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A Dirt(y) World in a Changing Climate: Importance of Heat Stress in the Risk Assessment of Pesticides for Soil Arthropods

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ABSTRACT

The rise in global temperatures and increasing severity of heat waves pose significant threats to soil organisms, disrupting ecological balances in soil communities. Additionally, the implications of environmental pollution are exacerbated in a warmer world, as changes in temperature affect the uptake, transformation and elimination of toxicants, thereby increasing the vulnerability of organisms. Nevertheless, our understanding of such processes remains largely unexplored. The present study examines the impact of high temperatures on the uptake and effects of the fungicide fluazinam on the springtail *Folsomia candida* (Collembola, Isotomidae). Conducted under non-optimum but realistic high temperatures, the experiments revealed that increased temperature hampered detoxification processes in *F. candida*, enhancing the toxic effects of fluazinam. High temperatures and the fungicide exerted synergistic interactions, reducing *F. candida's* reproduction and increasing adult mortality beyond what would be predicted by simple addition of the heat and chemical effects. These findings highlight the need to reevaluate the current ecological risk assessment and the regulatory framework in response to climate changes. This research enhances our understanding of how global warming affects the toxicokinetics and toxicodynamics (TK-TD) of chemicals in terrestrial invertebrates. In conclusion, our results suggest that adjustments to regulatory threshold values are necessary to address the impact of a changing climate.

1 | Introduction

1.1 | Threats to Arthropod Biodiversity in a World of Multiple Stressors

In the modern world, Earth and its inhabitants face a multitude of crises, primarily from accelerating climate change and anthropogenic pressures that threaten global ecosystems (IPCC et al. 2022; Richardson et al. 2023). The widespread use of pesticides and the impact of climate change have already affected many organisms and led to a decline in the global diversity of species (WWF 2022; Dornelas et al. 2019; IPBES 2019; Liess et al. 2021). Notably, arthropod diversity, biomass and abundance have declined by at least 30% in some habitats (Habel et al. 2016; Hallmann et al. 2017; Seibold et al. 2019). Soil arthropods are an integral part of soil biodiversity and key organisms in the ecosystem functions of soils (Potapov et al. 2023). In the face of a multitude of anthropogenic stressors,

it is therefore necessary to quantify their interaction and joint effect on soil arthropods. Understanding these effects and interactions will help us develop solutions to mitigate unwanted pesticide effects and better prepare for a warmer future.

1.2 | A Dirt(y) World of Climate Change

Climate change has a cascade of indirect effects bound to happen, possibly increasing already present pressures that soil organisms experience in the environment. The most prevalent is that the rise in ambient air temperatures also increases soil temperatures (Bradford et al. 2019; Hu and Feng 2003; Qian et al. 2011). Reshotkin and Khudyakov (2019) documented a rise in soil temperatures across various soil types and depths, observing an increase of 0.5°C–1.0°C over the past 50 years and recorded maximum summer soil temperatures of up to 22°C–23°C at depths down to 40 cm in Russian soils. In southern Europe, soil temperature changes during heat waves have been measured to be more extreme than changes in air temperature and are predicted to increase and outpace air temperatures by 0.7°C per decade in future climatic scenarios (García-García et al. 2023). Consequently, soil organisms will face both higher average temperatures and more frequent extreme heat events. Stressful high temperatures can lead to thermal injury and death in arthropods, including the model organism of the present study, the springtail *Folsomia candida* (Willem, 1902) (Jørgensen et al. 2021; Ørsted, Jørgensen, and Overgaard 2022; Wehrli et al. 2024; Xie, Slotsbo, and Holmstrup 2023). Sub-lethal effects, such as reduced reproduction and growth, are also important and vary with exposure duration (Ørsted et al. 2024). In the realm of soil arthropods, studies on *F. candida* and other springtails show that temperature influences longevity and reproduction (Roeben, Montoya-Tzschoppe, and Roß-Nickoll 2023; Xie, Slotsbo, and Holmstrup 2023). Another impact of climate change that is often overlooked in this context poses a challenge for agriculture and leads to yield losses (IPCC et al. 2022). These are not only caused by the direct effects of climatic changes, but also from increased pressure from pests and pathogens thriving in a warmer environment (Bale et al. 2002; Irlich et al. 2009). Increased pesticide use, as a response to increased pest pressure, further threatens the biodiversity of non-target organisms (Deutsch et al. 2018) and can impact communities of soil organisms (Knouft and Ficklin 2017; Parmesan and Yohe 2003; Walther et al. 2002). Lastly, increased temperature and chemical stressors occurring in combination have been shown to be a potent cocktail of stressors (Holmstrup et al. 2010; Hooper et al. 2013).

1.3 | Current Risk Assessment of Pesticides

Around the globe, different approaches to assess the risk of chemicals, in particular pesticides, are employed. These approaches are comparable, but here we focus on the approach used by the European Union (EU). The EU employs a well-defined risk assessment for pesticide use, based on single-species standardised laboratory tests with optimal conditions and generally single substance exposures (EC 2009; EC SANCO 2002). However, the laboratory and mesocosm tests do not account for co-occurring multiple stressors, such as the spatial, temporal or thermal conditions, or the combined effects of pesticide mixtures that organisms encounter in their natural environments. For example, the synergistic interaction of chemical mixtures has been quantified (Escher, Stapleton, and Schymanski 2020; Panico et al. 2022) and has led to calls for the integration of a mixture risk assessment factor to improve chemical risk assessment (Backhaus 2023). Similar interactions between toxicants and environmental stressors have been quantified (Liess et al. 2016), but have not yet been addressed in chemical risk assessment.

The importance of interactions of various stressors is reflected in retrospective assessments, such as monitoring pesticides and their effects on the ecosystem (Liess et al. 2021). For example, in 98% of tested French soils, up to 33 different pesticide residues were found (Froger et al. 2023), and these mixtures were predicted to exert a 'silent threat' to earthworms and possibly other soil organisms (Pelosi et al. 2021). Other studies in the aquatic environment with gammarids found that internal concentrations of pesticides in situ are higher than extrapolations from measured laboratory tests, possibly causing larger effects than previously accounted for in the prospective assessment (Lauper et al. 2022; Munz et al. 2018). The same pattern was shown in the risk assessment of tropical soils, where elevated temperatures increased the toxicity of pesticides for *Eisenia andrei* (an earthworm) and *F. candida* (Bandeira et al. 2020; Eijsackers et al. 2017). The interactions with elevated temperatures have not been monitored for soil arthropods yet, but the effects could be similar to the observed mixture effects. Thus, the inclusion of multiple stressors, in this case, the factor of thermal stress, could be a valuable adaptation to prevent future damage to the already vulnerable ecosystems of Earth (Eijsackers et al. 2017).

1.4 | Multiple Stressors

To understand the effects of multiple stressors, we need to investigate stressors individually and also in combination. For instance, heat stress can be lethal or induce species-specific sublethal effects (Ge et al. 2023; Roeben, Montoya-Tzschoppe, and Roß-Nickoll 2023; Wehrli et al. 2024). However, temperature also influences toxicokinetics and effects of chemicals in a wide range of organisms (Cairns, Heath, and Parker 1975; Heugens et al. 2001; Mayer and Ellersieck 1988). For these reasons, recent research emphasises the significance of multiple stressors and the need to predict the effects of co-exposure to low concentrations of pesticides and natural stressors (Liess, Henz, and Knillmann 2019). Especially, synergistic interactions between heat stress and other stressors (Holmstrup et al. 2010; Laskowski et al. 2010; Verheyen and Stoks 2019), affecting both toxicokinetics and toxicodynamics when the organisms are exposed to elevated temperatures and elevated pesticide levels are of interest (Camp and Buchwalter 2016; Chow et al. 2020; Lauper et al. 2022; Munz et al. 2018; Phillips and Bode 2004). For example, Raths et al. (2023) observed that higher temperatures increased the uptake of pesticides in the amphipod *Gammarus pulex*. Similar temperature-related increases in uptake and changes in toxicodynamics were measured in Gammarids (Huang et al. 2022, 2023; Mangold-Döring et al. 2022). Moreover, Dai et al. (2021, 2023) have shown altered phenanthrene toxicokinetics in the oligochaete *Enchytraeus albidus* and reduced detoxification rate in *F. candida* at high temperatures. The combination of environmental contaminants and

elevated temperatures appeared to hamper various life history traits and vital functions in *F. candida* (Dai et al. 2021, 2023; Ge et al. 2023), demonstrating the complex and detrimental effects of combined stressors on soil organisms.

To predict the outcome of these interactions in the present study, we employed the stressor addition model, SAM (Liess et al. 2016) and the ECx System Stress model, $EC_{x\text{-}SyS}$ (Liess, Henz, and Knillmann 2019). These models have been so far applied for assessing short-term acute mortality in aquatic species. Here, we extend their application to soil arthropods in a chronic context, aiming to refine risk assessment under elevated temperature scenarios, in order to illustrate how elevated temperature could influence prospective regulatory values.

1.5 | Research Hypothesis and Objectives

Our aim in this study was to decipher the combined impacts of high temperature and pesticide exposure on *F. candida*. We employed simultaneous exposure to elevated, suboptimal temperatures and exposure to a fungicide (fluazinam) in standard laboratory tests (OECD 2016), which measured internal concentrations of fluazinam and its primary metabolites in springtails as well as lethal and sublethal endpoints (reproduction and growth). Finally, we aimed to predict the combined effects of stressors with the common effect addition (Bliss 1939) and the novel mixture model stress addition model (SAM; Liess et al. 2016). Hereby, we aimed to identify suitable models for risk assessment that can predict the consequences of elevated temperature in combination with pesticide exposure.

2 | Materials and Methods

2.1 | Animals

The animals used for experiments originated from a population of *F. candida* that has been cultured in our laboratory for about 30 years (Simonsen and Christensen 2001). The animals were kept in Petri dishes with plaster of Paris and active charcoal (8:1 w:w). The cultures were held at 20°C (12:12 h light/darkness cycles), fed weekly with baker's yeast and kept at approximately 100% relative humidity (Wehrli et al. 2024).

Test animals were age-synchronised for the reproduction test, as described in OECD 232 and tests started when they were 10–12 days old (OECD 2016). Furthermore, for the toxicokinetics experiment, healthy adult organisms were chosen at random from the mass culture.

2.2 | Soil

Standard LUFA 2.2 soil (LUFA Speyer, Speyer, Germany) was used in all experiments; all relevant soil properties are described in Table S1. The soil for the toxicodynamics experiment was spiked with fluazinam according to OECD 232 (OECD 2016). Fluazinam (Sigma Aldrich, Darmstadt, Germany; 79,622–59-6, ≥ 98% purity) was dissolved in acetone (180 mL/kg soil) to obtain nominal concentrations of 0.5, 0.9, 1.6, 2.9, 5.0, 8.8, 15.3, and 25.8 mg/kg dw soil. For the toxicokinetics experiment, a separate batch of soil was spiked including only a single concentration of fluazinam (5.0 mg/kg dw soil). The soil was then mixed vigorously and left in a fume cupboard at room temperature to evaporate the acetone for at least 24 h before usage on Day 1; the remaining soil was stored at 5°C in air-tight glass containers. Subsequently, the desired amounts of soil were taken from storage at the start of each experiment day and moistened to 50% ± 5% water holding capacity (WHC) by adding deionised water. The soil pH was measured according to OECD 10390 Soil Quality—Determination with 1 M KCl (pH-KCl) and soil water content according to OECD 232 (OECD 2016). Exact values for pH and WHC are shown in Table S2.

