

Grant Proposal

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PoshBee: <u>Pan-European Assessment</u>, Monitoring, and Mitigation of Stressors on the Health of Bees

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1. Excellence

1.1 Objectives

PoshBee is a multi-actor, trans-disciplinary project whose overarching goal is to significantly enhance the sustainable health of bees and pollination services in Europe. We will: 1) provide the first pan-European quantification of the exposure hazard of chemicals to managed and wild bees; 2) determine how chemicals alone, in mixtures, and in combination with pathogens and nutrition, affect bee health; and 3) through interactive innovation meet the demand-driven need for monitoring tools, novel and innovative screening protocols, and practice- and policy-relevant research outputs to local, national, European, and global stakeholders. Our work will support healthy bee populations, sustainable beekeeping, and sustainable pollination across Europe.

Specific Objectives:

- 1. **Exposure hazard:** drawing on the complementary expertise of a diverse range of actors, we will quantify the exposure of honey bees, bumble bees, and solitary bees to chemicals within major agricultural cropping systems across Europe. This will fill a major knowledge gap in the exposure hazard of chemicals for bees.
- 2. **Ecotoxicokinetics:** through the development of innovative protocols and novel model systems, co-created with end-user partners, we will assess toxicity and dynamics of key agrochemicals, and their mixtures, in individual honey bees, bumble bees, and solitary bees. This will provide key information for the development of improved policy and regulations for the safe and sustainable use of agrochemicals in Europe.
- 3. **Health effects:** taking a trans-disciplinary approach, we will integrate across laboratory, semi-field, field, and landscape studies to provide a holistic understanding of how chemicals, their mixtures, and their interactions with pathogens and nutrition drive health in honey bees, bumble bees, and solitary bees. This will support beekeepers and other stakeholders improve bee health management, and fill a major knowledge gap on how hazards interact to threaten bee health.
- 4. **Modelling bee health:** PoshBee will develop the first mechanistically-underpinned holistic model of bee health. Building on current MUST-B approaches, and working with MUST-B, PoshBee will validate and develop agentbased, landscape-explicit models for risk assessment in honey bees, bumble bees, and solitary bees.
- 5. **Monitoring tools and protocols:** we will provide validated tools for the monitoring and assessment of bee health and exposure to stressors. We will develop and test an innovative 'air sensor' tool for assessing chemical exposure within honey bee hives. Using proteomics, we will produce a novel molecular monitoring tool, or 'health card' for bees, that measures chemical exposure, pathogens, immune capacities, and nutritional state.
- 6. **Driving policy and practice:** PoshBee will develop a European bee health knowledge exchange hub by working together with key stakeholders in the honey bee, agrochemical, farming, pollination service, research, EU policy and regulatory, and bee conservation sectors. Together, we will synthesize 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 sustainable beekeeping, bee health, and pollination services.

1.2 Relation to the work programme

POSHBEE will fully address, and go beyond, the specific challenge and scope of call SFS-16-2017:

Challenge and Scope	PoshBee
Hazard identification for	PoshBee will use a multi-actor approach – integrating academics, beekeepers and
the chemical exposure of	farmers – to establish the first pan-European site network across major cropping
bees, interactions with	systems for the assessment of chemical exposure in bees. Standardised state-of-the-
pathogens and nutrition,	art techniques will measure sources and levels of exposure at bee, food-intake,
and their relation to bee	hive-matrix, and environmental levels, and together with analyses of factors and
health in the field. Factors	processes driving chemical usage, will produce a holistic understanding of the
and processes leading to	causes and routes of chemical exposure across bee species. Ultimately, we will
contamination	demonstrate how continental-scale variation in chemical exposure, pathogens, and
	nutrition relate to bee health.
Effects and dynamics of	Taking a trans-disciplinary and multi-actor approach – incorporating academia,
common agrochemicals	government research institutes, and industry – PoshBee will determine the
and veterinary medicinal	ecotoxicology and toxicokinetics of major agrochemicals and their mixtures across
products in European bees,	individuals of different life-stages, sexes, and castes, and how this varies across
from individuals to	species to define species-specific thresholds and dose-response curves. We will
populations. Development	develop and validate new protocols to assess ecotoxicology across sex, caste, and

and validation of relevant	species. This will generate, for the first time, a comprehensive understanding of the
test protocols	effects of agrochemicals at the level of individuals. At a higher level, we will
	exploit the site network to determine how individual effects scale to
	colony/population effects of agrochemicals and other stressors, focusing on
	buffering and compensatory effects. This will produce a holistic understanding of
	the emergent effects of agrochemicals on colonies, hives, and populations of bees.
The effects of chemicals on	PoshBee will generate the first pan-European database of natural variation in
bee health. when	multiple chemical exposure, and how this relates to bee health. We will determine
modulated by pathogens	the key chemical-pathogen-nutrition interactions, their causal relationship with bee
and nutrition	health and produce a universal and predictive model of how such interactions
	affect bee health across solitary humble and honey bees as well as agent-based
	landscape explicit models for rick assessment of these effects in honey bees and
	humble bees
Develop 'omics as a tool to	PoshBee will develop a novel proteomics-based tool that generates a 'health-card'
measure exposure and	for individual bees, across a range of species, incorporating chemical exposure
health across bees in future	nathogens, and nutritional stress. It will identify molecular markers of chemical
monitoring plans	and pathogon exposure and putritional stress, which will be validated in the field
monitoring plans	Duilding on surrout local and national schemes. DeshDes will provide guidenes on
	Building on current local and national schemes, PosnBee will provide guidance on
	now this tool can be deployed in future long-term monitoring schemes.
A multi-actor project, with	PoshBee is a bottom-up multi-actor consortium that includes representatives of all
dissemination to	major stakeholder sectors. Stakeholders are integrated both as active research
stakeholders	partners, and on the advisory committees of the consortium, and their
	understanding of problems and opportunities around bee health have contributed to
	our overall project design. An integrated knowledge-exchange plan will translate
	our results into stakeholder-driven initiatives to change policy and practice, to
	enhance sustainable beekeeping and sustainable pollination.
Synergy and cooperation	Members of PoshBee are leaders or contributors to a range of past and on-going
with European projects	EU-funded projects on bee health, including MUST-B, enabling the development
1 1 0	of synergies and integration with current activities. Specifically, PoshBee will
	extend the modelling work of MUST-B across EU countries and across bee taxa.
	The EU reference laboratory (EURL) for bee health is a leading member of
	PoshBee enabling integration and cross-talk between PoshBee and EURL projects
	In addition, we aim to develop, through EIP-AGRI, a 'Bee Health and Sustainable
	Pollination' focus group. Integration and cooperation with the successful SFS-28-
	2017 project, and with the European Food Safety Authority (EFSA), will be
	achieved through their incorporation into our scientific and stakeholder
	committees
	commuces.

1.3 Concept and methodology

1.3 (a) Concept

Chemicals are an integral feature of modern agriculture, controlling pests and disease in crops and domesticated animals. However, agrochemicals can also have negative effects on non-target organisms, with ensuing environmental costs [Hallmann et al. 2014]. The potential effects of agrochemicals on bee health is a high profile, yet unresolved case [Godfray et al. 2015], as bees provide the essential ecosystem service of pollination, but are at risk around the globe [Potts et al. 2016, Vanbergen et al. 2013]. Here, we broadly define bee health as the ability of individuals/colonies/populations to self-perpetuate, whilst providing sustainable pollination services. Previous research has shown that agrochemicals affect behaviour, immunity, lifespan, physiology, and reproduction of individual bees and colonies, in honey bees, bumble bees, and solitary bees [Godfray et al. 2015]. At a population scale, correlational data associate neonicotinoid agrochemicals with declines in abundance and range of wild bees [Woodcock et al 2016]. Furthermore, the effects of chemicals can be modified by interactions among themselves [Traynor et al. 2016], with pathogens and disease [Doublet et al. 2015], and with the nutritional state of the bees themselves [Alaux et al. 2010]. However, there is a severe lack of both real-world exposure risk data for agrochemicals and bees, and insights into how such exposure translates into effects in the field [but see Rundlöf et

al. 2015, Tsvetkov et al. 2017, Woodcock et al. 2017]. In addition, our current knowledge is largely limited to honey bees and bumble bees, with little understanding of how important solitary bee pollinators (~95% of all bee species) are affected by these hazards.

To address these issues, PoshBee has developed from the ground-up as a demand-driven project co-created via a multi-actor approach. The coordinator (Brown, RHUL) convened a stakeholder meeting at ANSES (Paris) attended by all key sectors (May 2016). This built on the current knowledge base to identify stakeholder concerns (e.g., species-specific effects, field exposure effects, etc.), which have been incorporated into the PoshBee workplan. Stakeholders attending the meeting included: <u>Agrochemical industry</u> (BASF, Bayer, Syngenta); <u>Beekeeper organisations</u> (Apimondia, COLOSS, European Professional Beekeepers Association); <u>Conservation NGOs</u> (Bumblebee Conservation Trust, Pesticide Action Network (PAN) Europe); <u>Farming organisations</u> (COPA-COGECA – Committee of Professional Agricultural Organisations-General Committee for Agricultural Cooperation in the European Union, European Crop Protection Association); <u>Global health policy</u> (OIE – World Organisation for Animal Health); <u>Pollination industry</u> (Agrobio, Atlantic Pollination, Biobest, Red Beehive Ltd., Serbia Solitary Bees, with others, are integrated into the PoshBee structure as partners or members of the Stakeholder Advisory Committee (see Section 3.2), guaranteeing stakeholder commitment and participation in planning, research, dissemination, and knowledge exchange.

The PoshBee research concept rests on three integrated objectives: 1) determining the real-world agrochemical exposure profile of honey bees, bumble bees and solitary bees, and how this interacts with pathogens and nutrition to affect bee health, 2) using integrated and controlled laboratory, semi-field, and field experiments to characterise causal relationships between agrochemicals and bee health, incorporating exposure to pathogens and nutritional stress, and 3) developing novel protocols and tools to enable future monitoring of bee health (Fig. 1.1). These actions act to mitigate threats to bee health, and sustainably manage beekeeping and pollination across Europe.



