Year 1 | Year 2 | Year 3 | Year 4 | Year 5

PPP effects on human, animal and ecosystem – systematic scoping reviews

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Summary

Background - The impact of the exposure of plant protection products (PPPs) on human, animal and ecosystem health is described in numerous publications. This report provides an overview of this knowledge base using a systematic methodology to search, retrieve and select published research in this field.

Objective - The aim is to perform three systematic scoping reviews on the effects of PPPs on human, animal and ecosystem health.

Methods - The selection of all relevant studies from online databases is done according to a pre-defined strategy in line with the widely accepted PRISMA Extension for Scoping Reviews guidance. Because of the large number of publications on PPPs we followed a stepwise approach. This involved Systematic Online Living Evidence Summaries (SOLES) using a machine-learning supported pre-selection of potentially relevant citations from Pubmed and Embase bibliographic databases. The algorithm was built and tested as part of this exercise. The search strategy combined two components. The first component consisted of a search for pesticides in general as well as by PPP component based on the SPRINT list. The second component comprised the outcome definitions human, animal and ecosystem is in alignment with the goals of the SPRINT project. Using existing animal filters and existing and developed search strategies the selections were further narrowed down to sets of typically less than hundred publications that were then screened for eligible publications according to a predefined population, exposure, comparator and outcome (PECO) statements with corresponding sets of inclusion and exclusion criteria. The sets of included studies per subtopic were hand-selected by 23 trained experts who prepared scoping reports on each of the studied health endpoint. From these reports the main text of the report was compiled. The SPRINT SOLES is provided online and is publicly available.

Results - The search started with 402,270 unique citations and was narrowed down to 200,235 using the machine-learning algorithm. A total number of 56,626 publications contained information on PPPs with 8,492 publications describing human research 26,114 on animal research and 27,990 on ecosystem research. This resulted in 61 sets of included studies each described in a scoping report: 16 for effects on human health, 14 for effects on animal health and 31 for effects on ecosystem health.

For human health the included studies describe most studies published over the past 20 years. The largest evidence base covered reproductive and developmental effects indicating that maternal (and to some extent paternal) PPP exposures are associated with anomalies in birthweight, malformations, fertility and deviations from normal cognitive and behavioral development in early life. Limited evidence was provided for PPP effects on different types of blood cancers, respiratory symptoms/disease and chronic kidney disease. Inconsistent findings were reported for involvement of PPP exposures in the onset of neurogenerative and cognitive disease such as autism, ADHD, Alzheimer's disease, Parkinson's disease and mental health effects such as depression and sleeping problems. The evidence provided for effects on non-cancer blood disease, immune system and gut microbiome were not sufficient for a conclusive statement. Most important gaps in knowledge are related to involvement of non-persistent current use pesticides (CUP) and the lack of studies on mixtures. Future studies should have a focus on effects following perinatal effects of PPP exposure later in life.

For farm animal health the number of studies for cattle, goat, pig, chicken and sheep was too small to support any conclusions regarding the effect of PPP exposures through feed or from the environment. Most available research on PPP describes efficacy of parasite or infection control. In experimental animals' effects of pesticides on gut microbiome composition was observed.

Effects on aquatic ecosystem are mostly studied under controlled conditions in toxicity bioassays. For all the species studied, there strong evidence supporting connections between PPP exposure and effects on non-target aquatic species. Distinct endpoints were impaired, including lethal and sub-lethal effects at distinct levels of biological organization.The effects on terrestrial ecosystem were mostly studied using controlled laboratory experiments. For the different species and endpoints reported in the studies considered in this review, evidence was provided for PPP effects, especially on enzymatic activity in microbiota in controlled laboratory testing. Microcosm studies confirmed these effects and additionally suggested effects on plant growth inhibition, reproduction and community abundance in soil invertebrates. There is a lack of understanding of mixture effects of PPPs on terrestrial ecosystem. Field testing is not much practiced but requires further research to fully appreciate the complexity of PPP impact in real life.

