 2012b, 2012c, 2013a, 2013b, 2016). In a paper by Bénit et al. (2019), some concerns were raised against SDHIs, by showing an impact of different SDH fungicides on in situ enzyme activities in the mitochondrial respiratory chain of cells of the earthworm species Lumbricus terrestris. However, it remains unclear whether this physiological effect, measured at the cellular level, might lead to earthworm population-relevant effects resulting in population decline, which is the ecologically relevant endpoint needed to meet the environmental protection goals for crop protection products. Additional evidence from natural earthworm populations under realistic conditions in the field can further help to evaluate the protectiveness of the current environmental risk assessment procedure.

Some SDHI fungicides, including bixafen, show relatively slow degradation rates in soil (EFSA, 2008, 2012a, 2012b, 2012c, 2013a, 2013b, 2016). Soil dissipation half-life (DT50) values of bixafen under field conditions were studied under different pedoclimatic conditions and range from 30.6 to 1235 days (see EFSA, 2012a). With these values, there is potential for accumulation in soil and this is therefore routinely addressed in the risk assessment for soil organisms via a soil exposure evaluation (e.g., EC, 2002, 2009). Repeated application of the pesticide over multiple years is taken into account until a long-term background (plateau) concentration is reached. This plateau concentration is added to the PECsoil value arising from the annual applications to obtain the maximum PECsoil value for risk assessment (EU Commission, 2000; FOCUS, 1997). The estimation of the plateau concentration follows several conservative assumptions, for example degradation is described by the worst-case (longest) dissipation half-life observed from all field studies, the full application rate to be registered is assumed to be applied annually, and crop growth stage with the lowest crop interception during the potential use period thus provides the maximum fraction of the active substance reaching the soil (FOCUS, 1997, 2014b). Field exposure monitoring data can be used to quantify the realistic exposure in agricultural fields and to verify whether the exposure estimation considered in the risk assessment provides sufficiently conservative values. Therefore, the aims of this study were to

1. measure the exposure of bixafen at 10 locations representative for cereal-growing conditions in Germany after repeated application of bixafen in a long-term field exposure monitoring study and compare them with the modeled PECsoil,
2. measure the toxicity of the active substance bixafen and the end-use product Skyway XPro® in a standard laboratory earthworm reproduction test with E. fetida,
3. determine potential short- and long-term effects of bixafen (applied with Skyway XPro®) on the natural earthworm community in a 2.5-year field trial, and
4. conclude on the potential short- and long-term risks of bixafen for earthworms following the recommended use in cereal cropping systems using laboratory and field data.

MATERIALS AND METHODS

Exposure monitoring study on 10 agricultural fields

Field sites for exposure monitoring. To describe the fate and potential accumulation of the cereal fungicide bixafen under agricultural practice, a monitoring study on cereal fields in Germany was performed. Only sites with a known history of bixafen use were included. The selected 10 sites throughout Germany cover a wide range of environmental conditions and agricultural practices, with some bias to worst-case conditions regarding the quantity and persistence of bixafen. Environmental conditions that contribute to slower degradation and thus higher bixafen soil concentrations are low bulk density, high organic carbon content, low permeability, low soil moisture, and low temperatures. To consider these worst-case conditions, soil samples were taken from 0 to 30 cm layer prior to the final selection of the fields and analyzed for soil texture (USDA soil texture classes), percentage of organic carbon content, pH value, and bulk density. Soil textures at the sites were comprised of sand (one site), sandy loam (four), loam (one), clay loam (one), silt loam (two), and silty clay (one). The bulk density ranges from 950 to 1540 g/L (median 1190 g/L dry weight).

The spatial distribution of the selected fields to be monitored across Germany was intended to represent different soils, climatic conditions, and areas with a high percentage of cereal cultivation (Figure S1, full details on study site selection are provided in the Supporting Information).

Application schedule and history. The first bixafen product registrations in Germany were granted in autumn 2010 and the first sales took place in 2011. A complete list of bixafen-containing products with recommended application rates are provided in the Supporting Information (Table S1). The pesticide application history of the monitored fields was determined from at least 2015 onwards; in some cases, earlier applications from 2012 onward are also recorded (Table 1). Further information was available from farmer interviews comprising of application dates, the applied product, the application rate, and the crop. In addition to the pesticide application history, the soil preparation history was recorded, ideally with the date, the soil preparation type/machinery, and the depth.

Sampling depth and timing

To determine the residue status of bixafen in selected field plots, soil residue samples were taken at all study sites
in the spring of 2017, 2018, and 2019. These residues were sampled prior to the seasonal bixafen application. For study sites where an application with a bixafen-containing product was performed by the farmer in 2017, 2018, or 2019, a second soil residue sampling took place 10–20 days after the last application of the bixafen product.

Soil samples were taken to a 30 cm depth with a “Wacker Hammer.” Per site, 15 soil samples were taken at random locations in the field at each site. The soil cores were deep-frozen within 24 hours and then stored at −18 °C prior to analysis. The frozen soil cores were cut into soil segments. The first sample (prior to potential bixafen application) was segmented into three layers: 0–10, 10–20, and 20–30 cm. The second sample (10–20 day after last application of the season) was segmented into four layers: 0–5, 5–10, 10–20, and 20–30 cm (except for 2017, when three segments were separated [0–5, 5–10, and 10–30 cm]). To reduce random variation, 3 out of the 15 samples per site were bulked into one sample (for each soil horizon). These five composite samples were then carefully homogenized using cryomilling and stored at or below −18 °C until analysis. The soil samples taken in the earthworm field study were homogenized by cryomilling and stored at ≤−18 °C.

The dry matter of the soil samples was determined after complete evaporation of the dry ice.