2.3 | Combined Exposure to Fluazinam and Temperature Treatments

An overview of the experimental approach is shown in Figure 1.

The tests were conducted at five constant sublethal temperatures (20°C, 22°C, 24°C, 26°C, and 28°C) in Memmert ICP110 climate cabinets (Memmert, Schwabach, Germany). These temperatures were chosen because they represent a range of increasingly stressful, but not lethal (Wehrli et al. 2024), soil temperatures occurring in regions where *F. candida* is found (Hu and Feng 2003). Exposure temperatures were measured every 5 min with a Tinytag data logger precise to 0.1°C; for the toxicokinetics experiment, they were equipped with PB-5001 thermistors (Gemini Data loggers, Chichester, UK). Temperatures were accurate up to ± 0.17°C for the toxicokinetics experiment and ± 0.23°C for the reproduction experiment. Animals were exposed in air-tight glass jars (6.5 cm depth, 6 cm bottom diameter and 6.5 cm upper diameter) with spiked LUFA 2.2 soil (toxicokinetics experiment: 50 g dw; reproduction experiment: 35 g dw). For the toxicokinetics test, we added 100 adults to each jar, whereas for the toxicodynamics experiment, we added 10 age-synchronised springtails (10–12 days old).

For the reproduction test, we included eight solvent controls (acetone 180 mL/kg dw soil). For each concentration of fluazinam, we used four replicates. In addition, we included, for each concentration and temperature, jars dedicated to measurement of soil water content (WHC) and pH. For the toxicokinetics experiment, we used 12 jars per temperature and destructively sampled 3 replicates every 7 days. In addition, one jar at each temperature was dedicated to pH and soil water content that was measured at the last sampling. Blocks (including all treatment combinations) were started over 3 and 4 consecutive days in the toxicokinetics and toxicodynamics experiments, respectively, in order to reduce the daily workload. During the exposure, all jars were aerated twice a week and weighed every 7 days to calculate water loss and compensate for this if necessary. No water loss, however, was observed during the experiments. Also, every 7 days, the springtails were fed with 8 mg of dried baker's yeast per jar.

2.4 | Sampling in the Toxicokinetics Experiment

Springtails exposed to 5 mg fluazinam/kg dry soil were extracted by flotation, flooding the jars with deionised water and

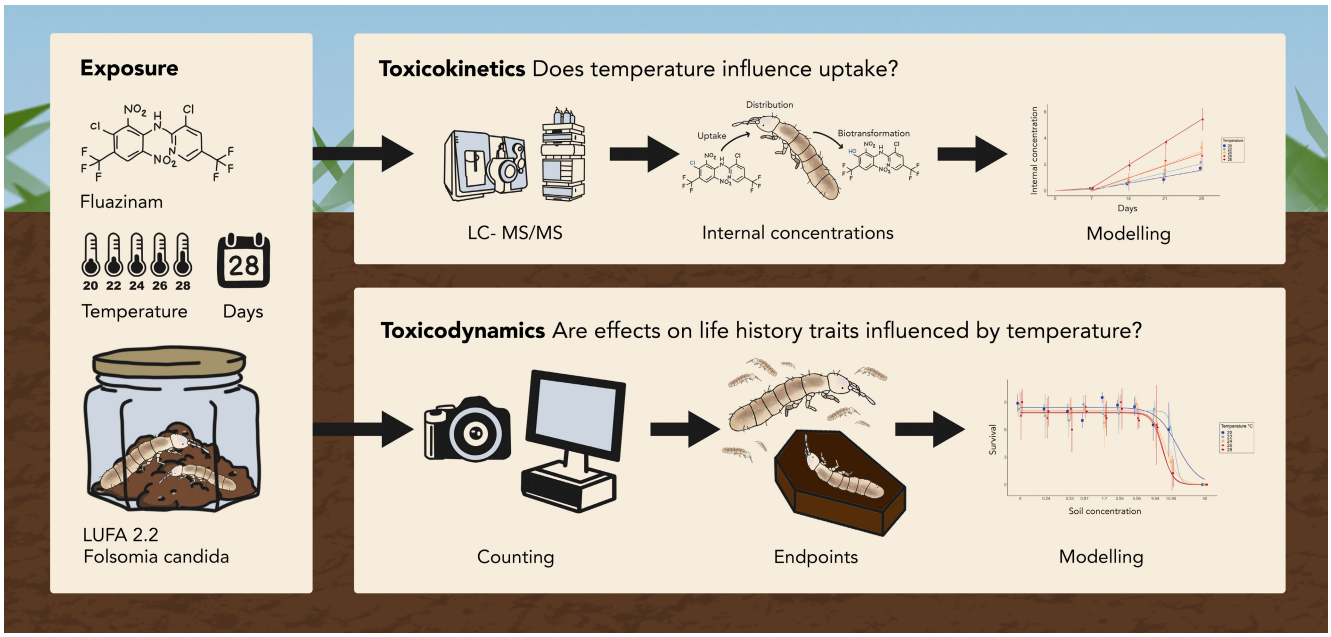

FIGURE 1 | Simplified overview of the major elements in the experimental approach. Springtails (*Folsomia candida*) were exposed to fluazinam mixed into a standard soil type. Exposure proceeded for 28 days at different temperatures. One experiment studied the body concentration of fluazinam and major metabolites (toxicokinetics). A second experiment studied the effects of fluazinam on life history traits of springtails (toxicodynamics). Statistical modelling evaluated the nature of interactions between effects of temperature and the pesticide.

stirring them gently. Springtails were subsequently removed from the water surface with a spoon and transferred to a clean petri dish with dry plaster of Paris to remove excess water. Finally, live springtails were sampled using an aspirator, transferred to a round-bottomed 2 mL centrifuge tube, weighed to the nearest 0.01 mg fresh mass using a Sartorius SC 2 microbalance (Sartorius AG, Goettingen, Germany) and snap-frozen at −80°C. At the start and end of the experiment, soil samples were taken to measure WHC (%), pH-KCl and the concentration of fluazinam and its primary metabolites. These measurements were done according to the methods described in the following paragraphs.

2.5 | Sampling in Reproduction Experiments

Sampling of animals in the reproduction experiment was done according to the flotation method described in OECD 232. Thus, adding deionised water to the jars, stirring gently, waiting 5 min to allow springtails to float to the surface and adding black ink to gain a better contrast between the white animals and the dark background. Finally, a picture was taken with a digital camera to record the number of adults and juveniles in ImageJ. Three replicate soil samples were taken at the beginning of the test for measurement of actual concentration of fluazinam in soil and stored at −80°C until analysis.

2.6 | Sample Preparation: Internal Concentrations

To extract fluazinam from springtails, we added 500 μL of acetonitrile to a 2 mL Eppendorf tube with 40 pre-weighed adult springtails and homogenised the sample in a Geno/grinder (SPEX SamplePrep, Metuchen NJ, USA) for 1 min. Homogenised samples were then ultrasonicated at 60°C for 45 min, followed by 10 min of centrifugation at 16,602 *g*. Subsequently, the supernatant was transferred to a clean amber glass vial. The pellet was then redissolved with 500 μL of acetonitrile, and the procedure was repeated. Finally, 500 μL of the combined supernatants was concentrated under a constant flow of nitrogen and resuspended in 100 μL of 25% acetonitrile before liquid chromatography with tandem mass spectrometry (LC–MS/MS) analysis. The internal concentrations are described in ng/mg fresh body mass.

2.7 | Sample Preparation: Soil Concentrations

Soil samples were extracted with a Dionex accelerated solvent extraction system 350 (ASE, Thermo Fisher Scientific Inc., United States). The system uses acetonitrile that penetrates the sample matrix, dissolves the analytes and transfers them to a collecting vial, where the extracts are then stored at −18°C until further usage. Samples were loaded in 33 mL extraction chambers with 5 g of dried Ottawa sand, followed by 1 g of soil sample and 5 g of Ottawa sand. A filter paper was pressed lightly on top, and the remaining space was filled with glass beads. The extraction cells were preheated to 80°C for 5 min and heated for 5 min. The chambers were kept static for 3 min, then flushed with 100% acetonitrile and then purged for 60 s. This cycle was repeated 4 times with a nitrogen pressure of 107 Pa. The soil concentrations are described in mg/kg dw soil.

2.8 | Analysis of Fluazinam and Metabolites by LC–MS/MS

To analyse internal concentrations of fluazinam and selected metabolites in springtails and in soil, we amended a method described for imidacloprid (Kristiansen et al. 2021). The mass transitions for the metabolites (Table S3) were taken from a

metabolomics paper that utilised the same mass spectrometer (Li et al. 2023). For parent compounds, the external calibration curve based on pure standards was used. Since no standards for the metabolites were available, metabolite concentrations had to be semi-quantified based on the parent compound, with a weighted linear curve fit and error estimation. Semi-quantification can cause uncertainties, due to differences in ionisation efficiencies, but can—as in this case—be the solution for the lack of pure standards.

The high-pressure liquid chromatography (HPLC) was an Agilent 1260 Infinity HPLC system (Santa Clara, CA, USA) coupled to a mass spectrometer with a quadrupole-linear ion trap (QTRAP) 4500 mass spectrometer (AB Sciex, Framingham, USA). Ionisation was performed with electrospray ionisation (ESI) in negative polarity. The QTRAP used MRM transitions. The system was run by Analyst software version 1.7.3 (AB Sciex, Framingham, USA), and settings are shown in Table S3.

Extracted samples were measured on a Kinetex 5 μ EVO C18 100 A 150 × 2.1 mm column with an injection volume of 10 μL. Chromatographic separation was achieved through a gradient elution at 20°C with the following eluents: Eluent A: 5 mM ammonium formate in Milli-Q Water; Eluent B: 5 mM ammonium formate in 100% methanol (MeOH), as shown in Table S4. The limit of detection (LoD) and limit of quantification (LoQ) were set to the lowest measurable standard at 0.024 ng/mL. All samples below 0.024 ng/mL were considered as below LoD and set to 0. Recovery was measured by spiking 40 untreated springtails with 100 μL of 40 ng/mL fluazinam-acetone solution. The spiked concentration in springtails was 0.8 μg/g. The measured concentration in springtails was around 0.005 μg/g. Recovery was determined twice (86.5% and 91.5%), which resulted in a mean recovery of 89% for internal concentrations. Recovery for soil samples was similar to springtail tissue and determined at a concentration level of 20 μg/g. Measured concentrations in experimental soil were 3–15 μg/g. Thus, the data was not corrected for recovery.

2.9 | Data Analysis

All the raw data was stored in Excel sheets (Wehrli 2024), and all data cleaning and treatment was performed in the R studio version, Desert Sunflower, 2023.09.1 + 494 (Posit team. 2023) and R version 4.3.2 (R Core Team 2023). The corresponding R-Script is available on DRYAD (Wehrli 2024). All datasets were analysed for normality with the Shapiro–Wilks test, visually with Q-Q plots and homogeneity of variance with Levene's test. The toxicokinetics experiment was analysed using general linear models with the model fitting function glm (R Core Team 2023).

