

Grant Proposal

WildPosh: Pan-European assessment, monitoring, and mitigation of chemical stressors on the health of wild pollinators

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Reviewable

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Received: 17 Apr 2025 | Published: 28 Apr 2025

Citation: Michez D, Bocquet M, Bulet P, Chauzat M-P, De la Rúa P, Karise R, Kiljanek T, Klein A, Laurent M, Leadbeater E, Mänd M, Martel A-C, Metodiev T, Miličić M, Osterman J, Paxton RJ, Potts SG, Reverte S, Rivière M-P, Schweiger O, Senapathi D, Tcheremenskaia O, Tosi S, Vujic A, Wintermantel D, Brown MJF (2025) WildPosh: Pan-European assessment, monitoring, and mitigation of chemical stressors on the health of wild pollinators. Research Ideas and Outcomes 11: e156185. <https://doi.org/10.3897/rio.11.e156185>

Abstract

Wild fauna and flora are facing variable and challenging environmental disturbances. One of the animal groups that is most impacted by this, concerns pollinators. Pollinators face multiple threats, but the spread of anthropogenic chemicals (i.e. pesticides) form a major potential driver of these threats. WildPosh is a multi-actor, transdisciplinary project whose overarching mission and ambition are to significantly improve the evaluation of risk to pesticide exposure of wild pollinators, and enhance the sustainable health of pollinators and pollination services in Europe. As chemical exposure varies geographically, across cropping systems, inside the crop system and among pollinators, we will characterise exposure by doing fieldwork in 4 countries representing the four main climatic European regions, Mediterranean, Atlantic, Continental and Boreal climate in Germany, England, Estonia and Spain. We will also develop experiments in controlled conditions on different species of bees, syrphid flies, moths and butterflies, and collect in silico data on their traits and on toxicity of pesticides. With WildPosh, we aim to achieve the following objectives:

1. Determining the real-world agrochemical exposure profile of wild pollinators at landscape level, within and among sites;
2. Using integrated and controlled laboratory and semi-field experiments to characterise causal relationships between pesticides and pollinator health;
3. Building an open database on pollinator traits/distribution and chemicals to define exposure and toxicity scenarios by developing databases on ecological traits and the spatial distribution of pollinators in relation to their potential exposure to pesticide;
4. Proposing integrated systems-based risk assessment tools for risk assessment for wild pollinators; and
5. Driving policy and practice through interactive innovation, meeting the need for monitoring tools, novel and innovative screening protocols for practice and policymaker use.

Keywords

Environmental impact assessment, Environmental stressors, Environmental toxicology at the population and ecosystems level, Fight against pollution, Fight against threats to the Environment, Protection of environment (before, during and after), Toxicology, pollution and climate, bees, syrphid flies, moths and butterflies

1. Excellence

1.1 Objectives and ambition

1.1.1 Background

Wild fauna and flora are facing dynamic challenging environmental perturbations induced by human action to such a degree that there is widespread scientific support to term the present time the Anthropocene (Wagner et al. 2021). This new human-dominated geological epoch is characterised by global environmental changes mainly driven by

1. atmospheric pollution and associated climate change,
2. soil pollution,
3. loss and fragmentation of natural habitats,
4. misuse/overuse of pesticides,
5. worldwide trade of managed species and their associated spread of pathogens, and
6. invasive exotic species (Butchart et al. 2010; Potts et al. 2010; Sala et al. 2000; Tylianakis et al. 2008).

These environmental stressors impact the health of living organisms, resulting in reduced fitness and even mortality, to such an extent that global biodiversity currently faces a major crisis, defined as the sixth mass extinction (Ceballos et al. 2015; IPBES 2019). Like the rest of biodiversity, pollinators are facing multiple threats (Goulson et al. 2015) of which anthropogenic chemicals (i.e. pesticides; henceforth, we treat them as synonyms) are a major potential driver (Woodcock et al. 2016). The effects, especially sublethal effects, of pesticides are not typically detected by current pesticide regulatory testing standards (Desneux et al. 2007). Moreover, the regulations have been based mainly on honey bees while protocols for wild pollinator fauna are essentially absent. Their relevance for wild pollinator health (i.e. ability of individuals/populations to self-perpetuate) is still vigorously contested and partly unresolved. We urgently need to understand the risks posed to wild pollinators by chemical exposure as they are a key element in the functioning of terrestrial ecosystems by pollinating flowering plants (Ollerton et al. 2011).

We are aware that chemicals are widespread across entire agro-ecosystems (Bonmatin et al. 2015) and accumulate through agricultural food chains (Douglas et al. 2014), but we still lack understanding of the routes of chemical exposure of wild pollinators in field realistic conditions through different matrices (i.e. soil, water and different parts of plants). New protocols need to be developed to tackle the sampling and analytical challenges, especially for pollen, the main source of nutrition for many pollinators. We also need to describe mechanistic links between these exposure routes and the health of wild pollinators. As wild insect pollinators are very diverse (> 12,000 species recorded in Europe), there is an important challenge in selecting and testing a representative set of

species that capture their diversity of ecological traits and concomitant routes of exposure. Which species have a higher risk of exposure is an important technical challenge when considering the well-being of wild animals in general and wild pollinators in particular. We need new protocols and new risk assessment tools to develop appropriate prevention and mitigation measures.

These challenges call for a highly ambitious, experienced and transdisciplinary scientific team, with a strong drive and shared vision towards wild pollinator conservation and sustainable pollination in Europe. Based on long-standing experience and level of excellence, the WildPosh consortium fully recognizes and embraces the magnitude of these challenges. It has been established and assembled specifically to achieve the objectives that are listed below. Overall, WildPosh aims to accelerate the rate of scientific and societal progress made so far to finally halt and reverse pollinator declines and maintain the EU's Natural Capital.

1.1.2 Objectives

WildPosh is a multi-actor, transdisciplinary project whose overarching mission and ambition are to significantly improve the evaluation of the risk to wild pollinators of pesticide exposure, and enhance the sustainable health of pollinators and pollination services in Europe. To fulfil our ambition, we aim to achieve the following objectives:

1. **Determine the real-world agrochemical exposure profile at landscape level, among and within at least 20 sites in 4 countries:** we will provide the first pan-European quantification of the exposure hazard of pesticides to representative wild pollinators while characterising their populations. We will answer one of the call's outcomes by defining routes of exposure, linked to ecosystem and biodiversity dynamics to pesticides (WP1 till WP6);
2. **Using integrated and controlled laboratory (in 15 species) and semi-field (in 4 species) experiments to characterise causal relationships between 2 pesticides and at least 4 pollinator health measures:** we will determine how major categories of pesticide alone and in mixtures affect pollinator health for 15 model species. We will answer to the call outcome on contamination of biodiversity in the natural environment by pesticides, including risks linked to existing contaminations (WP2-3);
3. **Building open database on pollinator traits/distribution (distribution for 2,138 bee species, >900 hoverfly species, >450 butterfly species, >2000 moths; traits for >25% of those species) and chemicals to define exposure and toxicity scenario in 4 countries:** we will develop databases on ecological traits and the spatial distribution of pollinators in relation to their potential exposure to pesticide. The database will also collect data on the pesticide toxicity and their use in 4 countries. These data will contribute to the outcome on predicting toxicological and ecological impacts of contaminants for relevant highly exposed species (WP4);

4. **Proposing one new toolbox for risk assessment on wild pollinators:** we will propose integrated systems-based risk assessment tools. This objective will contribute to the outcome on the development of prevention and mitigation measures. This will support farmers, land managers and other stakeholders to improve wild pollinator health, and fill a major knowledge gap on how hazards interact to threaten pollinators (WP4 till WP6);
5. **Drive policy and practice through developing and disseminating 3 policy briefings and 5 good practice guides:** through interactive innovation we will meet the need for monitoring tools, novel and innovative screening protocols, and practice- and policy-relevant research outputs to local, national, European, and global stakeholders (WP2 and WP6). Like the previous objective, this will contribute to the outcome on developing prevention and mitigation measures. Together with other funded European consortia (SPRING, SAFEGUARD), key stakeholders in farming, pollination service, research, EU policy and regulatory, and pollinator conservation sectors, WildPosh will contribute to a European pollinator health knowledge exchange hub. Together, we will synthesise and disseminate our research findings to improve knowledge exchange, and develop best practice protocols, tools, training resources, and policy support for stakeholders across Europe, thereby promoting conservation of pollinators and pollination services.

Our ambition is to support healthy pollinator populations and sustainable pollination across Europe. WildPosh will fully address, and go beyond, the specific challenges specified in the call HORIZON-CL6-2023-BIODIV-01. Based on previous experience from the H2020 PoshBee project, WildPosh will establish a pan-European site network across major cropping systems for the assessment of chemical exposure in pollinators (WP1) to contribute to characterising sources and routes of pesticide exposure in the key pollinator groups. We will determine the ecotoxicology and toxicokinetic of major pesticides and their mixtures across 15 pollinator species representing different taxonomical, ecological and physiological traits and across individuals of different life-stages (WP2-3). In this way, WildPosh will be able to define traits associated with sensitivity to pesticides, thereby identifying sensitive ‘umbrella’ species whose protection will benefit the broader community of pollinators. We will critically review all existing prediction methodologies (QSAR models, category approach, read-across), identify the most important gaps and sources of uncertainty (WP5) and propose improved strategies for increasing their ability to predict risk, facilitating the regulatory acceptance of *in silico* methods (WP4) and their integration the ERA process (WP5). For each of three agrochemical classes (insecticide, fungicide, herbicide), a dose-response relationship will be generated for model pollinator species, for both larvae/pupae and adults, in laboratory and semi-field condition (WP2), including toxicokinetic and toxicodynamic data and models for single and multiple chemicals.

During experiments on the impact of exposure on the 15 wild pollinator species, we will evaluate mortality and sublethal effects from single and multiple exposure (WP2-3) so that we may generate data on the combined toxicity of multiple chemicals, improving the

availability of baseline data. Based on residue data (from WP1 and PoshBee project) defining field realistic concentration and combination of pesticides, we will test the synergetic effect of pesticides on 15 representative wild pollinator species, investigating synergistic effects of typical combinations of pesticides. We will devise and test monitoring schemes for establishing the level of contamination of pollen/nectar/water/plant matrices/soil in order to develop new protocols to quantify contamination in pollen and nectar (WP1). These new protocols will take into account the challenge of the collection and analysis of pollen and nectar. We will compile a comprehensive trait database which will include morphological/ecological traits reflecting the sensitivity of European pollinators to pesticides and other stressors (e.g. nutrition, climate, parasite) combined with distributional data informing about the risk to pesticide exposure (WP4). We will additionally build an open-source database to include information on pesticide use, as well as on other stressors able to amplify the adverse effects (WP4). In this way, we develop an open-source curated database on pollinators and the use of pesticides.

Moreover, we will develop methodologies for risk assessment in open-source tools. WildPosh will integrate existing and WP1-5 data and models into an open-source user-friendly web-platform interface to produce a refined systems-based risk assessment output for stakeholders. It will include exposure, toxicity (sublethal, chronic), and risk of single and multiple pesticides at individual, population, and community level across landscapes and land-use scenarios. We will develop population models and landscape modelling for risk assessment of multiple chemicals in pollinators. To do this, pesticide risk maps will be developed for the WildPosh field sites based on information provided by WP1-5, the Pesticides Properties data base, documents provided by EFSA, and the Forum for Co-ordination of pesticide fate models and their Use (FOCUS) of DG SANTE. WildPosh will co-develop environmental scenarios for the risk assessments of pollinators together with the members of the Advisory Board and selected stakeholders from diverse sectors, covering agriculture and environment) so as to co-design scenarios of pesticide application.

WildPosh has an effective contribution from the social sciences and humanities (SSH) disciplines. Based on the results from WP1-5 that we will synthesise, we will identify appropriate response options to reduce pesticide risks to wild pollinators (e.g. adapt pesticide use to exposure risk of sensitive species). We will also develop good practice guides for practitioners to mitigate the impacts of pesticides on wild pollinators. Moreover, we will engage in science-policy dialogues to inform national and international policy on the development of mitigation measures. Finally, we will generate synergies and cooperation with other European projects. Members of WildPosh are leaders or contributors to the majority of past and ongoing projects and initiatives on wild pollinators in member states (MSs), Europe and globally (see section 1.2.2).

1.1.3 Beyond the state of the art

Pollinators supply the essential service of pollination ensuring the sexual reproduction of crops and wildflowers. They maintain pollination and reproduction of ca. 78% of

temperate wild plant species (Ollerton et al. 2011) and yield quantity and quality of 84% of major crops in Europe with an estimated value of between €3.1 and €17.7 billion Euros per year (Bauer and Sue Wing 2016; Gallai et al. 2009; Ollerton et al. 2011). In Europe, the four main groups of pollinators are insects: bees (2,138 species), syrphid flies (913 species), moths (>9,000 species) and butterflies (496 species) (Ascher and Pickering 2020; Breeze et al. 2016; Ghisbain et al. 2023; Middleton-Welling et al. 2020; Reverté et al. 2023; Vujić et al. 2022; Wiemers et al. 2018; Willmer 2011). Species and functional diversity of the pollinator community is an important factor in the efficiency of pollination (Garibaldi et al. 2013). Conservation of the diversity pollinators is therefore of major societal importance. WildPosh will go beyond the state-of-the-art regarding tools, knowledge and practices related to the evaluation of the chemical exposure and risks posed by pesticides for wild pollinators.

Chemical monitoring. The general lack of cheap and effective tools for quantifying pollinator exposure to chemical stressors, or for measuring pollinator health in general, holds back the development of large-scale multi-year pollinator health monitoring schemes and risk assessment. Standard methods applied in all previous studies of chemical exposure include the collection of physical materials (e.g., honey, pollen, wax) for laboratory screening using LC-MS/MS and GC-MS/MS technologies (Benuszek et al. 2017). In relation to first specific objective of WildPosh (i.e. determine the real-world agrochemical exposure profile of wild pollinators at landscape level, among and within sites), we will take two approaches to develop new scientific protocols for chemical monitoring of exposure (WP1) and impact (WP2-3). Direct sampling of key exposure matrices, like pollen and nectar, are highly challenging, and we will develop new methodologies for collecting these samples and proxies where such collection is too expensive on a time/money axis for viable monitoring protocols. We will extend to a higher diversity of wild pollinators the novel methods for lab-based testing of pesticide on wild bees developed in PoshBee (Dewaele et al. 2024). Moreover, to develop novel, scientific approaches to monitor chemical exposure and bee health, WildPosh will break new ground in the use of 'omics technologies e.g. combining MALDI and other proteomic approaches with traditional transcriptomics (WP3). Combining hyphenated mass spectrometry approaches (MALDI, LC-ESI-MS/MS) and MALDI imaging will reveal the specific mass fingerprints of the effects of stressors (chemical, nutrition, pathogen) on pollinators, and enable the selection of molecular markers for future monitoring of pollinator health. Inspired by MALDI BioTyping®, which is already approved by the FDA for clinical microbiology, a striking novelty of WildPosh will be to develop MALDI PolTyping® as a laboratory tool to monitor at the individual scale pollinator health through simple, non-lethal, field-collection of a pollinator's 'blood' (or hemolymph), an approach inspired from the MALDI BeeTyping® proposed in H2020 PoshBee (Cini et al. 2025). This is the first time such an approach will be used to explore wild pollinator responses to pesticides. We expect that this approach will become a fundamental component of future global solutions for health management plans at the national or EU level for pollinators, and become a referenced, fast, cost-effective and automatable analytical procedure to demonstrate the presence of stressors with the appropriate reliability and robustness required for official certification. Regulatory authorities will be

able to use our novel scientific methodologies as a standard means by which they can screen insect pollinators for exposure to pesticides, both those currently in widespread agricultural use and potentially for those yet to come on the market.