**Analytical methods**

Samples from the exposure monitoring were analyzed for bixafen. Soil samples of 5 g were extracted in a microwave extractor with a mixture of acetonitrile/water/cysteine-hydrochloride (0.8/0.2 L/50 mg). The extracts were centrifuged to remove fine particles of the soil. Possible matrix effects were eliminated using an internal standard solution of isotopically labeled reference items. Quantification of the analyte was performed by HPLC using MS/MS detection.

The limit of quantification (LOQ) of the analytical method was 0.005 mg/kg for bixafen.

For the analysis of soil samples taken in the earthworm field study, subsamples were extracted by mixing 5 ml of water and 5 ml of acetonitrile with 5 g of dry weight soil (dws) and shaken at 2500 rpm for 10 min. Analysis was conducted using reversed phase high performance liquid chromatography (RP-HPLC), coupled with electrospray ionization and mass spectrometric (MS/MS) detection. The LOQ was 0.01 mg/kg for bixafen and 0.005 mg/kg for tebuconazole (as contained in the product Skyway XPro® used in the earthworm field trial).

Additional details on analytical methods are provided in the Supporting Information.

**Models used for PECplateau estimation and extrapolation**

For each site, the information from the monitoring study was used to calculate site-specific PECsoil values over time. The application rates and dates provided by the farmers were considered as well as information on soil tillage. If no quantitative information on soil tillage was available, a default mixing depth of 20 cm was assumed. The soil bulk density was available from soil characterization carried out in the monitoring fields. Degradation kinetics were taken from the worst-case site (longest value) of the terrestrial field dissipation study (EFSA, 2012a). The dissipation of bixafen was described using the biphasic Hockey Stick kinetic model (FOCUS, 2014a)

\[
C = \begin{cases} 
C_0 \cdot e^{-k_1 \cdot t} & \text{for } t \leq t_0 \\
C_0 \cdot e^{-k_1 \cdot t} \cdot e^{-k_2 (t-t_0)} & \text{for } t > t_0
\end{cases}
\]

where \(C\) is the concentration of bixafen at time \(t\), \(C_0\) is the initial concentration of bixafen at time \(t=0\), \(k_1\) is the

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**TABLE 1** Known bixafen application history of monitoring sites in Germany (data provided by the farmers) given as the annual bixafen application rate in g/ha

| Monitoring site | 2012 | 2013 | 2014 | 2015 | 2016 | 2017 | 2018 | 2019 |
|-----------------|------|------|------|------|------|------|------|------|
| Laacher Hof     | 75   | –    | 60   | 52.5 | –    | 180  | 60   | –    |
| Höfchen         | 120  | n.a. | 93.75| –    | 56.25| 45   | 81.26| –    |
| Osnabrück       | 67.5 | –    | 93.75| 97.5 | –    | 120  | 168.75| –    |
| Schleswig       | n.a. | n.a. | –    | 26.25| 30   | –    | 26   | –    |
| Nordvorpommern  | n.a. | n.a. | n.a. | 112.5| 30   | –    | 56.25| –    |
| Nordsachsen     | n.a. | n.a. | –    | 56.25| –    | –    | 78   | 71.8 |
| Bayern Hof      | n.a. | n.a. | 82.5 | –    | 93.75| 67.5 | 71.25| 141  |
| Gotha           | n.a. | n.a. | n.a. | 100  | –    | 102  | –    | 140  |
| Schwäbisch Hall | –    | –    | 75   | –    | 90   | 75   | –    | 110  |
| Landshut        | n.a. | n.a. | n.a. | –    | 52.5 | 60   | –    | –    |

Abbreviations: –, no bixafen application; n.a., no information available.
dissipation rate until time $t = t_b$ (0.0081 d$^{-1}$), $k_2$ is the dissipation rate from time $t = t_b$ onwards (0.00023 d$^{-1}$), and $t_b$ is the breakpoint when degradation rate changes (52.7 days; EFSA, 2012a).

To account for the accumulation of bixafen in soil after repeated use, the dissipation was modeled using the Hockey Stick model for each application, and the PECsoil values from the individual applications were summed up at each time point. With this procedure, the fast decline until the breakpoint $t_b$ with rate $k_1$ is only accounted for in the newly applied bixafen, while residues from previous applications dissipate with the slow dissipation rate $k_2$.

The crop interception was based on the regulatory values for the product and its approved application timing, together with the application dates and the crop growth stage at these dates (FOCUS, 2014b). The phenological crop growth stages are described with the standardized BBCH-scales (Meier, 2018) and corresponding interception rates selected based on (EFSA, 2014a). For more details, please refer to the Supporting Information provided. Wash-off of bixafen from the cereal plants was taken into account as specified by EFSA (2017) for winter and spring cereals at different growth stages (38% and 58% for winter cereals and applications before and after BBCH30, respectively; 47% and 45% for spring cereals and applications before and after BBCH30, respectively).

To overcome uncertainties in the bixafen application history before 2017, additional calculations have been carried out, starting with background concentrations that were measured in 2017, before further bixafen applications were performed. From 2017 to 2019, the PECsoil in 0–10 cm resulting from applications in the 2017–2019 period was added to the measured background concentration. The PECsoil values used in soil risk assessment account for the potential accumulation of the compound in soil following repeated use over several years. The time until a plateau concentration is reached depends on the compounds’ soil DT50 and is 44 years for bixafen. In addition to the retrospective modeling, the future soil accumulation was modeled under repetitive use of bixafen with the same application pattern. This provided a calculation of the plateau concentration based on real-world field data. The exposure based on this plateau concentration, in addition to the concentration resulting from a new application, was then related to the ecotoxicological endpoints.