Most gaps in knowledge are related to effects of current use pesticides (CUP) that are more difficult to study than the more persistent organochlorine pesticides (OCP) because they are short-lived in the environment and in organisms. There is a paucity of research for effects of co-formulants in product formulations. For human health the use of biomonitoring in exposure assessment was a key method to demonstrate the health risk of OCP and should now be further developed and used to assess the health risk of exposures to CUP. Perinatal exposures should be further studied not only in early development but also for effects later in life. Human PPP exposures suggesting associations with health outcomes for worker's, neighbor's and consumer's health need to be confirmed in additional epidemiological studies using biomarkers and omics approaches as well as controlled exposure settings where toxicity can be studied *in vitro* or *in vivo* using the same biomarkers for confirmation of modes of action. For studies PPP effects on gut microbiome in vivo models have recently become available in rodents. For farm animals there is a paucity of studies with regard to effects of PPP residues in feed and from the environment. Effects of PPPs on ecosystem are currently mostly studied in controlled laboratory conditions on single species or micro and mesocosms. Effects of PPPs should be confirmed in field studies addressing potential effects of combined exposures to mixtures in concentration ranges reflecting real life conditions. Also here, experimental studies are needed to study toxicity mechanisms in the most sensitive species to understand how PPPs lead to adverse effects in vulnerable species.

In conclusion, PPPs is a pluriform group of biological active substances with a range of impacts on human health, animal health and ecosystem health that need confirmation specifically for the impact of new generation non-persistent PPPs that are currently used. Future research should target these CUPs based on well-established methods for exposure assessment in humans and farm animals supported by modelling. Effects on non-target species should be confirmed in field studies supported by laboratory-based studies involving new methods and disease models. To evaluate PPP effects on human, animal and ecosystem health the study of mixtures of PPPs at realistic doses is needed in future research. Different streams of evidence should be combined to reach conclusions regarding the effects of PPPs on human, animal and ecosystem health.

Cotents

Disclaimer: This report is part of a project that has received funding by the European Union's Horizon 2020 research and innovation program under grant agreement number 862568.

1. Report structure and general description

1.1. Introduction and aim

This deliverable covers three systematic scoping reviews of the available scientific literature on the effect of plant protection products (PPP). Scoping reviews follow a systematic review methodology to map existing evidence on a well-defined topic. Each of the three reviews follows the scoping review methodology using the Preferred Reporting Items for Systematic Reviews and Meta-Analyses Extension for Scoping Reviews (PRISMA ScR). The review will try to identify main concepts and theories and will attempt to identify knowledge gaps[1].

The deliverable is structured to support the three reviews in three sections each describing the current evidence for effects on humans (A), animals (B) and ecosystem (C), respectively. Plant health will be covered as part of ecosystem health and farm animals will be captured as part of animal health. Below the content of each of these reviews will be specified in more detail.

The overall aim of the scoping reviews is to map the available evidence and identify gaps in knowledge. One of the anticipated outcomes is a recommendation on the preparation of a full systematic review on selected topics that are currently not well covered by published literature reviews.

Note that D3.1 will not cover occurrence and levels in environmental compartments as this is covered in D2.2.

1.2. Methods

Below we will discuss the methodological approach to support the search and selection of relevant studies to support our systematic scoping reviews. For each of the three scoping reviews a PECO statement will be prepared. PECO is an approach to structure the search, formulate the search question and decide on eligibility of retrieved studies. It consists of the following components: population, exposure, comparator and outcome (Morgan et al., 2018)[2].

1.2.1. Search strategy

We designed and performed a comprehensive search in the online bibliographical databases Pubmed and Embase (via OVID). This search aimed to retrieve all literature describing primary research on PPPs (regardless of population or outcome) and consisted of two components, namely PPPs in general and by individual compound names. The former component consisted of identifiers such as 'pesticide' and narrower terms, such as 'fungicide', 'herbicide', 'insecticide', etc. The latter component used trivial names of PPPs currently approved for use in the EU based on the SPRINT list, which contained 206 PPP active ingredients identified by name. Search results were limited to full primary research articles as much as possible (e.g. by using "NOT review[ptyp]").

[1] Tricco et al. PRISMA Extension for Scoping Reviews (PRISMA-ScR): Checklist and Explanation. Ann Intern Med. 2018 169:467-473.