Effects of pesticides on a wide diversity of wild pollinators. Pesticides have not only lethal but also substantial sublethal effects on pollinators (Barascou et al. 2022; Williams et al. 2015). Lethal effects can be devastating but have become comparatively rare due to improved products and stakeholder training (Dainat et al. 2012). In stark contrast, sublethal effects are more cryptic, frequent, widespread, and have been experimentally shown to affect behaviour, immunity, physiology and reproduction of non-target insects like bees (Arena and Sgolastra 2014; Rundlöf et al. 2015; Tsvetkov et al. 2017; Woodcock et al. 2016). Experimental methods currently used to assess the toxicity of chemicals for pollinators mainly rely, at least for initial tests, on determining the acute toxicity through dose-response experiments (i.e. median lethal dose, LD50), which for a long time has only been performed on the western honey bee, *Apis mellifera* (OECD 1998; OECD 2017). Yet, considering their ecological, physiological and morphological variability, using *A. mellifera* to predict hazards of pesticides for wild pollinators could lead to a considerable underestimation of the adverse effects of pollinators (Uhl et al. 2016; Wood et al. 2020). Species-specific sensitivities of wild bees species have already been highlighted through meta-analysis, in which data related to LC50s (concentration at which 50% of individuals die) of multiple species were compared (Arena and Sgolastra 2014). Ninety five percent of wild bee species were less than a factor of 10 different from honey bees in their sensitivity, but there were outliers in both directions that could be attributed to species-specific life cycles, nesting activity and foraging behaviours. Moreover, the wide variation in size and body weight among wild bee species could be related to their variation in sensitivity to pesticides, which has been shown to increase with the body surface-to-volume ratio (Johansen 1977). So far, less than 20 wild bee species have been successfully kept under laboratory conditions for testing their sensitivity (Arena and Sgolastra 2014), mainly due to the difficulty in obtaining individuals for experimentation and in avoiding excess control group mortality. Therefore there are still major gaps in our knowledge on the lethal and sublethal effects of many chemicals across a range of pollinator groups. Furthermore, with local, national, and regional moratoriums and current bans on the use of neonicotinoids, our understanding of the effects of chemicals that may replace them is extremely limited. Finally, conflicting data that suggest no effects, and field studies that suggest results from laboratory or small-scale results may not necessarily scale up to real-world effects, also argue that our understanding is limited (e.g. Linguadoca et al. 2023). Thus, we still have poor knowledge of the real-world effects of pesticides on wild insect pollinator health.

In relation to our second and third specific objectives, WildPosh will consider an ambitious range of pollinator species (section 1.2), characterised by various genetic, physiological, morphological and ecological traits, to provide a step-change in our understanding of the effects of chemicals on pollinator health. WildPosh is the first project to take a fully integrated experimental approach, in laboratory conditions, supported by semi-field experiments. By developing an innovative model of pollinator exposure and

health, we will provide a framework to enable understanding of future threats from novel chemicals. These studies will explicitly consider the modifying roles of host genetics (Sandrock et al. 2014). As effects are known to vary with the resilience of host species (Rundlöf et al. 2015; Woodcock et al. 2017), WildPosh will break new ground by using multiple model systems across a range of high to low resilience: namely:

1. big and small species;
2. eusocial and solitary species,
3. pollen specialist and generalist,
4. saprophagous, zoophagous, saproxylic, phytophagous and pollinivore larvae;
5. no nest, ground nesting and stem nesting species;
6. spring and summer flying species.

In addition, WildPosh proposes innovative scientific approaches to understanding effects, including the first proof of concept for using histo-proteomics studies by MALDI molecular imaging to

1. decipher the molecular cross talk/interplay during host and pesticide (and other stressor) interactions and
2. for toxicodynamic investigations.

In conclusion, by investigating the sublethal effects of chemicals and their interactions with traits and host genetics, WildPosh has clear and truly ground-breaking scientific objectives. Our results, which will push understanding significantly beyond the current state-of-the-art, will provide a future framework to mitigate the undesired effects of chemicals and pesticides on wild pollinators and crop yields. This will be especially relevant for EU policies for pesticide use and nature conservation, and will contribute significantly to a more sustainable agriculture sector.

Modelling environmental risk assessment at species and fauna level. Predicting the response of pollinators to agricultural pesticide use under field conditions requires a multi-factorial approach (Tosi et al. 2022). This needs to consider multiple routes of pesticide exposure (Kopit and Pitts-Singer 2018) within the entire foraging range of a pollinator at the landscape level (Knapp et al. 2023), the environmental fate of the chemicals (Bonmatin et al. 2015), and the relative importance of uptake from different environmental compartments (Ward et al. 2022). To ultimately assess the resulting impacts on population and community performance, species-specific toxicity of the active ingredients accounting for lethal and sublethal, acute and chronic effects (Tosi et al. 2022), species traits related to pesticide risk, and potentially confounding effects within the given environmental and biogeographical context (Knapp et al. 2023) need to be included within a systems approach. There is a range of theoretical, mechanistic, and statistical models available, but they mostly address single components, such as the fate and behaviour of active substances in the environment (e.g. FORum for Co-ordination of pesticide fate models and their Use [FOCUS] of DG SANTE), pesticide application rates at large to regional scales (Maggi et al. 2019), honey bee colony performance (Becher et al. 2014; More et al. 2021), pollinator foraging patterns at the landscape scale (Häussler

et al. 2017), or combined impacts of multiple factors (Schweiger et al. 2005). However, an overarching framework combining those different aspects is currently missing.

To meet our fourth specific objective of providing innovative and reliable risk assessment for wild pollinators and our fifth objective of driving policy and practices, WildPosh will develop a user-friendly Toolbox integrating sound models for the evaluation of pollinator's exposure, toxicity, and risk of single and multiple pesticides at individual, population, and community level. WildPosh will overcome the limits of the current models and risk assessment methods by refining the assessment of

1. the exposure of pollinators across a gradient of environmental and agricultural intensity scenarios,
2. the lethal and sublethal, single and combined, short- and long-term toxicity and risk of pesticides, and
3. the uncertainties in published results.

The ToolBox will build upon existing models and can fruitfully extend them as a supportive instrument for risk assessment and management of pesticide impact on wild pollinators, finally benefitting wider society.

1.1.4 Research & Innovation Maturity

WildPosh encompasses the development of tools and protocols from basic principles to advance, high-resolution technologies. Key examples include

1. a proteomics based tool, or 'health card', that monitors exposure to and effects of chemicals, pathogens, and nutritional stress in wild pollinators (predicted progression: TRL3-TRL6);
2. 15 new model pollinator species for ecotoxicology (predicted progression: TRL2-TRL5);
3. new methods and tested protocols (including an OECD protocol) for ecotoxicology of wild pollinators (predicted progression: TRL2-TRL5);
4. open-source user-friendly web-platform interface to produce a refined systems-based risk assessment (predicted progression: TRL2-TRL5).

The Research and Innovation of this project will contribute to the UN's Sustainable Development Goals and accelerate the ecological transition required by the European Green Deal. Of particular relevance is the SDG 2 (zero hunger) as sustainable pollination is related to crop production (Klein et al. 2007) and SDG 15 (life on land) as wild pollinators are an important part of biodiversity and ecosystem functioning (Michez et al. 2019).

1.2 Methodology

1.2.1 Overall methodology

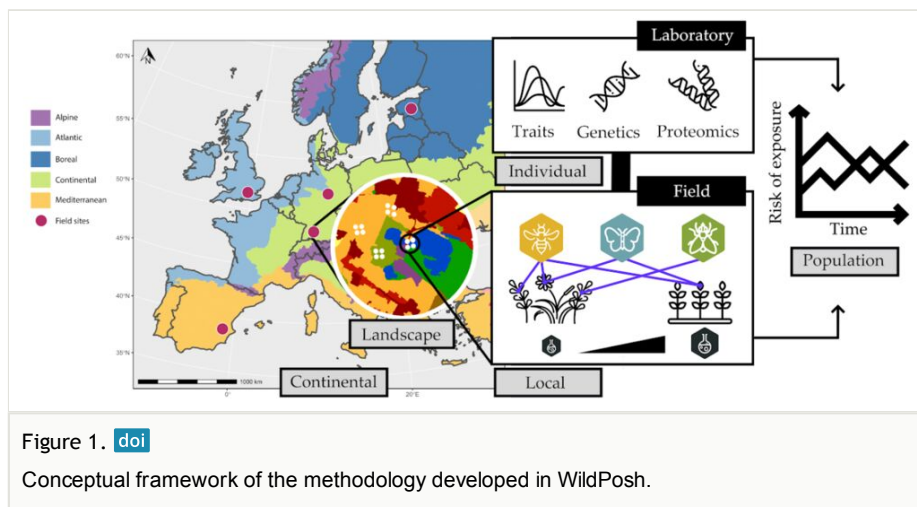
Pollinator research is at the forefront in terms of understanding the complex interplay between biodiversity, ecosystem functioning, socioeconomic and political drivers, environmental pressures, and impacts on human societies (Isbell et al. 2017; Potts et al. 2016). This research has tremendous importance per se, to safeguard both pollinators and the values they generate, but can also serve as a blueprint that guides public and private initiatives to halt and reverse biodiversity decline in general and thus restore and enhance ecosystem services and their resilience. Pesticides are now an integral feature of modern agriculture, controlling pests and disease in crops and domesticated animals. However, when pesticides are overused, or have adverse effects on yields by depressing the abundance of beneficial organisms (e.g. pollinators, pest predators), their use can reduce farm profitability (Sargent et al. 2023). The potential effects of pesticides on wild insect pollinators is a high profile, yet poorly resolved, case (Godfray et al. 2015), as insects (including bees) provide the essential ecosystem service of pollination, but are at risk around the globe (Tsvetkov et al. 2017; Vanbergen and the Insect Pollinators Initiative 2013). Here, we broadly define pollinator health as the ability of individuals/populations to self-perpetuate, whilst providing sustainable pollination services. Previous research has shown that pesticides affect the behaviour, immunity, lifespan, physiology, and reproduction of bees (Godfray et al. 2015; Tsvetkov et al. 2017; Woodcock et al. 2017), and deleterious effects of exposure to pesticide was described in other wild pollinators, syrphid flies (Jansen et al. 2011), butterflies (Olaya-Arenas and Kaplan 2019) and moths (Pisa et al. 2015) all may reduce pollination efficiency (Stanley et al. 2015). Most of the studies on the wild pollinator species consider correlational data (Van Dyck et al. 2009; Woodcock et al. 2016) [e.g. for butterflies].

For example, at a population scale, correlational data associate neonicotinoid pesticides with declines in abundance and range of wild bees (Woodcock et al. 2016). Furthermore, the effects of pesticides can be modified by interactions with other pesticides (Traynor et al. 2016). However, there is a severe lack of both real-world exposure risk data for pesticides and wild pollinators, and insights into how such exposure translates into effects in the field (Rundlöf et al. 2015; Tsvetkov et al. 2017). In addition, current knowledge is largely limited to the honey bees, a bumble bee and a mason bee, with little understanding the degree to which solitary bee pollinators (~95% of all bee species), syrphid flies, butterflies and moths are affected by these hazards, and how this impact is related to pollinator traits (i.e. morphology, ecology, physiology, genetics), spatial and temporal distribution, chemicals property or landscape configuration. To address these issues, WildPosh has developed an expert-driven project co-created via a multi-actor approach. Members of the PoshBee consortium have developed, in the last 5 years, a strong expertise on ecotoxicological testing of established model bee species, *Apis mellifera*, *Bombus terrestris* and *Osmia bicornis* and initiated studies of some wild bee species (e.g. *Anthophora plumipes*, *Bombus hypnorum*, *Colletes hederæ*, *Osmia brevicornis* and *Osmia cornuta*). Based on our expertise developed in monitoring tools,

analytical tools, eco-toxicokinetics, transcriptomics and proteomics, wild pollinator breeding, modelling and risk assessment, and with additional expertise from new partners (e.g. toxicologists, specialists on syrphid flies and Lepidoptera), we propose here a project focussing on wild pollinators. WildPosh, a research concept, rests on five integrated objectives to mitigate threats to pollinator health and pollination across Europe:

1. determining the real-world agrochemical exposure profile of wild pollinators at landscape level, within and among sites;
2. using integrated and controlled laboratory and semi-field experiments to characterise causal relationships between pesticides and pollinator health;
3. building an open database on pollinator traits/distribution and chemicals to define exposure and toxicity scenarios;
4. proposing new tools for risk assessment for wild pollinators;
5. driving policy and practice.

Pesticide exposure varies geographically, as well as, at finer scales, within and between cropping systems. Bees and syrphids can be exposed while they are foraging on crops while they, along with butterflies or moths, inhabiting structures adjacent to pesticide-impacted areas, can be affected by drift of spraying during and after application (Sinha et al. 2008). We know that systemic pesticides also appear in plants growing alongside crops, via leaching through soil water, and larvae of bees, syrphids, butterflies, and moths could be exposed in this way. To embrace this range of exposure routes, we use a pan-European site network (4 countries representing the four main climatic European regions, Mediterranean, Atlantic, Continental and Boreal; Fig. 1), derived from the experience in PoshBee project (Hodge et al. 2022), which focuses on national key crops for pollinators, and one anemophilous crop (wheat). These are chosen on the basis of their importance for the EU agro-economy, their dependency on animal pollination, and value as forage to pollinators (high/zero). We will collect samples across and within sites at different times of the year to capture temporal variation of exposure at the landscape level. The monitoring protocol across the site network will provide a large number of variables measured at the same sites. These variables are organised in blocks related to contamination by pesticides in different matrices. More precisely, the datasets related to pesticide contamination - in soil/mud, nectar, pollen from flowers, plant tissues, aggregated pollen on bumble bees and water - will be considered as blocks of response variables. They will be analysed with explanatory variables, namely the pollinator communities, pesticide usage (farmer practices), agricultural management (crops present close to the sites), habitats (quantity and spatial configuration), floral resources (plant communities) and landscape management (presence of managed bees). A multiblock analysis will be applied to all these data sets to quantify the weight of each explanatory block on the presence of pesticides in the different matrices. This will enable us to elucidate the drivers of pesticide contamination levels in different environmental compartments.



To produce an in-depth mechanistic understanding of effects, we will use an integrated set of laboratory and semi-field experiments. We face a double challenge in this goal:

1. Chemicals are hugely diverse and ever-expanding,
2. European wild pollinators comprise many thousands of species from major insect taxa.

They represent diverse life history traits as well as habitat and floral resource requirements. The scientific challenge of understanding this complex “exposure-toxicity-sensitivity” path can be only reached by a European-wide, transdisciplinary collaboration of scientists from across environmental, molecular, chemical, social and economic disciplines, working together with a stakeholder group to develop new knowledge and evidence to underpin and drive concerted policy and practitioner actions. WildPosh will strategically focus on

1. exemplars (chosen on the basis of widespread use and economic value) from 3 major groupings of pesticides (insecticide, fungicide and herbicide), and
2. 15 key species from the three main groups of pollinators (i.e. bees, butterflies/moths and syrphid flies).