Figure 1.1 The conceptual structure of PoshBee, which integrates and incorporates different levels of structure and analysis to address the challenges and scope of call SFS-16-2017

Chemical exposure varies geographically and across cropping systems. Consequently, we use a pan-European site network (8 countries, 128 sites; Fig. 1.3, see below), developed with our multi-actor partners, which focuses on two key crops for bees, oilseed rape and apples, chosen on the basis of their importance for the EU agro-economy (production in 2015: oilseed rape – 16 million tonnes from >6.5 million ha, apples – 12.7 million tonnes from >35% EU orchard area – source: Eurostat), their dependency on animal pollination, and value as forage to bees. We will use a sentinel colony methodology, combined with analyses of the factors and processes leading to contamination, to provide a landscape-level quantification of chemical exposure, pathogen levels, nutrition, and bee health across Europe.

To produce an in-depth mechanistic understanding of effects, we will use an integrated set of laboratory, semi-field, and field experiments. Faced by a hugely diverse and ever-expanding array of chemicals and pathogens, we will focus on: (i) exemplars (chosen on the basis of widespread use and economic value) from 3 major groupings of agroand veterinary-chemicals (insecticide/acaricide, fungicide, herbicide – henceforth, agrochemicals), and (ii) the key honey bee pathogens *Varroa destructor*, *Nosema* spp., American Foulbrood, and Deformed Wing Virus (DWV), and key pathogens for bumble bees (e.g., *Nosema* spp.) and solitary bees (currently unknown, to be determined in project). Using these arrays of chemicals and pathogens, together with nutritional stress (defined by quantity and quality of food), we will develop and use innovative experimental designs to assess how different groups of chemicals affect bee health at the individual, colony, and population levels. Field experiments will exploit the chemical exposure site

773921, PoshBee, Part B

network, enabling us to assess potential buffering mechanisms and thus scale from laboratory experiments to field-realistic effects. We will integrate our results into a holistic model of 'bee health' to enable extrapolation of our results to other chemicals, pathogens and nutritional stresses, crops, and bees. Combining our causal effects data with the exposure profiles generated in the site network, we will be able to characterize the risk that chemicals pose to bee health.

Finally, practice and policy are currently held back by important evidence gaps and a lack of novel protocols and tools. We will provide new model 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, pathogens, and nutritional stress in the field and the hive, and an 'air screen' tool to assess chemical exposure. We will work with MUST-B and build on their current work to produce models that enable environmental risk assessment for honey bees, bumble bees, and solitary bees across European landscapes. Together with key European stakeholders, we will broker knowledge exchange to enhance and support future policy and practice to foster bee health and sustainable pollination.

PoshBee takes a fundamentally trans-disciplinary approach, incorporating the academic disciplines of apiculture, chemistry, modelling, nutritional ecology, parasitology, proteomics, and social science, with industrial and NGO 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 the expertise of apiculture through local stakeholders, environmental chemistry, nutritional biology, parasitology, and proteomics. Such an approach underlies our entire proposed work programme.

Positioning of PoshBee with respect to Technology Readiness Levels

PoshBee encompasses the development of tools and protocols from basic principles to technology. Key examples include (i) a novel 'air screen' tool to assess chemical exposure in-hive (predicted progression: TLR2-TLR5); (ii) a proteomics based tool, or 'health card', that monitors exposure to and effects of chemicals, pathogens, and nutritional stress in managed and wild bees (predicted progression: TLR3-TLR6); (iii) two new model solitary bee systems for ecotoxicology (predicted progression: TLR2-TLR5); (iv) new methods and tested protocols for ecotoxicology of bumble bees and solitary bees (predicted progression: TLR2-TLR5).

Links to national and international research and innovation activities

PoshBee will benefit from links with a significant array of international research and innovation, and policy activities. Our partners lead/led or play(ed) key roles in global, European, and national projects, and PoshBee will benefit uniquely from the outputs of these projects as a result (Table 1.1).

Activity	Programme	Partners	Relevant outputs
MUST-B	EFSA multi-	ANSES, AU	State-of-the-art knowledge of risk
	annual project		assessment for stressors; capturing relevant
			data for models; development of
			environmental risk assessment models for
			bees in European landscapes
EPILOBEE	EC & 17 EU	ANSES (lead)	Regional and annual variation in honey bee
	nation states		mortality; pathogen drivers of mortality
SMARTBEES	EU-FP7	ANSES, SLU,	Solutions to prevent honey bee colony losses
		UNIUD	
STEP	EU-FP7	UREAD (lead),	Status and trends of bee populations;
		BERN, Pensoft, SLU,	potential drivers of these trends
		UFZ	
BEEDOC	EU-FP7	BERN, INRA, MLU,	Effects of pathogens and agrochemicals on
		SLU	honey bee health
COLOSS	EU-COST	BERN (lead),	Patterns in honey bee mortality; protocols;
	Network (now	ANSES, CREA,	dissemination methodology
	NGO)	INRA, MLU, UM,	

Table 1.1 Key recent & on-going international research, innovation, and policy activities that feed into PoshBee.

		SLU, TCD, UNIUD,	
		UREAD	
SUPER-B	EU-COST	ANSES, BERN,	State-of-the-art knowledge on pollination;
	Network	MLU, RHUL, TCD,	drivers of pollinator loss; mitigation of
		UFZ, UREAD	drivers
IPBES Pollinators,	UN	UREAD (co-lead)	Status and trends of pollinators; value of
Pollination and Food	Environment		pollinators and services; threats and response
Production	Programme		options

In addition to these international programmes, PoshBee partners are involved in a large number of national programmes across Europe addressing bee health and sustainable pollination. Overall, these diverse and strong links mean that PoshBee will benefit hugely from integration with past, current, and future activities at national, European, and global levels.

1.3 (b) Methodology

Summary PoshBee will determine the exposure of honey bees, bumble bees, and solitary bees to agrochemicals across Europe. It will combine these profiles with data on pathogen prevalence and nutritional state to explain how chemical exposure affects bee health across different crops and biogeographical zones. Through laboratory, semi-field, and field experiments we will determine the ecotoxicology and causal effects of chemicals, both single and mixed, on bee health for exemplar managed and wild, social and solitary bees, incorporating interactions with pathogens and nutritional state. Finally, we will develop new protocols for ecotoxicological studies of bees, and new tools and models to monitor exposure and predict the effects of chemicals, pathogens, and nutritional stress in bees in the wild. All of these outputs will be shared, including through the EIP-AGRI system, with the broadest stakeholder community, to enable changes in policy and action on the ground (Fig. 1.2).

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



Figure 1.2 The PoshBee work flow – see text below for detail on contents of individual work packages

WP1: A Site Network for Assessing Exposure of Bees to Chemical, Nutritional, and Pathogen stressors (TCD lead) Together with multi-actor partners, we will develop a European-wide sentinel approach to identify exposure to chemical hazards. WP1 will build a site network that spans Europe's main biogeographical regions (Fig. 1.3), with 128 sites distributed across two of Europe's major arable and horticultural cropping systems dominated by apple and oilseed rape across a range of predicted chemical contamination, based on landscape-level agricultural intensity. Site design within countries will be developed together with our local farming organisation partners. We will use sentinel hives of honey bees (*Apis mellifera* – the four major European subspecies), sentinel colonies of bumble bees (*Bombus terrestris*) and sentinel populations of solitary bees (*Osmia bicornis*) (with the latter two representing wild bees) to harvest samples for the measurement of chemical exposure across a range of bee developed with our local beekeeping organisation partners, for bumble bees with the SME partner Biobest, and for solitary bees with the SME partners

ATPOLL, RBH, and WILD. We will also collect bee samples for determining pathogen load, and pollen samples for dietary analysis. Land-owners/users will be surveyed to assess past and current chemical usage at sites. Measures of bee health will be taken, including colony growth and reproductive fitness. WP1 will deliver samples for analysis to WP2 and WP9, field sites and landscape data to WP7 and WP8, and farmer and beekeeper survey data to WP2 and WP10.

WP2: Measuring Chemical Exposure, Pathogens and Aspects of Nutrition in Honey Bees, Bumble Bees, and Solitary Bees (ANSES lead) Samples from WP1 for chemical analysis will be screened using LC-MS/MS and GC-MS/MS technology, to determine their relative chemical contamination. We will analyse these data in conjunction with records from farmers and beekeepers of chemical usage inside and outside of hives, to determine the driving factors and processes leading to chemical contamination. Together, these data will provide a unique dataset:



Figure 1.3 Top-level distribution of the 8 study regions, each with 16 sites

the chemical exposure profile of social and solitary bees for two major cropping systems across Europe, against a range of chemical use. Measuring the hazard of chemical exposure is not the same as demonstrating cause-and-effect relationships between chemicals and bee health. Nevertheless, we will use the bee health data from WP1, together with nutritional data and pathogen/disease-load, to determine how varying levels of chemical contamination correlate with bee health, and how interactions with pathogens/disease and nutrition modulate health effects. Finally, we will develop a novel 'air screen' tool to measure chemical exposure in and outside the hive.

WPs3-8: Effects of Chemicals, Chemical Mixtures, and Interactions with Pathogens and Nutrition (MLU oversight) In an array of integrated tasks, we will generate a comprehensive experimental assessment of the causal effects of the EU's most commonly used agrochemicals, their mixtures, and their interactions with pathogens and nutrition, on bee health, including males and for social species, queens and workers. Males and queens are significantly under-represented in research on chemical effects, despite their vital role in mating and reproduction; PoshBee will address this shortfall by incorporating them into comprehensive testing. These WPs will be crowned by the development of holistic and agent-based models that will enable the development of environmental risk assessment tools for honey bees, bumble bees, and solitary bees. The WPs below represent integrated WPs, providing clarity of scientific and organisational structure. MLU will provide oversight to all of these WPs, to enhance and ensure integration across their activities.

WP3: Toxicokinetics, Toxicodynamics and Interactions Among Agrochemicals (EMU lead) We will use controlled laboratory approaches to identify lethal, chronic, and sub-lethal toxicological effects of chemicals on bee health. Building on the current state of knowledge, and together with our SME partner Vita Europe, we will determine dose-response curves, LD50s, species-specific thresholds, and toxicokinetics/dynamics for three groups of chemicals (insecticide/acaricide, fungicide, herbicide), and mixtures thereof, on individual honey bees (all four major European subspecies), bumble bees (*B. terrestris*), and solitary bees (*O. bicornis*). We will do this for workers, queens, males, and larvae/pupae, reaching beyond the current state of the art with its focus on female worker honey bees. We will develop and validate novel protocols for toxicological testing in both bumble bees and solitary bees, as current guidelines have been developed primarily for honey bees.