The reproduction data was analysed using the DRC R package (Ritz et al. 2015). A three-parameter log-logistic dose–response model (LL.3 models [Finney 1971]) was fitted to the data for lethality and a three-parameter Weibull model (W1.3 models [Seber and Wild 1989]) to the number of juveniles, both based on the best model fit. Since the highest tested concentration of fluazinam did not result in full lethality, we decided to constrain the model to zero survival and reproduction at 50 mg/kg, which greatly improved the model fit without changing the effect values. This constraint was deemed sound based on previous experiments (Wehrli, 2022).

Body length measurements (measured from the end of the posterior abdominal segment to the anterior margin of the head) showed that the 10–12 days old springtails added to jars at the outset of the experiment had a body length of 0.09 ± 0.02 mm (mean ± SD, $n = 3089$) with no difference between blocks. Hence, the body length of the adults at the end of the experiment was used as a proxy for body growth. Measurements were done in ImageJ with the line tool. Data for adult body length was analysed with general linear models with the model fitting function glm (R Core Team 2023), while the juvenile size was analysed using a 2° polynomic (splines package) glm function with a log gamma distribution (R Core Team 2023). Parameters and statistics of all glm tests are compiled in Table S5.

Combined effect models were applied to predict the simultaneous stress exerted by temperature and fluazinam and whether interactions between effects were deviating from the independent action model as either synergistic or antagonistic. Since antagonism was not observed, we focused on synergistic interactions. As a null model, we used the widely used model of joint EA of independently acting stressors, which assumes the additivity of two effects in the form of a probabilistic sum (Bliss 1939). The interaction was considered synergistic if the combined effect of temperature and fluazinam was greater than the joint stressor effect predicted by EA. We used the 'SAM' to predict the combined stress effects (Liess et al. 2016). The SAM assumes that each individual has a general stress capacity towards all types of stress that should not be exhausted. Using stress-related mortality, specific stress levels are converted into general stress levels—the common currency of stress. These general stress levels of independent stressors are added determining the total stress exerted on a population. This approach quantitatively predicts the highly synergistic direct effects of independent stressor combinations. For the non-monotonic dose–response relation on reproduction, we used the extension of SAM, the EC_{x-SyS} model (Liess, Henz, and Knillmann 2019) This related approach enables to consider hormetic responses (Schulz 1877) often observed in long-term investigations. The SAM and the EC_{x-SyS} were calculated using the stress addition R package (Henz and Liess 2020). The measured model fits of the LL.3 models were extracted using the method described by Ritz and Streibig (Ritz and Strebig 2016) and inserted into the Indicate interface. For mortality, 20°C was used as non-stressing reference temperature because the effects increased monotonically from 20°C to 28°C. For the endpoint reproduction, an optimal temperature of 24°C was observed at which the highest population growth was recorded. Accordingly, this endpoint showed a non-monotonic cause–effect relationship with a maximum at 24°C. Thus, 24°C was chosen as reference temperature for this endpoint. To identify synergism, we used the EA model (Bliss 1939) as null model. The SAM was used as a novel model to predict the combined effects of environmental stressors and toxicants and to identify synergism compared to the null model (Liess et al. 2016) and the EC_{x-SyS} (Liess, Henz, and Knillmann 2019). The model parameters were extracted and plotted in R. Model deviation was calculated by dividing the fitted model by the measured model. The closer the result is to 1, the better is the prediction of the actual effects.

2.10 | Risk Assessment

The risk assessment was done according to the toxicity exposure ratio (TER) and hazard quotient (HQ) approach described in the technical guidance document (EC SANCO 2002), where we added the interaction with elevated temperatures. As the predicted environmental concentration (PEC), we used the $PEC_{Global\ Max}$ obtained from the EFSA assessment report of fluazinam (EFSA 2019).

3 | Results

3.1 | Toxicokinetics Experiment: Concentrations of Fluazinam in Soil

In the toxicokinetics experiment, the nominal fluazinam concentrations were aimed at 5 mg/kg dw, but the mean measured soil concentration was 3.54 ± mg/kg dw soil ($n = 2$) (Table S6). During the experiment, there was apparent degradation in the fluazinam concentration, which was reduced to about 45% of the start concentrations after 4 weeks. However, the degradation rate did not correlate with temperature (glm; $p = 0.65$; Figure S1).

In addition to the parent compound, the primary metabolite, hydroxyl-fluazinam, was detected in low concentrations. While fluazinam degraded over time, the concentration of hydroxyl-fluazinam increased from being undetectable to about 0.5 mg/kg dw soil after 4 weeks (Table S6).

3.2 | Toxicokinetics Experiment: Concentration of Fluazinam in Springtail Tissue

Survival of adults was not affected at temperatures up to 26°C, whereas at 28°C, survival was about 40% at the latest sampling (data not shown). Thus, we recovered enough animals to measure internal concentration of fluazinam and metabolites at all temperatures. Results of the toxicokinetics experiment showed a slightly increasing trend over time (from Day 7 and onwards) of fluazinam concentrations at 20°C, while this trend reversed, and concentrations decreased (from Day 7) with increasing temperature (Figure 2a). Time (glm, $p = 0.005$), temperature (glm, $p < 0.001$) and the interaction of temperature and time (glm, $p = 0.003$) all showed a significant effect on the internal concentrations of fluazinam. In the first 14 days of the exposure experiment, high temperature resulted in up to 10-fold higher internal concentrations of fluazinam (Figure 2a, insert).

Contrary to fluazinam, we observed a continuous, increasing trend of internal hydroxyl-fluazinam (Figure 2b) over time (glm, $p = 0.002$). While the overall temperature effect was not significant here (glm, $p = 0.1$), the interaction of temperature and time was highly significant (glm, $p < 0.001$). Internal concentrations of hydroxyl-fluazinam were consistently much higher than the parent compound, up to 8000 times higher. The second metabolite, sulfhydryl-fluazinam, was detected in low concentrations but was not influenced by time or temperature (Figure S2).

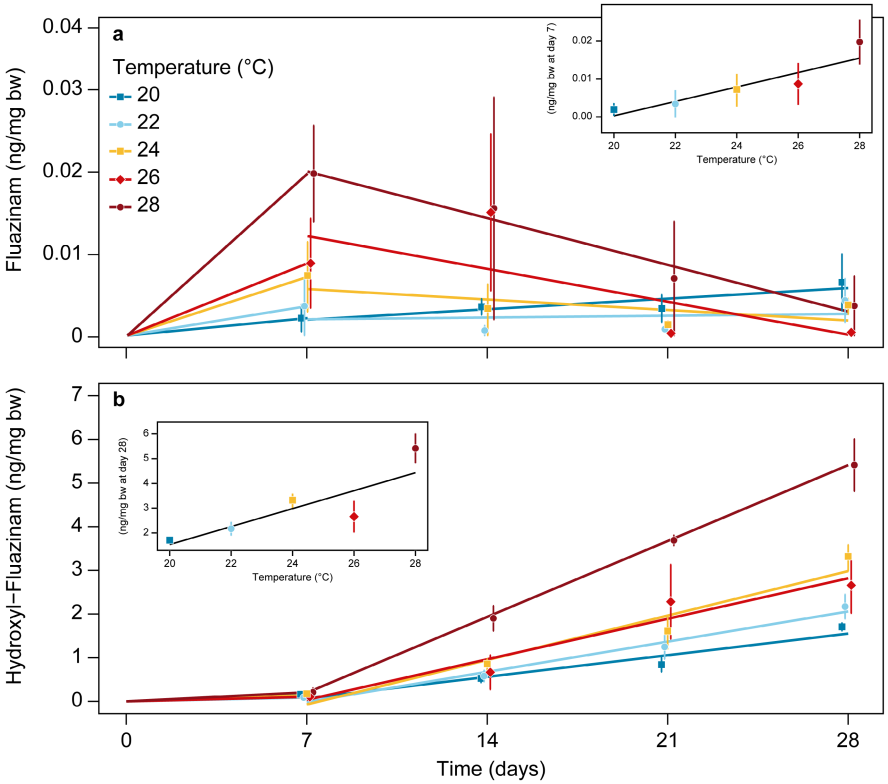

FIGURE 2 | The influence of temperature on the internal concentration of fluazinam (a) and hydroxyl-fluazinam (b) in *Folsomia candida* adults during 28-day exposure to soil spiked with 5 mg fluazinam/kg dry soil. Symbols at Days 7, 14, 21 and 28 indicate mean ± SE ($n = 3$). Lines from Days 0 to 7 are hypothetical trends shown for illustration. Linear models have been fit to mean values of Days 7–28. Inserts in (a) and (b) are the means ± SE ($n = 3$) from Days 7 and 28, respectively, plotted against exposure temperature.

3.3 | Toxicodynamics Experiment: Concentration of Fluazinam in Soil

Measured start concentrations of fluazinam were 48%–65% lower than the nominal concentrations (Table 1). Therefore, all analyses of dose responses were based on the measured concentrations of fluazinam in soil. Hydroxyl fluazinam was measured at very low concentrations from 0.01 to 0.06 mg/kg dw soil and was therefore ignored in the further analysis.

3.4 | Toxicodynamics: Mortality of Adult Springtails

In the reproduction experiment, we assessed the adult mortality at the end of the 28 days experiment. Adult mortality in the controls was negligible and not affected by elevated temperature (Figure 3).

Dose–response curves depicted in Figure 3 indicated that the LC_{50} of fluazinam was 19.8 ± 2.4 mg/kg dw at the control temperature (20°C). We did not find any decrease in adult mortality due to the interaction of temperature and fluazinam at 20°C–22°C (LC_{50}: 17.9 ± 5.9 mg/kg dw soil). However, comparing 20°C with 24°C, 26°C, and 28°C indicated a synergistic interaction between temperature and fluazinam concentration; the adult mortality increased when exposed jointly compared to when exposed to the stressors separately. This interaction caused a significant ($p = 0.016$) decrease in LC_{50} values by 51%, from 19.8 ± 2.4 mg/kg dw soil to 12.0 ± 1.0 mg/kg dw soil (Table S7), where the LC_{10} at 20°C (9.1 ± 2.1 mg/kg) was similar to the LC_{50} at 28°C (12.0 ± 1.0 mg/kg).

Predictions of the combined stressor effects with the SAM were performed for the mortality data. The SAM model predicted well the synergistic interaction of fluazinam and elevated temperatures, as depicted in Figure 4a,b. To compare the accuracy of the models, the model deviations (Model LCx/Measured LCx) for each model were calculated (Table S8). The results showed that the SAM model accurately predicted the toxicity values in the synergistic range with a model deviation of 0.9 for LC_{50} values (Table S9).

TABLE 1 | Nominal and measured soil concentrations of fluazinam at the start of the reproduction experiment.

Nominal concentration (mg/kg dw soil)	Measured concentration (mg/kg dw soil)	Difference measured/nominal (%)
0	0 ± 0	—
0.5	0.24 ± 0	48
0.9	0.53 ± 0.01	58
1.6	0.87 ± 0.03	54
2.6	1.69 ± 0.08	65
5	2.85 ± 0.06	57
8.8	5.06 ± 0.57	57
15.3	9.44 ± 0.18	61
25.6	16 ± 0.58	62

Note: Values are mean ± SE ($n = 3$).