Laboratory reproduction tests with *E. fetida*

To assess the impact of bixafen on earthworms, laboratory earthworm reproduction studies with *E. fetida* (OECD, 2004) were conducted with the active substance bixafen (purity 95.8%) and the end-use product Skyway XPro® (containing [measured] 76.28 g bixafen/L, 101.5 g prothioconazole/L, and 101.1 g tebuconazole/L). Five different concentrations of bixafen or Skyway XPro® (each n = 4 replicates) were considered: 25, 50, 100, 200, and 400 mg bixafen/kg dws, and 8, 14, 25, 45, and 80 mg Skyway XPro®/kg dws, respectively. Water controls with eight replicates were run in parallel. In both tests, artificial soil was used containing 74.8% quartz-sand, 20% kaolin clay, 5% sphagnum peat, and 0.2% CaCO$_3$. Further soil and environmental properties are presented in Table S2. Ten synchronized adult earthworms per replicate were randomly selected, weighed, and placed on the soil surface. In the first 4 weeks, 5 g of fine-ground cattle manure per replicate was added weekly as a food source for the earthworms. After 28 days, surviving adult earthworms per replicate were taken out, counted, and weighed. After further 28 days, juvenile earthworms that hatched from the cocoons laid by the adult earthworms were counted per replicate. The results were statistically evaluated using Dunnett’s multiple t tests (one-sided for reproduction, two-sided for growth, p-value = 0.05) using the software ToxRatPro Version 2.09.

Two-and-a-half-year earthworm field study

A 2.5-year field study was performed to assess the short- and long-term impact of the fungicide Skyway XPro® (end-use product containing the active substances bixafen [75.04 g/L], prothioconazole [100.7 g/L], and tebuconazole [100.6 g/L]) on natural earthworm populations in a cereal cropping system after multiple applications. The study was conducted in an arable field in Eastern Germany, Saxony, near Leipzig. The design of the study was in line with International Standards Organisation (1999) and took into account the recommendations of Kula et al. (2006). The experiment was set up with five treatment groups: Control, Low rate (LR) with 2 x 1.25 L/ha/year (recommended field rate); Medium Rate (MR) with 2 x 2.5 L/ha/year, and High Rate (HR) with 2 x 3.75 L/ha/year, and a toxic reference treatment with 1 x 10 kg carbendazim/ha/year (applied as 20 L Maypon Flow/ha [500 g carbendazim/L]), each with four plot replicates following a randomized block design (Figure S3). The control was left untreated. Each of the 20 plots had a size of 10 x 14 m. In November 2016, 4.8 kg bixafen/ha (applied as an experimental suspension concentrate formulation containing 501.8 g bixafen/L, density 1.213 g/ml) and 41.7 g tebuconazole/ha (applied as a wettable granule formulation containing 25.4% tebuconazole) were applied in a tank mix to all test item treatment groups (LR, MR, and HR, 10 $\times$ 10 cm depth; based on the assumption of total annual application of 187.5 g bixafen/ha, 20% crop interception, dissipation according to the Hockey Stick kinetic described above and 20 cm soil mixing) and for tebuconazole 0.0043 mg a.s./kg dws (0–20 cm depth; based on the assumption of total annual application of 250 g tebuconazole/ha, 20% crop interception, dissipation according to a nonnormalized worst-case field DT50 of 91.6 days, and 20 cm soil mixing [EFSA, 2014b]). The application rate of 4.8 kg a.s./ha for the simulation of the plateau concentration for bixafen was
expected to lead to a concentration of 1.6 mg a.s./kg dws (0–20 cm) immediately after application. This off-label experimental high rate was selected to generate worst-case conditions for the plateau and to account for the possible degradation of bixafen in the period between the autumn of 2016 and the spring of 2017. Soil samples (5 cm diameter) were taken 10 per plot immediately after the plateau application at 0–10 cm depth and combined into one sample per plot for analytical verification of the simulated bixafen and tebuconazole plateau concentration. The plots were then ploughed to a depth of 0–20 cm, followed by a shallow grubber to mix the substances for the plateau as homogeneously as possible into the soil. During this step, winter wheat (Triticum aestivum) was drilled according to standard agricultural practice. The plateau concentration was applied in the autumn of 2016 (about 6 months before the product applications in May 2017) to give earthworms time to recover from the mechanical mixing of the soil (i.e., ploughing to 20 cm depth). In the following spring (March 2017), soil samples were taken again to verify the simulated bixafen plateau concentration in soil after the winter period (80 subsamples [5 cm diameter] per plot at 0–20 cm depth combined into one sample per plot). All samples for verification of the plateau concentration were taken from the 10 × 10 m area where the plateau was applied. In May 2017 and April–May 2018, Skyway XPro® was applied two times per season with a single rate of 1.25, 2.5, and 3.75 L/ha considering an application interval of 14 days in the respective year. The product was applied at the winter wheat crop stages of BBCH 22 and 23 in 2017 and BBCH 24 and 25 in 2018. The selected application timing is earlier than the recommended crop stage of BBCH 25+ to simulate a high soil exposure with low crop interception as a worst-case approach. All plots were irrigated with at least 10 mm of water after each product application to ensure maximum exposure of the test substance to earthworms as recommended by Kula et al. (2006). In 2017, the Skyway XPro® was applied to a 10 × 12 m plot area (a 2 × 10 m area thereof was formerly not applied with the plateau application) to verify the bixafen concentration from the first two product applications only (excluding the plateau). Soil samples were taken from this 2 × 10 m strip, which was only treated by these two product applications. On the day of the second product application, per season, 10 soil samples were taken per plot from different soil depths: 0–1, 1–5, 5–10 cm, and combined into one sample per plot for each layer. In 2018, the product was applied twice to the total plot area (10 × 14 m). Soil samples were taken from the same layers as in 2017 on the 2 × 10 m strip, which was not treated before in 2016 (plateau) and 2017 (first two product applications). All soil samples taken for analytical verification were stored deep frozen after sampling, homogenized by adding dry ice, and measured for their bixafen and tebuconazole contents in soil. After the product applications and corresponding analytical samplings, the field was irrigated with 12 and 10 mm of water after the first and second applications (May 2017), respectively, and 10 mm each after the third and fourth applications each to aid the exposure of the test item to earthworms in soil (Kula et al., 2006). Earthworms were sampled using a combination of hand sorting and formaldehyde extraction (DIN ISO 23611-1, 2006). Four holes per plot (each 1/4 m² area at a depth of 20 cm) were dug and earthworms were manually sorted from this soil. Formaldehyde solution (2.5 L of 0.2%) was poured into each hole and all the earthworms visible during the following 30 min were sampled. All earthworms were stored for a maximum of 48 h in water until they were counted, weighed, and determined to the species level according to the identification key published by Sims and Gerard (1999) and the nomenclature according to Easton (1983). Earthworms were sampled 12 days before plateau application (first presampling), 40 days before the first product application (second presampling), and at six dates after the first product application: 37, 168, 358, 393, 547, and 742 days.