WP4: Development of Novel Wild Bee Species for Risk Assessment (MLU lead) Currently, model systems for ecotoxicological testing in solitary bees are limited to a few species of stem-nesting bees (e.g., *O. bicornis*). However, most solitary bees (~65%) are ground-nesters, and consequently will have a very different exposure profile to chemicals, as recognised explicitly by EFSA [2013]. Together with an SME (WILD), we will develop new model species of ground-nesting solitary bees, and carry out preliminary ecotoxicological studies to produce new protocols for testing agrochemical effects. The SME will lead on developing these species as managed crop pollinators, a further step towards sustainable pollination in Europe.

WP5: Effects of Agrochemical-Nutrition Interactions on Bee Health in the Laboratory (UMONS lead) We will conduct laboratory experiments to determine how chemical exposure and nutritional stress interact to affect

bee health. Using variation in the quality, quantity, and diversity of food sources, we will develop novel protocols for assessing the interactive effects of chemicals and nutrition on honey bee, bumble bee, and solitary bee health, and assess the affect of chemical exposure on nutritional intake. This WP will deliver a step-change in our understanding of how chemical effects are modulated by nutrition across a range of bee species.

WP6: Effects of Agrochemical-Pathogen Interactions on Bee Health in the Laboratory (BERN lead) Pathogens can also modify the effects of chemicals on bee health. Focusing on *Varroa destructor*, *Nosema spp.*, American Foulbrood, and DWV (for honey bees), and key pathogens (e.g., *N. bombi*) of bumble bees and solitary bees, we will conduct laboratory experiments to determine how they modulate the effects of chemicals on bee health. Together with WP5, this will provide a comprehensive dataset for the determination of semi-field and field experiments (WP7), and a base for generalising how such interactions affect bee health (WP8).

WP7: Effects of Chemicals and their Interactions with Other Stressors on Bees Tested in Semi-field and Field Experiments (ALU-FR lead) Chemical effects elucidated in laboratory trials do not necessarily scale-up when exposed to field testing. From our chemical exposure profile (WP1,2), and chemical effects (Tasks 3.1-4) work, we will identify key chemicals, their mixtures, and chemical/pathogen combinations for semi-field and field-testing. Together with SME partners (ATPOLL, RBH) we will develop innovative protocols for testing chemical effects in honey bees, bumble bees, and solitary bees across our site network (WP1). We will determine how effects on individuals scale up to effects on colonies/populations, and the buffering mechanisms that might mitigate these effects, using state-of-the-art, semi-automated techniques.

WP8: Systems and Agent-based Modelling Approaches to Assess the Synergistic Effects of Multiple Stressors on Bee Health (UNIUD lead) We will use data from bees exposed to natural stressors to define the parameter space for health in honey bees, bumble bees, and solitary bees. From this we will produce a holistic model for bee health, under the pressures of chemical exposure, in order to develop an operational definition of bee health. This mechanistic framework will provide the first tool for the predictive analysis of future threats from novel chemicals and changes in chemical usage. Building on this, and on current work by MUST-B (the lead of which, AU, is a partner in PoshBee), we will develop agent-based models for environmental risk assessment for honey bees, bumble bees, and solitary bees across representative European landscapes. These will provide standardised environmental risk assessment models for European bees.

WP9: OMICS of Agrochemical Responses in Bees (Biopark/CNRS lead) We will develop a new, 'omics-based tool – BeeTyping® – that uses molecular markers to monitor chemical and pathogen exposure, immune function, and nutritional state in bees. Taking advantage of approaches in medical science (biotyping by Mass Spectrometry, MS) we will develop a proteomics-based barcoding system (by MALDI-MS) to provide a 'health card' for bees that is cheap and easy to use. We will use laboratory studies validated with field samples, to build models that provide an integrated and dynamic database of proteome changes in response to chemical and pathogen exposure and nutritional stress. This will provide a multi-scale monitoring tool for sanitary policy and beekeeper best practice.

WP10: Knowledge Exchange and Impact Strategy (UREAD lead) At the start of the project, we will develop a strategy for overall knowledge exchange and conduct a stakeholder mapping exercise. For each expected PoshBee output linked to relevant end-users, a detailed knowledge exchange plan will be developed to ensure effective impact. The incentives for, and barriers to, the adoption of PoshBee tools by beekeepers and growers will be assessed in order to maximise the impacts of the project outputs. This WP will synthesize project findings, as well as external knowledge, in three main areas: (i) Exposure; (ii) Effects; and (iii) 'Omics, to provide stakeholders with the best quality information. The synthesis work will underpin the development of policy briefs on high priority topics identified by the policy community in the stakeholder mapping exercise. WPs 2-9 will produce a set of protocols, tools, and best practice guides; a key task in WP10 will be to develop and make these outputs available for the stakeholder community in a timely manner and available for knowledge exchange activities. We will establish a horizon scanning expert group to forward scan for upcoming threats and opportunities, building on previous innovative work by consortium members [Brown et al. 2016].

WP11: Dissemination, Communication and Knowledge Transfer (Pensoft lead) We will establish and implement a project-wide Data Management Plan that ensures research data quality, sharing and security, thereby providing a platform for integration and knowledge exchange activities. We will develop, with our stakeholders, a multi-pronged approach to develop and disseminate the results of our project as actionable information. Stakeholder partners, and members of the Stakeholder Advisory Committee (see Section 3.2), will disseminate information and novel practice

and tools to their communities. Our project website (<u>http://www.poshbee.eu/</u>) will serve as a central hub for this, and for broader dissemination to the wider EU and global communities. Bee health is a complex and controversial topic, and provides a perfect opportunity to develop tools and mechanisms for educating and informing the wider European public. Together with WP10, we will develop policy briefs and disseminate them to relevant local, national, and European stakeholders.

WP12: Project Management and Scientific Coordination (RHUL lead) PoshBee will be led by RHUL, which has long-standing experience in coordinating EU projects and will provide overall coordination to the consortium. This central administration, supported by WP leaders, will enable progress in research and fully integrate stakeholders across the length and breadth of the project. An explicit equality and diversity plan will underlie management and recruitment, ensuring the highest levels of equality within the consortium.

WP13: Ethics Requirements (RHUL lead) The consortium will comply with the ethical requirements identified in the ethics screening– with reference to protection of personal data, environmental protection and safety, humans, and third countries. See also below section 5.1 Ethics and deliverables linked to WP13.

Across these work packages we will examine the interactions between agrochemicals, other stressors, and bees from the level of the individual to the colony. Specifically, we will examine direct effects of agrochemicals, other stressors, and their interactions, on solitary bees at the level of individuals and populations. We will assess effects on bumblebees and honey bees at the level of individuals and colonies. We will examine experimentally the toxicokinetics and dynamics of agrochemicals at the level of individuals. We will determine the accumulation and dynamics of agrochemicals in populations of solitary bees, and in colonies of bumblebees and honey bees. Accumulation and dynamics will be measured in a range of substrates, including bees and hive products.

1.4 Ambition

Overall Ambition

Bees supply the essential service of pollination to our crops and wildflowers, but their health and population status face multiple threats. Anthropogenic chemicals are a potential driver of this threat, but their relevance for bee health is vigorously contested and unresolved. PoshBee will break new ground in determining the importance of chemicals for bee health across Europe, from managed honey bees to wild solitary bees, and across key chemical-chemical, chemical-nutrition, and chemical-parasite interactions. We will develop much-needed models, tools, and protocols to underpin future assessment and monitoring of threats to bee health. The complexity of stressors, and their interactions, coupled with the wide range of bee taxa, geographic variability, and multiple stakeholders, calls for a highly ambitious, multi-actor, transdisciplinary community, with a strong drive and shared vision towards bee health and sustainable pollination in Europe. PoshBee fully recognizes and embraces the magnitude of these challenges, and has been established and grown specifically to achieve its stated objectives.

State of the art: Exposure to chemicals

The exposure of bees (both managed and wild bees, including honey bees, bumble bees, and solitary bees) to chemicals at the landscape-scale depends upon a range of factors, including chemical use, physico-chemical characteristics of both the chemicals and the soil matrix, plants, time of year, landscape composition, weather and climate, and how the bees themselves use the landscape. Specific pathways of exposure include treated crops, drift during application, wildflowers, and standing water, amongst others. In addition to agricultural chemicals, honey bees are directly exposed to chemicals used to manage honey bee health within the hive. Chemicals have been shown to contaminate bees (including larvae, pupae, adults), nesting materials (e.g., wax), and products of foraging (e.g., beebread, honey). In fact, residue analyses in individual samples of beebread, honey, and bees have revealed the presence of a cocktail of chemicals accumulating concurrently, in honey bees, bumble bees, and solitary bees (although evidence for the latter is limited to one study)[Mullin et al. 2010, Sanchez-Bayo and Goka 2014, Simon-Delso et al. 2014, David et al. 2016]. In particular, beebread in honey bees has been shown to be an excellent indicator for pollution by agrochemicals [Daniele et al. 2017, McArt et al. 2017]. The accumulation of chemicals in honey bee hives has been demonstrated at local, regional, and national scales [e.g., Porrini et al. 2016]. In contrast, such accumulation in bumble bee nests or solitary bees (O. bicornis) has only been documented in the UK, Germany, and Hungary [Goulson 2015, Woodcock et al. 2017]. However, despite these excellent earlier studies, we still have no quantitative understanding of how exposure hazard varies at a European scale, across key crops (all previous controlled studies have focused on a single crop, oilseed rape), and across a range of bee species at such a continental, multi-crop scale. Such data are key to explaining the potential threat of chemicals to bee health.