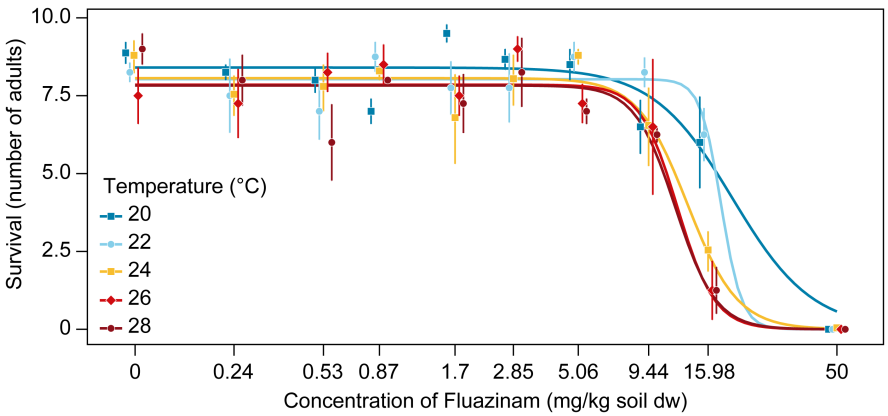

FIGURE 3 | Dose–response curves fitted to the number of surviving adult *Folsomia candida* per replicate (out of 10 adults added) after 4 weeks exposure to fluazinam at five temperatures. Symbols represent mean ± SE ($n = 4$). Concentrations of fluazinam are measured concentrations at the start of the test.

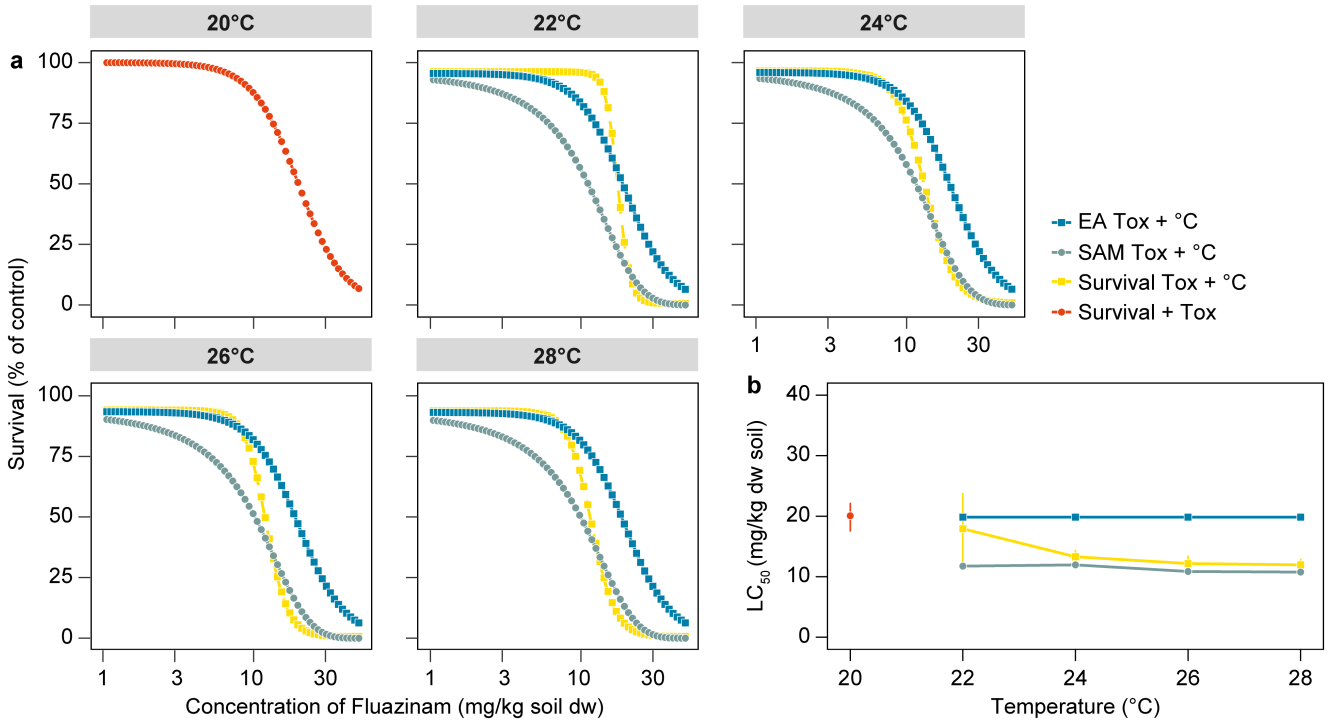

FIGURE 4 | (a) Model fits of measured survival of adult *Folsomia candida* at each temperature in red (at control temperature; 20°C) and yellow (elevated temperature; taken from Figure 3), effect addition (EA) in blue, and the stressor addition model (SAM) in green. (b) LC50 values with standard errors for the measured toxicity at different temperatures (taken from Figure 3; yellow and red symbols) and modelled LC50 values for the EA and stressor addition model (SAM) shown in blue and green, respectively. Values of fluazinam represent concentrations in soil at the start of the test.

3.5 | Toxicodynamics: Growth of Springtails

Growth of adults was reduced at temperatures above 20°C (Figure 5a; glm, $p < 0.001$). The concentration of fluazinam did not have an effect on adult growth (glm, $p = 0.972$), and there was no interaction between temperature and fluazinam concentration (glm, $p = 0.998$).

Body length of juveniles was increased at temperatures above 20°C (Figure 5b; glm, $p = < 0.001$). Fluazinam decreased juvenile size (glm, $p < 0.001$), and there was a significant interaction between temperature and fluazinam concentration (glm, $p < 0.001$).

3.6 | Toxicodynamics: Reproduction of Springtails

All validity criteria set out in the guideline were passed with adult survival better than 80% and reproduction in controls higher than 100 per replicate (Table S10). Additionally, pH and soil water content were in the acceptable range (Table S2).

Temperature, fluazinam and the interaction influenced reproduction in different ways. Adult springtails exposed to 20°C produced fewer juveniles than those exposed to 22°C, 24°C and 26°C, while at 28°C, they did not reproduce at all (Figure 6a; Table S10). The temperatures 22°C–24°C showed the highest number of juveniles, and this range was defined as optimal for the reproduction of *F. candida*, as previously reported (Ge et al. 2023). This led to a unimodal distribution of the data that influenced the modelling of the mixture models.

Fluazinam also affected reproduction with an EC_{50} at 10.4 ± 1.1 mg/kg dw soil at 20°C (Table S11). When organisms were exposed to increased temperatures from 22°C to 24°C, the respective EC_{50} was unaffected with a maximum reproduction at 24°C. We therefore defined this concentration as the one with the least temperature stress, that is, the reference temperature for reproduction. At 26°C, where reference growth was reduced, the combined effect of stressors resulted in a reduced EC_{50} at 7.2 ± 1.4 mg/kg dw soil (although not statistically significant; $p = 0.07$), which was a 31% decrease from 20°C to 26°C (Table S11).

For 24°C and 26°C, we were able to fit the $EC_{x\text{-}SyS}$ model (Figure S3) and the SAM to the data (Figure 6b), describing the hormesis for pesticide concentration–response relationship at the reference temperature (24°C) and also for the combined stressors where the pesticide and the temperature stress (26°C) interact. This reduced EC_{10} and EC_{50} values for the EC_{x-SyS} model (Table S12) and was able to predict accurately the effect on reproduction with SAM (Table S13), which was superior to the classic dose–response analysis with the LL.3 model. At 28°C, no population growth was observed.

3.7 | Risk Assessment

We did a risk assessment for Collembola based on effect values generated for fluazinam and *F. candida* and added the interaction with elevated temperature. In this case, we disregard that other, more sensitive, organisms would normally be used for the TER

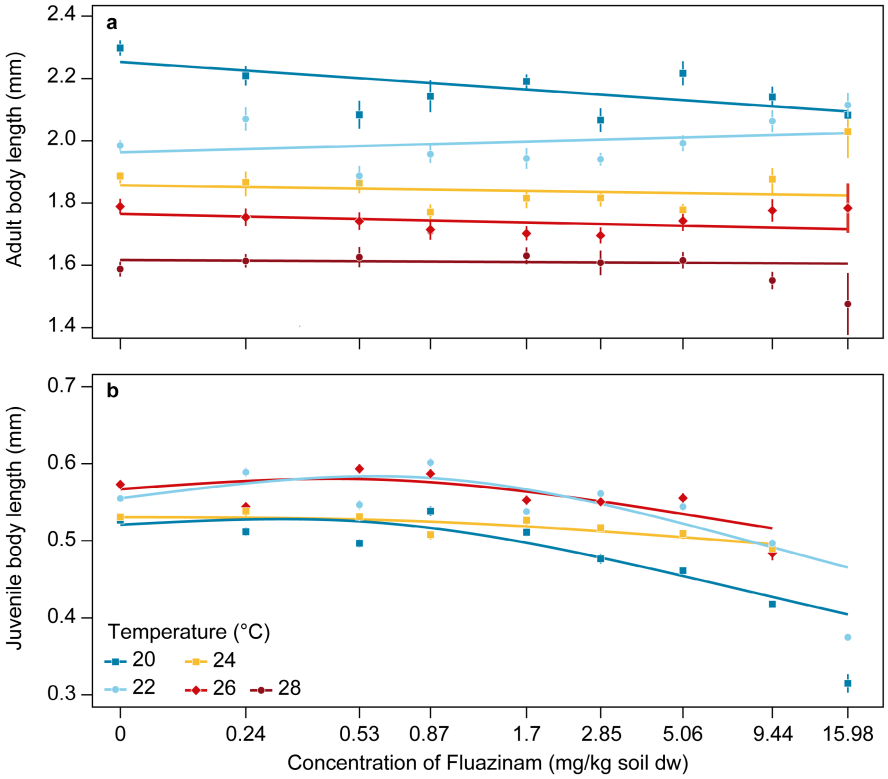

FIGURE 5 | The effects of exposure temperature and fluazinam concentration on body length of adults (a) and juveniles of *Folsomia candida* (b). Symbols are mean ± SE ($n = 4$). Lines represent a polynomic regression of the log gamma glm analysis. Note that no juveniles were produced at 28°C. Values of fluazinam represent concentrations in soil at the start of the test.

calculation (EFSA 2019; Marti-Roura et al. 2023) and hypothesise that endpoints of these organisms would also decrease with the increase in temperature. Due to the lipophilic nature of fluazinam, the EC_{10} values are divided by 2 to account for Log_{kow} of 4.04 as previously done (EC SANCO 2002; Marti-Roura et al. 2023). The calculated TER values, based on the lipophilic corrected EC10 values, decreased by a factor > 2 from 6.7 at 20°C to 2.93 at 26°C (Figure 7). In addition to the TER, a ficticious HQ was calculated as described in the European Commission Technical Guidance Document (EC 2003) and using an assessment factor of 50 based on a previous assessment (Marti-Roura et al. 2023). This resulted in the HQ being above the trigger value of 1 for both temperature exposures of 3.7 at 20°C to 8.5 at 26°C.

4 | Discussion

In our study, we predicted interactive effects of increased temperature and a commonly used fungicide on soil-dwelling springtails. Indeed, we found distinct synergistic interactions in which the effects of the fungicide were exacerbated at high, but realistic, soil temperatures that these organisms already experience in their environment and increasingly will do in the near future. Although this springtail species is merely a surrogate species representing many soil animals, it is likely that the results can be extrapolated broadly, including the important ecosystem functions that soil animals have (Fountain and Hopkin 2005).