The abundance and biomass of total earthworms and single species in the test item and reference treatment groups were statistically compared with those in the control treatment by t-test statistics (Williams’, Dunnett’s, Welch, or Student t test, one-sided smaller, p = 0.05; for further details, see the Supporting Information). Data from posttreatment samplings were analyzed using Shapiro–Wilk’s test or the Kolmogorov–Smirnov test for normal distribution, Levine’s test for homogeneity, and the Trend analysis by Contrasts for monotonicity. Data from the pretreatment sampling were analyzed by a two-factorial analysis of variance (p = 0.05) with treatment as the fixed factor and block as the random factor. The software ToxRat version 3.2 was used for statistical analysis. ECx values were calculated for the laboratory studies using probit analysis for the bixafen study and a three-parameter Type II Weibull dose–response model with normal error assumption for the Skyway XPro® study.

A more detailed description of the materials and methods is presented in the Supporting Information.

RESULTS

Field exposure monitoring

The measured soil concentrations at the 10 sites show a concentration distribution based on five samples each for different soil depths over 3 years (Table 2, Figures 1 and 2). In general, a vertical concentration gradient was observed for the samplings before and after applications. On average, more than 50% of the residues remained in the upper 10 cm, whereas 12% were found below 20 cm. However, the vertical residue distribution showed strong variation between the fields (30%–80% found at 0–10 cm depth and 0%–24% below 20 cm depth). The maximum measured concentration was 0.288 mg a.s./kg dws in the upper 5 cm after bixafen application (Table 2).

The PECsoil modeling based on application and soil tillage history for the individual fields in comparison to monitored soil concentrations underestimated the
PECsoil for those sites where application history was incomplete (Figure S2). Therefore, the first monitored soil concentrations were considered as the background concentration and the information provided by farmers used from 2017 onward.

Modeled maximum concentrations for the upper 10 cm range from 0.0375 to 0.2158 mg a.s./kg dws. These values cover the mean observed concentration averaged over the top 10 cm at all sites. Monitored concentrations from individual samples are higher in two cases (Figure 3, Table S3).

For the regulatory risk assessment, the long-term accumulation of soil residues is considered, and the background plateau concentration is added to the PECsoil value resulting from the current application. The maximum PECplateau value for bixafen based on the Hockey Stick kinetic parameters and the assumption of annual use at the highest possible rate with the lowest crop interception is 0.375 mg/kg.

From the 10 monitored fields, the site Bayern Hof was the site with the highest application frequency. At this site, bixafen was applied for 5 out of 6 years. The applied rates ranged from 67.5 to 141 g/ha/year. The modeled accumulation based on the repeated known use history and soil bulk density from this site resulted in a PECplateau of ~0.083 mg/kg after 44 years (Figure 4). An additional application on top of the background PECplateau of 141 g bixafen/ha with 80% crop interception (highest rate during the monitoring study duration) resulted in a PECaccu (sum of PECplateau [0–20 cm] and PECinitial [0–5 cm]) of 0.142 mg/kg.

Ecotoxicological studies

The earthworm species *E. fetida* was tested in a 56-day laboratory reproduction test with bixafen (active substance) concentrations between 25 and 400 mg a.s./kg dws. All adult earthworms survived the 28-day exposure to the

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**TABLE 2** Range of measured bixafen concentrations (mean and median) in different soil depths from 10 fields over a period of 3 years

| Sampling time          | Soil depth (cm) | Bixafen concentrations (mg/kg dws) |
|------------------------|-----------------|-------------------------------------|
|                        |                 | Mean     | Median                      |
| Pre-application sampling | 0–10            | 0.005–0.127 | 0.026–0.036               |
|                        | 10–20           | 0.000–0.039 | 0.019–0.025              |
|                        | 20–30           | 0.000–0.027 | 0.006–0.008              |
| Post-application sampling | 0–5             | 0.031–0.288 | 0.065–0.076              |
|                        | 5–10            | 0.008–0.145 | 0.021–0.042              |
|                        | 10–20           | 0.000–0.054 | 0.024–0.037              |
|                        | 20–30           | 0.000–0.028 | 0.007–0.009              |

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**FIGURE 1** Measured bixafen concentrations at 10 fields in early spring before potential bixafen application from 2017 to 2019 for soil depths 0–10, 10–20, and 20–30 cm. Boxes show the median with the first to third quartile and whiskers show the minimum and maximum values.
compound in soil at all tested rates. The NOEC for mortality was \( \geq 400 \text{ mg a.s./kg dws} \) (Table S4). Adult earthworms showed an increase in weight by 53.2\% \pm 8.6\% in the control group and between 46.2\% \pm 9.7\% and 53.6\% \pm 9.1\% in the bixafen treatment groups, with no significant differences compared to the control group. The NOEC for growth amounts to \( \geq 400 \text{ mg a.s./kg dws} \), the highest concentration tested (Table S4). At the two highest concentrations (200 and 400 mg a.s./kg dws), statistically significant differences compared to the control were observed with the number of juveniles. Therefore, the NOEC for reproduction was concluded to be 100 mg a.s./kg dws (Table S4). EC10 and