773921, PoshBee, Part B

Scientific ambition: Exposure to chemicals

PoshBee will move significantly beyond previous studies, which have examined exposure in a single landscape [Goulson 2015, David et al. 2016, Rundlöf et al. 2015], or across 3 countries in a single crop [Woodcock et al. 2017]. We will establish a unique site network, covering eight countries across all of the EU's major biogeographical regions, with two key crops for bees in each country, and eight independent sites for each crop across a range of chemical application regimes. We aim to make this site network a semi-permanent feature of the European research landscape, to support and facilitate future research and innovation with respect to bee health. The site network is being codeveloped with multi-actor partners – national and regional farming and beekeeping organisations – to maximise the value and impact of our study, but also to benefit from the synergies between transdisciplinary academic and stakeholder knowledge sets. At each site we will deploy sentinel colonies of honey bees (A. mellifera), bumble bees (B. terrestris), and solitary bees (O. bicornis). PoshBee will evaluate indirect routes of exposure by intensive landscape analysis around sentinel bees, including crops and treatments applied to crops. All crops, both attractive and non-attractive to bees, will be included in this survey. This analysis will incorporate a temporal component to take into account previous crops and treatments used in the site area, given the high persistence of chemicals (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, both outside – nectar, pollen, standing water – and inside – beebread, honey, wax, royal jelly, bees – hives or nests. Finally, air sensors will be developed, to be placed inside and outside hives and nests to complete the contamination assessment of the bee environment. Combining data on agrochemicals coming from three different sources (landscape use and agricultural practices; quantification of agrochemical residues in various matrices; and air sampling) 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.

State of the art: Effects of chemicals

Agrochemicals (including veterinary chemicals, as defined above) have both lethal and sublethal effects on bees [Godfray et al. 2015, Neumann 2015]. Lethal effects can be devastating and are obvious to stakeholders, but have become comparatively rare due to improved products and stakeholder training [e.g., Dainat 2014]. In stark contrast, sublethal effects are more cryptic, frequent, widespread, and have been experimentally shown to affect behaviour, immunity, physiology and reproduction of individual bees, colonies, and populations in honey bees, bumble bees, and solitary bees [Godfray et al 2015, Rundlöf et al 2015, Woodcock et al. 2017, Tsvetkov et al. 2017]. At a larger landscape scale, correlative studies suggest an association between neonicotinoid use and population declines in solitary bees [Woodcock et al. 2016]. However, despite this body of literature on the effects of chemicals, particularly neonicotinoids, there are still significant gaps in our knowledge on the lethal and sublethal effects of many chemicals across a range of bee types. Furthermore, with local, national, and regional moratoriums on the use of neonicotinoids, and the potential of future bans, our understanding of the effects of chemicals that may replace them is extremely limited. This holds especially true for the sublethal effects of chemicals on bee reproduction. In addition, whether and how chemicals interact with each other, and with other stressors (pathogens: e.g., Doublet et al. 2015; nutrition: e.g., Alaux et al. 2010), and how their effects may vary with environment [Woodcock et al. 2017] and/or bee genetics [Sandrock et al. 2014a] remains relatively unknown. Finally, conflicting studies that suggest no effects, and field studies that suggest laboratory or small-scale results may not necessarily scale up to real-world effects [Retschnig et al. 2015], also argue that our understanding is limited. Thus, the question remains as to the real-world effects of agrochemicals on bee health.

Scientific ambition: Effects of chemicals

PoshBee will take a multi-actor approach, across an ambitious range of scales, to provide a step-change in our understanding of the effects of chemicals on bee health. Academic and veterinary partners, and agrochemical company stakeholders, will work together to define a portfolio of chemicals that will be used to assess (i) individual chemical effects, (ii) chemical-chemical interactive effects, (iii) chemical-nutrition interactive effects, and (iv) chemical-pathogen interactive effects on bee health. We will be the first project to take a fully integrated experimental approach that scales from laboratory, to semi-field, and finally to field-scale studies. PoshBee will produce the first integrated analysis of the effects of chemicals and their mixes on bee health across a range of species, from the laboratory to the field, that incorporates their interaction with both pathogens and nutritional stress. By developing an innovative holistic model of bee health, we will provide a framework to enable understanding of future threats from novel chemicals and pathogens. These studies will explicitly consider the modifying roles of host genetics (honey bee subspecies, e.g., Sandrock et al. 2014a) and environmental variation in the field on effects. Effects are

773921, PoshBee, Part B

also known to vary with the resilience of host species [Rundlöf et al. 2015; Woodcock et al. 2017], and so PoshBee will break new ground by using multiple model systems across a range of high to low resilience [Straub et al. 2016]: eusocial honey bees, *A. mellifera*, primitively eusocial bumble bees, *B. terrestris*, and solitary bees, both *O. bicornis* and new model ground-nesting solitary bee species (to be developed in PoshBee). As successful reproduction is the key parameter for all managed and wild bees, and as reproductive individuals represent a weak point in otherwise buffered social species [Sandrock et al. 2014a,b, Straub et al. 2016, Fauser et al. 2017, Baron et al. 2017] we will have a special focus on effects on reproductive traits, and thus we will move beyond the current focus on honey bee workers in ecotoxicological studies. In addition, PoshBee proposes innovative scientific approaches to understanding effects, including the first proof of concept for using histoproteomics studies by MALDI molecular imaging to (i) decipher the cross talk/interplay during host and agrochemical (and other stressor) interactions and (ii) for toxicodynamic investigations.

In conclusion, by investigating the sublethal effects of chemicals and their interactions with nutrition, pathogens, host genetics, and environmental variability, with a focus on insect reproduction over the whole life cycle of highly eusocial, primitively eusocial, and solitary bees, PoshBee 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 on bees. This will be especially relevant for EU policies for chemical use and conservation, and will contribute significantly to a sustainable agriculture sector.

State of the art: Chemical monitoring

The general lack of cheap and effective tools for quantifying bee exposure to chemicals, pathogens, and nutritional stress, or for measuring bee health in general, holds back the development of large-scale multi-year bee health monitoring schemes. Standard methods applied in all previous studies of chemical exposure include the collection of physical materials (e.g., honey, pollen, wax, etc.) for laboratory screening using LC-MS/MS and GC-MS/MS technology [Benuszak et al. 2017].

Scientific ambition: Chemical monitoring

PoshBee will take two approaches to develop new scientific protocols for chemical monitoring. First, it will develop a new air sensor tool, which can be deployed by individual beekeepers at the single hive level, to provide immediate assessment of chemical exposure within and outside hives. This will enable easier and real-time analysis of chemical threats to bees, enabling beekeepers to modify practice on the ground in response to immediate threats. This approach will still rely on standard LC-MS/MS and GC-MS/MS techniques for analyses, but will avoid issues caused by complex matrices that integrate historical and current exposure.

In order to develop novel, scientific approaches to monitoring chemical exposure and bee health, PoshBee will break new ground in the use of 'omics technologies. 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 bees, and enable the selection of molecular markers for future monitoring of bee health. Inspired by MALDI BioTyping, which is already approved by the FDA for clinical microbiology, a striking novelty of PoshBee will be to develop MALDI BeeTyping® as a laboratory tool to monitor bee health through simple field-collection of a bee's 'blood' (or haemolymph). This is the first time such an approach will be used to explore honey bee, bumble bee, and solitary bee responses to agrochemicals, nutrition, and pathogens. We expect that this approach will provide a fundamental component of future global solutions for health management plans at the national or EU level for bees, 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 definitively screen bees for exposure to agrochemicals, both those currently in widespread agricultural use and potentially for those yet to come on the market.

State of the art: Nutritional monitoring

The spatio-temporal composition and availability of floral resources in the landscape affects the quality, quantity, and diversity of food available to bees, and thus their health [Dolezal et al. 2015]. For example, the ECOBEE monitoring project in France found that honey bee colony dynamics were affected by mass-flowering crop phenology, resulting in food shortages in late spring between the flowering of oilseed rape and sunflowers [Odoux et al. 2014]. However, there is a general lack of monitoring of patterns of food availability to, and use by bees. At large temporal scales, increased land-use intensification contributed to declines in nectar resource availability in the UK between 1930-1970 [Baude et al. 2016], and conversely lower intensity of cultivation across the landscape is linked with

higher lipid levels (and better health) in honey bees [Dolezal et al. 2015]. Similarly, landscapes providing pollen diversity benefit bee health and reproduction [Carvell et al. 2017]. On the flip side, patterns in bumble bee colony growth rate can be used to indicate a general lack of food in the environment [Rotheray et al. 2017]. However, despite the obvious fact that the availability of diverse floral resources can have direct effects on bee health through better nutrition, and indirect effects on individual and colony level health through amelioration of bee diseases [Koch et al. 2017], there has been no Europe-wide assessment of food quality and availability for bees (but see Baude et al. 2016 for nectar in the UK). Furthermore, mass flowering crops have increased in area across Europe [Breeze et al. 2014], but the consequences for bee health are unknown. Finally, there is limited understanding of how nutrition (quantity, quality and diversity of nectar and pollen) influences health in non-*Apis* bees, and whether this is affected by pathogen load or agrochemical contamination.

Scientific ambition: Nutritional monitoring

In PoshBee, we will generate the first European-wide database on the nutritional quality of key agricultural landscapes, using three species of bee as highly efficient and effective sampling agents. By examining the resources they actually collect in relation to what is available, we will determine 1) the quality of resources available in landscapes planted with mass-flowering perennial and annual crops, in terms of nutritional quality and agrochemical contamination, 2) the diet choice of bees in these landscapes, 3) the nutritional value (sterols and amino acids) of collected pollen, and 4) how diet choice is related to pathogen loads and agrochemical residues. This will produce the first comprehensive understanding of bee nutrition, across honey bees, bumble bees, and solitary bees, across key agricultural landscapes at the European scale. This will enable the first large-scale assessment of agrochemical exposure through ingestion. In addition, these results will, to the best of our knowledge, provide a global benchmark for understanding nutrition in bees in real-world landscapes.

State of the art: Pathogen monitoring

Pathogens are a key on-going and emerging threat to bee health [Vanbergen et al. 2013]. Europe-wide initiatives to monitor the spread of exotic pests of honey bees have been moderately successful to date, e.g., the Varroa mite (Varroa destructor) arrived in the 1960s or earlier in Europe and subsequently spread to every EU country by 1998 [Rosenkranz et al. 2010]. Yet systematic monitoring of pathogens has been sporadic at best and usually national in scope; an example is the German bee monitoring scheme in which 10,000 colonies of honey bees have been monitored across Germany for their health and pathogen presence for more than 10 years [Genersch et al. 2010], with only Varroa mites being identified as a major factor related to poor colony health. Similar studies have been conducted in other countries within the EU (e.g., Italy, Porrini et al. 2016). EPILOBEE, an EC-funded programme, monitored honey bee colony losses and associated pathogens for two years (2014, 2015) [Jacques et al. 2017]. Similar continent-scale monitoring programmes of honey bee health have been run in North America [Seitz et al. 2016]. All these programmes monitored colonies of only one species, the honey bee, thus providing no information on other major pollinating bee species. To our knowledge, only two studies have examined pathogens in bumble bees at national scales [USA, Cameron et al. 2011; UK, Fürst et al. 2014]. To address this imbalance, the EU COST-Action SUPER-B (of which PoshBee partners are key members) initiated monitoring of viral pathogens in honey bees and wild bees collected from multiple localities across the EU. The initiative, coordinated by PoshBee partner MLU, has currently generated data from 4 localities, though the voluntary nature of the initiative and its sampling in one of three years (2014-2016) mean that our picture of viral pathogens across bee species from this initiative will be patchy at best. To conclude, we are still lacking, at regional, national, and European-levels, key information on pathogen prevalence across honey bees, bumble bees, and solitary bees.