4.1 | High Temperature Perturbs Metabolism of Pesticides in Springtails

As hypothesised, temperature significantly influenced the toxicokinetics of fluazinam in *F. candida*. Fluazinam was incorporated into soil where it binds primarily to soil organic matter (SOM) and attains equilibrium partitioning between soil water and SOM (Ma et al. 1998). From here, the main uptake route is likely across the springtail skin, from where it will partition into other body compartments, but a small contribution from dietary uptake is also possible (Schmidt et al. 2013). It should be noted that we did not measure the full toxicokinetics since this would have required measurements of internal concentration during a depuration phase, which was beyond the scope of the present study. Instead, we have shown the effect of high temperature on the body burden of fluazinam and primary metabolites. Hence, in the short term (7 days), the springtails had an increased body concentration of fluazinam at raised temperatures (10-fold higher at 28°C than at 20°C), as shown for organic compounds in other aquatic and terrestrial organisms (Dai et al. 2021; Huang et al. 2022, 2023). However, the springtails seemed to regulate internal concentrations of fluazinam to a similar internal concentration over all temperatures during the following 21 days. The peak of fluazinam after 7 days followed by a steady decrease in the following weeks suggests that detoxification mechanisms were rapidly induced, raising the ability of the animals to metabolise fluazinam, as observed for other contaminants in *F. candida* (Ardestani, Oduber, and van Gestel 2014; Nota et al. 2009;

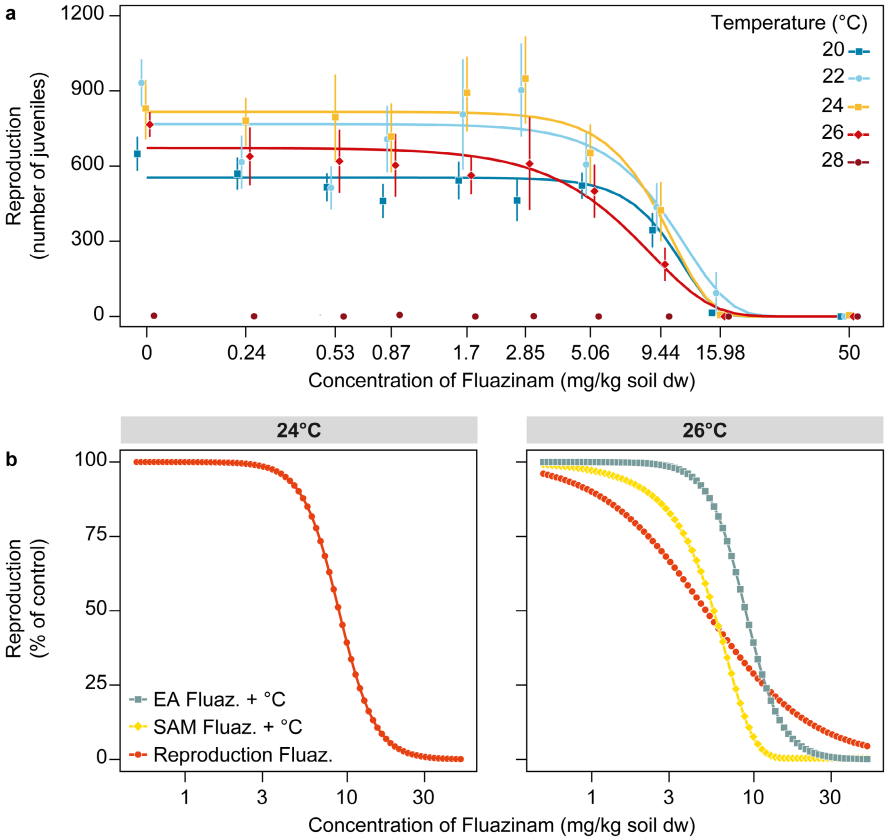

FIGURE 6 | (a) The number of juvenile *Folsomia candida* produced in the reproduction test. Animals were exposed to fluazinam for 4 weeks at five different temperatures. Values of fluazinam represent concentrations in soil at the start of the test. Values are mean ± SE ($n = 4$). Lines are dose–response curves fitted to data using a three-parameter log-logistic model (LL.3). (b) Model fits of the measured number of juveniles at each temperature in red at the reference temperature (24°C) and at elevated temperature (26°C); data from Figure 3, effect addition (EA) in green, and the stressor addition model (SAM) in yellow.

Wang et al. 2024). Fluazinam was observed to degrade in soil, exposing springtails to a decreased load of fluazinam over time, which might also have contributed to the decreased levels of internal fluazinam. In the case of hydroxyl-fluazinam, we found negligible low concentrations in soil, about 0.03 mg/kg dw compared to 3.69 mg/kg of fluazinam. These concentrations increased over time and reached 0.5 mg/kg dw at its highest. This suggests that springtails may have taken up both fluazinam and hydroxyl-fluazinam directly from the soil.

Contrary to the parent compound, we measured a consistent increase in tissue concentration of the hydroxy-metabolite with time, which indicates that springtails can carry out the initial degradation step but cannot degrade it further or excrete metabolites sufficiently. This is also supported by other observations since we could not measure the metabolite in the soil at the start of the experiment but were able to find increased metabolite concentrations in the organisms already after 1 day in preliminary experiments (Figure S4). Additional studies on the transcription of cytochrome p450 genes could be a valuable addition to validate the hypothesis that hydroxyl-fluazinam is produced internally (Nota et al. 2009). As previously shown, p450 transcription is usually induced with increasing concentrations of a toxicant, leading to an upregulation of excretion processes in springtails (Bakker et al. 2023; Dai et al. 2023; Holmstrup et al. 2014; Wang et al. 2024). It is possible that hydroxyl-fluazinam might not be metabolised further or excreted and therefore accumulates in the body. This notion is supported by the difference in $\log K_{ow}$, which is 4.04 for the parent compound (PubChem 2024), and likely slightly less lipophilic for hydroxyl-fluazinam as judged from soil sorption studies (EFSA 2008), which could indicate some bioaccumulation of hydroxyl-fluazinam in tissue and membranes. However, this cannot be confirmed without conducting further studies that include both the uptake and excretion phase (Wang et al. 2024). Additional gene expression studies on phase II and phase III enzymes could be of value to detect if genes related to excretion, such as glutathione-s-transferase or similar pathways are upregulated (Bakker et al. 2023; Dai et al. 2023; Nota et al. 2008, 2009), or testing activities of the targeted enzymes. Additionally, heat shock protein regulation was previously shown to be upregulated when springtails were exposed to sub-optimal high temperatures, which could delay the detoxification of the parent compound or metabolite due to receiving a priority production, thus reducing energy availability for homeostasis and detoxification processes (Dai et al. 2023). Detoxification mechanisms of phases I, II or III are energy-intensive and can be shut down, as shown in the mosquito *Culex pipiens molestus* when exposed to pesticides in combination with heat stress (Delnat et al. 2020; Siddique, Shahid, and Liess 2021). Therefore, the organisms must choose between upregulating HSPs or detoxification mechanisms when subjected to stressful high temperature, both energy-intensive processes. These previous findings could explain why we saw an increase in internal concentration of hydroxyl-fluazinam and, therefore,

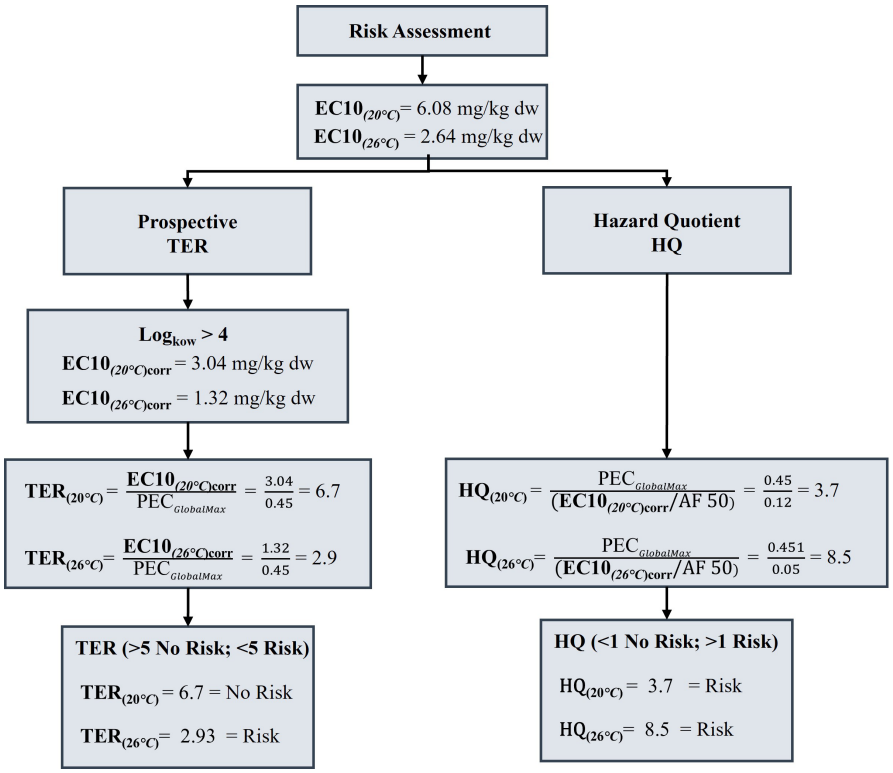

FIGURE 7 | Risk assessment of fluazinam based on data from *Folsomia candida* reproduction tests at 20°C and 26°C, respectively. The EC_{10} values are divided by 2 to account for the $Log_{kow} > 4$. The prospective toxicity exposure ratio (TER) and the hazard quotient (HQ) is calculated at 20°C and 26°C, respectively. Finally, a risk comparison is made, where a temperature increase from 20°C to 26°C doubled the TER and the HQ, making it conceived as a risk when temperature is 26°C in the case of the TER and at both temperatures for the HQ.

a delay or complete stop of detoxification in phase II or III of the hydroxy-metabolite. Our study also highlights the importance of measuring not only the parent compound, but also the most important metabolites, which may also exert cellular damage. We note that the metabolite hydroxyl-fluazinam was always found in much larger quantities than the parent compound and could potentially exert increased toxicity to the organism; however, this would need further experiments to verify.

4.2 | High Temperature Increases the Effects of Pesticides

The EC_{50} value at the reference temperature related to mortality and reproduction (20°C) was similar to previously reported data and unpublished data available to us (EFSA 2019; Wehrli, 2022). The values were also in line with results from tests using another springtail species (*Folsomia fimetaria*) that reproduced sexually, ruling out that reproductive strategy could change the toxicity values (Wehrli et al. 2022). We confirmed the hypothesis of synergistic interactions and decreasing lethal (LCx) and sublethal endpoints (ECx) due to high temperature, thus increasing sensitivity. This could mean that in a world of increasing temperatures, the influence of pesticides on non-target soil arthropods can have more detrimental effects on populations than expected from traditional environmental risk assessment (ERA) (Bandeira et al. 2020, 2021; Dai et al. 2019; Eijsackers et al. 2017). This is the case not only for soil, but also for freshwater invertebrates, as shown in experimental systems (Liess, Henz, and Knillmann 2019), outdoor microcosms (Knillmann et al. 2013) and at the ecosystem level (Russo, Becker, and Liess 2018). Recent studies have shown that this problem is even increased when adding environmental stress to mixtures of pesticides typical of field exposures (Shahid, Liess, and Knillmann 2019).