![Figure 2](image-url) Measured bixafen concentrations at 10 fields after bixafen application from 2017 to 2019 for soil depths 0–5, 5–10, 10–20, and 20–30 cm (10–30 cm in 2017). Boxes show the median with the first to third quartile and whiskers show the minimum and maximum values

![Figure 3](image-url) Comparison of monitored residue concentrations in upper 10 cm before (red dots) and after (green dots) bixafen applications at the 10 monitored fields with predicted soil concentrations (background concentration based on measured concentrations in spring 2017 is in gray and concentration resulting from recent applications in years 2017, 2018, and 2019 is in blue). For detailed values, please refer to Table S3
EC20 values (reproduction) were calculated using the Probit analysis and resulted in an EC10 = 103.7 mg a.s./kg dws (lower and upper 95% confidence limits: 46.2 and 148.5 mg a.s./kg dws, respectively) and EC20 = 215.4 mg a.s./kg dws (lower and upper 95% confidence limits: 151.3 and 284.2 mg a.s./kg dws, respectively). An EC50 was not calculated as the maximum effect was < 50%.

The laboratory earthworm reproduction study with Skyway XPro® was performed with concentrations between 8 and 80 mg product/kg dws and revealed no effects on survival up to the highest tested concentration (Table S4). The NOEC for mortality was therefore ≥ 80 mg product/kg dws. The growth of adult earthworms was significantly increased at the highest test concentration, leading to a NOEC for growth of 45 mg product/kg dws. The number of juveniles was negatively affected by the two highest concentrations of 45 and 80 mg product/kg dws. Therefore, the NOEC (reproduction) was determined to be 25 mg Skyway XPro®/kg dws (Table S4). ECx values (with upper and lower 95% confidence limits) for reproduction were calculated, being EC10 = 26.0 (18.7–33.3) mg Skyway XPro®/kg dws, EC20 = 36.5 (27.8–45.2) mg Skyway XPro®/kg dws, and EC50 = 80.8 (64.1–97.5) mg Skyway XPro®/kg dws.

In the 2.5-year earthworm field study, bixafen concentrations were measured at different times and depths. Immediately after the plateau application in November 2016, bixafen concentrations amounted to 1.575, 1.769, and 1.828 mg/kg dws (scaled to 0–20 cm depth) in LR, MR, and HR, respectively (Table S5). Before both product applications, the plateau concentration was measured again in March 2017 and showed lower values. In LR, MR, and HR, plateau concentrations reached 0.823 ± 0.189, 0.860 ± 0.303, and 0.908 ± 0.297 mg/kg, respectively (Table S5, Figure 5). The plateau concentrations of tebuconazole were analytically verified immediately after application in November 2016. The mean concentrations amounted to 0.014, 0.014, and 0.015 mg/kg dws (scaled to 0–20 cm; data not shown) in LR, MR, and HR, respectively. In March 2017, tebuconazole concentrations were reduced to values of 0.006, 0.007, and 0.020 mg a.s./kg. Both product applications in May 2017 led to a strong vertical stratification of bixafen in soil. High concentrations were observed in the top 1 cm from both product applications (no plateau included), with concentrations of 1.121 ± 0.063, 1.581 ± 0.138, and 2.264 ± 0.519 mg/kg dws in LR, MR, and HR, respectively. The increasing values from LR to HR correspond well to the rates applied in the three Skyway XPro® treatment groups in this study. From 0–1 cm over 1–5 cm to 5–10 cm soil depth, the concentrations decreased consistently in LR, MR, and HR, demonstrating that a major part of the substance from product applications in 2017 was located the upper top-soil layer (Table S6). Calculated peak concentrations at 0–1 cm depth (sum of the plateau concentrations at 0–20 cm depth [March 2017] and the concentrations at 0–1 cm depth only from the product applications in May 2017) amounted to 1.944, 2.441, and 3.172 mg/kg dws in LR, MR, and HR, respectively. Bixafen concentrations after both Skyway XPro® applications in May 2018 did not show this strong vertical stratification as observed in 2017. Concentrations were lower at 0–1 cm depth (0.385, 1.012, and 1.326 mg/kg dws in LR, MR, and HR, respectively) compared to values in 2017 and showed a less pronounced vertical gradient. In 2017, the largest part of the measured bixafen mass applied with Skyway XPro®, for
example, 82.4% in LR, was located in the upper 1 cm soil layer, whereas in 2018, the majority of the bixafen mass (59.6%) was measured at layers deeper than 1 cm (see Table S6). In MR and HR, this tendency was less pronounced. The field site of the 2.5-year earthworm field study contained a diverse earthworm community, with total earthworm abundances up to 288.5 individuals/m² in the control (Figure 5, Table S7). Seven different earthworm species were found, that is Lumbricus castaneus, L. terrestris, Aporrectodea longa, Aporrectodea caliginosa, Aporrectodea rosea, Allobophora chlorotica, and Octolasion cyanum. Three species L. castaneus, A. longa, and O. cyanum were not considered in the single-species evaluation as these species were found only sporadically and at low densities. A. chlorotica was also not evaluated in this study as this species showed a highly uneven distribution on the field (see Figure S3). Earthworm abundances showed strong seasonal variations. The strongest temporal abundance fluctuations were observed between March and May 2017, which was more visible for the endogeic species A. caliginosa and A. rosea compared to the anecic species L. terrestris (Figure 5). Abundances of ranged between 2.5 and 32.3 individuals/m² for L. terrestris, between 25.8 and 201.5 individuals/m² for A. caliginosa, and between 2.8 and 32.5 individuals/m² for A. rosea. Abundances in the control treatments were below 10 individuals/m² for A. rosea in May 2017 and October 2018 and for L. terrestris between April and October 2018.