[2] Morgan RL, Whaley P, Thayer KA, Schünemann HJ. Identifying the PECO: A framework for formulating good questions to explore the association of environmental and other exposures with health outcomes. Environ Int. 2018 Dec;121(Pt 1):1027-1031.

There were no restrictions regarding publication date or language. The searches were performed on 19-10-2021 (Pubmed) and 09-11-2021 (EMBASE). The full search strategy can be found in Appendix Z. Searches strategies on specific outcomes are provided in Annex A, B and C.

1.2.2. Development of SOLES

For the specific aim of this data mining effort, we have developed a Systematic Online Living Evidence Summaries (SOLES) to retrieve published articles that describe the effect of PPP on health outcomes. The development process is shown in **Figure 1**. For the development of SPRINT SOLES, we enrolled 24 experts from the SPRINT project consortium. These experts covered all fields of interest to SPRINT (human health, animal health and ecosystem health). All experts were invited to conduct a mandatory exercise with annotations from a set of 20 publications pre-selected as 'challenging' for a decision on relevance. From this step it became clear that a distinction should be made between studies that report on an actual health outcome whereas studies that (just) provide only indirect evidence in the context of health outcome (but not report any primary research data) were not considered relevant.

Of the enrolled experts 15 completed 500 or more annotations. Conflicts were reconciliated independently by two experts (MM and PS). The annotation process was carefully monitored and meetings were organized to discuss the use of the annotation guide. Feed-back resulted in clarifications of the instructions and some resulted in simplifications of the guidance. E.g., it became clear that sometimes experimental animal studies can be distinguished from observational studies in animals on farms by using further selections of relevant farm animals using a filter consisting of a list of relevant domestic animal species. Animal studies overall may inform human, animal and ecosystem health and sometimes it is difficult to tell. Therefore, it was decided to place all experimental animal studies in a separate category and to search for mammalian toxicity studies and also consider non-mammalian models such as the zebrafish model for studies on reproductive and developmental health.

A similar solution was used regarding plant health. Our preliminary search indicated that it would be difficult to decide that effects on plants as in 'crops' would be difficult to distinguish from the effect on non-target plants species. Similar as in the case of farm animals we supposed that selections on plant health could be made by the researcher in the category 'effects on ecosystem health' applying free text searches for specific crops of interest.

Some studies clearly reported on several relevant outcomes, e.g. a study could report on the toxicity of a specific species and the same study could report on population dynamics of that species in an ecosystem. Then this study could be classified in both categories 'effect on animal' and 'effect on ecosystem' If studies describe results that cover more than one category the machine learning algorithm was adapted to allow one study to be added in two categories.