While synergistic negative interactions were most obvious in mortality and reproductive traits, we also observed a decrease in growth in adults with higher temperature, which is congruent with previous studies of this species (Ge et al. 2023). Contrary to this, juveniles increased in size at a higher temperature. However, this was probably due to an earlier hatching of juveniles due to sped-up embryonic development and hence the growth of juveniles for a longer period during the experiment at the higher temperatures. The fungicide does not affect the growth of adults, but at high concentrations, juveniles were clearly smaller than in uncontaminated control soil. Fungicide-induced reductions of springtail biomass could lead to shifts in their functional role in ecosystems, for example, as grazers of fungi and role in nutrient cycles, hunter–prey relationships, or changes in trophic niches (Filser et al. 2016; Lux et al. 2024).

4.3 | New Approaches for Multiple Stress Modelling Are Needed

The approach we used to account for synergistic interactions could be reached by embedding the novel SAM model in ERA

(Liess et al. 2016). Predictions for the chronic lethality of fluazinam were precisely predicted with the SAM model by ± 2 mg/kg dw compared to the measured toxicity data. On the contrary, the conventional IA model (Bliss 1939) could not predict lethality with sufficient precision. SAM was also able to predict the reproductive inhibition in the range of the EC_{50} and fit the combined stress effects more accurately. However, the non-monotone EC_{x-SyS} model was more precise at lower concentrations, as a non-monotone hormetic cause–effect relationship generally occurs in the range of concentrations well below the EC_{50} (Liess, Henz, and Knillmann 2019). This is due to the observed maximum population growth (T_{opt}) at 22°C–24°C. Such a unimodal distribution of performance in the control needs to be recognised in order to identify the least stressful conditions for the reference cause–effect relationship for any combined stress–effect model as SAM or EA. If this were incorporated into the classic risk assessment approach that was utilised before, the effect values as well as the regulatory values would be drastically reduced compared to the classic approach.

4.4 | Risk Assessment Adapted to a Changing Climate

The previously discussed changes in toxicodynamics due to elevated temperatures led to changes in the risk assessment. Observations of soil temperatures further support its importance and relevance. For example, July soil temperatures (10 cm depth) in the United States will regularly reach up to 28°C (Hu and Feng 2003) in a region where *F. candida* is found (GBIF 2024), showing that our experimental conditions reflect realistic scenarios.

In our risk assessment, we determined a decrease in the TER at 26°C by a factor of 2. In this case, the synergistic interaction between the effects of increasing temperature and fluazinam was clear and changed the outcome of the TER approach, which would not have triggered a higher-tier assessment at $TER_{20°C}$ but does so when calculated for $TER_{26°C}$. The reduction of the TER by a factor of two is concerning and not limited to the TER only. In the simulated HQ approach ($3.7_{20°C}$–$8.5_{26°C}$), both are above the accepted trigger value of 1 stated by the European Commission (EC 2003). The same holds true for other risk assessments since all are based on the lowest effect value at higher temperature ($EC_{10(26°C)}$). Liess et al. reported up to 100-fold higher effects than at benign temperatures (Liess et al. 2016). These effects have been observed both as toxicant-induced climate susceptibility (TICS) or vice-versa as climate-induced toxicant sensitivities (CITS), where previous exposure to environmental stressors results in increased sensitivity to a toxicant (Polazzo et al. 2022; Roth et al. 2022; Shahid, Liess, and Knillmann 2019). Studies on this report significant changes in toxicokinetics and dynamics using TK-TD and GUTS models explaining the change in toxicities (Huang et al. 2022, 2023; Mangold-Döring et al. 2022). We therefore argue that these shifts in EC/LC values should be considered in the risk assessment and authorisation to account for warming temperatures (Bandeira et al. 2020, 2021; Bandow, Karau, and Römbke 2014; Eijsackers et al. 2017; Noyes et al. 2009; Polazzo et al. 2022).

For more than two decades, many investigations addressing heat stress and chemical exposure reported synergistic interactions both on toxicokinetics and dynamics (Bandeira et al. 2020; Bandow, Karau, and Römbke 2014; Eijsackers et al. 2017; Hooper et al. 2013; Huang et al. 2022, 2023; Mangold-Döring et al. 2022; Polazzo et al. 2022; Raths et al. 2023; Verheyen and Stoks 2023). Some papers called explicitly for including environmental factors in the risk assessment. These synergistic interactions, which also include the mixture of multiple chemical stressors, pose a relevant threat to populations and ecosystems (Bale et al. 2002; IPCC et al. 2022; Noyes et al. 2009; Shahid, Liess, and Knillmann 2019; Siddique, Shahid, and Liess 2021; Sigmund et al. 2023). Based on previous studies and the results of our present study, it appears necessary to incorporate multiple stressors into the risk assessment of harmful substances.

Author Contributions

Micha Wehrli: conceptualization, data curation, formal analysis, investigation, methodology, project administration, visualization, writing – original draft, writing – review and editing. **Martin Holmstrup:** conceptualization, funding acquisition, resources, supervision, writing – original draft, writing – review and editing. **Matthias Liess:** formal analysis, supervision, writing – review and editing. **Inge S. Fomsgaard:** methodology, supervision, writing – review and editing. **Stine Slotsbo:** conceptualization, formal analysis, supervision, writing – review and editing. **Jonas Gröning:** formal analysis, writing – review and editing. **Bente B. Laursen:** methodology, supervision, writing – review and editing.

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Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

The data that support the findings of this study are openly available in DRYAD at https://doi.org/10.5061/dryad.np5hqc03h. The code that supports the findings of this study are openly available in ZENODO at https://doi.org/10.5281/zenodo.13886166.

References

Ardestani, M. M., F. Oduber, and C. A. M. van Gestel. 2014. "A Combined Toxicokinetics and Toxicodynamics Approach to Assess the Effect of Porewater Composition on Cadmium Bioavailability to *Folsomia Candida*." *Environmental Toxicology and Chemistry* 33, no. 7: 1570–1577. https://doi.org/10.1002/etc.2585.

Backhaus, T. 2023. "The Mixture Assessment or Allocation Factor: Conceptual Background, Estimation Algorithms and a Case Study Example." *Environmental Sciences Europe* 35, no. 1: 55. https://doi.org/10.1186/s12302-023-00757-w.

Bakker, R., J. Ellers, D. Roelofs, et al. 2023. "Combining Time-Resolved Transcriptomics and Proteomics Data for Adverse Outcome Pathway

Refinement in Ecotoxicology." *Science of the Total Environment* 869: 161740. https://doi.org/10.1016/j.scitotenv.2023.161740.

Bale, J. S., G. J. Masters, I. D. Hodkinson, et al. 2002. "Herbivory in Global Climate Change Research: Direct Effects of Rising Temperature on Insect Herbivores." *Global Change Biology* 8, no. 1: 1–16. https://doi.org/10.1046/j.1365-2486.2002.00451.x.

Bandeira, F. O., P. R. L. Alves, T. B. Hennig, J. Brancalione, D. J. Nogueira, and W. G. Matias. 2021. "Chronic Effects of Clothianidin to Non-Target Soil Invertebrates: Ecological Risk Assessment Using the Species Sensitivity Distribution (SSD) Approach." *Journal of Hazardous Materials* 419: 126491. https://doi.org/10.1016/J.JHAZMAT.2021.126491.

Bandeira, F. O., P. R. Lopes Alves, T. B. Hennig, T. Toniolo, T. Natal-da-Luz, and D. Baretta. 2020. "Effect of Temperature on the Toxicity of Imidacloprid to *Eisenia andrei* and *Folsomia candida* in Tropical Soils." *Environmental Pollution* 267: 115565. https://doi.org/10.1016/j.envpol.2020.115565.

Bandow, C., N. Karau, and J. Römbke. 2014. "Interactive Effects of Pyrimethanil, Soil Moisture and Temperature on *Folsomia candida* and *Sinella curviseta* (Collembola)." *Applied Soil Ecology* 81: 22–29. https://doi.org/10.1016/j.apsoil.2014.04.010.

Bliss, C. I. 1939. "The Toxicity of Poisons Applied Jointly." *Annals of Applied Biology* 26, no. 3: 585–615. https://doi.org/10.1111/j.1744-7348.1939.tb06990.x.

Bradford, J. B., D. R. Schlaepfer, W. K. Lauenroth, et al. 2019. "Climate-Driven Shifts in Soil Temperature and Moisture Regimes Suggest Opportunities to Enhance Assessments of Dryland Resilience and Resistance." *Frontiers in Ecology and Evolution* 7: 358. https://doi.org/10.3389/fevo.2019.00358.

Cairns, J., A. G. Heath, and B. C. Parker. 1975. "The Effects of Temperature Upon the Toxicity of Chemicals to Aquatic Organisms." *Hydrobiologia* 47, no. 1: 135–171. https://doi.org/10.1007/BF00036747/METRICS.

Camp, A. A., and D. B. Buchwalter. 2016. "Can't Take the Heat: Temperature-Enhanced Toxicity in the Mayfly *Isonychia bicolor* Exposed to the Neonicotinoid Insecticide Imidacloprid." *Aquatic Toxicology* 178: 49–57. https://doi.org/10.1016/j.aquatox.2016.07.011.

Chow, R., R. Scheidegger, T. Doppler, A. Dietzel, F. Fenicia, and C. Stamm. 2020. "A Review of Long-Term Pesticide Monitoring Studies to Assess Surface Water Quality Trends." *Water Research X* 9: 100064. https://doi.org/10.1016/j.wroa.2020.100064.

Dai, W., M. Holmstrup, S. Slotsbo, R. Bakker, C. Damgaard, and C. A. M. van Gestel. 2023. "Heat Stress Delays Detoxification of Phenanthrene in the Springtail *Folsomia candida*." *Chemosphere* 311: 137119. https://doi.org/10.1016/J.CHEMOSPHERE.2022.137119.

Dai, W., S. Slotsbo, C. Damgaard, X. Ke, L. Wu, and M. Holmstrup. 2019. "Synergistic Interaction Between Effects of Phenanthrene and Dynamic Heat Stress Cycles in a Soil Arthropod." *Environmental Pollution (Barking, Essex: 1987)* 254: 113071. https://doi.org/10.1016/j.envpol.2019.113071.

Dai, W., S. Slotsbo, C. A. M. Van Gestel, and M. Holmstrup. 2021. "Temperature-Dependent Toxicokinetics of Phenanthrene in *Enchytraeus albidus* (Oligochaeta)." *Environmental Science and Technology* 55, no. 3: 1876–1884. https://doi.org/10.1021/acs.est.0c06182.

Delnat, V., J. Swaegers, J. Asselman, and R. Stoks. 2020. "Reduced Stress Defence Responses Contribute to the Higher Toxicity of a Pesticide Under Warming." *Molecular Ecology* 29, no. 23: 4735–4748. https://doi.org/10.1111/mec.15667.

Deutsch, C. A., J. J. Tewksbury, M. Tigchelaar, et al. 2018. "Increase in Crop Losses to Insect Pests in a Warming Climate." *Science* 361, no. 6405: 916–919. https://doi.org/10.1126/science.aat3466.

Dornelas, M., N. J. Gotelli, H. Shimadzu, F. Moyes, A. E. Magurran, and B. J. McGill. 2019. "A Balance of Winners and Losers in the Anthropocene." *Ecology Letters* 22, no. 5: 847–854. https://doi.org/10.1111/ele.13242.

EC. 2003. "Technical Guidance Document on Risk Assessment."

EC. 2009. "Regulation (EC) No 1107/2009."