Field studies will exploit the pesticide exposure site network, enabling us to assess the impact of pesticide at the population level and thus scale from laboratory experiments to field-realistic effects. We will also build a comprehensive database on pesticide use, pollinator trait, pollinator distribution and toxicity of pesticides to cover, *in silico*, the potential variation in sensitivity and exposure of wild pollinators to the diversity of pesticides. Although active ingredients are authorised at the EU level by EFSA panels, the commercial preparations authorisation of use falls under national laws. This is why commercial products including insecticides, herbicides and fungicides are different from country to country in terms of names, usages, dosages and formulations. To date, no database gathers all information on commercial preparations in a single dataset

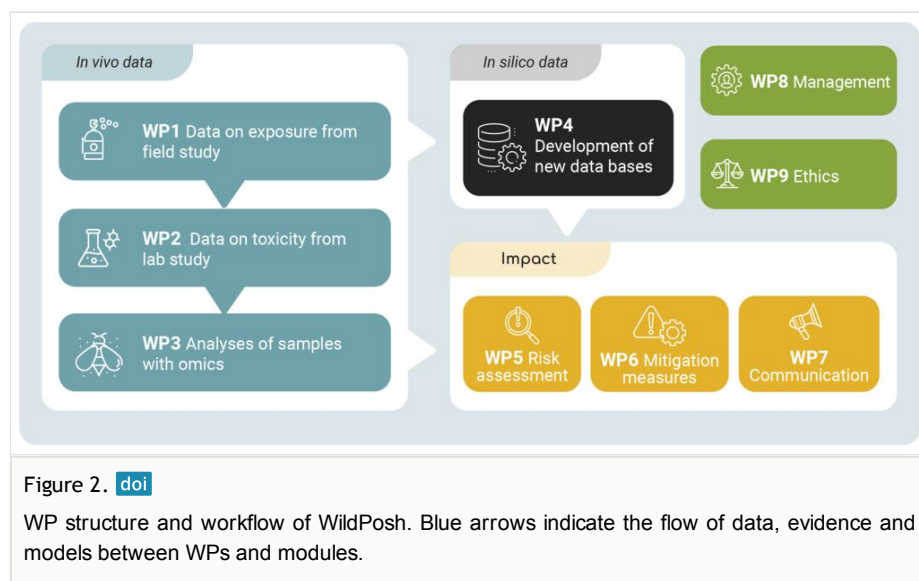
(Mesnage et al. 2021). This lack of centralisation of the large diversity in pesticides makes it difficult for the statistical analyses to explain the routes of contamination of various matrices by active ingredients. To fill this practical gap, WildPosh will develop a centralised pesticide database restricted to the four countries of WildPosh site network (WP1), to the honey bee and bumble bee model species and to the active ingredients quantified in residue analysis (WP1). Data on commercial products, together with exposure data (from the PoshBee, SAFEGUARD and other projects), ecotoxicological data collected from literature and other sources (repositories, other databases). Other stressors that change the effect of pesticides on model species (*A. mellifera*, *B. terrestris*) will also be recorded. Knowing the scarcity of ecotoxicological data for pollinators other than honey bees and bumble bees, the dataset on the two model species will support the extrapolation of sensitivity to other species. Pollinators respond in various ways to global threats (including pesticide exposure), which can be partly explained by their individual and specific ecological and morphological traits (e.g. Scheper et al. 2014), in a simple causal relationship trait / function. We aim to capture these trait data, which is much easier to collect than those on physiological response. From these global datasets, we will be able to detect the species which are the most at risk. Thereafter, we will integrate our results into a holistic model of 'pollinator health' to enable extrapolation of our results to all European pollinators. By combining our causal effects data with the exposure profiles generated in the site network, we will be able to characterise the risk that chemicals pose to pollinator health at the field level.

Practice and policy on pesticide use are currently held back by important evidence gaps and a lack of novel protocols and tools. We will provide new model test species and protocols for chemical exposure testing, to build on current OECD/EFSA approved testing practices in honey bees, which are currently extrapolated to bumble bees and solitary bees (EFSA 2013). To enable long-term monitoring, we will provide a novel proteomics-based tool that will use molecular markers to assess bee health and exposure to chemicals in the field and the wild. Together with key European stakeholders with diverse expertise (e.g. nature conservation, agro-company, academic, farming), we will broker knowledge exchanges to enhance and support future policy and practice to foster pollinator health and sustainable pollination. WildPosh takes a fundamentally transdisciplinary approach, incorporating the academic disciplines of entomology, chemistry, modelling, nutritional ecology, proteomics, and social science, with industrial partners and stakeholders. We have combined an array of world-class partners - academic, government, industry, NGO - who provide expertise across these disciplines. The structure of our consortium deliberately bridges disciplinary boundaries to maximise outputs and impacts of this work. For example, the chemical exposure site network approach integrates environmental chemistry, nutritional biology, and proteomics, an approach that underlies our work programme.

Structure of the project

WildPosh is organised in nine work packages (see Fig. 2) to determine the exposure of wild pollinators to pesticides across Europe, across biogeographical zones- and across crops (WP1). Based on previous data from PoshBee and the broader literature, and

where possible WP1 data on exposure, we will organise in WP2 laboratory and semi-field experiments on the ecotoxicology and causal effects of chemicals, both single and mixed, on pollinator health for 15 model species. We will develop new protocols for ecotoxicological studies of pollinators, and new tools and models to monitor exposure and predict the effects of chemicals in the wild at different spatial scales. We will also collect samples of a different nature (i.e. hemolymph, fat body, DNA, peptides and proteins) in WP2 to feed multiple Omics analyses in WP3. At the same time, WP4 will develop full databases *in silico* on traits and the distribution of pollinators. WP4 will also develop open-source databases on toxicity of chemicals and their distribution. By combining *in vivo* and *in silico* data from the four first work packages, we will propose an integrated systems-based risk assessment for all wild pollinators recorded in Europe and develop ambitious proposals for mitigation actions (WP5). These outputs will be shared with the broad stakeholder community (scientific community, policy makers, NGO, practitioners related to pesticide production and use), to enable changes in policy and action on the ground (WP6-7).



Below, we detail the methodologies that will put the concepts of WildPosh into action.

WP1: A monitoring scheme to determine sources and routes of pesticide exposure in environmental matrices (EMU lead). WP1 aims to develop the first specific objective of the project, determining the real-world agrochemical sources and exposure profile of wild pollinators. First, we aim to optimise sampling protocols to assess agrochemical contamination in environmental compartments/matrices. The minimum mass of a sample (~0.3g) for residue analysis is a limiting factor to screen matrices like pollen and nectar (Kiljanek et al. 2021) as flowers of plant families like Fabaceae produce a limited amount of pollen (Müller et al. 2006). We will develop new protocols to collect pollen, and new proxies for this environmental compartment when direct collection is not viable. New

methods for the determination of pesticide residues by LC-MS/MS and/or GC-MS/MS techniques will be developed that will allow assessment of the actual exposure of pollinators to pesticides. The targeted analyses will be based on pesticides that have been found as part of the PoshBee project, while expanding the analyses to include pesticides currently used for the protection of plant species selected within the site network. Moreover, we will develop a new site network in four countries representing the 4 main biogeographic regions of Europe (i.e. Atlantic, Continental, Boreal and Mediterranean). WildPosh will evaluate direct and indirect routes of exposure among sites and within sites, by intensive landscape analysis. Crops and wild plants, both attractive and non-attractive to pollinators, will be included in this survey. This analysis will incorporate a temporal component to take into account previous crops and pesticide treatments used in the site area, which is important given their high persistence (particularly neonicotinoids and some fungicides) in the environment (Van Lexmond et al. 2015). Direct chemical residue screening will be conducted on a range of exposure sources, specifically vegetative parts of the plants, nectar, pollen, standing water and mud. Combining data on pesticides coming derived two different sources (landscape and agricultural practices; quantification of agrochemical residues in various matrices) is an innovative approach that, combined with our pan-European site network, will for the first time provide an EU-level understanding of the driving factors and processes leading to bee environmental contamination.

WP2: Effects of exposure to single pesticides single exposure and their mixtures on wild pollinators as novel models in laboratory and semi-field experiments (MLU lead). WP2, associated with WP3 (see below), aims to develop the second specific objective of the project indicated in section 1.1.2: organise integrated and controlled laboratory and semi-field experiments to characterise causal relationships between pesticides and pollinator health. First we will develop new protocols to test in controlled conditions a wide range of wild pollinators at adult and larval stages. We will base our protocols on the experimental paradigm developed in PoshBee (Dewaele et al. 2024) and stakeholders (e.g. WILDBIENE). One of the major problems in testing pesticide on wild insect pollinators is to keep them alive; PoshBee experience has shown that adults of many wild bee species in particular do not accept a laboratory set-up and do not feed, invalidating current protocols for assessing sensitivity to pesticide. This is clearly problematic for testing oral sensitivity but is also an issue for contact sensitivity because mortality of untreated individuals can be very high. Following an initial proposal by (Ladurner et al. 2005; Dewaele et al. 2024) have overcome these issues over laboratory accommodation of wild bees by incorporating a feeding lure into cages used to house wild pollinators. WP2 will develop this approach, along with improved cage design, to boost the acceptance of wild pollinators to feed within a controlled laboratory environment. For juvenile stages, feeding of larval and housing of pupae in microwell plates has functioned very well in PoshBee and will be employed in WildPosh, where relevant (e.g. wild bee species, wild syrphid fly species). With a set of improved and efficient protocols for housing wild pollinators in the laboratory, WildPosh will generate data on the sensitivity of wild insect pollinator species to pesticides to which those pollinators are commonly exposed in European agro-ecosystems: the insecticide sulfoxaflor (i), the fungicide azoxystrobin (a)

and the herbicide glyphosate (g), all as commercial formulations to reflect field-realistic exposure. If one or more of these pesticides lose their approved status, we will change to other related pesticides. Pesticides will be tested individually plus as mixtures (all 2-way interactions: i+a, i+g, a+g; and the 3-way interaction: i+a+g) to test for synergistic interactions (or potentiation) between pesticides. The insecticide dimethoate will be used as a positive control.

To determine wild pollinator sensitivity in our laboratory paradigm, we will quantify both lethal and sublethal effects of pesticides in comparison to a model species, the commercially available *B. terrestris*, with which we have excellent experience within PoshBee (e.g. Barraud et al. 2022) and for which we possess or will generate the LD50 (the dose at which 50% of individuals die after 48 hours) for the chosen pesticides. Pesticides will be administered to wild pollinators by contact and by feeding to adults and juvenile (larva, pupa) stages, and acute effects will be quantified as mortality after 48 hours using the LD50 dose for *B. terrestris* and corrected for body size i.e. a fixed dose ($\mu\text{g-pesticide/mg-bee}$) per species/life-stage. Developing an LD50 for each species would be challenging because of the large number of specimens this would require we follow ethical guidelines to limit the removal of wild insects from their natural habitats^{84,85} Sublethal impacts following pesticide treatment will be assessed as survival to emergence and fluctuating asymmetry of wing veins (for treated larvae and pupae) and longevity and behavioural disruption (for treated adults). Complementary experiments will be undertaken with a subset of pollinator species and pesticides in a semi-field set-up to evaluate the extent to which lab-based assays capture the full impact of pesticides in the field. Choice of wild insect pollinator species to test is central to our goal of assessing the most sensitive of species that could act as umbrella species for wider biodiversity. To ensure we capture variation in pesticide sensitivity across species, we will use 5 species each of the 3 most important insect pollinator taxa in Europe, the bees (Table 1), the butterflies and moths (Table 2) and the syrphid flies (Table 3). Species are chosen to vary in their ecological traits that may impact their sensitivity to pesticides. All are widespread in Europe and we have experience in collecting all from the field, where they are not of conservation concern. Some of the syrphid fly species can already be reared in the laboratory. Species selection may be changed (to a related species) after Year 1 if we find it is too difficult to test one or more in the laboratory.

Table 1.

Initial selection of European wild bee (Hymenoptera) species for testing sensitivity to pesticide.

Species	Taxonomic Family	Nest site	Flight period	Social behaviour	Size	Pilosity	Resource specialisation
<i>Andrena vaga</i>	Andrenidae	soil	Spring	Solitary	large	Hairy	Oligolectic
<i>Anthophora plumipes</i>	Apidae	soil	Spring	Solitary	large	Hairy	Polylectic
<i>Colletes hederæ</i>	Colletidae	soil	Autumn	Solitary	medium	Hairy	Oligolectic

Species	Taxonomic Family	Nest site	Flight period	Social behaviour	Size	Pilosity	Resource specialisation
<i>Lasioglossum malachurum</i>	Halictidae	soil	Spring & summer	Social	small	Bare	Polylectic
<i>Osmia brevicornis</i>	Megachilidae	stem	Late Spring	Solitary	medium	Hairy	Oligolectic

Table 2.
Initial selection of European wild butterfly and moth (Lepidoptera) species for testing sensitivity to pesticides. Uni.= Univoltine (i.e. one generation per year). Mul. = Multivoltine (i.e. more than 2 generation per year).

Species	Clade	Taxonomic Family	Larval food plant	Adult habitat	Voltinism	Overwintering stage	Flight period	Migratory behavior
<i>Macroglossum stellatarum</i>	Moth	Sphingidae	Rubiaceae	Meadows and gardens	Bivoltine	Adult	Spring-autumn	Long range
<i>Papilio machaon</i>	Butterfly	Papilionidae	Apiaceae	Meadows	Bivoltine	Pupa	Spring-summer	None to very short range
<i>Pieris brassicae</i>	Butterfly	Pieridae	Brassicaceae	Meadows	Mult.	Pupa	Spring-autumn	Short range
<i>Vanessa cardui</i>	Butterfly	Nymphalidae	Asteraceae	Sunny and open areas	Mult.	Adult	Spring-summer	Long range
<i>Zygaena filipendulae</i>	Moth	Zygaenidae	Poaceae, Fabaceae	Edges and meadows	Uni.	Larva	Summer	None

Table 3.
Initial selection of European wild syrphid fly (Diptera) species for testing sensitivity to pesticides.

Species	Tribe	Larval habitat	Larval food type	Flight period	Inundation tolerance of larvae	Size of adults	Pilosity
<i>Eristalis Tenax</i>	Eristalini	Aquatic	Saprophagous	Spring-autumn	Tolerant with long breathing tube	Large	Medium hairs
<i>Eristalinus aeneus</i>	Eristalini	Aquatic	Saprophagous	Spring-autumn	Tolerant with long breathing tube	Large	Medium hairs
<i>Episyrphus balteatus</i>	Syrphini	Leaves	Zoophagous	Spring-autumn	Not tolerant	Medium	Short hairs

Species	Tribe	Larval habitat	Larval food type	Flight period	Inundation tolerance of larvae	Size of adults	Pilosity
<i>Myathropa florea</i>	Eristalini	Dead wood	Saproxyllic	Late spring-autumn	Tolerant with long breathing tube	Medium	Medium hairs
<i>Cheilosia canicularis</i>	Rhingiini	Plant stems	Phytophagous	Mid-summer	Tolerant with short breathing tube	Large	Medium hairs

Wild bees, with over 2,000 species native to Europe (Ghisbain et al. 2023) rank as the most diverse and most important insect pollinators in European agro-ecosystems that, at the same time, vary markedly in their sensitivity to pesticides (Arena and Sgolastra 2014). We select five species that cover much of the taxonomic breadth of European wild bees and therefore also cover a range of life-history and morphological traits potentially of relevance for their exposure and sensitivity to pesticides: nesting site, flight period, social behaviour, size, pilosity and resource specialisation (Table 1). We particularly focus on ground-nesting bees (4 of our 5 selected species) because exposure through soil is a major lacuna in current pesticide testing of model bee species like the honey bee (Sgolastra et al. 2019) and those used in PoshBee (*B. terrestris* and *O. bicornis*). The one above-ground stem-nesting species in our list, *Osmia brevicornis*, is selected because

1. we have recently succeeded in rearing it and testing it in the laboratory and
2. it preferentially forages on oilseed rape (*Brassica napus*) and could therefore easily be developed into a model wild bee species for this widely grown and bee-attractive crop plant (Hellström et al. 2023).