The toxic reference treatment (10 kg carbendazim/ha) induced significant reductions in the total earthworm abundances by 57.1% (May 2017), 26.5% (October 2017), 67.8% (October 2018), and 59.1% (May 2019; Figure 5A), proving the sensitivity of this field study (Kula et al., 2006). The strongest impact of the toxic reference was observed on abundances of L. terrestris, with significant reductions of between 82.1% and 100% (Figure 5D).

The results from the Skyway XPro® treatments revealed little overall effects on natural earthworm populations in this study. The abundances of total earthworms and of the individual species L. terrestris, A. caliginosa, and A. rosea were not significantly reduced up to the highest application rate of 2 × 3.75 L product/ha/year (HR) during the study, except for the total earthworm count at the highest application rate (HR) 1.5 years after the first product application (October 2018; Figure 5). Here, the total earthworm abundances were significantly reduced by 43.3% compared to the control. Therefore, the no-observed effect rate (NOER) is 2 × 2.5 L Skyway XPro®/ha/year and the lowest-observed effect rate (LOER) = 2 × 3.75 L Skyway XPro®/ha/year. However, the results demonstrate that the earthworm population was no longer impacted 2 years after the first product application (1 year after applications in the second year) in LR, MR, and HR (Figure 5A). The biomass of earthworms was not significantly impacted during the study up to the highest application rate HR (Tables S7–S11). Thus, the no-observed ecologically
adverse effect rate (NOEAER) in the study is $2 \times 3.75\text{ L}$ Skyway XPro®/ha/year.

**Risk assessment**

The earthworm Tier 1 risk assessment indicated a potential risk for the product Skyway XPro® considering the NOEC and EC10 (reproduction) from the laboratory study with *E. fetida*. The TERs for Skyway XPro® were slightly below the trigger value of 5 (Table S12). However, with TERs of 87 and 90, the active substance bixafen did not show a risk for earthworms at Tier 1 considering the NOEC and the EC10, respectively. In the higher tier risk assessment, an acceptable risk was identified for earthworms for the use of Skyway XPro® in cereals up to the highest dose rate (HR). Comparison of the measured peak concentration in the 2.5-year earthworm field study at 0–5 cm depth in the highest application rate (HR; no-observed ecologically adverse effect concentration [NOEAE] = 1.508 mg a.s./kg) with the PEC soil (PECaccu = 0.575 mg a.s./kg) shows that the NOEAE exceeds the expected exposure after the use of Skyway XPro® in cereals by a factor of 2.6. The observed margin of safety in the higher tier risk assessment is even higher when the measured bixafen exposure concentrations after multiple years of treatment in cereals (from exposure monitoring study) are taken into consideration. The NOEAE in the field study exceeds the maximum measured peak concentration after realistic use of bixafen-containing products in cereals by a factor of 5.2 and the modeled (extrapolated) PECsoil by a factor of 10.6 if a continuous repetition of the real application and soil tillage history (from 2012 to 2019 at the field site Hof) for 44 years is assumed (Table S12).

**DISCUSSION**

**Field exposure monitoring and PEC calculations**

It has been shown that the model assumptions, in particular, the kinetic parameters describing the background accumulation, provide a conservative description of the observed residue data. Deviations between the modeled exposure of the realistic use patterns from the exposure monitoring study and the measured residue data that were observed can likely be attributed to the spatial variability of field conditions. The 10 monitored fields represent a large variety of different real-world cereal cropping scenarios with different crop rotation cycles, application rates, soil tillage practices, canopy processes, weather, and soil conditions. This is reflected in the monitored residue distribution and magnitude. Depending on soil tillage practices, bixafen application rates, and frequency during the years 2012–2019 in the individual fields, the concentrations vary with depth and time.

The soil accumulation plateau calculation used in the regulatory context considers a concentration that is built up after repeated applications over succeeding years. It introduces an additional level of conservatism and hence protection in the risk assessment. The calculations are based on several conservative assumptions: the degradation is described by the worst-case dissipation half-life observed in a field study, the full application rate to be registered is assumed to be applied annually, and the crop growth stage with the lowest crop interception during the potential use period is assumed when estimating the maximum fraction of the active substance reaching the soil.

This monitoring study showed that these assumptions, in particular, the assumption of the full application rate to be applied every year, are highly conservative. To account for the accumulation of bixafen in soil after repeated realistic application patterns over several decades, PECs were calculated based on an extrapolation of the known use history from the sites for a period of 44 years. This results in a plateau concentration that is more than four times lower compared to the PECplateau values used in risk assessment. In addition to uncertainties related to application rates and frequency, soil mixing and canopy processes play an important role in long-term extrapolations. For the long-term modeling of the PECs following the repeated use history, the assumption was that soil was homogeneously mixed. It can be reasonably assumed that mechanical mixing of the soil down to 0–20 cm depth will occur within a period of 44 years on cereal fields. This is reflected in lower extrapolated long-term PECs compared to the maximum measured concentrations, that is, at the field site Bayern Hof (Table S3, Figure 4), where reduced soil tillage was performed for several years.

**Ecotoxicological effects on earthworms**

Chronic laboratory studies with *E. fetida* were conducted for the active substance bixafen and the end-use product Skyway XPro®. In addition to bixafen (nominally 75 g/L), Skyway XPro® contains two additional active substances (prothioconazole and tebuconazole, both nominally at 100 g/L) as well as additional formulation additives. EFSA reported chronic NOECs for *E. fetida* of 1.33 mg a.s./kg dws (tested as a product) and 10 mg a.s./kg dws for prothioconazole and tebuconazole, respectively (EFSA, 2007, 2014b). The presence of two additional active substances and formulation additives in Skyway XPro® may increase the toxicity to earthworms compared to bixafen alone.