EC SANCO. 2002. "SANCO Terrestrial Guidance 2002. Guidance Document on Terrestrial Ecotoxicology."

EFSA. 2008. "Conclusion Regarding the Peer Review of the Pesticide Risk Assessment of the Active Substance Fluazinam." *EFSA Journal* 6, no. 7: 1–82. https://doi.org/10.2903/j.efsa.2008.137r.

EFSA. 2019. "Draft Renewal Assessment Report Prepared According to Commission Regulation (EU) N 1107/2009 Fluazinam." Volume 3—B.9 (PPP)—MCW 465 500 SC.

Eijsackers, H., A. Reinecke, S. Reinecke, and M. Maboeta. 2017. "Threatened Southern African Soils: A Need for Appropriate Ecotoxicological Risk Assessment." *Environmental Impact Assessment Review* 63: 128–135. https://doi.org/10.1016/j.eiar.2016.12.003.

Escher, B. I., H. M. Stapleton, and E. L. Schymanski. 2020. "Tracking Complex Mixtures of Chemicals in Our Changing Environment." *Science* 367, no. 6476: 388–392. https://doi.org/10.1126/science.aay6636.

Filser, J., J. H. Faber, A. V. Tiunov, et al. 2016. "Soil Fauna: Key to New Carbon Models." *Soil* 2, no. 4: 565–582. https://doi.org/10.5194/soil-2-565-2016.

Finney, D. J. 1971. "Probit Analysis." *Journal of Pharmaceutical Sciences* 60, no. 9: 1432. https://doi.org/10.1002/jps.2600600940.

Fountain, M. T., and S. P. Hopkin. 2005. "*Folsomia candida* (Collembola): A "standard" soil arthropod." *Annual Review of Entomology* 50: 201–222. https://doi.org/10.1146/annurev.ento.50.071803.130331.

Froger, C., C. Jolivet, H. Budzinski, et al. 2023. "Pesticide Residues in French Soils: Occurrence, Risks, and Persistence." *Environmental Science and Technology* 57: 7818–7827. https://doi.org/10.1021/acs.est.2c09591.

García-García, A., F. J. Cuesta-Valero, D. G. Miralles, et al. 2023. "Soil Heat Extremes Can Outpace Air Temperature Extremes." *Nature Climate Change* 13, no. 11: 1237–1241. https://doi.org/10.1038/s41558-023-01812-3.

GBIF. 2024. "*Folsomia candida* V. Willem, 1902." https://www.gbif.org/species/2120383.

Ge, J., S. Slotsbo, J. G. Sørensen, and M. Holmstrup. 2023. "Copper-Contaminated Soil Compromises Thermal Performance in the Springtail *Folsomia candida* (Collembola)." *Science of the Total Environment* 897: 165334. https://doi.org/10.1016/j.scitotenv.2023.165334.

Habel, J. C., A. Segerer, W. Ulrich, O. Torchyk, W. W. Weisser, and T. Schmitt. 2016. "Butterfly Community Shifts Over Two Centuries." *Conservation Biology* 30, no. 4: 754–762. https://doi.org/10.1111/cobi.12656.

Hallmann, C. A., M. Sorg, E. Jongejans, et al. 2017. "More Than 75 Percent Decline Over 27 Years in Total Flying Insect Biomass in Protected Areas." *PLoS One* 12, no. 10: e0185809. https://doi.org/10.1371/journal.pone.0185809.

Henz & Liess. 2020. "Stressaddition: Modelling tri-Phasic Concentration-Response Relationships." R package version 3.1.0. https://CRAN.R-project.org/package=stressaddition.

Heugens, E. H., A. J. Hendriks, T. Dekker, N. M. van Straalen, and W. Admiraal. 2001. "A Review of the Effects of Multiple Stressors on Aquatic Organisms and Analysis of Uncertainty Factors for Use in Risk Assessment." *Critical Reviews in Toxicology* 31, no. 3: 247–284. https://doi.org/10.1080/20014091111695.

Holmstrup, M., A.-M. Bindesbøl, G. J. Oostingh, et al. 2010. "Interactions Between Effects of Environmental Chemicals and Natural Stressors: A Review." *Science of the Total Environment* 408, no. 18: 3746–3762. https://doi.org/10.1016/j.scitotenv.2009.10.067.

Holmstrup, M., S. Slotsbo, S. N. Schmidt, P. Mayer, C. Damgaard, and J. G. Sørensen. 2014. "Physiological and Molecular Responses of Springtails Exposed to Phenanthrene and Drought." *Environmental Pollution* 184: 370–376. https://doi.org/10.1016/j.envpol.2013.09.005.

Hooper, M. J., G. T. Ankley, D. A. Cristol, L. A. Maryoung, P. D. Noyes, and K. E. Pinkerton. 2013. "Interactions Between Chemical and Climate Stressors: A Role for Mechanistic Toxicology in Assessing Climate Change Risks." *Environmental Toxicology and Chemistry* 32, no. 1: 32–48. https://doi.org/10.1002/etc.2043.

Hu, Q., and S. Feng. 2003. "A Daily Soil Temperature Dataset and Soil Temperature Climatology of the Contiguous United States." https://journals.ametsoc.org/view/journals/apme/42/8/1520-0450_2003_042_1139_adstda_2.0.co_2.xml.

Huang, A., A. Mangold-Döring, A. Focks, C. Zhang, and P. J. Van Den Brink. 2022. "Comparing the Acute and Chronic Toxicity of Flupyradifurone and Imidacloprid to Non-target Aquatic Arthropod Species." *Ecotoxicology and Environmental Safety* 243: 113977. https://doi.org/10.1016/j.ecoenv.2022.113977.

Huang, A., A. Mangold-Döring, H. Guan, et al. 2023. "The Effect of Temperature on Toxicokinetics and the Chronic Toxicity of Insecticides Towards *Gammarus pulex*." *Science of the Total Environment* 856: 158886. https://doi.org/10.1016/j.scitotenv.2022.158886.

IPBES. 2019. "Summary for Policymakers of the Global Assessment Report on Biodiversity and Ecosystem Services." Zenodo. https://doi.org/10.5281/zenodo.3553579.

IPCC, H.-O. Pörtner, D. C. Roberts, et al. 2022. "Climate Change 2022: Impacts, Adaptation and Vulnerability." In *Contribution of Working Group II to the Sixth Assessment Report of the Intergovernmental Panel on Climate Change*. Cambridge, UK and New York, NY, USA: Cambridge University Press.

Irlich, U. M., J. S. Terblanche, T. M. Blackburn, and S. L. Chown. 2009. "Insect Rate-Temperature Relationships: Environmental Variation and the Metabolic Theory of Ecology." *American Naturalist* 174, no. 6: 819–835. https://doi.org/10.1086/647904.

Jørgensen, L. B., H. Malte, M. Ørsted, N. A. Klahn, and J. Overgaard. 2021. "A Unifying Model to Estimate Thermal Tolerance Limits in Ectotherms Across Static, Dynamic and Fluctuating Exposures to Thermal Stress." *Scientific Reports* 11, no. 1: 12840. https://doi.org/10.1038/s41598-021-92004-6.

Knillmann, S., N. C. Stampfli, Y. A. Noskov, M. A. Beketov, and M. Liess. 2013. "Elevated Temperature Prolongs Long-Term Effects of a Pesticide on *Daphnia* Spp. Due to Altered Competition in Zooplankton Communities." *Global Change Biology* 19, no. 5: 1598–1609. https://doi.org/10.1111/gcb.12151.

Knouft, J. H., and D. L. Ficklin. 2017. "The Potential Impacts of Climate Change on Biodiversity in Flowing Freshwater Systems." *Annual Review of Ecology, Evolution, and Systematics* 48, no. 1: 111–133. https://doi.org/10.1146/annurev-ecolsys-110316-022803.

Kristiansen, S. M., K. Borgå, J. T. Rundberget, and H. P. Leinaas. 2021. "Effects on Life-History Traits of *Hypogastrura viatica* (Collembola) Exposed to Imidacloprid Through Soil or Diet." *Environmental Toxicology and Chemistry* 40, no. 11: 3111–3122. https://doi.org/10.1002/etc.5187.

Laskowski, R., A. J. Bednarska, P. E. Kramarz, et al. 2010. "Interactions Between Toxic Chemicals and Natural Environmental Factors—A Meta-Analysis and Case Studies." *Science of the Total Environment* 408, no. 18: 3763–3774. https://doi.org/10.1016/j.scitotenv.2010.01.043.

Lauper, B. B., E. Anthamatten, J. Raths, M. Arlos, and J. Hollender. 2022. "Systematic Underestimation of Pesticide Burden for Invertebrates Under Field Conditions: Comparing the Influence of Dietary Uptake and Aquatic Exposure Dynamics." *ACS Environmental Au* 2, no. 2: 166–175. https://doi.org/10.1021/acsenvironau.1c00023.

Li, N., Y. Xia, Y. Li, et al. 2023. "Untargeted Screening, Quantitative Analysis, and Toxicity Estimation of Degradation Products of Fluazinam in Vegetables." *Microchemical Journal* 190: 108584. https://doi.org/10.1016/j.microc.2023.108584.

Liess, M., K. Foit, S. Knillmann, R. B. Schäfer, and H.-D. Liess. 2016. "Predicting the Synergy of Multiple Stress Effects." *Scientific Reports* 6, no. 1: 1. https://doi.org/10.1038/srep32965.

Liess, M., S. Henz, and S. Knillmann. 2019. "Predicting Low-Concentration Effects of Pesticides." *Scientific Reports* 9, no. 1: 15248. https://doi.org/10.1038/s41598-019-51645-4.

Liess, M., L. Liebmann, P. Vormeier, et al. 2021. "Pesticides Are the Dominant Stressors for Vulnerable Insects in Lowland Streams." *Water Research* 201: 117262. https://doi.org/10.1016/j.watres.2021.117262.

Lux, J., Z. Xie, X. Sun, D. Wu, and S. Scheu. 2024. "Trophic Niches of Collembola Communities Change With Elevation, but Also With Body Size and Life Form." *Oecologia* 204, no. 3: 491–504. https://doi.org/10.1007/s00442-023-05506-7.

Ma, W., A. van Kleunen, J. Immerzeel, and P. G.-J. de Maagd. 1998. "Bioaccumulation of Polycyclic Aromatic Hydrocarbons by Earthworms: Assessment of Equilibrium Partitioning Theory in In Situ Studies and Water Experiments." *Environmental Toxicology and Chemistry* 17, no. 9: 1730–1737. https://doi.org/10.1002/etc.5620170913.

Mangold-Döring, A., A. Huang, E. H. Van Nes, A. Focks, and P. J. Van Den Brink. 2022. "Explicit Consideration of Temperature Improves Predictions of Toxicokinetic–Toxicodynamic Models for Flupyradifurone and Imidacloprid in *Gammarus pulex*." *Environmental Science & Technology* 56, no. 22: 15920–15929. https://doi.org/10.1021/acs.est.2c04085.

Marti-Roura, M., G. Dell'Ambrogio, S. Campiche, et al. 2023. "Part 1-Review and Comparison of International Methodologies." In *Methodology Proposal for the Derivation of Soil Guideline Values for Plant Protection Product Residues*. Dübendorf and Lausanne, Switzerland: Swiss Centre for Applied Ecotoxicology.

Mayer, F. L., and M. R. Ellersieck. 1988. "Experiences With Single-Species Tests for Acute Toxic Effects on Freshwater Animals." *Ambio* 17, no. 6: 367–375.