Lepidoptera (butterflies and moths) are significant flower visitors with high public appeal; many of Europe's >10,000 species are in serious decline (Warren et al. 2021). For testing their sensitivity to pesticides, we select 5 Lepidoptera species that are widespread and which generally have a long adult flight period (with 2 or more generations per year), facilitating laboratory-based analyses (Table 2). We include moths as well as butterflies so as to capture a wider taxonomic range of species as well as a night active species in our testing. Among the flies (Diptera), syrphid flies (Syrphidae or syrphid flies) in particular are widely considered regular flower visitors (Rader et al. 2016), though little is known of their sensitivity to pesticides. Syrphid species vary in where their larvae live and what they eat, which likely impact their exposure and sensitivity to pesticides in the environment. To capture this breadth of ecologies, we initially select a range of syrphid flies covering this diversity of life-history traits (Table 3). With these and potentially other wild insect pollinator species, we thereby aim to reveal how ecology and life-history relate to pesticide sensitivity so as to identify traits or taxa that may act as umbrella species in the field and in future risk assessment.

WP3: Omics of Agrochemical Responses in wild Pollinators (BioPark/CNRS and UM lead). WP3 aims to apply proteomics and transcriptomics strategies to the samples collected in WP2 in order to decipher molecular changes (genes, transcripts, and proteins) that occur in wild pollinators in response to pesticide exposure. To reduce the

overall sampling of pollinators (ethical consideration), the multi-omics analyses will be performed on the same samples. Using mass spectrometry (MS), we will deliver dedicated tools to collect hemolymph and fat bodies with respect to the developmental stages of the wild pollinators (adult versus juvenile). Using MALDI MS molecular mass fingerprints (MFPs) (Arafah et al. 2019), a technique having transfigured the routine analyses in clinical microbiology (Kostrzewa et al. 2019), we will establish MFPs of peptides (including immune peptides) in the experimental scenario described in WP2. The MFPs generated on the individual hemolymph and fat body will define peptide signatures for each pollinator. Using a statistical algorithm, these signatures will be scrutinised to reveal sets of peptide markers that reflect the consequences of pesticide exposures in adults versus juveniles. Using off-gel bottom-up proteomics and high-resolution mass spectrometry (nanoLC-ESI-MS/MS) applied on hemolymph (Askri et al. 2024; Askri et al. 2023; Bournonville et al. 2023; Chantaphanwattana et al. 2023; Piou et al. 2023; Houdelet et al. 2021) and fat body samples (pools of individual tissues from a same individual selected according to the MALDI MFPs protein changes will be quantified by label free quantification (LFQ). The altered physiological/cellular pathways, will be described and ranked following the pesticides for each species of wild pollinator provided by WPs 1 and 2. A wider monitoring of the tissues that may respond to the pesticides and according to their routes of exposure, will be performed by MALDI imaging mass spectrometry (MSI) (Houdelet et al. 2022) to track the proteomic changes throughout body sections of entire wild pollinators.

To investigate how pesticides modify detoxification and innate immunity based on differential expression of immune genes, we will perform experiments combining a global RNA-seq transcriptomic approach with screening of selected gene expression by quantitative RT-PCR (Aufauvre et al. 2014). Pollinator midguts will be pooled for RNA-seq analysis. RNA-seq libraries will be generated with two libraries per experimental group. The R DESeq package will be used to normalise the data and determine which genes were differentially expressed between treatments. To assess the distribution of samples according to their expression profiles, principal component analysis (PCA) will be performed. Modifications of the midgut transcriptome induced by pesticide exposure, acting alone or in combination, will be translated into protein sequences to be linked with data resulting from proteomics analyses. We will collect whole-genome sequencing data from individuals with well-defined phenotypes (pesticide sensitivity/resistance) to understand genomic responses to the strong selective force that pesticide exposure imposes (Trapp et al. 2017). The umbrella species of each group will be used as a model to investigate the evolution of pesticide resistance and to perform comprehensive assessments of variation between species. Sequencing the genomes of insects considered as non-models can be challenging but we will follow the most current protocols already being applied to pollinator species (Falk and Wawman 2024). Genomic libraries will be sequenced on an Illumina short read platform, with an approximate theoretical coverage per genome of ~ 30X (assuming that the size of all sequenced genomes is ~ 300 Mb) with an estimate of around 9 Gbases generated per species. Quality in terms of gene content will be assessed following the proposed Benchmarking Universal Single-Copy Orthologues (BUSCOs) (Feron and Waterhouse 2022). BUSCO is

based on the expectation that single-copy orthologues present in most species of a taxonomic lineage should be traceable in any new genome from a species of the same clade. These approaches will be performed on the most sensitive and responsive pollinator species using datasets gathered from WP2 in concert with proteomics and transcriptomics analyses. We will use laboratory studies validated with field samples, to build models that provide an integrated and dynamic database of proteome and transcriptome changes in response to pesticides and the collected results as a “health card” will feed into WP5. This multi-omics study will provide a multi-scale monitoring tool for health policy and wildlife conservation best practice.

WP4: Global data to feed risk assessment (BIOS lead). WP4 aims to develop our fourth specific objective on building open databases related to pollinators and pesticides. There has been substantial progress already in compiling trait datasets for both pollinators (bees (Gerard et al. 2018); syrphid flies (Speight et al. 2020); Lepidoptera (Roine 2000), and, similarly as with distribution data (bees (Nieto et al. 2014); syrphid flies (Vujić et al. 2022); Lepidoptera (Van Swaay et al. 2010)). We will continue this effort by focussing on traits related to pesticide exposure and sensitivity (e.g. pollen diet including crop, body size). Collection of information on traits will be conducted based on literature search, but also using direct measurements of specimens. Moreover, as many data linked to pesticides (i.e. field exposure to pesticides; toxicological data like LD50, NOAEL, NOAEC; and data on pesticide use like authorised products, commercial preparations, usages, doses recommended in the field) are needed for risk assessment analyses and are available across literature, various databases, and repositories, there is a need to centralise the information. This effort will be combined with our work on improving QSAR predictivity/robustness and facilitate chemical grouping and toxicity extrapolations for single chemicals and component-based mixtures. Finally, all these data will be organised in an open-source data base.

WP5: Integrated systems-based risk assessment (UNITO lead). WP5 aims at using existing and original data from WildPosh (WP1-4) to develop an open-access integrated systems-based risk assessment and provide an open-access user-friendly Toolbox for stakeholders. The Toolbox will integrate advanced RA frameworks, *in vitro*, *in vivo*, and *in silico* data (WP1-5) to evaluate pollinator’s exposure, toxicity, and risk of single and multiple pesticides at individual, population, and community level across representative European landscapes. The Toolbox will propose an integrative assessment including sublethal and chronic effects of single and combined pesticides using toxic unit approaches and validated *in silico* models for a predictive ERA with multiple risk scenarios and mitigation options.

WP6: Assessing the effectiveness and feasibility of mitigation measures (ANSES lead). The overall aim of WP6 is to assess the effectiveness and feasibility of mitigation measures in response to pesticide pressures on wild pollinators and to ensure this information is collated and disseminated in an accessible manner to relevant stakeholders including researchers, policy makers and practitioners. In order to achieve this ambition, we will tailor our tasks and deliverables to synthesise evidence and findings across the various WildPosh work packages alongside external knowledge;

utilise this synthesised evidence alongside expert opinion to identify effective response options to reduce pesticide risks to wild pollinators; develop good practice guides for practitioners to mitigate the impacts of pesticides on wild pollinators and engage in science-policy dialogues and produce policy briefs to inform national and international policy on the development of mitigation measures.

1.2.2 Relation with national and international research and innovation activities

WildPosh will benefit from links to national and international research and innovation activities. Our partners lead/led or play(ed) key roles in global, European, and national projects, and WildPosh will as a result benefit uniquely from the outputs of these projects (Table 4).

<p>Table 4.</p> <p>Summary of the most relevant international research, innovation and policy activities feeding into WildPosh. Coordinators/leads are indicated in bold.</p>		
Projects	Relevant outputs and skills feeding WildPosh / How WildPosh will extend these projects	WildPosh partners
POSHBEE (2018-2023)	Data set on pesticide, pathogen and nutritional stressors on domesticated bees and tools to mitigate these stressors. Expertise in the development of protocols to evaluate pesticide exposure and to test wild bees in laboratory condition. WildPosh will extend knowledge of this project to wild pollinators.	RHUL , UMONS, UFZ, UM, EMU, MLU, PIWET, PENSOFT, ANSES, UREAD, UFR, CNRS, BIOPARK
SAFEGUARD (2021-2025)	Data on the spatial distribution and trait of European pollinators, status & trends of wild pollinators with a special focus on emerging threats, developing assessment and decision toolkit. Expertise in databasing. WildPosh will bring additional information on the mechanism associated to the impact of pesticides.	UREAD , UMONS, UFZ, UNSPMF, BIOS, EMU, PENSOFT
ORBIT (2021-2024)	Data and illustrations on morphological and ecological traits of European bees. Expertise in determination of European bees. WildPosh will extend knowledge on trait and distribution of wild bees.	UMONS , UREAD
TAXOFLY (2021-2024)	Data and illustrations on morphological and ecological traits of European syrphids. Expertise in ID of European syrphids. WildPosh will extend knowledge on trait and distribution of Syrphid flies.	UNSPMF, BIOS
SPRING (2021-2023)	Expertise in testing, piloting, capacity-raising for an implementation of the EU-wide Pollinator Monitoring Scheme. WildPosh will prolong this project by determining the most sensitive species to pesticides which will help to determine the species to focus the monitoring on.	UFZ , UMONS, UREAD, UNSPMF, BIOS
European Pollinators Initiative (2022-...)	Definition and implementation of strategic objectives and actions for EU and Member States to address pollinator declines. WildPosh will determine the most sensitive species of pollinators and their distribution to help to design the most efficient actions of conservation.	UREAD and many partners

Projects	Relevant outputs and skills feeding WildPosh / How WildPosh will extend these projects	WildPosh partners
EU PoMS & STING (2021-2024)	Expertise in designing and refinement of EU monitoring scheme and pollinator indicators for CAP. WildPosh will also prolong this project by determining the most sensitive species to pesticides which will help to determine the species to focus the monitoring on.	UREAD , UFZ, UNSPMF
IPBES (2014-2019)	Expertise in assessment of drivers, state, impacts and responses for pollinators, wider biodiversity and ecosystem services. WildPosh will complete this project by bring new information for Europe.	UREAD ('Pollination' chair), UFZ ('Global' chair), UFR
FAO IPI 2.0 (2021-)	Expertise in the development of a coordinated global pollinator strategy to conserve pollinator and sustainably manage pollination services.	UREAD, UFR
Red Lists (2021-2024)	Data set on spatial distribution and ecological trait of European pollinators. Expertise in conservation status, data and maps of EU pollinators. WildPosh will complete this project by bring new information for European pollinators	UMONS, UM, UNSPMF, BIOS, UREAD, UFZ
RestPoll (2023-2027)	Expertise to develop, test, evaluate and refine cross-sectoral pollinator restoration approaches to conserve biodiversity and to benefit nature and society. WildPosh will determine the most sensitive species of pollinators and their distribution to help to design the most efficient actions of conservation.	UFR , UFZ, UREAD

1.2.3 Multidisciplinary character of the Project

The multiple and ambitious goals of WildPosh can be only reached by a European-wide, transdisciplinary collaboration of scientists from across environmental, social and economic disciplines, working together with multiple stakeholder groups to develop new knowledge and evidence to underpin and drive concerted policy and practitioner actions. WildPosh also takes a fundamentally transdisciplinary approach in terms of scientific fields. We will consider chemistry, modelling, nutritional ecology, proteomics, and social science. We have combined an array of world-class partners - academic, government, and stakeholders from industry and NGOs - who provide expertise across these disciplines. The structure of our consortium bridges disciplinary boundaries to maximise outputs and impacts of this work. For example, the pesticide exposure site network approach follows the same successful approach as PoshBee: integrate the expertise of academic partners with that of local stakeholders, environmental chemistry, nutritional biology, and proteomics. Such an approach underlies our entire proposed work programme.

1.2.4 Integration of social sciences and humanities

Social science methods and principles will be applied where relevant throughout the project most notably in the areas of interactions with stakeholders, identifying response options to mitigate impact of multiple stressors (T6.1), identifying and developing guides for practitioners (T6.2 and T6.3) and facilitating science-policy dialogues (T6.4).

Established methods from social sciences will be utilised including expert elicitation processes for e.g. modified Delphi, methodology to integrate and share best practice and co-develop practical guides. The integration of social science methodology with the evidence and findings of WildPosh will be invaluable in ensuring and enabling knowledge exchange across sectors, feeding directly into collaborative approaches for implementation, and collectively contributing to effective policy and practice at the local, national, and European and international levels.

1.2.5 Gender dimension

WildPosh and partners are well aware that diversity in nationality, and gender and sexual identity play a key role in ensuring comprehensive perspectives and the quality of project outcomes. The Gender Action Plan of the Convention on Biological Diversity provides a framework for gender mainstreaming in commitments and actions, which may be useful for the WildPosh research project. Failure to understand gender issues in policy formulation, on the other hand, may result in a lack of support from important parts of society for innovative policy approaches and solutions. The diversity of sources of knowledge expands the range of perspectives and options for decision-makers to tackle environmental and sustainability issues, including those related to risk evaluation of chemical substances on pollinator health. Comprehensive risk evaluations ensure and enhance its legitimacy and likelihood to be included in risk management programs as well as policies (Díaz-Reviriego et al. 2019). WildPosh consortium partners will consider the gender dimension across five distinct axes:

1. Balancing gender in research activities, such as surveys, to include gender in analysing research results and facilitate community and market uptake;
2. Considering gender and equality in sex identity as designing criteria of WildPosh's conferences and initiatives facilitating equal involvement and engagement with all gender groups, including LGBTQI+. We will achieve this goal through concrete measures such as conference codes of conduct and providing female, male, and non-binary options in gathering demographic data;
3. Contributing to avoid bias diversity in science through social media initiatives (e.g. Facebook, Twitter, LinkedIn) and presentation of careers and activities of participants;
4. Fostering a quota-based balancing hiring to ensure overall gender equality in the consortium and in the stakeholder advisory board;
5. Implementing working methods in line with a work-life balance. For instance, virtual meeting options will be exploited to allow participants higher flexibility.