The 2.5-year earthworm field study with Skyway XPro® revealed little effects on natural earthworm populations. No statistically significant reductions in earthworms were observed during the study, except for the total earthworm population in the highest, threefold field application rate (HR) 1.5 years after the first product application (which is 6 months after Skyway XPro® applications in the second year). Therefore, the NOER in this study is concluded being the twofold field rate and the LOER is threefold the field rate. However, at the end of the study (1 year after the last Skyway XPro® treatments), no treatment-related deviations from the control were observed up to the threefold field application rate, demonstrating that Skyway XPro® has no negative long-term impact on the natural earthworm community. Therefore, the threefold application rate (HR) is considered to be the NOEAER. The significant reduction in
the total earthworm abundance 1.5 years after the first application (6 months after product applications in the second year; October 2018) for the HR is caused by reductions observed for the endogeic species A. caliginosa (34.1% reduction) and A. rosea (76.3% reduction), which were both not statistically significant. No significant reductions compared to the control were observed for the total earthworm count, A. caliginosa, and A. rosea in MR at this sampling date. The limited statistical sensitivity 1.5 years after the first application for that is A. rosea is due to a high relative variabilty (CV = 139% in the control). Following the Welch t test after Bonferroni–Holm, the minimum detectable difference (MDD, Brock et al., 2015) for A. rosea was calculated to be 223.5%, 165.4%, and 262.6% for LR, MR, and HR, respectively, and only 47% for A. caliginosa using the t test according to Dunnett (Tables S13–S22). For A. rosea, the control abundances were relatively low at this sampling date (<10 individuals/m²). According to Kula et al. (2006), only taxa showing abundances >10 individuals/m² should be statistically evaluated and alternatively (if <10 individuals/m²) evaluated after being grouped with other species (here, at the level of total earthworms). With this measure, the number of non-robust evaluations in the data set can be reduced, which, however, includes the risk of obtaining false negatives in the evaluation. A transient treatment-related effect on A. rosea and A. caliginosa 6 months after the Skyway XPro® applications of the second year at the threefold field rate cannot be excluded. The observed differences in abundances between Skyway XPro® treatments and the control for the other sampling dates and species were small and not related to the treatment with Skyway XPro®. Hence, the inherent variability in this study and corresponding MDD values do not mask unacceptable effects on earthworms. Generally, MDD values can provide useful information on the robustness of a statistical univariate comparison; however, care needs to be exercised on how these values are interpreted. Field studies even with high MDD can reliably demonstrate acceptable risks of a pesticide to earthworms, for example if low-effect magnitudes or no dose–response relationships (in multidose studies) are observed.

An evaluation of the impact of Skyway XPro® on A. chlorotica was not possible in this study because of its uneven distribution in the test field (see Figure S3). This uneven distribution could be due to spatially changing soil properties on the field site or different agricultural use or management history in parts of the field.

The development of the earthworm abundances in the control was characterized by pronounced temporal fluctuations. Total abundances were decreased in May 2017 as well as in May and November 2018, probably due to higher temperatures and low precipitation in the period before earthworm samplings (Figure S4) and hence a lower soil moisture content. This is in line with Lee (1985) and Curry (2004), who showed that weather conditions can substantially influence earthworm abundances in agricultural fields. L. terrestris showed less reaction in dry weather conditions in May 2017 compared to A. rosea or A. caliginosa, which might be due to its lifeform and specific burrowing behavior (Lee, 1985). This species forms deep vertical burrows and therefore may more easily tolerate dry phases by resting in the deeper soil layers with lower temperatures and higher soil moisture content during warm and dry periods.

Risk assessment

To determine the risks to earthworms at the Tier 1 risk assessment, the ecotoxicological endpoints are compared with the PECsoil, leading to TER values. For various SDHI fungicides, TER values of 18–299 for the active substances and 9.35–292 for the representative products and uses are reported by EFSA, which exceed the trigger value of 5 and leads to the conclusion of no unacceptable risks in the Tier 1 risk assessment for all SDHI, including bixafen (EFSA, 2008, 2012a, 2012b, 2012c, 2013a, 2013b, 2013c, 2015, 2016). The Tier 1 level risk assessment for earthworms indicated an acceptable risk at Tier 1 for the bixafen active substance following the use of Skyway XPro® in cereals (2×1.25 L/ha at BBCH 25+). However, for the product Skyway XPro®, a TER of 4.6 indicates a potential risk at Tier 1 that is due to the higher toxicity of the product containing additional active substances. Taking the outcome of the earthworm field study with artificially high overdosing into consideration in the higher tier risk assessment, the risk for earthworms can be considered to be low. No unacceptable effects were observed up to the highest application rate of 2×3.75 L Skyway XPro®/ha/ year, which is three times the recommended field application rate. The simulated bixafen plateau concentrations measured in March 2017 at 0–20 cm depth (0.823–0.908 mg a.s./kg) exceeded the estimated PECplateau for bixafen (0.375 mg a.s./kg) by a factor of 2.2–2.4. When compared with modeled bixafen plateau concentrations assuming a repeated application history at the field site Hof in the exposure monitoring study (0.083 mg/kg; 0–20 cm), the simulated measured plateau concentrations in the earthworm field study were higher by a factor of ~10. Measured peak concentrations after product applications in 2017 were determined to be 1.508 mg/kg (scaled to 0–5 cm depth), which exceeds the modeled peak PECsoil value by a factor of 2.6. This mirrors the clear overdosing of bixafen in this study compared to the expected exposure according to the recommended use pattern.