Munz, N. A., Q. Fu, C. Stamm, and J. Hollender. 2018. "Internal Concentrations in Gammarids Reveal Increased Risk of Organic Micropollutants in Wastewater-Impacted Streams." *Environmental Science & Technology* 52, no. 18: 10347–10358. https://doi.org/10.1021/acs.est.8b03632.

Nota, B., M. Bosse, B. Ylstra, N. M. Van Straalen, and D. Roelofs. 2009. "Transcriptomics Reveals Extensive Inducible Biotransformation in the Soil-Dwelling Invertebrate *Folsomia candida* Exposed to Phenanthrene." *BMC Genomics* 10, no. 1: 236. https://doi.org/10.1186/1471-2164-10-236.

Nota, B., M. J. T. N. Timmermans, O. Franken, et al. 2008. "Gene Expression Analysis of Collembola in Cadmium Containing Soil." *Environmental Science & Technology* 42, no. 21: 8152–8157. https://doi.org/10.1021/es801472r.

Noyes, P. D., M. K. McElwee, H. D. Miller, et al. 2009. "The Toxicology of Climate Change: Environmental Contaminants in a Warming World." *Environment International* 35, no. 6: 971–986. https://doi.org/10.1016/j.envint.2009.02.006.

OECD. 2016. "OECD/OCDE 232 OECD Guidelines for Testing Chemicals: Collembolan Reproduction Test in Soil." http://www.oecd.org/termsandconditions/.

Ørsted, M., L. B. Jørgensen, and J. Overgaard. 2022. "Finding the Right Thermal Limit: A Framework to Reconcile Ecological, Physiological

and Methodological Aspects of CTmax in Ectotherms." *Journal of Experimental Biology* 225, no. 19: jeb244514. https://doi.org/10.1242/jeb.244514.

Ørsted, M., Q. Willot, A. K. Olsen, V. Kongsgaard, and J. Overgaard. 2024. "Thermal Limits of Survival and Reproduction Depend on Stress Duration: A Case Study of *Drosophila suzukii*." *Ecology Letters* 27, no. 3: e14421. https://doi.org/10.1111/ele.14421.

Panico, S. C., C. A. M. van Gestel, R. A. Verweij, et al. 2022. "Field Mixtures of Currently Used Pesticides in Agricultural Soil Pose a Risk to Soil Invertebrates." *Environmental Pollution* 305: 119290. https://doi.org/10.1016/J.ENVPOL.2022.119290.

Parmesan, C., and G. Yohe. 2003. "A Globally Coherent Fingerprint of Climate Change Impacts Across Natural Systems." *Nature* 421, no. 6918: 37–42. https://doi.org/10.1038/nature01286.

Pelosi, C., C. Bertrand, G. Daniele, et al. 2021. "Residues of Currently Used Pesticides in Soils and Earthworms: A Silent Threat?" *Agriculture, Ecosystems & Environment* 305: 107167. https://doi.org/10.1016/J.AGEE.2020.107167.

Phillips, P. J., and R. W. Bode. 2004. "Pesticides in Surface Water Runoff in South-Eastern New York State, USA: Seasonal and Stormflow Effects on Concentrations." *Pest Management Science* 60, no. 6: 531–543. https://doi.org/10.1002/ps.879.

Polazzo, F., S. K. Roth, M. Hermann, et al. 2022. "Combined Effects of Heatwaves and Micropollutants on Freshwater Ecosystems: Towards an Integrated Assessment of Extreme Events in Multiple Stressors Research." *Global Change Biology* 28, no. 4: 1248–1267. https://doi.org/10.1111/gcb.15971.

Posit team. 2023. *RStudio: Integrated Development Environment for R*. PBC, Boston, MA: Posit Software. http://www.posit.co/.

Potapov, A. M., C. A. Guerra, J. van den Hoogen, et al. 2023. "Globally Invariant Metabolism but Density-Diversity Mismatch in Springtails." *Nature Communications* 14, no. 1: 674. https://doi.org/10.1038/s41467-023-36216-6.

PubChem. 2024. "3-((3-Chloro-5-(trifluoromethyl)pyridin-2-yl)amino)-2,4-dinitro-6-(trifluoromethyl)phenol." https://pubchem.ncbi.nlm.nih.gov/compound/13044390.

Qian, B., E. G. Gregorich, S. Gameda, D. W. Hopkins, and X. L. Wang. 2011. "Observed Soil Temperature Trends Associated With Climate Change in Canada." *Journal of Geophysical Research-Atmospheres* 116, no. 2: D02106. https://doi.org/10.1029/2010JD015012.

R Core Team. 2023. *R: A Language and Environment for Statistical Computing*. Vienna, Austria: R Foundation for Statistical Computing. https://www.R-project.org/.

Raths, J., V. Švara, B. Lauper, Q. Fu, and J. Hollender. 2023. "Speed It Up: How Temperature Drives Toxicokinetics of Organic Contaminants in Freshwater Amphipods." *Global Change Biology* 29, no. 5: 1390–1406. https://doi.org/10.1111/gcb.16542.

Reshotkin, O. V., and O. I. Khudyakov. 2019. "Soil Temperature Response to Modern Climate Change at Four Sites of Different Latitude in the European Part of Russia." *IOP Conference Series: Earth and Environmental Science* 368, no. 1: 12040. https://doi.org/10.1088/1755-1315/368/1/012040.

Richardson, K., W. Steffen, W. Lucht, et al. 2023. "Earth Beyond Six of Nine Planetary Boundaries." *Science Advances* 9, no. 37: eadh2458. https://doi.org/10.1126/sciadv.adh2458.

Ritz, C., F. Baty, J. C. Streibig, and D. Gerhard. 2015. "Dose-Response Analysis Using R." *PLoS One* 10, no. 12: e0146021. https://doi.org/10.1371/journal.pone.0146021.

Ritz, C., and J. Strebig. 2016. "Package "Drc" Title Analysis of Dose-Response Curves."

Roeben, V., L. Montoya-Tzschoppe, and M. Roß-Nickoll. 2023. "Long Life at Low Temperatures – The Life-History of *Folsomia candida* at Ecological Relevant Temperatures." *Pedobiologia* 96: 150847. https://doi.org/10.1016/j.pedobi.2022.150847.

Roth, S. K., F. Polazzo, A. García-Astillero, L. Cherta, A. Sobek, and A. Rico. 2022. "Multiple Stressor Effects of a Heatwave and a Herbicide on Zooplankton Communities: Implications of Global Climate Change." *Frontiers in Environmental Science* 10: 920010. https://doi.org/10.3389/fenvs.2022.920010.

Russo, R., J. M. Becker, and M. Liess. 2018. "Sequential Exposure to Low Levels of Pesticides and Temperature Stress Increase Toxicological Sensitivity of Crustaceans." *Science of the Total Environment* 610–611: 563–569. https://doi.org/10.1016/j.scitotenv.2017.08.073.

Schmidt, S. N., K. E. C. Smith, M. Holmstrup, and P. Mayer. 2013. "Uptake and Toxicity of Polycyclic Aromatic Hydrocarbons in Terrestrial Springtails—Studying Bioconcentration Kinetics and Linking Toxicity to Chemical Activity." *Environmental Toxicology and Chemistry* 32, no. 2: 361–369. https://doi.org/10.1002/etc.2051.

Schulz, H. 1877. "Zur Lehre von der Arzneiwirkung." *Virchows Archiv* 108: 427.

Seber, G. A. F., and C. J. Wild. 1989. *Nonlinear Regression*. 1st ed. Auckland, New Zealand: Wiley. https://doi.org/10.1002/0471725315.

Seibold, S., M. M. Gossner, N. K. Simons, et al. 2019. "Arthropod Decline in Grasslands and Forests Is Associated With Landscape-Level Drivers." *Nature* 574, no. 7780: 671–674. https://doi.org/10.1038/s41586-019-1684-3.

Shahid, N., M. Liess, and S. Knillmann. 2019. "Environmental Stress Increases Synergistic Effects of Pesticide Mixtures on *Daphnia magna*." *Environmental Science & Technology* 53, no. 21: 12586–12593. https://doi.org/10.1021/acs.est.9b04293.

Siddique, A., N. Shahid, and M. Liess. 2021. "Multiple Stress Reduces the Advantage of Pesticide Adaptation." *Environmental Science & Technology* 55, no. 22: 15100–15109. https://doi.org/10.1021/acs.est.1c02669.

Sigmund, G., M. Ågerstrand, A. Antonelli, et al. 2023. "Addressing Chemical Pollution in Biodiversity Research." *Global Change Biology* 29, no. 12: 3240–3255. https://doi.org/10.1111/gcb.16689.

Simonsen, V., and P. G. Christensen. 2001. "Clonal and Genetic Variation in Three Collembolan Species Revealed by Isozymes and Randomly Amplified Polymorphic DNA." *Pedobiologia* 45, no. 2: 161–173. https://doi.org/10.1078/0031-4056-00077.

Verheyen, J., and R. Stoks. 2019. "Shrinking Body Size and Physiology Contribute to Geographic Variation and the Higher Toxicity of Pesticides in a Warming World." *Environmental Science & Technology* 53, no. 19: 11515–11523. https://doi.org/10.1021/acs.est.9b03806.

Verheyen, J., and R. Stoks. 2023. "Thermal Performance Curves in a Polluted World: Too Cold and Too Hot Temperatures Synergistically Increase Pesticide Toxicity." *Environmental Science & Technology* 57, no. 8: 3270–3279. https://doi.org/10.1021/acs.est.2c07567.

Walther, G.-R., E. Post, P. Convey, et al. 2002. "Ecological Responses to Recent Climate Change." *Nature* 416, no. 6879: 389–395. https://doi.org/10.1038/416389a.

Wang, Y., S. Slotsbo, P. B. Sørensen, and M. Holmstrup. 2024. "Low Soil Moisture Slows Uptake and Elimination Rates of Phenanthrene in Springtails." *Bulletin of Environmental Contamination and Toxicology* 112, no. 2: 25. https://doi.org/10.1007/s00128-024-03854-5.

Wehrli, M. 2022. "Generating Ecotoxicity Data for Integration in Consoil – Soil Guidance Values." BSc thesis, University of Applied Sciences Northwestern Switzerland.

Wehrli, M., S. Slotsbo, J. Ge, and M. Holmstrup. 2024. "Acclimation Temperature Influences the Thermal Sensitivity of Injury Accumulation in *Folsomia candida* at Extreme Low and High Temperatures." *Current Research in Insect Science* 6: 100089. https://doi.org/10.1016/j.cris.2024.100089.

Wehrli, M. 2024. "Toxicokinetics and Toxicodynamics Data for Fluazinam in the Springtail *Folsomia candida* [Dataset]." *Dryad*. https://doi.org/10.5061/dryad.np5hqc03h.

Almond, R. E. A., T. Petersen, D. Juffe Bignoli, and M. Grooten, eds. 2022. "WWF." In *Living Planet Report 2022 – Building a Nature- Positive Society*. Gland, Switzerland: WWF.

Xie, L., S. Slotsbo, and M. Holmstrup. 2023. "Tolerance of High Temperature and Associated Effects on Reproduction in Euedaphic Collembola." *Journal of Thermal Biology* 113: 103439. https://doi.org/10.1016/j.jtherbio.2022.103439.

Supporting Information

Additional supporting information can be found online in the Supporting Information section.