Scientific ambition: Pathogen monitoring

PoshBee will provide a sea-change in our approach to pathogen monitoring. First, it will monitor pathogens not only in honey bees but also in bumble bees and solitary bees, which are known to harbour some of the same pathogens as honey bees, e.g., DWV [Fürst et al. 2014]. Second, it will monitor pathogens across bee species in a systematic manner in all of the EU's biogeographical regions at the same time during the spring/summer flight season of bees. Third, rather than relying on the haphazard presence of different bee species, it will use sentinel colonies of honey bees, bumble bees and solitary bees, placed in the field to provide an unbiased estimate of pathogen prevalence. Fourth, it will use qPCR to quantify pathogen loads of sentinel colonies and individual bees, providing a much higher resolution to pathogen prevalence and risk of disease across the EU; qPCR primers have already been refined and tested for their sensitivity and specificity in the SUPER-B protocol (see above). Finally, it will be able to correlate pathogen prevalence with agrochemical exposure and bee nutrition to allow more meaningful interpretation of pathogen loads across bee species. Together, this will provide the first EU-wide assessment of parasites and

pathogens in honey bees, bumble bees, and solitary bees.

State of the art: Modelling for environmental risk assessment

Understanding the affect of changes in management or natural stressors on bee health and bee distribution requires a multi-factorial approach. For honey bees, this has given rise to the production of new models based on simulation approaches. The best known is BEEHAVE [Becher et al. 2014], a systems model of a honey bee colony, written in NetLogo and capable of integrating weather, phenology, resource availability and biological agents into predictions of colony development through time. Recently this model has been expanded to include pesticide effects [Thorbek et al. 2017a,b]. However, BEEHAVE has limitations, primarily due to the lack of design flexibility resulting from the programming system used [EFSA PPR 2015b]. To avoid this constraint, a second model – Bee++ – has been written in the programming language C++ using extensible object oriented design [Betti et al. 2017]. This approach potentially allows for much more complex and larger simulations with the capability of integrating a larger number of factors. However, currently the Bee++ model is still of the same scale of complexity as BEEHAVE. Models of other bees are less advanced [Ulbrich et al. 1999, Faruq et al. 2013, Dyer et al. 2014] and are not yet capable of placing the bees in complex landscapes, nor of integrating multiple stressors, since they lack the capability to represent or integrate these details. These models are therefore unable to predict landscape-context effects of management on bee populations. Overall, we currently lack models that can incorporate explicit sources of contamination, as identified by chemical monitoring projects (see above), and models for bumble bees and solitary bees are largely absent.

Scientific ambition: Modelling for environmental risk assessment

In order to provide effective risk assessment, models need to incorporate the detailed landscape component of bee stressors [Simon-Delso et al. 2017]. PoshBee will use a dynamic landscape representation, which is not present in current models for honey bees. This will enable the incorporation of detailed and dynamic representations of pesticide availability, and their intregration with bee foraging behaviour, to predict exposure. Such a dynamic 'systems' approach has already been taken for predicting effects of pesticides on non-target arthropods [EFSA PPR 2015a, Topping et al. 2015], using the ALMaSS modelling system [Topping et al. 2003]. This dynamic systems approach is now being extended to honey bees by the EFSA MUST-B project, utilising the ALMaSS framework (PoshBee partner Topping, AU). The forthcoming EFSA model (ApisRAM) will extend current capabilities of the model to include dynamic landscapes and pesticide fate, as well as much greater detail of in-hive pesticide behaviour, colony structure and an extended set of simulated parasites, diseases and predators than is possible in current models. This systems approach of detailed dynamic environmental models integrated with individual (agent)-based simulation also provides the other key component of dynamic behaviour, i.e., feedback loops based on local context. This is most often associated with social simulation [Miyasaka et al. 2017], but is also critical in social insects, and must be a key feature of the bee/management/weather complex socio-ecological system. PoshBee will advance beyond the state of the art by providing detailed landscape data to expand the coverage of the EFSA ApisRAM model, as well as a related model being developed for *Osmia* spp. More importantly, it will develop a sister-model for bumble bees using the same framework. Together, this will provide the first integrated, dynamic suite of models for assessing environmental risk across bee species in Europe.

Technological and innovation ambition

PoshBee aims to enhance monitoring of bee health through the provision of new technological tools. Our air sensor tool (WP2) will provide a cheap and easy way to assess chemical contamination inside and outside honey bee hives, and as such we expect it to become the tool of choice for environmental monitoring by individual beekeepers, bee farmers, and national/international monitoring projects. This will provide a dramatic and positive change in our knowledge and understanding of environmental conditions for bees at local through to continental scales. We expect this tool to be used not just within Europe, but at a global scale, due to its potential ease of use and cost-effectiveness. Complementing the air sensor approach, the bee 'health card' or MALDI BeeTyping® (WP9) provides a new technological approach to assess exposure to and effects of chemicals, nutrition, and pathogens in honey bees, bumble bees, and solitary bees. Given its expected cost-effectiveness and efficiency, we expect it to become the go-to tool for monitoring actual exposure and effects of stressors in bees, at both European and global scales. This will enable large-scale and sustainable monitoring of European bee health, and thus strengthen the resilience and sustainability of beekeeping, wild bees, and the delivery of pollination services.

PoshBee aims to enhance the management of bee health. The environmental risk assessment models produced by PoshBee (WP7), and in collaboration with MUST-B, will provide new tools for EFSA and other policy-setting bodies to assess the risks of environmental chemicals and land-use change for bee health. This will enable evidence-based

773921, PoshBee, Part B

decision making to manage and maintain sustainable bee communities across Europe, and thus the provision of sustainable pollination services. We envision these tools being used outside Europe, with the local provision of relevant landscape data, to further maintain global bee health and the sustainable pollination this provides.

PoshBee aims to translate knowledge into bee health, sustainable beekeeping, and sustainable pollination. This will improve the livelihoods of beekeepers, growers, and the agri-food sector, whilst bolstering food security, underpinning the pollination services for European crops that are currently worth more than 22 billion Euro [Gallai et al. 2009]. PoshBee will assess the attitudes of beekeepers, farmers, SMEs, and the agrochemical industry towards novel tools and practices for bee health, in order to understand objective and subjective incentives and barriers to the adoption of new tools and practices. For the first time, this will be done at both regional and European scales, looking across the full set of costs and benefits to these stakeholder groups, and across national economies (WP10). Our unique, multi-actor structure, combined with partners who are global leaders in knowledge exchange (e.g., UREAD, ANSES, BERN), will enable the translation of our novel and innovative research, protocols, and tools into ground-breaking changes in practice and policy at local, national, and European scales. Together with the work of the successful SFS-28-2017 project, with whom we will closely collaborate, this will support the health of wild bee populations. Dissemination and workshops with our local beekeeper partners, and our stakeholder committee member Apimondia (the global beekeeping organisation), will enhance sustainable beekeeping. Together, healthy wild and managed bees will provide sustainable pollination across Europe.

To conclude, PoshBee will strive to provide world-leading evidence (WPs1-10) to strengthen national and EU policies and regulations for risk assessment, bee health monitoring, and the breeding, trade, and movement of managed pollinators. Together, this will facilitate bee health, from honey bees to wild bees, and the sustainable pollination services that these essential insects provide.

2. Impact

2.1 Expected Impacts

2.1.1 How PoshBee will contribute to the expected impacts

The SFS-16-2017 Bee Health and Sustainable Pollination call states four expected impacts, and we demonstrate how POSHBEE meets and exceeds these below:

<u>Expected Impact 1:</u> Filling gaps of knowledge on hazards, focusing mainly on (chronic and sub) lethal effects of mixtures of chemicals on both healthy and infected bees at population and/or colony levels, while also taking into account the aspect of bee nutrition and its effect on bee health

Impact. Chronic and sublethal effects of agrochemicals on the health of bees have been poorly defined to date (see Section 1.4 above). Moreover, potential antagonistic interactions among two or more agrochemicals on the health of bees, and modulation of that agrochemical affect by bee nutrition and bee pests/pathogens, remain major holes in our understanding. By taking an explicitly empirical approach from highly controlled laboratory settings through to full field-scale experiments, and from individual bees through to the colony and population level, PoshBee will provide novel, cutting edge findings that provide definitive answers to these pressing, open questions.

PoshBee will (i) define chronic and sublethal effects for individual agrochemicals and for combinations of agrochemicals across castes and sexes of bee, including the major managed, commercial pollinators: honey bees (A. *mellifera*) and bumble bees (*B. terrestris*), as well as solitary bees (*O. bicornis* and other European wild bee species) (WPs3,4), (ii) determine how the responses of European bees to agrochemicals are modulated by nutrition and pathogens/pests (WP4-W7), (iii) develop and run novel semi-field and field experiments to determine the real-world effects of chemicals in the presence of other stressors (environmental variation, nutrition, pathogens)(WP7), (iv) develop holistic models of bee health that inform our understanding of how stressors perturb healthy bees (WP8), and (v) develop agent-based models to simulate the effects of pesticide regimes, and their interaction with nutrition and pathogens, on bee health (WP8).

Enhanced knowledge of these hazards will have direct benefit to: agrochemical industry (to inform the use and deployment of agrochemicals); policy-makers (enhanced evidence base for policy making); farmers (to enable decisions about usage of agrochemicals); beekeepers (to enhance maintenance and management of healthy bees); public (cutting edge information about pollinators).

Impact indicators. First and foremost, filling knowledge gaps means publication of research results in the peerreviewed scientific press, where results can be independently scrutinized and verified. Secondly, our research results

need to reach our target audience and end-users, which can be most successfully achieved by targeted conference presentations and EIP practice abstracts submitted to lead institutes and user groups (see Table 2.1).