All consortium institutions in WildPosh have gender equality plans (GEP) or minimally gender policies in place as described in Part A. Specific, measurable, attainable, realistic, and time-related measures (SMART) for GEP will be used as a baseline for monitoring the implementation of gender and nationality equality throughout the course of the

project. WildPosh coordinating partner, UMONS, is developing strong pro-gender equality efforts. It founded the Genre.S discussion group in May 2014 to address gender issues. Since 2016, UMONS has actively participated in the Wallonia Brussels Federation's Women and Science Committee. This advisory body creates suggestions for French-speaking academic policies in Belgium, is organising exchanges of best practises, and holds awareness-raising activities on a variety of gender equality-related topics. UMONS provides a number of courses in the Master in Gender Studies that this committee established and launched in 2017. With the goal of promoting equitable chances, the Rectoral team has since 2018 reinforced, extended, and established many working groups. Three areas have seen the implementation of numerous projects: the struggle against precarity, gender inequality, and improved awareness of the unique needs of those with disabilities. The Board of Directors of the University has approved a "Gender and Diversity" strategy, which sets a road map for the advancements to be pursued in the next years.

1.2.6 Open Science

Key elements of Open Science are:

1. Open Access,
2. Open Data,
3. Open-Source Code,
4. Open Reproducible Research,
5. Open Science Policies,
6. Open Funding,
7. Open Science Evaluation,
8. Open Science Tools, and
9. Open Education.

Longevity and availability of WildPosh-produced research, materials and guidelines will be of outmost importance to ensure that results are exploited by the target audiences. While providing all results openly via its web-based project portal, the project will also add an additional layer to its exploitation plan by launching a unique Open Science Pilot, as well as depositing and sharing project results and information via already established thematically linked resources such as the COLOSS BEEBOOK. The WildPosh Open Science Pilot will start with the open access publication of the project Description of Work in the Research Ideas and Outcomes (RIO) journal (organised by PENSOFT). Similarly to Pilots already launched for other EU projects, unconventional research outputs, such as policy briefs, policy recommendations, factsheets, inventories, case studies and data management plans, will be added to the collection. This will ensure that all project outputs are published openly, with a stable DOI assigned, and comprehensively collected in one place. We will favour transparency of the publication process preferentially choosing open peer-review journals (e.g. *Scientific Reports*, PENSOFT journals) for WPs producing and analysing data (WP1-6). WildPosh will ensure reproducibility by providing extended documentation on the methodologies employed as

well as the raw data of individual studies. The citizen science methodology will be a key point for the involvement of farmers in providing information on pesticide application (WP1) and for the identification of appropriate response options to reduce pesticide risks (WP6). Overall, WildPosh will adhere rigorously to the EC (2022) Guidelines on Open Access to Scientific Publications and the HORIZON Programme Guide 2022 Version 2.0. WildPosh will publish results under the Creative Commons Attribution License 4.0 (CC-BY) (Gold open access). In addition to providing all results openly via the web-based project portal, data will be stored in the EU Pollinator Hub, a trusted repository for EC funded research, ensuring that all output will be identifiable and findable through a digital object identifier (DOI) and comprehensively stored in one place.

1.2.7 Data Management

WildPosh will adhere strictly to the EC (2013) Guidelines on Open Access to Scientific Publications and Research Data in Horizon Europe and to the EC guidelines on FAIR data management in Horizon Europe. We will pursue publication of results under the **Creative Commons** Attribution License 4.0 (CC-BY) (Gold open access) and publication of databases under the **Open Data Commons Attribution License** (ODC-By). As a rule, data and software will also be published as data papers and software description papers in appropriate journals. As presented in the previous section 1.2.6 and WP8 for the data generated during the project, a Data Management Plan (DMP, D8.3) will be set in place following the **FAIR data principles**: Findable, Accessible, Interoperable, Reusable. WildPosh will produce a wide array of data that will be generated through *in vitro*, *in vivo*, *in silico* methods via laboratory, semi-field, field, and modelling approaches. We will also use existing data from published peer-reviewed publications and open-access databases (e.g. Bartomeus et al. 2022; Roine 2000). Data will be stored throughout the collecting process to permit an anticipated sharing timeline. We will pursue the distribution of the tools for risk assessment in **open-source tools** (WP5) under licenses that follow Open Source Initiative criteria. Interoperability of research outputs will follow format and vocabulary standards already set during the PoshBee project and descriptor categories by [EFSA](#) financed platform EU Pollinator Hub. Together with the consortium reference for data management and quality assurance, all WildPosh partners will have one reference person for data management and quality assurance. The project website repository will include research outputs as well as unconventional project outputs such as policy briefs, policy recommendations, factsheets, inventories, case studies and data management plans. WildPosh will start with the open-access publication of the project Description of Work in the Research Ideas and Outcomes (RIO) journal. WildPosh will produce within the first six months a detailed data management plan (DMP) setting out project output requirements, taking advantage of the online tool created by a Belgian consortium including UMONS called DMP Online. We will also deliver a Mid-term DMP (M24) and a Final DMP (M47). Wherever we see fit, the WildPosh consortium will seek ethical approval at UMONS for the collection, use and storage of personal data that will be acquired through focus groups, surveys, and interviews. This Ethical approval will be sought at the beginning of the project to ensure that it is in place before data collection. This ethical review will include the informed consent procedure for use of personal data.

2. Impact

2.1 Contributions of the project results

2.1.1 Contribution of the project results towards the outcomes specified in this topic

The HORIZON-CL6-2023-BIODIV call states five interrelated outcomes. WildPosh is in prime position to deliver fully on these outcomes and their related broader impacts. During the project lifetime, we will provide the necessary knowledge and process to engage with key stakeholders (i.e. NGO, associations, policy makers, land managers, agrichemical businesses), to develop pathways to impact. By coupling this to an exit-strategy (i.e. strategy for impact after the end of the project), and links to other ongoing initiatives (e.g. EU PI), we will ensure a genuine legacy of ongoing impacts. Below we demonstrate how WildPosh meets and exceeds these outcomes, and how we use SMART indicators (Specific, Measurable, Achievable, Relevant and Time-bound) to evaluate progress.

Expected outcome 1. Routes of exposure, linked to ecosystem dynamics, of flora and fauna to chemicals are better understood.

Outcome: There is an important knowledge gap in the description of the route of exposure and level of exposure of wild pollinators (see Section 1.1). Science-based protocols are essential to identify and characterise evidence-driven routes and levels of pesticide exposure of pollinators. WildPosh will develop new standardised protocols and run novel field experiments to determine the real world effects of chemicals in the presence of other stressors (WP1). Further, WildPosh will capture the important variation in the routes of exposure associated to different ecosystem dynamics related to different climates, different plant communities, different wild pollinator communities and different crop systems (including different pesticide management regimes) in Europe. Thanks to the diversity and distribution of the partners of our consortium, we will capture this variation by implementing a site network covering 4 countries and the four main biogeographical regions (WP1). WildPosh will transform our understanding of pesticide exposure of wild pollinators across a diversity of matrices capturing spatio-temporal variation (WP1). **Outcome indicators:** WildPosh will significantly close knowledge gaps, and make scientific contributions, evidenced by the anticipated publication of research results in the peer reviewed scientific press, where results can be independently scrutinised and verified (e.g. *PNAS*, *Nature*, *Scientific Reports*, *Global Change Biology*, *Science of Total Environment*). We target the broad readership associated with these global and generalist journals (i.e. the scientific community at worldwide scale). As scientific experts are part of groups of discussion and commissions evaluating quality of many legislations, from local to global, we believe that our publications will also have an impact on driving legislation to an evidence-based approach. In the short term, the indicator of success for this outcome will be measured by the citations of these articles in the international platforms Web of Science and Scopus (Table 5). Secondly, moving

beyond the scientific community we also intend to make a societal impact. We will engage directly with multiple European policy and practice stakeholders through the co-development of multiple good practice guides (targeted at risk assessors, growers, researchers, land managers), policy briefs (targeted at EFSA, DG AGRI, DG SANTE, DG ENV, national agencies) and science-policy dialogues (WP6). The WildPosh Advisory Board comprises multiple actors representing our target who will provide guidance and support in promoting the uptake of WildPosh outputs with multiple end users. Indicators of success include the number of good practice guides, policy briefs and science-policy meetings emerging from the project and the number of policy and practice organisations involved in an active uptake of these.

Table 5. Key elements of the impact section.		
SPECIFIC NEEDS	EXPECTED RESULTS	D&E&C MEASURES
<div>1. Chemicals are spreading through entire agro-ecosystems and travel up agricultural food chains, but we need to understand the routes of chemical exposure of wild pollinators in field realistic conditions through different matrices (i.e. soil, water and different parts of plants). New protocols need to be developed to tackle the sampling and analytical challenges.</div> <div>2. As wild insect pollinators are very diverse (> 12,000 species recorded in Europe), there is an important challenge in selecting and testing a representative set of species and their associated ecological traits. We need to describe mechanistic links between these exposure routes and the health of wild pollinators.</div> <div>3. We do not know which species have a higher risk of exposure; there is a technical challenge to monitor and assess the risk of exposure of wild animals in general and wild pollinators in particular.</div>	<div>Scientific articles in international, peer reviewed journal on exposure to pesticide, and their impact on wild pollinators.</div> <div><ul style="list-style-type: none">• New data sets in open-source database on pollinator traits and distribution and sensitivity to pesticide, on pesticide use and toxicity;• Development of new protocols to characterise pesticide exposure and the impact of pesticides (including BeeTyping® tool);• Development of prognosis/ diagnosis markers of chemical stress available for monitoring and research;• Development of mass spectrometry tissue imaging in toxicodynamic studies and risk assessment;• New models associated to risk assessment, including for species most at risk.</div>	<div>Tailored and targeted Dissemination, Exploitation and Communication activities for a maximized impact in each stakeholder group. To accelerate outreach, synergies will be established with relevant project, networks and initiatives.</div> <div><ul style="list-style-type: none">• Dissemination to policy makers & scientific community: policy briefs, policy workshops, targeted Twitter posts, scientific publications, international conferences, Horizon;• Dissemination to industry and practitioners: training videos, practice abstracts, workshops for practitioners;• Exploitation by users: Open access to all key exploitable results in WildPosh;• Communication to the general public: one pager, awareness-raising materials, website, non-technical newsletter, press releases, social media, non-specialist videos, infographics.</div> <div>All activities will be supported by the EC platforms Horizon Results Platform, Horizon Results Booster, Open Research Europe.</div>
TARGET GROUPS	OUTCOMES	IMPACTS
<div><ul style="list-style-type: none">• Scientific community (SC) - Public and private sector research institutions, national and EU projects, academic fora and networks;• Policymakers (PM) - Local, national, EU and global policymakers and policy advisors;</div>	<div><ul style="list-style-type: none">• Development of new protocols through novel field experiments to determine the real-world effects of chemicals in the presence of other stressors;</div>	<div><ul style="list-style-type: none">• WildPosh will help understand the mechanisms of population trends of wild pollinators, the drivers behind the threats and how to respond to the drivers. This will strengthen the resilience of wild flower pollination and supplies of fruits, seeds and nuts from pollinator-dependent plants. This provides key links in food chains containing many of Europe's iconic birds and mammals and help understand questions on biodiversity decline.</div>

<ul style="list-style-type: none"> • Industry (IN) - Agrochemical businesses, growers, suppliers, processors and retailers; • Practitioners (PR) - Wider agri-food sectors, land managers, reserve managers, conservation NGOs; • General public (GP) - Citizen organisations, amateur societies and recording schemes, media and the wider public. 	<ul style="list-style-type: none"> • Definition of lethal and sublethal effects for individual pesticides and for combinations of pesticides across stages (i.e. adult, larvae), tax a (i.e. bees, syrphid flies, butterflies, moths), ecological groups (e.g. specialist versus generalist pollen diet), morphological groups (e.g. small versus big species); • Assessment of how the European pollinators to pesticides, as community, are at risk of exposure; • Assessments at landscape level of the distribution of new chemical in different matrices (i.e. pollen, nectar, soil, water); • Datasets regarding pesticides distribution and their toxicity and pan-European maps of risks for pollinators; • Development of improved and novel methodologies and protocols for testing pesticide effects across life-stages and groups of pollinators; • Development of multiple models of wild pollinators for regulatory testing; • Development of novel protocols and end-points for regulatory testing in wild pollinators. • Development of improved protocols for semi-field experiments for pollinator regulatory testing schemes; • Provision of these novel science-based protocols, to enable uptake and incorporation into pollinator regulatory testing schemes; • Providing EIP best practice abstracts and policy briefs for pollinator health monitoring and mitigation responses; • Enhancing the resilience of wild pollinators underpinning pollination service delivery. 	<ol style="list-style-type: none"> 1. WildPosh will drive longer-term innovation capacity through establishment of successful communities of researchers and practitioners co-developing and refining monitoring tools. This strengthens competitiveness in the global market for pollinator health tools by providing a suite of next-generation tools, protocols and applications. 2. A key impact of WildPosh will be enhanced food security by contributing to more robust and sustainable wild pollinator populations, which will safeguard crop pollination services. 3. WildPosh will support the development of new policy related to pesticide use. New protocols will be available after the lifetime of Wild Posh <ul style="list-style-type: none"> • for industry and regulatory bodies to test the toxicity of new pesticides on wild pollinators, • for policy makers to adjust strategic plans to the real needs of the actors, improving efficiency of policies; • other connected actors and general public to get reliable information on pollinator health status and better understand how to contribute to food security, biodiversity protection and high-quality environment.
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Expected outcome 2. Issues raised by the contamination of wild fauna and flora are better known, including risks linked to existing contaminations (legacy) and accumulations in nature.

Outcome: With regard to this outcome, WildPosh will have both scientific and societal impact. Chronic and sublethal effects of pesticides on the health of wild pollinators have been poorly defined to date (see Section 1.1). By taking an explicitly empirical approach from highly controlled laboratory settings through to full field-scale experiments, and from individual bees through to the population level, WildPosh will provide novel, cutting edge findings that provide definitive answers to these pressing, open questions. WildPosh will

1. define lethal and sublethal effects for individual pesticides and for combinations of pesticides across stages (i.e. adult, larvae), taxa (i.e. bees, syrphid flies, butterflies, moths), ecological groups (e.g. specialist versus generalist pollen diet), morphological groups (e.g. small versus big species) (WPs 1-3),
2. based on trait and distribution data, determine how the European pollinators to pesticides, as community, are at risk of exposure (WP4).

All the in vivo and in silico data will be publicly available databases for a wide range of data users. **Outcome indicators:** In combination with expected outcome 1, WildPosh will fill critical knowledge gaps on sensitive species demonstrated by publication of research results in the peer reviewed scientific press. Better knowledge on the sensitive species will help to define evidence-based conservation strategies. We aim to feed “zero-pesticide” policy from local level to global level by giving arguments to our readership, from local structures to global institutions. We will evaluate the quality of our outcomes based on the same indicators but also on the development of better practices of pesticide use (Table 5). To have an indication of the quality of the impact of our databases, we will consider the number of downloads of data set from the website hosting the open-source databases (Table 5). In addition, WildPosh will embed the key scientific findings in the policy briefs, practice guides and science-policy dialogues (WP6) using the same indicators of success.

Expected outcome 3. Environmental fate of new chemicals of emerging concern is better understood.

Outcome: WildPosh will have both societal and scientific contributions. WildPosh will generate novel assessment tools, maps and models to advance our understanding on the environmental fate of pesticides, including new chemicals of emerging concern. For example, we will determine at landscape level the distribution of the new chemical in different matrices (i.e. pollen, nectar, soil, water) (WP1). We will compile state-of-the art information and datasets regarding pesticides distribution at continental level and their toxicity (WP4) and pan-European maps of risks for pollinators (WP5). **Outcome indicators:** In line with previous two expected outcomes, the publication and validation of data and models in peer-reviewed scientific journals will evidence the filling of knowledge gaps about the environmental fate of pesticides (using indicators in Table 5). Further, to ensure accessibility by non-scientific stakeholders, WildPosh will co-develop key messages, maps and tools targeted at specific stakeholders including EFSA, and national risk assessors. These will underpin the production of briefs and guides (WP6) and disseminated via the website and targeted stakeholder workshops (WP6) (Table 5). The development of global and user friendly assessment tools will potentially help any pesticide end-user to consider the risk of exposure of the wild pollinators, and adapt their practices to protect the pollinators. It will also give evidence based arguments to policy makers, associations, and citizens to motivate healthier practices regarding pesticide use. This outcome targets European entities but it has the potential to inspire the development of the same tools in other continents.