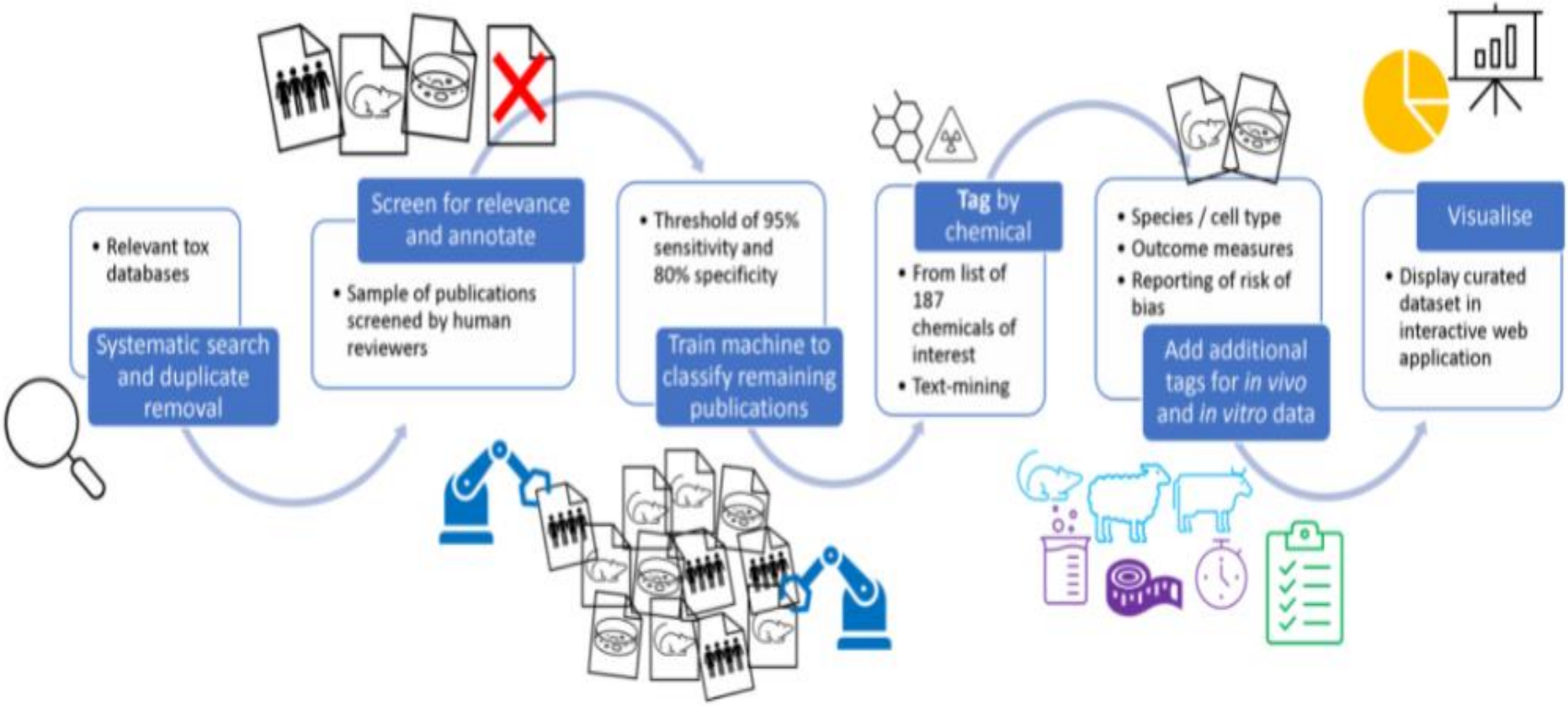


Figure 1. Step-wise development process of SPRINT SOLES.

To address the increasing body of evidence from the toxicity testing of PPPs in in vitro systems we added a separate category for this as it was considered to be difficult to a priory decide on the relevance of an in vitro study outcome for either human, animal and/or ecosystem health. Instead, we suppose that experts would use this subset of studies and decide (based on the type of endpoints reported) how the study and reported endpoint(s) will inform one or more fields of interest. This refined judgment is difficult to implement in machine learning specifically if testing is done on non-mammalian cells. Annotation of 5,000 entries and reconciliations of conflicts resulted in 4,957 fully annotated unique publications. This set was used to generate the machine learning algorithm.

1.2.3. Using SPRINT SOLES

The SPRINT SOLES database can be searched for any terms in title and abstract and keywords field of study records. There is no need to put quotation marks around search terms e.g. if searching for Alzheimer's disease OR dementia, this would be entered in the input box as: Alzheimer's disease, dementia. Wildcards can be used: Parkinson.* to search for Parkinsons, Parkinson's OR Parkinsonian. Search terms can be entered separated by commas. Boolean operators can be used to determine whether a study must contain all terms (AND) or any of terms (OR). Searches were made in one out of four pre-defined subsets: 'effect on human health', 'effects on animal health', 'effect on ecosystem health' and 'effects on in vitro models'. Results can be filtered by pesticide, animal species.

1.2.4. Eligibility of studies

Studies that were considered eligible are studies describing any observed effect on health that can be considered adverse. For example, in toxicology it is very common to make a distinction between physiological responses that do not indicate adversity and endpoints that indicate adverse effects that can be confirmed by histological/pathological methods, e.g. on molecular level (e.g. DNA-damage), cellular level (damage to cell structure or cell organelles and subcellular structures), organ level (cell necrosis and loss of organ functional capacity), organism level (effects morphology, physiology or behavior) and population level (changes in certain diseases, survival and/or reproduction/fertility on the level of species or community).