Expected Impact 2: Provide science-based protocols for improvement of bee regulatory testing schemes

Impact. Science-based protocols are essential to provide and support evidence-driven bee regulatory testing schemes. PoshBee will realise this impact through: (i) the development of improved and novel methodologies and protocols for testing chemical effects across life-stages and castes/genders of bees, (ii) developing new ground-nesting solitary bee species for regulatory testing, (iii) the development of novel protocols and end-points for regulatory testing in ground-nesting solitary bees, (iv) the development of novel protocols for assessing how nutrition modulates chemical effects, (v) the development of novel protocols for semi-field and field experiments for bee regulatory testing schemes, and (vi) the provision of these novel science-based protocols to industry and regulatory bodies, to enable uptake and incorporation into bee regulatory testing schemes.

(i) PoshBee will develop and validate novel and improved science-based protocols for testing the effects of chemicals on the life-stages (larva/pupa/adult) and castes/genders (worker/male/queen) of social and solitary bees (WP3). These will be developed through a multi-actor approach, incorporating input from industry partners and stakeholders (e.g., Bayer, Syngenta), as well as regulatory bodies (EFSA), to ensure that they significantly improve bee regulatory testing schemes. (ii) Currently, regulatory testing ignores the majority of solitary bee species. To fill this gap, PoshBee will develop new model systems of ground-nesting bees for regulatory testing (WP4). This will be done in collaboration with our industry partner WILD, to maximise value and facilitate industrial uptake. (iii) Using these new model systems, PoshBee will provide the first science-based protocols for regulatory testing in ground-nesting solitary bees (WP4). Again, using the multi-actor approach will maximise the value and uptake of these protocols. (iv) Regulatory testing schemes largely ignore interactions between chemicals and nutrition, and their effects on bee health. PoshBee will rectify this situation through the development of novel protocols for testing the affect of chemicals under controlled variation in nutritional state (WP5). As before, the multi-actor approach will be followed in the development of these protocols. (v) Finally, in a collaboration between industry (ATPOLL, RBH), public research organisation (WBF-Agroscope), and academia (ALU-FR), PoshBee will provide improved protocols for semi-field and field regulatory testing of honey bees, bumble bees, and solitary bees (WP7). (vi) While all of these protocols will be developed through the multi-actor approach, incorporating industry, academia, government institutes, and regulatory organisations, PoshBee will further drive the uptake of these novel protocols through focused delivery to industry and regulatory bodies, at European and global scales (WP10, WP11).

Enhanced science-based protocols for bee regulatory testing schemes will have direct benefit to: agriculture (lower environmental impact); agrochemical industry (better/safer products); beekeepers (healthier colonies); policy-makers (evidence to support effective regulation of agrochemicals); veterinary industry (better/safer products for bee health); wider society (the protection of wild and managed pollinators and the ecosystem service of pollination).

Impact indicators: Success of these impacts will occur partially within the lifetime of the project. However, uptake of new protocols into regulatory schemes is a long-term process that will extend beyond the time-line of PoshBee (see below). 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 industry 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 2.1).

<u>Expected Impact 3:</u> Contribute to the consolidation of validated monitoring plans on bee health with the development and use of molecular markers

Impact. PoshBee will realise this impact through the development of novel, proteomics-based tools that can be rolled out as cheap and effective mass monitoring tools for bee health.

PoshBee will (i) develop a novel 'health-card' for bees (WP4) using the BeeTyping® tool to assess both the stressors involved and their effects. This will enable rapid and affordable testing of honey bees, bumble bees, and solitary bees for chemical exposure, pathogens, and nutritional state, as well as for general health status. BeeTyping® can play a central role in future validated monitoring plans. In addition to BeeTyping®, PoshBee will (ii) produce an integrated and dynamic EU proteomics database enabling a coherent understanding of the effects of stressors on metabolic pathways. This will feed directly into supporting knowledge on bee health and immunity for the selection of prognosis and diagnosis molecular biomarkers. These molecular markers will be selected based upon their relevance

for the development of field tests, and their applicability for the general market. (iii) PoshBee will integrate mass spectrometry imaging (MSI) for toxicodynamic studies in bees. The dynamic interaction of a toxicant with its bee target will be evaluated through histoproteomics studies by mass spectrometry, a novel method never before used on bees. This will be of immediate benefit to environmental risk assessment and therapeutic drug monitoring by implementing conventional toxicokinetic-toxicodynamic evaluations. This will consolidate our knowledge on bee health by defining spatio-temporal images of the effects of environmental stressors ranging from the tissue scale (i.e. nervous system, digestive tract, reproductive organs), through individuals and the colony, to the population level. MSI represents a novel approach to fulfill pharmacokinetic and pharmacodynamics studies during the development of new agrochemicals or to follow the effects of drug treatment.

These novel molecular 'omics tools will have direct benefit to: local, national, and EU-level monitoring schemes (fast, cheap monitoring of bee health); policy-makers (enabling rapid, cheap, large-scale monitoring); agrochemical industry (rapid, cheap assessment of bee health in developing new chemistry); beekeepers (individual-based tools for managing bee health); wider society (provision of healthier bees, and thus more sustainable pollination).

Impact indicators: 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 2.1).

Expected Impact 4: Contribute to sustainable pollination and sustainable beekeeping

Impact. PoshBee will realise this impact through the following interlinked steps: (i) developing state of the art monitoring tools, (ii) providing EIP best practice abstracts and policy briefs through multi-actor approaches for bee health monitoring and mitigation responses, (iii) improving bee health and the sustainability of beekeeping, and (iv) enhancing the resilience of managed and wild bees underpinning pollination service delivery.

(i) PoshBee will develop a portfolio of novel and innovative datasets, evidence-based protocols and toolkits (as described in Expected Impacts 1-3). These will have been co-developed with end-users, thereby ensuring the outputs are all fit for purpose and matched to requirements. PoshBee tools will allow early and accurate detection of biologically relevant thresholds of stressors on bee health (including agrochemicals, pathogens, malnutrition, and combinations thereof) as an integral part of bee health monitoring programmes. (ii) Coupled to these tools, PoshBee will produce, and deliver to stakeholders, multi-media best practice guides, EIP practice abstracts, and policy briefs to allow the application of tools to assess risks, monitor bee health and implement appropriate response actions at the local, national and European levels. The tools and guidelines will form the basis of improved monitoring programmes tailored to a range of stakeholders ranging from individual beekeepers and beekeeper groups through to national and international monitoring schemes. (iii) This will contribute to a strengthened multi-actor framework for the sustainable management of wild and managed bees to improve bee health, which in turn will (iv) underpin healthier, more resilient pollinator communities, thereby improving the sustainability of pollination service provision to European crops and wild flowers.

Greater sustainability in beekeeping and pollination services will have direct benefit to: beekeepers and the managed pollinator industry (healthier colonies, reduced costs, improved income); growers and suppliers (reliable pollination services for dependent crops, improved yield, quality and income); policy-makers (evidence to support the development of policy related to bees in the agricultural, environment and business sectors); consumers (secured access to a variety of pollinator-dependent produce, such as fruit, vegetables, seeds and honey); wider society (public goods including the safeguarding of pollinator and wild flower biodiversity).

Impact indicators: Some impacts will be achieved within the lifetime of the project, and we will measure the uptake and adoption of monitoring tools across stakeholders, the development of new monitoring frameworks using PoshBee tools, and the extent of engagement the project has with policymakers. However, other impacts will take several years to be realised and so will not be directly measurable during PoshBee. For these longer-term impacts (e.g., sustainable pollination) we will use indicators reflecting travel along the route towards these, including commitment of national beekeeping and farming groups to adopt frameworks to improve beekeeping and pollination sustainability. Our indicators, targets and methods of measurement are summarised in Table 2.1.

 Table 2.1 Summary of measurable impact indicators

Expected	Indicator of impact	Measurement method	Total
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Impact			target value
1	Scientific articles in international, peer- reviewed journal	Web of Science (Science Citation Index) entry	25
1	Presentations of research results at major EU/world conferences addressing bees: Apimondia, EurBee, IUSSI	PoshBee Coordinator, verified by conference proceedings	10
1	Face-to-face presentations of research results to major stakeholders: PoshBee stakeholder group, IC-PBR (Bee Protection Working Group), national beekeeper meetings	PoshBee Coordinator, verified by conference proceedings and www proceedings	40
1	Downloadable EIP practice abstracts of major research findings for policy-makers, NGOs, and the public/press	Number of downloads from PoshBee website	400
2	Incorporation of protocols into the literature	Number of downloads, reads and citations	200
2	Ring-testing of protocols	Uptake by ICPPR for ring-testing	Yes
2	Incorporation of protocols into regulatory regime	Integration of novel protocols into EFSA discussion documents	Yes
3	Use of BeeTyping [®] as a new analytical tool	BIOP/CNRS, survey of end-users on satisfaction with tool	>75% satisfied
3	Adoption of BeeTyping® for bee health monitoring	Partner feedback on number of monitoring schemes using the tool	5
3	Set of prognosis/diagnosis markers available for monitoring and research	Standardized protocols developed and kit ready for marketing.	1
3	Adoption of mass spectrometry tissue imaging in toxicodynamic studies and risk assessment by authorities (EFSA) and industry	Reporting by partners on number of industries adopting/committing to adopt the kit in their drug development process	3
4	Number of local/national beekeeper associations adopting the PoshBee toolkit	Beekeeping association surveys	20
4	Number of downloads of EIP practice abstracts: Response options for multiple stressors on bee health	Number of downloads from website	100
4	Number of Member State governments directly engaged in dialogue on developing improved bee health monitoring frameworks	Reporting by project partners	10
4	Wider interest in PoshBee toolkits by farming groups	Number of downloads of PoshBee factsheets	150
4	Number of project workshops/meetings disseminating PoshBee tools	Reporting by PoshBee partners	25

2.1.2 How PoshBee will contribute to additional substantial impacts

Enhancing innovation capacity, creating new market opportunities, strengthening competitiveness and growth of companies. PoshBee 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. The project will strengthen European competitiveness in the rapidly expanding global market for bee health tools by providing a suite of next generation tools, protocols and applications. This would also be expected to open up new markets within Europe and outside, as the demand from the pollination supplier and 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). PoshBee would also help grow a new generation of honey beekeepers using high technology tools to safeguard honey bees and ensure top quality and highly efficient honey production for domestic and international markets.