Expected outcome 4. Toxicological and ecological impacts are better understood and risk assessments for relevant highly exposed species are strengthened.

Outcome: WildPosh will have both scientific and societal impact. As for the characterisation of pesticide exposure, standardised science-based protocols are essential to provide and support evidence-driven pollinator regulatory testing schemes, including on highly exposed species. WildPosh will realise this outcome in the following ways.

1. It will develop and validate novel and improved science-based protocols for testing the effects of chemicals on the life-stages (larva/adult) and various groups of wild pollinators (WP2). These will be developed through a multi-actor approach, incorporating input from stakeholders including regulatory bodies (EFSA), to ensure that they significantly improve pollinator regulatory testing schemes (including bees).
2. Currently, regulatory testing ignores the majority of pollinator species. To fill this gap, WildPosh will develop new model systems of wild pollinators for regulatory testing (WP2), to maximise value and facilitate industrial uptake.
3. Using these new model systems, WildPosh will provide the first science-based protocols for regulatory testing other than honey bee, bumble bee and mason bee (WP2). Again, using the multi-actor approach will maximise the value and uptake of these protocols.
4. Finally, in a collaboration between industry (WILDBIENEN), public research organisation (WBF-Agroscope), and academia (ALU), WildPosh will provide improved protocols for semi-field regulatory testing of wild pollinators (WP2) which will be made available to national and EU risk assessors. This will facilitate better assessment of pesticide effects (before approval) and improved protection from pesticide effects of over ~2,000 wild bee species, ~1,000 syrphid fly species and ~8,000 butterfly/moth species in Europe.
5. While all of these protocols will be developed, WildPosh will further drive the uptake of these novel protocols through focused delivery to industry and regulatory bodies, at European and global scales (WP6). Moreover, we will produce cutting-edge omics-based-tools to identify potential molecular markers of wild pollinator health, to facilitate the ranking of consequences of pesticides on pollinator health indicators (WP3).

Sequence information from genomic, transcriptomic, and peptidomics/proteomics will provide a data-dense, comprehensive view of the molecular health status of wild pollinators. Similarly, spectrometry-based fingerprinting of the juvenile and adult females will reveal sensitivity-level responses to pesticides and provide valuable datasets for understanding and evaluating the health status of the different pollinator species investigated according to their biotope. Above the simple traditional following of insect presence, WildPosh will use individual blood-like tests (MALDI-BeeTyping) to characterise the health status of a large range of wild pollinators, with a set of specific markers (e.g. immune peptides) (WP3). On the basis of minimal air/soil/water insect sampling, Health authorities, veterinarians and environmental actors will get a set of new

holistic solutions for evaluating and prescribing more accurate and effective preventive and curative measures. Finally, by compiling *in vivo* data (WP1-3) to *in silico* data, including data on pollinators (WP4) and pesticide toxicity (WP4), and by developing holistic risk assessment for pollinators that inform our understanding of how stressors perturb healthy pollinators (WP5), we will connect land managers and citizens to better recommendations for managing their habitat / properties to favour pollinator health (including sensitive species), pollination capacity, biodiversity and environmental protection. **Outcome indicators:** Success of these impacts will occur partially within the lifetime of the project in Europe. However, uptake of new protocols into regulatory schemes is a long-term process that will extend beyond the geographical framework and time-line of WildPosh. Publications of protocols and methodologies will act as indicators for science-based regulatory protocols. Indicators for the longer-term uptake of these protocols will include the commitment of national agencies, agrichemical business, the EFSA and policy-makers to incorporate these new protocols into regulatory schemes, and further ring-testing of these protocols for bodies such as the OIE and OECD (see Table 5). The number of actors which uptake this guidance will be recorded. Moreover, we will assess the satisfaction level of individual end-users implementing the BeeTyping® tool and also the extent to which it is adopted within bee health monitoring schemes. A further measure of success will be the development of a set of prognosis/diagnosis markers within a kit ready for marketing. Finally, we will assess how far our MSI in toxicodynamic studies is adopted for risk assessment by authorities (e.g., EFSA) and industry (see Table 5).

Expected outcome 5. Prevention and mitigation measures are developed.

Outcome: WildPosh will expand our understanding on the impact of pesticide on wild pollinators and propose novel prevention and mitigation strategies for pollinator conservation in Europe. Building on the standardised protocols and novel field experiments as well as the assessment tools, maps and models developed across WPs 1-3, WildPosh will engage with stakeholders across sector to

1. identify the best response options to help mitigate the effect of these stressor in WP6. Further collaborations with stakeholders will also identify co-design solutions to enable possible implementation of the best response options and ensure the outputs are all fit for purpose and matched to requirements. WildPosh tools will allow early and accurate detection of biologically relevant thresholds of pesticide stress on pollinator health (including sensitive species) as an integral part of pollinator health monitoring programmes.
2. Coupled with these tools, WildPosh will produce, and deliver multi-media best practice guides, EIP practice abstracts, and policy briefs across a wide range of stakeholders to allow the application of tools to assess risks, monitor pollinator health and implement appropriate response actions at the local, national and European levels. This will contribute to a strengthened multi-actor framework for the conservation of a diverse community of wild pollinators, which in turn will
3. underpin more resilient pollinator communities, thereby preventing the decline of the sustainability of pollination service provision to European crops and wild

flowers. Greater sustainability in pollination services will have direct benefit to growers and suppliers (reliable pollination services for dependent crops, improved yield, quality and income); policy-makers (evidence to support the development of mitigation/prevention strategy related to pollinators in the agricultural, environment and business sectors); consumers (secured access to a variety of pollinator-dependent produce, such as fruit, vegetables and seeds); wider society (public goods including the safeguarding of pollinator and wild flower biodiversity).

Outcome indicators: Identification of a key set of response options that are deemed both effective and feasible to implement to help mitigate detrimental impact of stressors. These will inform Good practice guides aimed at practitioners and policy briefs designed to convey key findings to policy makers will also be made available during the course of this project (Table 5). WildPosh will track the citation of peer reviewed publications arising from the project and simultaneously co-develop with stakeholders infographics and summaries aimed at non-scientific audiences. We will measure the uptake and adoption of monitoring tools across stakeholders, the development of new monitoring frameworks using WildPosh tools, and the extent of engagement the project has with policymakers and practitioners during the lifetime of the project. However, other impacts may take several years to be realised and will therefore not be directly measurable during WildPosh. For these longer-term impacts (e.g., sustainable pollination) we will use indicators reflecting progress along the potential pathways to impact. All indicators, targets and methods of measurement are summarised in Table 5.

2.1.2 Contribution of the project results towards the wider impacts, in the longer term

Overall, the development of the four expected outcomes will participate to longer and broader interrelated scientific and societal impact introduced in the call for the Horizon cluster 6:

Understand and address direct drivers of biodiversity decline. The main driver targeted in WildPosh is pollution, particularly pesticides. Pesticides are one of the main threats to pollinator decline, WildPosh will help to understand the mechanisms of population trends of wild pollinators but also how they respond to the driver. We believe that the generated evidence will positively influence the general public and farmers. People will better understand the potential negative impact of the spread of pesticides. As a consequence, policy makers will adapt the current policy toward a sustainable use of pesticide: more efficient, better targeted and protection of the sensitive species. Safeguarding healthier European pollinator communities by protecting sensitive species (i.e. conservation of a high number of species) will strengthen the resilience of wild flower pollination and supplies of fruits, seeds and nuts from pollinator-dependent plants providing key links in food webs containing many of Europe's iconic birds and mammals. The outcomes from the project will be used by stakeholders and extrapolated to other living organisms, through which WildPosh will therefore help to understand wider

questions on drivers of biodiversity decline than the strict question on pollinator conservation. Towards the end of the project WildPosh will seek opportunities to explore ways to bring in other key environmental drivers (e.g. nutrition, climate, pathogens) into the models and tools developed by WildPosh. For instance, we will make contact with other institutions and projects developing risk assessments and look to develop collaborations to adapt WildPosh tools and approaches to include other stressors.

Mainstream biodiversity, ecosystem services and natural capital in the society and economy: integrate them into public and business decision-making; build approaches for enabling transformative changes to tackle societal challenges including through the deployment of nature-based solutions (NBS). WildPosh has the potential to drive longer-term innovation capacity through its establishment of successful communities of researchers and practitioners co-developing and refining monitoring tools. Europe is the first continent to develop these tools. The project will strengthen European competitiveness in the rapidly expanding global market for pollinator health tools by providing a suite of next generation tools (e.g. characterisation of pollinator hemolymph like a blood test for human), protocols (e.g. new pollinator species as model to test the impact of new molecules) and applications (e.g. risk assessment). This would also be expected to open up new markets within Europe and outside, as the demand from the agricultural sector for more sustainable production approaches intensifies. The markets would be both for products (monitoring and analytical tools) and services (training in tool application, extension service advisors).

Develop and improve practices in agriculture to support and make sustainable use of biodiversity and a wide range of ecosystems services. WildPosh will develop studies about wild pollinator species living in agro-ecosystems. Based on the *in vivo* and *in silico* data we will develop environmental scenarios for pesticide risk assessment and mitigation options. We will work closely with the Promote Pollinators platform (included in the Advisory Board) to ensure effective interaction between WildPosh and global treaties as well as with national governments to identify priority needs and opportunities through workshops and surveys. This will provide a clear understanding of the different policy maker's perspectives of the target SDGs. Based on this, we will adapt the narrative of the proposed mitigation options to develop for sure a sustainable use of pollinator diversity for a sustainable agriculture. Evidence of the wide range of co-benefits of pollinator conservation throughout the value chain facilitate mainstreaming of biodiversity into farming. WildPosh will further demonstrate how interventions to improve pollinator health can be integrated into the management of agricultural landscapes and what the societal and private consequences are, such as links to specific UN Sustainable Development Goals (for e.g. SDG 2 and SDG 15). By this, a key impact of WildPosh will be enhanced food security by contributing to more robust and sustainable wild pollinator populations, which will safeguard crop pollination services.

Interconnect biodiversity research and support policies and processes at EU and global levels, making use of advanced digital technologies where appropriate.

WildPosh will support the development of new policy related to pesticide use. New protocols will be available long after the lifetime of WildPosh

1. for industry and regulatory bodies to test the toxicity of new pesticides on wild pollinators,
2. for policy makers who will be able to adjust their strategic plans to the real needs of the actors, improving the efficiency of policies;
3. other connected actors and general public to get reliable information on pollinator health status and better understand how they can contribute to food security, biodiversity protection and high-quality environment.

As a consequence, pollinators will be better integrated in conservation strategy, and more than 12,000 species of insects will be better protected in Europe.

2.1.3 Requirements and barriers

Policy on pesticide use. Currently there is a moratorium in the European Community on some uses of three neonicotinoids and, in a recent call, European Commission aims to reduce pesticide use in every EU country by a 50% reduction in the use and risk of chemical pesticides and a 50% reduction in the use of more hazardous pesticides. In the case of regulation modifications at EU or national levels, the WildPosh site network will be able to capture these changes as the coverage of the European biogeographical regions is sufficiently extended and complete to allow for the comparison of exposed/non exposed populations. WildPosh will ensure an active dialogue with policy and regulatory experts (WP7) in order to anticipate and actively respond to a changing policy environment. Several partners of WildPosh are part of groups and processes where new standards are presented, discussed and ring tested (EFSA, OECD, ICPBR). Therefore, any actual or planned changes in analytical/test standards will become quickly known to the consortium. If new/modified standards are put in force, WildPosh partners will rapidly include them as part of the protocols tested.

Stakeholder fatigue or lack of interest in participating in research projects targeted at biodiversity may present a risk to WildPosh's impact. We will minimise this risk by close targeting and tailoring of engagement to the key pollinator-related interests of particular stakeholders. Other incentives to participation include tackling knowledge gaps including those associated with land management for wild pollinators and pollination service provision, addressing national or regional policy targets, or working with societal leaders with aligned agendas integrating biodiversity into management. WildPosh partners' long-standing relationships across the stakeholder spectrum have established a high level of trust that will help ensure stakeholders recognise the concrete benefits of working with the project.

Changes in country-specific policies and the EU Common Agricultural Policy (CAP), will modify the framework within which WildPosh is aiming to achieve impact. Proposals for the new CAP give member states more freedom to target agri-environmental management through enhanced conditionality, eco-schemes and agri-environment

climate schemes. This could alter incentives for farm biodiversity management and may differ between member states. WildPosh views this an opportunity to improve policy impact by assessing and informing the CAP decision making process and actors and providing knowledge and methods for the EC, MS and civil society to improve designs of interventions. WildPosh will engage with policy and regulatory experts from the beginning to respond to the changing policy environment (Green Deal, Farm to Fork).

Emerging threats to pollinators. Interventions to enhance pollinators may need to be adapted to account for emerging threats across Europe. For example, wildflower strips have been associated with enhanced parasite transmission from honey bees to wild bees, and future deployment of this intervention may need to account for the risk of new invasive pathogens and pests. Such developments may significantly change the relative importance of different threats, and they may shift the needs of relevant stakeholders and mitigation strategies. WildPosh partners are at the forefront of monitoring current and emerging threats to pollinators in the EU and we will proactively ensure that our research accounts for these to maximise relevance of outcomes.

Economic concerns driven by war in Ukraine and the Covid-19 crisis may override the interest of local, regional and national actors in engaging with biodiversity-relevant measures. Economic recession might shift private and policy priorities away from pollinator conservation and global change mitigation to promoting economic recovery. However, sustainable development of European economies is central to the EU Green Deal and requires reconciling biodiversity protection and economic welfare. WildPosh will actively support this strategy by demonstrating the direct and indirect economic values of pollinators. WildPosh's policy solutions will explicitly account for economic concerns, both in terms of costs of solutions and by the private and public values of ecosystem services.

2.2 Measures to maximise impact - Dissemination, exploitation and communication

2.2.1 Plan for the dissemination and exploitation including communication activities

Purposefully designed communication, dissemination and exploitation (CDE) activities are key components for maximising the impact of WildPosh. The project's CDE activities will be streamlined in a Communication Plan (CP) and Plan for the Exploitation and Dissemination of Results (PEDR), which will be regularly updated. The plans will serve as a management tool for defining how the project's progress and results are shared with stakeholders and target audiences. These updates will include any necessary modification and adapt appropriately to project progress and new circumstances, including feedback from stakeholders and target audiences. The activities will be led by WP7 leader PENSOFT and co-designed by all project partners in order to accelerate the project impact and ensure the uptake of its results on a large scale. Each team member will bring local, national and international contacts to the project through which the results

will be efficiently disseminated. Successful implementation of this plan will support systemic change for wild pollinator management.