After both product applications in 2017 and 2018, bixafen was analytically measured in samples taken at different soil depths: 0–1, 1–5, and 5–10 cm. In HR, the mean concentrations of 2.264, 0.184, and 0.018 mg/kg were determined at these depths, respectively. A clear vertical stratification of bixafen in soil was demonstrated in 2017, with the highest values in the top 1 cm. In 2018, this vertical gradient was less pronounced, that is in the lowest treatment group LR. This could be due to specific weather conditions after both product applications in the respective year. However, a higher amount of precipitation was not recorded between the first application and the date for soil residue sampling in...
2018 (1.7 mm total natural rainfall and in total 20 mm irrigation) compared to 2017 (22 mm irrigation), which could have led to an enhanced bixafen translocation to deeper layers. EFSA proposed in its Scientific Opinion (Ockleford et al., 2017) to consider 0–1 cm as the relevant soil depth in the risk assessment for in-soil living organisms. From an exposure point of view, a PECsoil calculated for 0–1 cm depth can lead to a realistic initial peak exposure estimate under certain environmental conditions and application scenarios (see results for 2017), as observed by Fent et al. (1999). However, vertical movement and distribution of pesticides in soil can vary between years. Furthermore, earthworms move and feed at a range of soil depths and thereby have a large range of different exposures over space and time (Roeben et al., 2020). As pointed out by Christl et al. (2016), the level of conservatism needed at the Tier 1 earthworm risk assessment is determined on the one hand by the relevant soil layer for PECsoil calculation and on the other on the selection of the TER trigger value. A PECsoil calculated for 0–1 cm could lead to an over-conservative Tier 1 risk assessment with a high rate of false positives if combined with a TER trigger value of 5 (Christl et al., 2016). The regulatory relevant PECsoil depth for the risk assessment needs to be calibrated in combination with the Tier 1 assessment factor by comparing the Tier 1 risk assessment with the results from field studies. With this approach, Christl et al. (2016) showed that a PECsoil calculated for the 0–5 cm soil layer in combination with the assessment factor of 5 (EC, 2009) provides a sufficiently conservative Tier 1 earthworm risk assessment.

Bénit et al. (2019) measured inhibition of in situ enzyme activities within the mitochondrial respiratory chain in cells of the earthworm species L. terrestris, that is, SCCR (succinate cytochrome c reductase), GCCR (glycerol-3-phosphate cytochrome c reductase), and QCCR (quinol cytochrome c reductase). Inhibitory concentration (IC50) values for bixafen (the concentration at which 50% inhibition is observed) of 6, 450, and >500 μM were measured, respectively. While acknowledging that a comparison of these physiological effects with the realistic exposure in the field is difficult, reference is made to potential exposure directly under the spray nozzle in the context of human exposure, that is, 0.6–1.8 mM corresponding to the recommended field rate of 75–125 g bixafen/ha (Bénit et al., 2019). In the case of SCCR, the IC50 values were by a factor 100–300 lower than this theoretical exposure level under the nozzle. However, processes like crop interception, degradation, adsorption, and transformation processes in soil, vertical distribution of the substance in soil, and biological factors like movement of earthworms in soil, feeding behavior, and uptake kinetics lead to a decrease in the real exposure and potential accumulation in the bodies and cells of earthworms (Roeben et al., 2020). Earthworm metabolic capability is described for a range of pesticides in Katagi and Ose (2015) and is assumed to metabolize SDHIs as well through enzymatic processes. The inhibition of SDH observed in the studies presented by Bénit et al. (2019) using earthworm cell lines is to be expected in an experimental model that has no metabolic capability and places SDHIs in close proximity to their intended biological target. The relevance of in vitro studies of SDHI activity against SDH in systems that lack metabolic capability for predicting effects in intact living organisms is therefore questionable.

The present study demonstrates that earthworms (E. fetida) were not affected up to 100 mg a.s./kg dws, which is a factor of 147 higher than the expected exposure in soil. Hence, the population relevance of the finding of Bénit et al. (2019) for earthworms under realistic conditions is low. This is confirmed by the outcome of the 2.5-year earthworm field study with Skyway XPro®, where no unacceptable adverse long-term effects on earthworms (including L. terrestris, which was used in the study of Bénit et al., 2019) were observed at up to three times the recommended field rate or 5.2 times the maximum measured bixafen concentrations in soil in the exposure monitoring study after realistic use in agriculture.

CONCLUSIONS

Bixafen concentrations in field soils after multiyear use in cereals were below the estimated PECsoil value used in the risk assessment in most of the cases. The PECplateau for bixafen calculated under assumption of the worst-case observed field dissipation kinetic parameters and annual application of the highest registered rate with the lowest crop interception represents a conservative exposure estimate that describes the background accumulation in soil after repeated long-term use of bixafen-containing products.

The Tier 1 earthworm risk assessment based on the laboratory reproductions test with E. fetida indicated an acceptable risk for bixafen (active substance), whereas for the product Skyway XPro®, a potential risk was indicated that triggered the conduct of a higher tier assessment under field conditions. The 2.5-year field study revealed no unacceptable effects on earthworms even at unrealistically high simulated bixafen exposure in soil that was clearly above the realistic bixafen concentrations observed in the long-term exposure monitoring study. It is concluded that bixafen does not pose a long-term risk for natural earthworm populations when applied according to the recommended use pattern in cereals. This monitoring program confirms that the current earthworm risk assessment scheme is fully protective for earthworms including multiyear use of bixafen on a long-term basis.

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CONFLICT OF INTEREST
The authors work for a chemical company that produces pesticides. The presented work reports the results of an environmental monitoring program and a field study of one of its fungicides conducted under principles of good laboratory practice and discusses the outcomes in the context of the current European risk assessment regulation.

DATA AVAILABILITY STATEMENT
Most relevant data of this project are presented as figures and tables in the manuscript and supporting information. Data, associated metadata, and calculation tools are available from the corresponding author (gregor.emst@bayer.com).

SUPPORTING INFORMATION
The supporting information file contains a more detailed description of the results and covers additional data on side aspects related to presented study.

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