Wider societal benefits flowing from PoshBee. These would include enhanced food security through more

sustainable beekeeping and pollination service management, which would safeguard crop pollination services. POSHBEE will also support wider biodiversity conservation through strengthening the resilience of wild flower pollination underpinned by healthier European pollinator communities. Further, the fruits, seeds and nuts from pollinator-dependent plants are key links in food webs that include many of Europe's iconic birds and mammals. Together, these benefits would contribute to the UN Sustainable Development Goal 15 (protect, promote, restore sustainable use of terrestrial ecosystems).

2.1.3 Barriers, obstacles, and any framework conditions that may determine whether and to what extent the expected impacts will be achieved

Currently (12 Sep 2017) there is a moratorium in the European Community on some uses of three neonicotinoids. The restriction could be maintained, dropped or extended to all usages and/or more chemicals. In the meantime, new active ingredients could also be authorised for use in the field at EU level. In the case of regulation modifications at EU or national levels, the PoshBee 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. PoshBee (Tasks 10.1, 10.6 and 10.7) will ensure an active dialogue with policy and regulatory experts in order to anticipate and actively respond to a changing policy environment.

Several partners of PoshBee 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, PoshBee partners will rapidly include them as part of the protocols tested. Furthermore, PoshBee will be proactive on this issue, as we will develop new validated protocols, building on those that already exist and current 'gaps' in available tools.

Bees are threatened by a range of invasive and emerging pathogens and pests. As new threats emerge across the European landscape – e.g., the Asian hornet, *Vespa velutina*, the small hive beetle, *Aethina tumida* – beekeepers and farmers may need new management regimes and monitoring tools. While it is unlikely that new threats will significantly decrease the importance of current major threats (DWV, the Varroa mite), they may shift the relative needs of relevant stakeholders. However, PoshBee partners are at the forefront of monitoring pathogens and pests in the EU (see Section 1.4 above), and we will take a proactive approach to ensure that new threats are incorporated where possible into our research and outputs, to maximise value for key stakeholders.

The successful transfer and adoption of the knowledge created by PoshBee for the beekeeping and pollination supply community, and its acceptance by end-users, requires adjustments to local environmental and political beekeeping conditions. PoshBee will provide these adaptations via the network of regional sites managed by local beekeeper associations. The consortium also includes lead representatives of local, national and international beekeeping communities as partners and on advisory groups, thereby ensuring continuous dialogue on the pathways, opportunities and barriers to adoption of PoshBee tools.

2.2 Measures to maximise impact

2.2 (a) Dissemination and exploitation of results

General plan: Two fundamental documents, drafted in the first 6 months of the project implementation, will guide and schedule PoshBee's dissemination and exploitation activities. The project's plans for (i) dissemination and (ii) exploitation of results will draw on a multi-channel targeted approach tailored for the specific needs of each stakeholder group. A preliminary outline of the project's stakeholders alongside the planned dissemination and exploitation routes are identified below.

Channels and Methods
 EIP practice abstracts, guidelines and training based on findings and developed knowledge on bee health hazards, focused on agrochemicals, pathogens, and nutrition Multi stakeholder discussions, workshops and training Training video series Farmer & beekeeper newsletters, Annual newsletter <i>PoshBee BUZZ</i> Open days and demonstrations at farms Social media targeted to relevant stakeholders
• Policy briefs, factsheets, guidelines and training based on findings and developed knowledge on bee health hazards, focused on agrochemicals, pathogens, and nutrition

(apicultural and	Multi stakeholder discussions, workshops and training		
conservation	• Involvement with COLOSS and Apimondia via conferences, newsletter etc.		
organisations)	• Annual newsletter <i>PoshBee BUZZ</i>		
	Social media targeted to relevant stakeholders		
Policy (local	• Policy briefs based on factsheets, guidelines and training based on findings and developed		
governments,	knowledge on bee health hazards, focused on agrochemicals, pathogens, and nutrition		
policy and	Multi stakeholder discussions, workshops and training		
decision makers	• Dialogue with the European Food Safety Agency (EFSA)		
at local, EU and	• Involvement with COLOSS and Apimondia via conferences, newsletter, etc.		
global levels)	Annual newsletter PoshBee BUZZ		
	Social media targeted to relevant stakeholders		
Citizens and	• Print materials written in popular language on bee health hazards		
general public	Website news posts and e-newsletter to interested parties		
	Training video series		
	Open days and demonstrations at farms		
	Social media targeted to relevant stakeholders		

<u>Expected Impact 1:</u> Filling gaps of knowledge on hazards, focusing mainly on (chronic and sub) lethal effects of mixtures of chemicals on both healthy and infected bees at population and/or colony levels, while also taking into account the aspect of bee nutrition and its effect on bee health

Our main target groups are (i) scientists addressing the effects of agrochemicals on bees; (ii) agrochemical industry, farmers, and farm advisory services; (iii) commercially managed pollinator industry; (iv) national and international regulatory authorities, including EFSA, EU DG AGRI and DG ENV; and (v) wider public and press.

Our main outputs in filling knowledge gaps represent our research results, which we will communicate in the first line to scientists via scientific publications and conference presentations; for the major bee-related conferences Apimondia (2019 in Montreal, 2021 and 2023, venues to be voted upon) and EurBee (2020 in Belgrade, 2022 and 2024, venues to be voted upon), we shall aim to stage focused symposia on agrochemical effects, where PoshBee research outputs will be showcased. We will engage with the agrochemical industry and the managed bumble bee industry via these same conferences and via formal presentation at PoshBee Stakeholder Group meetings, to be held annually in conjunction with PoshBee's annual workshop; these meetings will include a discussion of additional avenues to pursue to reach industry and farm advisory bodies, beyond our current partners and stakeholders. National and international regulatory authorities will be provided with executive summaries of our major research findings, translated into Partner languages; time will be allocated at the annual PoshBee workshop to identify key institutes and individuals. In addition, we will present our findings to the IC-PBR (International Commission on Plant-Bee Relationships) 'Bee Protection Working Group' so as to help shape improved methods for measuring sub-lethal and interactive effects of agrochemicals on bees. To reach beekeepers with our research results, we will - in addition to Apimondia presentations - address all national beekeeper meetings of Participant nations toward the end of PoshBee (2021) as well as in the year following its formal end (2023) to provide continuity of information transfer. In addition, our executive summaries of major research papers available on the PoshBee website, translated into Participant national languages and publicised by Participant institute press offices, will help inform not only beekeepers and farmers, but also the wider public and press.

Expected Impact 2: Provide science-based protocols for improvement of bee regulatory testing schemes

The main target groups for this expected impact are: (i) agrochemical industry (e.g., BASF, Bayer, Syngenta); (ii) policy-makers (including policy-makers at European and national levels, EFSA, member state ministries for agriculture and environment); (iii) veterinary industry (including industries focused on bee health).

The main outputs of PoshBee from WPs 3-8 will underlie the key messages that will be communicated to each target group. While our previous stakeholder meeting identified most members of the relevant stakeholder groups, the stakeholder mapping exercise (Task 10.1) will ensure that we incorporate all relevant stakeholders from the start of the project, new stakeholders as they emerge, and that key messages will be focused clearly on each stakeholder group. Key messages for the agrochemical and veterinary industries will be structured around the benefits of novel species and protocols for regulatory testing regimes, which will deliver safer and more sustainable chemicals and chemical practices for pest control. For policy-makers, we will focus on the value of these new science-based

protocols to deliver quantifiable and reliable evidence for the assessment of chemicals in regulatory schemes.

To ensure that our key messages reach the targeted stakeholder groups, we will use a range of methods. Publication and communication of novel protocols in the scientific literature, as EIP practice abstracts, at relevant conferences, through websites, and through focused outreach activities (including the PoshBee annual meeting and stakeholder advisory committee), will effectively inform researchers within agrochemical and veterinary industries. We will invite EFSA to our stakeholder advisory committee, which will facilitate seamless exchange of information between PoshBee and the main European body for chemical regulatory schemes. Together with dissemination tools described in section 2.2.2, this will enable rapid and effective dissemination of key messages to the target groups for this expected impact.

<u>Expected Impact 3:</u> Contribute to the consolidation of validated monitoring plans on bee health with the development and use of molecular markers

The main target groups for this expected impact are: (i) beekeepers and managed pollinator industry (including individual honey beekeepers and associations, bumble bee and solitary bee suppliers); (ii) agrochemical industry; (iii) veterinary industry (including industries focused on bee health); (iv) local, national, EU-level monitoring schemes (including policy-makers, bodies such as EFSA).

The main outputs from PoshBee in WP9 will provide novel molecular tools for the consolidation of validated monitoring plans, as well as individual-level assessment of bee health. The PoshBee health-card and health markers (BeeTyping®, marker selection) will be tailor-made for both field and semi-field testing and laboratory detection. After proof-of-concept, these novel prognosis and diagnosis kits will be validated via a PoshBee ring test and certified prior to recommendation as a reference test for the EU authorities and other stakeholders (industry, beekeepers, NGOs). The reliable and cost effective prognosis/diagnosis PoshBee markers will fill urgent gaps in our knowledge of bee health, enabling monitoring of both wild and managed bee populations. The toxicodynamic imaging will be ready for immediate usage by EU and national reference laboratories, private industry, including SMEs and NGOs, and research laboratories. Its integration as a reference tool into on-going environmental risk assessment schemes and therapeutic drug monitoring/development will complement and potentially replace conventional toxicokinetictoxicodynamic evaluations. This robust, reliable and user-friendly PoshBee test system will provide a valuable, costeffective tool, which has considerable potential to eventually replace conventional identification techniques (i.e. PCR, ELISA). Our 'omics strategy, BeeTyping® (molecular marker selection for prognosis and/or diagnosis and toxicodynamic studies by mass spectrometry imaging), provides a corner-stone to optimize decision-making for concerted and controlled actions to validate monitoring and research plans on bee health. It will provide an immediate benefit to beekeepers, who will utilise it to monitor the health and status of honey bees, and support NGOs in monitoring the health status of wild bees. This enhanced understanding of bee health will enable better management, and consequent positive effects on the environment and the economy through sustainable pollination services.