The CDE efforts are planned in four consecutive phases, following the AIDA (Attention-Interest-Desire-Action) principles, to reach the target groups and decide on the most appropriate instruments to reach them. The phases are designed as followed:

1. **Attention (M1-6).** This stage will raise awareness about the project and its aims, objectives and activities to attract the attention of the public at large, but also introduce the project to its main target groups in order to lay the foundation of results emergence.
2. **Interest (M6-18).** The project will intensify its communication and dissemination actions at European, national, regional and local levels to promote its progress and results. This phase will encourage potentially interested parties to participate in the project and receive knowledge on chemical exposure and effects.
3. **Desire (M19-36).** During this exploitation-oriented phase, which will focus on the dissemination and exploitation of the findings and results, the project will use workshops, training events and scientific publications to move the mindset from “I like the concept” to “I want to use the results”.
4. **Action (M37-48+).** It is important to make sure that results find their way into society and that relevant stakeholders are taking action. Exploitation of the results to ensure sustainability will be facilitated in this phase in order to facilitate young people’s engagement in curriculum making after the project ends.

The consortium has identified a significant list of target groups to which the communication and dissemination activities will be directed to, as outlined in Table 6.

Table 6.

Stakeholders and target groups of WildPosh.

Nr.	Stakeholder group	Description
1	Scientific community (SC)	Public and private sector research institutions, national and EU projects, academic fora and networks
2	Policymakers (PM)	Local, national, EU and global policymakers and policy advisors
3	Industry (IN)	Agrochemical businesses, growers, suppliers, processors and retailers
4	Practitioners (PR)	Wider agri-food sectors, land managers, reserve managers, conservation NGOs
5	General public (GP)	Citizen organisations, amateur societies and recording schemes, media and the wider public

2.2.1.1 Communication

The communication strategy aims to present the project from its very beginning, promote its development and announce its results to the general public. Therefore, the communication plan covers the entire lifespan of the project. The plan will

1. ensure effective communication of the project,
2. raise project visibility and awareness among the stakeholder groups about the project's goals,
3. reach a variety of audiences including the general public,
4. promote the sustainability of the project and its results, as well as the overall dissemination and exploitation strategy, and
5. raise awareness on how EU funding contributes to the health of wild pollinators.

WildPosh communication instruments will be designed in a user-friendly way, ensuring accessibility by the wider public. The project website, along with the dissemination measures, will ensure that the project will reach the appropriate target groups, permitting fruitful discussion and exchange of ideas throughout the lifespan of the project. WildPosh is aiming to be a 'green' consortium, so therefore we will aim to minimise the use of printed materials as a communication measure.

2.2.1.2 Dissemination

The main objective of WildPosh's dissemination strategy is to promote results as they arise and make them available in the best possible format, thus contributing to the project's scientific and economic impact. Successful dissemination of the project will allow the consortium and the target groups to better understand and ultimately benefit from the research, methodology and tools developed within the project. This might both precede and evolve together with the exploitation phase, focused on the active use (or re-use) of the project results. As the project brings a new scope of information and knowledge, the dissemination plan aims to ensure a wide transfer of knowledge for all stakeholder groups. The dissemination measures will be used to enable stakeholder groups to become familiar with project results. This will be done by the consortium members themselves as well as their aggregated community of networks presented in Section 3.2, which details how the partners are active in the different initiatives and how they can exploit a multiplier effect. WildPosh will identify and plan collaboration paths with existing projects, networks, programmes and initiatives working to protect pollinators (e.g., ORBIT, SPRING, SAFEGUARD, Voodoo, Sting, Pulse, RestPOLL, PollinERA; IPBES; IPCC). Moreover, the results of WildPosh will be fed into Biodiversa+ projects. On social media alone, the collective outreach of WildPosh-involved institutions amounts to 601,170 followers on Twitter and 768,300 followers on LinkedIn. All further opportunities offered for dissemination, for example through other EC programs, will be analysed and used for the advantage of this project to enable an effective and Europe-wide dissemination of the project's experiences and results. All communication and dissemination measures and their relevant target groups, key performance indicators and stage of application are identified and described in Table 7.

Table 7.

WildPosh communication and dissemination tools. C = communication. D = dissemination.

Activity	Tool	Target	KPI for outreach	Application stage
C	Promotional materials: Project one-pager, introductory presentation, roll-up banner, stickers	ALL	500 copies of one-pager distributed; 300 downloads; presentation shared at 20 events	1,2,3
C & D	Website: The main platform for general information about the project, its objectives, news, articles and public deliverables	ALL	Number of news items>1/month; Number of visits>10,000/project duration; average session duration>120s; returning visitors>30%; geographical distribution: worldwide	1,2,3,4
C & D	E-newsletters: Bi-annual online updates about the project, its progress and results with specialised content for each target group (e.g. non-technical version for general public)	SC, IN, PM, GP	Number of subscribers + 50/year, number of opens >35%	1,2,3,4
C&D	Presentations of research results and findings	SC, PM	Presentations of research results at major EU/world conferences addressing pollinators: Apimondia, EurBee, IUSSI, International >10	2,3,4
C	Press releases: Bi-annual to annual communiqués on key project milestones (project launch, WildPosh-organised event) distributed via top science news portals EurekAlert! and AlphaGalileo	GP	>1500 views/press release	1,2,3,4
C & D	Social media: Accounts in Twitter, Instagram and LinkedIn, which will help disseminate the project results and can be used to stimulate youth participation	ALL	Number of followers/subscribers and "likes" > +100/year, number of impressions on Twitter>100 000/project duration	1,2,3,4
C	Videos: Short interviews with WP leaders will broadcast the specific activities and outcomes trying to give them visibility and engage stakeholders.	GP	Number of videos>8; Number of views>200	1,2,3,4
D	Training videos: Practitioner videos providing hands on demonstration of WildPosh tools	PR	Number of videos>5; number of viewers>200	3,4
D	Practice abstracts: Short and to-the-point summaries with practical information published in EIP-AGRI	PR	Number of abstracts>10; statistics of EIP-AGRI not available to date	3,4
D	Workshops: Practice and policy informing events in hybrid format focused on project findings	PR, PM	Number of workshops=5; Number of attendees>50	3,4

D	Policy briefs: Evidence-based policy recommendations compiled into persuasive collection of briefs	PM	Number of briefs>3;Number of distributed copies>250; Number of downloads from website>250	3,4
D	Scientific publications: A key outlet for scientific insights obtained in WildPosh	SC	Number of publications>30Average impact factor of journals > 3-30	2,3,4
C & D	Data sets from the open-source database	ALL	Number of downloads from website > 100	1, 2, 3, 4
C&D	Incorporation of protocols in literature	ALL	Number of downloads, reads and citations > 200	
C	BeeTyping® as a new analytical tool	ALL	BIOP/CNRS, survey of end-users on satisfaction with tool >75% satisfied Feedback from partners on number of monitoring schemes using the tool >5	
C&D	National government engagement	PM	Number of Member State governments directly engaged in dialogue on developing improved bee health monitoring frameworks > 10	
D	Set of prognosis/diagnosis markers available for monitoring and research	SC	Standardised protocols developed and kit ready for marketing > 1	
C & D	Adoption of mass spectrometry tissue imaging in toxicodynamic studies and risk assessment by authorities (EFSA) and industry	PM	Reporting by partners on number of industries adopting/committing to adopt the kit in their drug development process > 3	

2.2.1.3 Exploitation

The exploitation activities will be closely attuned to the dissemination measures and aim to help stakeholders utilise the project results. The research findings and project results will be synthesised into reusable Key Exploitable Results (a preliminary list is available in Table 8) . More specifically, the insights and knowledge collected from WPs will be turned into concrete tools and models that can be applied by professionals and shared with the scientific community. The policy briefs will be shared with policymakers, and the exchange hub will be explicitly with the Scientific Community at large, also beyond pollinators and pesticides research. This will maximize the project's impact and ultimately make clear scientific and societal impact far beyond the duration of WildPosh.

Table 8. WildPosh Key Exploitable Results and their route of exploitation to target groups.		
Key exploitable results	Targets	Route to exploitation
Open source databases	SC	Databases will be freely available online and become the go-to place for data on pollinator traits and distribution and sensitivity to pesticides, on pesticide use and toxicity.
Integrated systems-based risk assessment tools	SC, PR	Farmers, land managers and other stakeholder will be tutored on the usage of the risk assessment tools via workshops and demonstrations.

Key exploitable results	Targets	Route to exploitation
Monitoring tools and models	SC, PR	The protocols developed to monitor pesticides in a range of environmental matrices will be disseminated and promoted for use by national and European-level monitoring schemes.
Collection of policy briefs	PM	This collection will be a social innovation used by local, national, European-level policymakers for step change in the direction of CAP and the changing policy environment.
European pollinator health knowledge exchange hub	SC	This hub will ensure that the project community will transcend the project duration. It will be solidified by new HORIZON projects and extended consortia.

To maximise exposure of project results and their potential for exploitation, the project will take advantage of the EC's Horizon Results Platform. This platform will serve as a bridge towards policymakers and researchers, giving access to the project's main and prioritised results with a high potential value. In addition, WildPosh will consider the Horizon Results Booster for dissemination and exploitation of results so that the added value of the Key Exploitable Results is amplified.

2.2.2 Intellectual Property Rights management strategy

A Consortium Agreement in accordance with EU and national legislation has been compiled in which (amongst others) the IP rights are arranged. Key principles of this arrangement are that:

- Project results shall be the property of the Partner(s) whose employees, researchers, research fellows, individuals equivalent to those persons or Subcontractors making the inventive step, or the creative step (in case of non-patent IP), carrying out the work generating the results.
- In the case of joint ownership of the results between multiple partners, a joint-ownership agreement should be established detailing the allocation and terms of such joint ownership.
- Ownership of results can be transferred as far as the rights of other co-owners are not affected.
- The Project Management Team will be supported by the Technology Transfer Office (AVRE) of the Coordinator institution (UMONS).

Additional to the Intellectual Property Rights the partners agree that each participant may propose ancillary studies, using the data collected by the entire consortium. The Project Management Team will decide on the allocation of the various proposals. All scientific publications originating from this project will be made available to the public by ensuring Open Access.

3. Quality and efficiency of the implementation

3.1 Work plan and resources

The WildPosh project is composed of nine integrated and interconnected work packages (Fig. 2, section 1.2), where each WP has a set of specific and clearly defined goals. The flow of outputs between WPs is designed to generate trans-disciplinary synergies in the endpoints of the project. In order to maximise the impact of the research, we have dedicated two WPs to disseminating knowledge and generating stakeholder uptake of new approaches, methodologies, and technologies (WP6-7). The temporal structure of the work packages has been designed to enhance both the outputs and the integrated nature of the consortium, with nearly all WPs starting at the beginning of the project, and running across the majority of the proposed duration of WildPosh (48-month period). We have carefully considered risks, and associated contingency plans. Here, we show the temporal structure of WildPosh tasks (Fig. 3).

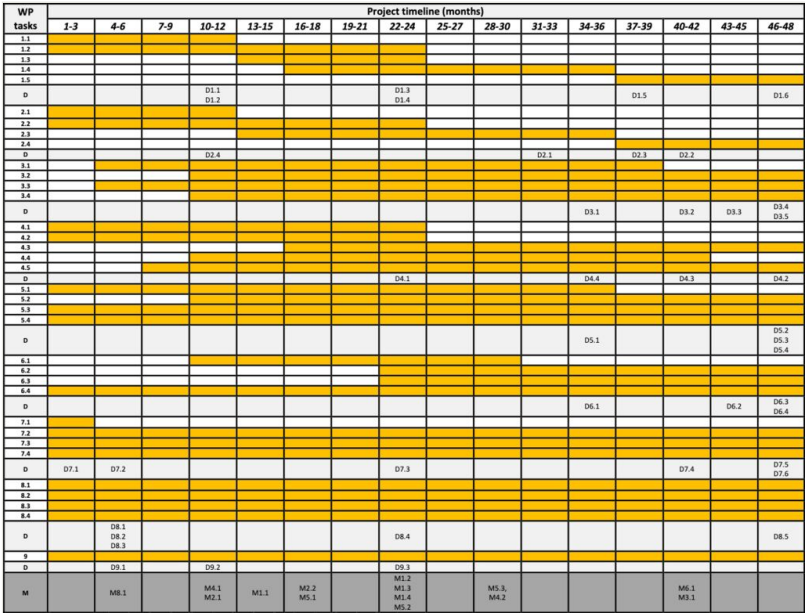


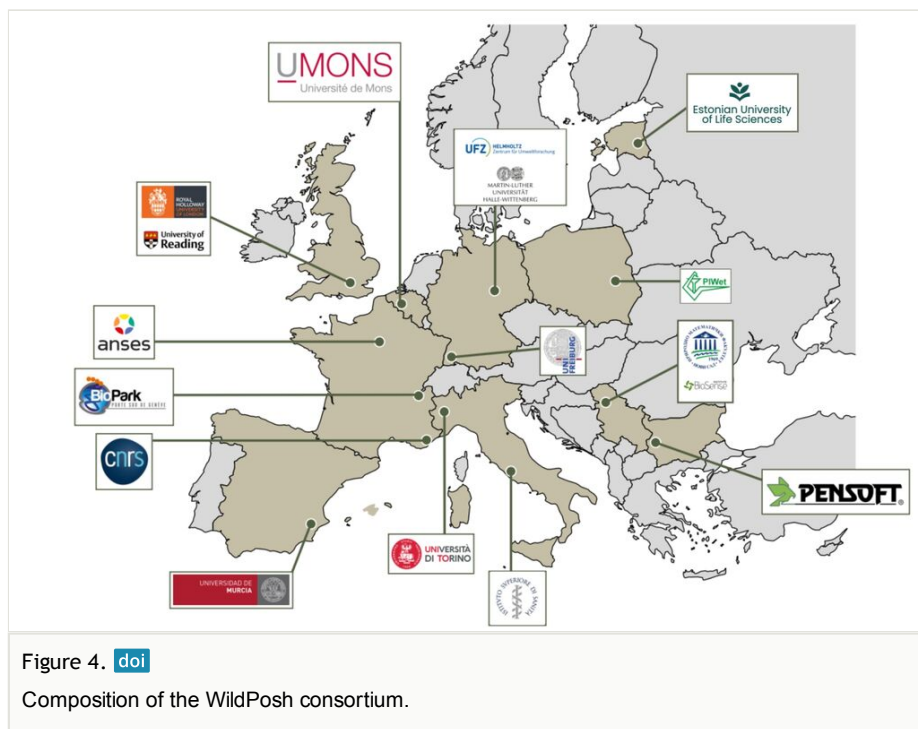
Figure 3. [doi](#)
Gantt chart of tasks, deliverables and milestones.

3.2 Capacity of participants and consortium as a whole

3.2.1 The consortium

The consortium consists of 15 partners widely distributed in Europe (Fig. 4), with extensive experience in Research and Innovation projects under Horizon, with excellent

knowledge on chemistry, modelling, nutritional ecology, proteomics, local stakeholders, environmental chemistry, nutritional biology. There are also two Associated Partners from the United Kingdom: The University of Reading, and Royal Holloway and Bedford New College. These two Associated Partners have extensive experience with European programmes and possess key knowledge on pesticides and pollinator research that is crucial for the implementation of the project. The University of Reading, The Helmholtz Centre for Environmental Research, Albert-Ludwigs-Universität Freiburg, Eesti Maaülikool, and Universidad de Murcia all provide us access to test locations and possess top notch laboratory facilities to conduct pollinator research. Below the specific roles and expertise of these partners is explained.