To ensure that these novel tools reach key stakeholder and user groups, we will work with the PoshBee partners and stakeholders to provide a roadmap of how to integrate the bee 'health-card' into a range of ongoing (e.g., COLOSS) and future monitoring schemes (i.e., European Bee Partnership initiated by EFSA), from field to continental scale (WP10. Through this innovative 'omics 'health card', PoshBee will put Europe at the cutting-edge of monitoring tools, with potential economic and ecological impacts through the roll-out of this tool at national, European, and even global scales for monitoring schemes. It will enable holistic bee health monitoring in the field (as envisaged by EFSA), thereby providing immediate benefit to beekeepers, veterinarians, industry and researchers. The stakeholders will be able to use it routinely to monitor the health and status of managed bees (honey bees, bumble bees and solitary bees) and support NGOs and researchers in monitoring the health status of wild bees. This will foster not only conservation efforts, but also sustainable economic development in the EU for honey bee and bumble bee producers, pharmaceutical companies, and agriculture.

Expected Impact 4: Contribute to sustainable pollination and sustainable beekeeping

The main target groups for this expected impact are: (i) beekeepers and the managed pollinator industry (including individual honey beekeepers and associations, bumble bee and solitary bee suppliers); (ii) farmers (including farmer's unions, federations, associations and cooperatives; producer organisations and unions; suppliers; chambers of agriculture); (iii) agricultural consultants (including agronomists; public advisory and extension services); (iv) policy makers (including policy makers at European and national level, EU DG AGRI, DG ENV; member state ministries

for agriculture and environment; public environmental monitoring authorities); (v) environmental advocacy groups (including conservation organisations, trusts and charities); (vi) public.

Main outputs of PoshBee from WP10 will underpin the tools and key messages that will be tailored to each target group. Our stakeholder mapping exercise (Task 10.1) and identification of incentives and barriers to adoption of new tools (Task 10.2) will ensure we have all relevant stakeholder groups involved in the project from the very start, and that key messages are appropriately formulated and effectively delivered. Key messages for beekeepers and the managed pollinator industry will be focussed around the quantified benefits and concrete opportunities to both enhance bee health through adoption of innovative tools and practices, and at the same time provide improved pollination services. For farmers and agricultural consultants, messaging will identify opportunities to work with beekeepers and other stakeholders collectively to achieve more sustainable pollination services. For policy makers, the messages will capture how the knowledge generated by PoshBee can support improved evidence-based decision-making for new and existing environmental and agricultural policies. Environmental advocacy groups will be informed about practices that beekeepers and farmers can implement as part of their businesses, but which can also provide important opportunities for wider pollinator biodiversity conservation. Finally, the messages for the public will aim to provide a simple and clear understanding of how partnerships between beekeepers, farmers, policymakers and other stakeholders can believe not only food security but also a high quality environment.

To ensure that our key messages reach the target groups, we will use a combination of multi-media materials and innovative dissemination tools as described above. The dissemination and exploitation plan for this expected impact is designed to have a multiplicative effect by targeting those users within stakeholder groups who will spontaneously further disseminate PoshBee key messages beyond the initial reach of the project. In addition, ensuring close links to other H2020 projects (e.g., SFS-28-2017 and SFS-07-2018) through inviting the successful coordinators to join the PoshBee Scientific Steering Committee (see Section 3.2) will facilitate this process.

Follow-up to PoshBee

PoshBee will produce a range of outputs and deliverables within the life of the project, but to maximise the impact of PoshBee, a longer time-line is necessary. We will actively explore opportunities for perpetuating the project at local, national and international levels. The partners and Advisory Committees will be specifically tasked with making recommendations to achieve this in years 4 and 5 of the project. We envisage the follow-up and exploitation of PoshBee outputs occurring in the following general and specific ways: (i) new science-based protocols for regulation will need to be incorporated into practice and regulatory processes. PoshBee's advisory bodies contain key industry (e.g., Bayer, Syngenta), regulatory (OIEE) and policy-making (EFSA) bodies. Towards the end of the life of PoshBee, we will consult these stakeholders to agree mechanisms to encourage further ring-testing of our novel protocols, and their incorporation into the process of developing modified regulatory processes; (ii) new 'omics tools will need to be brought to market, which will require development and investment. The relevant partners in PoshBee will work together with our industry partners to apply for follow-on funding to develop these tools for market roll-out; (iii) new model bee species for regulatory testing and pollination will be developed within PoshBee in an explicit academic-industry partnership. This multi-actor approach will facilitate the further development and roll-out of these new model bee species by the industrial partner after PoshBee has ended; (iv) PoshBee will produce numerous practical and policy outputs to support stakeholders. While remaining available on the PoshBee website, our links to key stakeholder groups (e.g., Apimondia, COLOSS, industry, policy-makers, etc.), and the presence of our partners on international (e.g., IPBES) bodies and their action as national and international advisors to policymaking bodies (e.g., EFSA), will facilitate the continued communication of our outputs to relevant stakeholders. In addition, PoshBee has proposed an EIP-AGRI focus group on 'Bee health and sustainable pollination', and if this bid is successful it provides a unique route for the longer-term distribution of PoshBee outputs through the shared work spaces on the EIP-AGRI website, and a thematic network with Operational Groups which should continue to operate beyond the formal end of the project.

Data management

As the Research Infrastructures Work Programme requires, PoshBee will participate in the Pilot on Open Research Data in Horizon 2020, in line with the Commission's Open Access to Research Data policy for facilitating access, re-use and preservation of its research data. To coordinate data management within the project, PoshBee will develop a guiding Data Management Plan (DMP) within Task 6.4 (Establish and implement a project-wide Data Management Plan). The DMP will specifically cover: handling of research data during and after the project; data collection and processing; methodologies and standards; data sharing and open access; and curation and preservation. The DMP will also provide the dataset metadata specification that will be used in the data registry, following an appropriate

relevant standard (<u>http://www.dcc.ac.uk/resources/metadata-standards</u>). It will specify the recommended licensing schemes, preferably using the Creative Commons Public Domain (CC0) and Attribution (CC-BY) licenses as suggested by H2020. In cases where the datasets cannot be publicly shared, the reasons will be mentioned in its metadata description (e.g. ethical, rules of personal data, intellectual property, commercial, privacy-related, security-related). Project outcomes will be integrated with European knowledge hubs and initiatives, e.g., EFSA, COLOSS, OpenAire, BISE, and OPPLA. Each WP will follow agreed DMP protocols and contribute data to the overall project database hosted by RHUL. Selected key datasets deriving from POSHBEE will be published in the form of data papers in peer-reviewed open access journals such as <u>Biodiversity Data Journal</u>. The project proposal, methods, protocols and workflow will be published as a topical collection in the journal <u>Research Ideas and Outcomes</u>. This will provide a stable scientific record and citable publication for the data creators, as well as acting to motivate experts to engage with data curation after the expiration of the project.

Strategy for knowledge management and protection

On 17 July 2012, the European Commission (EC) outlined measures to improve access to scientific information, with a vision to ensure the widest possible access to publicly funded research results. PoshBee will adhere strictly to the EC (2013) Guidelines on Open Access to Scientific Publications and Research Data in Horizon 2020 Version 3.2 (2017) (<u>https://goo.gl/ACK9aC</u>) and to the EC guidelines on FAIR data management in Horizon 2020. 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.

Key elements of Open Science and FAIR data management 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 PoshBee-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 PoshBee Open Science Pilot will start with the open access publication of the project Description of Work in the Research Ideas and Outcomes (RIO) journal. Similarly to Pilots already launched for other EU projects, unconventional research outputs, such as policy briefs, policy recommendations, factsheets, inventories, case studies, data management plans, etc., 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.

2.2 b) Communication activities

Practice, Policy, Umbrella organization (UO), Citizens and General Public (GP)

Tool	Target	Contribution to impact	Verification of use		
Core activities					
General project website and Internal Communication Platform (ICP)	Project partners, ALL	Inform and discuss specific topics of common interest; engage interested parties through provision of general information access to the project's main outcomes	Number of users, session lengths and depths		
Website Information Corner	ALL	Provide EIP practice abstracts, fact sheets, guidelines and inventories relevant to decision- making, practice and policy implementation	Number of visits, requests, unique visitors and document downloads		
RIO Journal Open Science pilot collection	Practice, Policy, UO	Publish unconventional research outputs, including policy briefs, recommendations, factsheets, posters, infographics, data management plans, in gold open access as a part of a comprehensive project collection	Number of visits, requests, unique visitors and document downloads,		

			Altmetrics		
Presentations at meetings, webinars	Practice, Policy, UO	Direct raising of awareness among stakeholders	Participants at meetings, feedback forms		
PoshBee training workshops, webinars	Practice, GP	Direct raising of awareness and knowledge transfer among stakeholders	Participants at meetings, feedback forms		
Project-relevant mailing lists and networks	Practice, Policy, UO	Dissemination and discussion of specific topics of interest; facilitate collaboration/uptake	Account of mailing lists and networks		
Training video series	Practice, UO, GP	Project findings and results translated in an easy to digest format for practitioners and interested private persons.	Number of views on YouTube, engagement and comments on social media		
Social media (Twitter, Facebook, LinkedIn, YouTube, etc.)	ALL	Create communities and inform members about project developments, results and recommendations	Number of users, engagement		
Supporting materials &	Supporting materials & publications				
Posters	Practice, Policy, UO	Promotion and raising awareness of the project	Number of downloads of		
Leaflets	Practice, Policy, UO	Increase awareness about the topics dealt with by the project	number of distributed printed copies		
e-Newsletter <i>PoshBee</i> BUZZ	ALL	Disseminate ongoing activities, results, and other project relevant news and events	Number of clicks and links opened		
Scientific publications	Policy, UO	Presentation of project research	Number of reads, downloads, citations		
Policy briefs and white papers	Practice, Policy, UO	Knowledge transfer from the project to policy- makers for key issues	Number of visits, downloads		
Factsheets, guidelines	Practice, UO	Transferring key results directly to end-users to ensure maximum uptake and re-use.			
Infographics & visuals	ALL	Enhance communication of core project outputs to facilitate knowledge transfer			
Wider outreach activities					
Press releases	Journalists, mass media, ALL	Announcement of significant project results	Number of press releases issued, downloads, visits		

External blogs, e- newsletters, websites	ALL	Dissemination and discussion of specific topics of interest; facilitate collaboration/uptake, contributions to newsletters and blogs to be targeted to the specific stakeholder group.	Number of posts, number of readers, engagement and feedback.
Publications in specialist and popular media, interviews, broadcasts	ALL	Raising public awareness	List of publications or broadcasts