University of Mons

The University of Mons (UMONS) comprise 1000 researchers in some 100 Departments across its 10 faculties and schools. There are currently 10 autonomous Research Institutes in which the university is organised. Each Institute brings together the expertise of many researchers from all the faculties and schools of UMONS. UMONS is one of the top Belgian research-led universities, comprising more than 700 researchers in some 100 Research departments across its 10 faculties and schools. Through its research and close links with industry, UMONS is also actively involved in regional development through its Research Centers, spin-off and start-up companies that surround the university. Since 2011 UMONS is HR Excellence in Research Award holder (Human Resources Strategy for Researchers (HRS4R)). UMONS has recently become a

European university in the EUNICE alliance. EUNICE aims to be an inter-university campus linking students, teachers, researchers, and administrative staff in a multi-core university campus creating an alternative to nearby, traditional and often perspective-less universities. The Laboratory of Zoology has broad expertise in bee biology, with areas of strength in bumblebee and solitary bee nutrition and health.

Martin Luther University Halle-Wittenberg

The Martin-Luther-University Halle-Wittenberg (MLU) is a world-renowned research institution with a university-wide research focus is 'biodiversity'. With the hire of Prof Robert Paxton (2010) into the department of General Zoology, MLU has become a strong research centre for insect ecology, evolution and pathology. The broad and dynamic intellectual environment of MLU lends itself well to biological research. The membership of MLU and of Prof Paxton with the world-renowned German Centre for Integrative Biodiversity Research Halle-Jena-Leipzig provides additional academic stimulus and infrastructure to undertake cutting-edge research in biodiversity science.

Plateforme Biopark d'Archamps

The Association Plateforme BioPark of Archamps (BIOPARK), is a 1901 association (Non-Profit Organizations Law of 1901) created in 2008. The BioPark, located in the French Genevois region, offers a state-of-the-art technology platform, providing support for Life Sciences research teams, a business nursery specially designed for young biotech and medtech companies, industrial services, expertise and machine time rental. It is composed of a growing community of researchers from academic, and industrial laboratories.

Universidad de Murcia

Universidad de Murcia (UM) is a public university that has more than 31 015 students, with a teaching staff of about 2,553, spread over five campuses. EU programmes represent an important source of funding for the University, which has taken part in more than 185 projects belonging to those programmes. Related to this, the European and International Research Project Office -Operum- was set up in 2007, and it offers support to proposal preparation and submission of international projects, as well as management and control of these projects once these have been approved. It also has a Finance Unit to ensure efficient financial management. Additional services and infrastructures are available to conduct state-of-the-art research in Science.

Università degli Studi di Torino

The University of Turin (UNITO) is a public university with around 80,000 students. The Department of Agriculture Forest and Food Science is a leading research institution covering all crucial disciplines of agriculture science, including pollinator's and environmental health. The Bee Health and Behaviour Lab (BeeLab) coordinated by Prof. Simone Tosi has significant expertise in managed and wild pollinators rearing, behaviour, monitoring, ecotoxicology, and assessment of environmental and

anthropogenic risks. The BeeLab has long-lasting collaborations (i.e., EFSA, EU DG-AGRI) aimed at developing refined estimations of the health and the impact of stressors on key pollinator species.

Pensoft Publishers

Pensoft Publishers (PENSOFT) is an SME specialising in academic book and journal publishing, software development, web design, dissemination and publicity of science news. PENSOFT is well known among academics worldwide for its technologically advanced peer-reviewed Open Access journals, such as Nature Conservation, NeoBiota, ZooKeys, PhytoKeys, Comparative Cytogenetics, Biodiversity Data Journal. The company is actively developing new tools, workflows and methods for text and data publishing, dissemination of scientific information and technologies for semantic enrichment of articles' content. PENSOFT is actively looking to expand the subject-scope of its publishing towards open science publishing practices with the launch of Research Ideas and Outcomes (RIO) - an open science journal that publishes all research ideas & outcomes that constitute the research cycle.

Agence Nationale de la Sécurité Sanitaire de l'alimentation, de l'environnement et du Travail

The French Agency for Food, Environmental and Occupational Health & Safety (ANSES) is a public agency reporting to French ministries for health, agriculture, environment, work, and consumer affairs. The Sophia-Antipolis laboratory employs 25 staff members. The laboratory has a history of 35 years in the field of honey bee biology and pathology. It was nominated as the European Union reference laboratory (EURL) for bee health in 2011, is the French National Reference Laboratory for bee diseases and also WOA reference laboratory for honey bee diseases. The Unit of Honeybee Pathology has a broad expertise in pesticide search in different matrices using robust methods with low level of detection and quantification. The laboratory also gained expertise in database design and management over the years. The laboratory activities are linked to some extent to risk assessment for the protection of bees.

Albert-Ludwigs-Universität Freiburg

The University of Freiburg (UFR) is one of the nation's leading research and teaching institutions, evidenced by its membership in the League of European Research Universities. With the Faculty of Earth and Environmental Sciences and the Faculty of Biology the University has leading experts in biology, ecology, and various disciplines in natural and social environmental sciences. The faculty of Earth and Environmental Sciences engages with stakeholders in the fields of forestry, nature conservation and agriculture. The Chair of the Department of Nature Conservation and Landscape Ecology coordinates and is involved in different leading bee and pollination projects including strong interactions with farmers and beekeepers.

Eesti Maaülikool

The Estonian University of Life Sciences (EMU) is a leading institution for research, survey and monitoring, and training for agricultural and environmental sciences in the Baltic Region in the field of agriculture, biodiversity and landscape ecology. EMU provides independent research to supply National governmental institutions with information on agricultural and apicultural policymaking, natural resource management, environmental protection, biodiversity and to raise public awareness of environmental issues. The university provides world leading research facilities in the Centre of the Renewable Natural Resources. The Institute of Agricultural and Environmental Sciences has been responsible for carrying out research in areas of current public concern such as the Agriculture Programme, Environment Programme, Integrated Pest Management Programme, Apiculture Programme and agri-environmental measures.

Panstwowy Instytut Weterynaryjny - Panstwowy Instytut Badawczy

The National Veterinary Research Institute (PIWET)'s main mission is scientific research in food safety and zoonotic disease diagnosis and control. The Department of Pharmacology and Toxicology has a dedicated Pesticide Residue Analysis Team, which serves as the National Reference Laboratory (NRL) for pesticide residues in food of animal origin and honey, implementing and supervising nationwide monitoring in this area. Since 2014, the Team has also been assessing the exposure of bees in Polish apiaries to pesticides and acting as a nationwide diagnostic centre for bee poisoning incidents. We have deep experience in developing miniaturised analytical methods using gas and liquid chromatography coupled to tandem mass spectrometry (LC-MS/MS, GC-MS/MS) dedicated to sensitive and reliable assessment of exposure and risk of pesticide residues on bees.

University of Novi Sad Faculty of Sciences

The University of Novi Sad Faculty of Sciences (UNSPMF) is one of the leading higher education institutions in Serbia. The Department of Biology and Ecology focuses on taxonomic analyses of autochthonous fauna and flora, monitoring of biodiversity (including the genetic diversity), causes, trends, extent of changes in ecosystems, conservation actions that mitigate and prevent processes of alteration. The Department has well-equipped facilities for insect identification, field work, genetic and molecular research, biochemistry and ecotoxicology: insect reference collections, facility for DNA extraction, amplification (PCR) and analyses, microscopy facility, other equipment for molecular and taxonomic analyses, laboratory for biochemistry and insect ecotoxicology.

Helmholtz-zentrum Fuer Umweltforschung GmbH

The Helmholtz Centre for Environmental Research (UFZ) is one of the world's leading research centres in the field of environmental research. It demonstrates ways in which sustainable use of natural resources is possible for the benefit of both mankind and the environment. The UFZ currently focusses on the following research areas in a highly integrative manner: (i) Environment and Society, (ii) Ecosystems of the Future, (iii) Water

Resources and Environment, (iv) Chemicals in the Environment, (v) Environmental Engineering and Biotechnology, (vi) Smart Models and Monitoring. The Department of Community Ecology merges animal and plant ecology, and population and community ecology, to ask how biodiversity and ecosystem stability are interrelated, and how land use and global change will impact biodiversity.

Istituto Superiore di Sanità

Istituto Superiore di Sanità (The Italian National Institute of Health) (ISS) is the main research institution in the field of public health in Italy and the technical and scientific body of the Italian National Health Service. The Environment and Health Department, participating in this proposal, is involved in research and regulatory activities in the field of toxicology, toxicity, environmental and human health risk assessment and in the development and promotion of new methodologies (e.g., (Q)SAR models, toxicokinetics and toxicodynamics, integrated approaches for testing and assessment) supporting risk assessment procedures. The Department provides scientific consultancy services at national and international level with international regulatory Agencies (EFSA, ECHA, JRC, OECD).

Centre Nationale De La Recherche Scientifique

The joint research Unit IAB-Institute for Advanced Biosciences University Grenoble Alpes (UGA) UMR is set up by the French National Centre for Scientific Research (CNRS), INSERM and is part of the Medicine Faculty of UGA. IAB is composed of 18 research teams and groups and supported by 5 technical core facilities. The CNRS is a government-funded research organisation under the responsibility of the French Ministry of Research. With 32,000 people and 1,115 research units spread throughout the country, CNRS carries out research in all scientific fields of knowledge. Moreover, CNRS conducts interdisciplinary programs, one major objective being to promote interdisciplinarity to improve knowledge, ensure economic and technological development or solve complex societal needs.

University of Novi Sad, BioSense Institute-Research Institute for Information Technologies in Biosystems

BioSense Institute - Research and Development Institute for IT in Biosystems (BIOS), is a pioneer in digital transformation for a sustainable environment in Serbia, founded in 2015, as a part of the University of Novi Sad. Exploring scientific and technological frontiers regarding the application of IT in biosystems, the Institute strives to deliver state-of-the-art digital solutions to a range of sectors, from agriculture to nature conservation. The Center for Biosystems of BioSense Institute is devoted to understanding the diversity and functioning of biosystems, and research of morphology, genetics, distribution, and ecology of insect pollinators, particularly syrphid flies, is in the focus of several researchers of this Center.

Royal Holloway and Bedford New College

Royal Holloway (RHUL) is one of the UK's leading research-intensive universities. The Department of Biological Sciences is a research-led department, with 30 PIs whose work ranges from gene therapy to the health of bees, and an annual research income of ~€4.5m. It contains dedicated bee research laboratories and rearing rooms, and a new apiary to facilitate research in this area. In addition to our expertise, as described above, we have deep expertise in bee-pesticide interactions, and led the H2020 consortium PoshBee, which was a precursor to the current project.

The University of Reading

The School of Agriculture, Policy and Development at the University of Reading (UREAD), is a world leader in both teaching and research, maintaining a reputation developed since the 1800's. Our focus is to provide knowledge to address the major challenges and opportunities in our sector for the 21st century, including sustainable food production, food and nutritional security, adaptation and mitigation to climate change, food chains and health, animal welfare and behaviour, poverty alleviation, and international development. Our co-developed research has a well-established track record for impact, with long-term partnerships with a wide range of local, national and international stakeholders from industry, policy and NGO's. We have broad experience in running and delivering at the international level in terms of impact and knowledge (e.g., we led the UN IPBES Global Pollinator Assessment).

4. Ethics Self-Assessment: Ethical dimension of the objectives, methodology and likely impact

A number of activities in WildPosh have been identified which may raise ethical concerns. All ethical, legal, social and safety issues that may arise from the WildPosh project are addressed below:

Protection of data. The research does not intend to focus on the processing of personal data. However, it is possible that during this project personal data will be collected. If it is decided that personal data is relevant and needs to be processed for the sake of the project and for achieving project objectives, participants are asked to consent to their data being used for the project. The templates of the informed consent forms and information sheets covering the voluntary participation and data protection issues (in language and terms intelligible to the participants) will be submitted as a deliverable before the relevant recruitment commences. Detailed information on the procedures for data collection, storage, protection, retention, and destruction, and confirmation that they comply with national and EU legislation will be submitted as a deliverable. Personal data will not be shared as part of project results, but will only be published as anonymized metadata.

Animals. The project intends to test and monitor the impact of pesticides on the most predominant pollinators in the EU: bees (species: *Andrena vaga*, *Anthophora plumipes*, *Colletes hederæ*, *Lasioglossum malachurum*, *Osmia brevicornis*), moths and butterflies (species: *Macroglossum stellatarum*, *Papilio machaon*, *Pieris brassicae*, *Vanessa cardui*, *Zygaena filipendulae*) and syrphid flies (species: *Eristalis tenax*, *Eristalinus aeneus*, *Episyrphus balteatus*, *Myathropa florea*, *Cheilosia canicularis*). With WildPosh, we aim to characterise the exposure of wild pollinators to pesticides in field conditions and test in controlled conditions their toxicokinetic, lethal and sublethal effects. At the same time we aim to develop ambitious databases on pollinator traits and distribution, and on pesticide toxicity to predict risk of exposure and sensitivity at multiple levels (i.e. population, landscape, global). The testing of these pollinator species is therefore necessary and an important requirement for the success of the project. Some of these species are largely impacted by pesticides already. That is why the project intends to focus on insect species who are not endangered and still abundant. In WP9, it is explained that our Ethics Summary Report will be dedicated to a Document of Good Practices in laboratory and field work, in order to make an active effort to reduce the number of specimens killed during the experiments. For the moment this is not a legal requirement, as the legislation about ethics on manipulation of vertebrates and other invertebrates does not apply to insects at the moment. However, a growing body of scientific literature shows that insects may have consciousness, thus they probably feel pain as a subjective sensation. We will make active efforts to reduce the numbers of killed specimens to the minimum necessary. The protocols developed will be critically evaluated in order to understand where the numbers of live specimens needed can be reduced while not endangering the success of the data collection, and to pinpoint where non-lethal methods can be used. This way the impact of the experimental work of this project can be reduced to the minimum, and use only the number of specimens that are necessary in order to answer the scientific questions.

Non-EU countries. Part of the work that is performed in the project will be done in the United Kingdom (England) and Serbia. The aim of the project is to study the impact of different pesticides on pollinators in real life environment conditions and specific laboratory tests. Given that the test site in the UK is one of 5 test sites, with all different environmental and pesticide conditions, and unique selections of insects and insect species, it is necessary that these different types of sites are included in the project. The experiments in Serbia are related to Syrphid flies as our Serbian partners are leaders in the research on the conservation of this group of flies.

Environmental protection and safety. Because the proposal will use agrochemicals that will harm bees, moths, syrphid flies and butterflies, the research thus involves elements that may cause harm to animals and humans. We confirm that appropriate health and safety procedures conforming to relevant local/national guidelines/legislation are followed for staff involved in this project. We do not intend to do any harm to people in our project and besides. To prevent harm to animals or might have a negative effect on the environment, we will develop very critical and strict protocols for testing, to make sure that the impact is as limited as possible.

Compliance with ethical principles and relevant legislations. This project will comply with the following EU legislation: All data will be handled and processed under applicable international, EU and national law, with particular adherence to the General Data Protection Regulation (EU regulation 2016/679). There is no legislation applicable to insect experiments in laboratory conditions.

Grant title

WildPosh (Pan-European assessment, monitoring, and mitigation of chemical stressors on the health of wild pollinators) receives funding from the European Union's Horizon Europe research and innovation programme under grant agreement No. 101135238. Views and opinions expressed are those of the author(s) only and do not necessarily reflect those of the European Union or the European Research Executive Agency (REA). Neither the EU nor the REA can be held responsible for them.

Conflicts of interest

The authors have declared that no competing interests exist.

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