Studies that are included report on

- Any effect-related outcome indicating relevant changes in health status such as a disease or mortality on any level (individual/group/population/community)

Studies that excluded are studies that report on

- Environmental fate and exposure;
- Biotransformation/metabolism of PPP active ingredients on cellular, organ or organism level;
- Biomarkers informing on exposure; and
- Purely theoretical simulation studies.

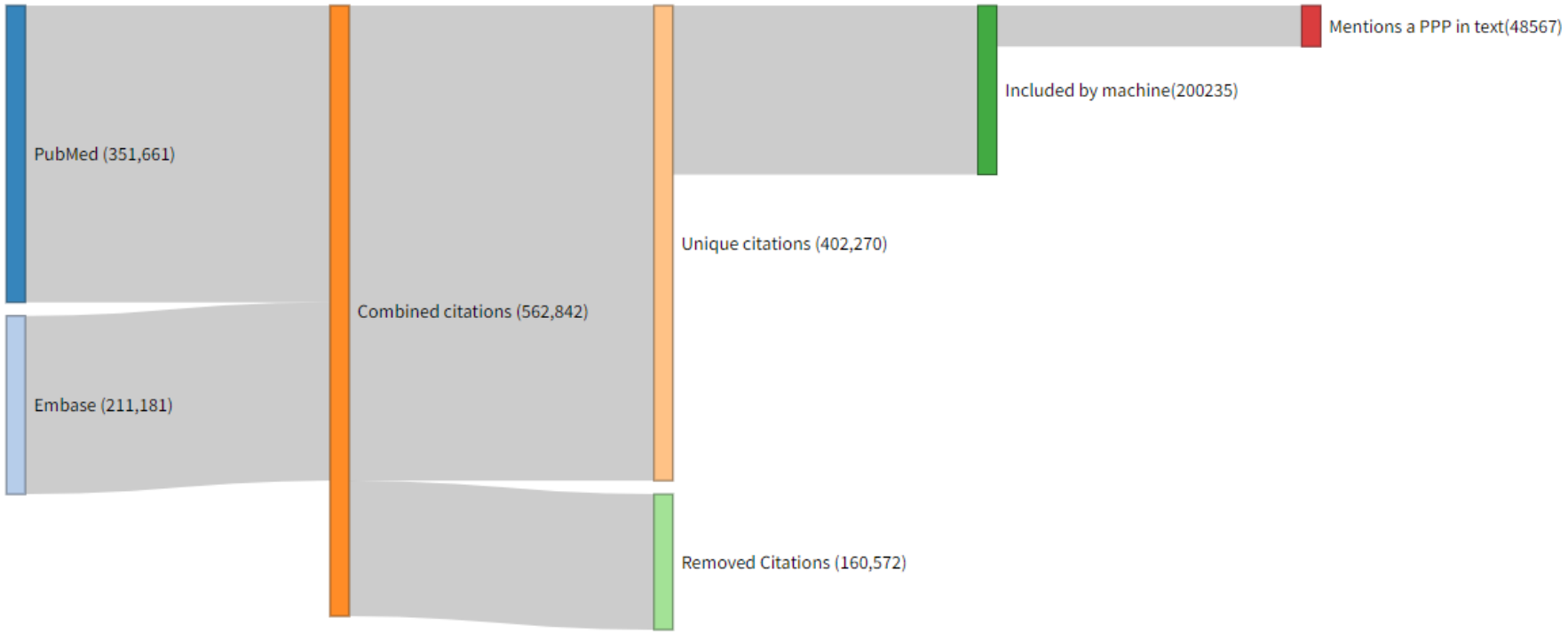


Figure 2. The stream of publications in our workflow. PubMed and EMBASE were searched for relevant citations on 20 November 2021 and identified over 500,000 potentially relevant publications using the search strategy in Supplementary file 1.

1.3. Results and discussion

The search resulted in approx. 562.842 retrieved citations in Pubmed and Embase (status 20-11-2021 (**Figure 2**). After deduplication the total number of studies retrieved by data mining was approximately 402,270 (status 20-11-2021. Using the machine learning it was possible to reduce to a selection of approximately xx relevant citations (approximately 18.8 %) which amounted to 48.567 publications considered relevant (**Figure 3**).

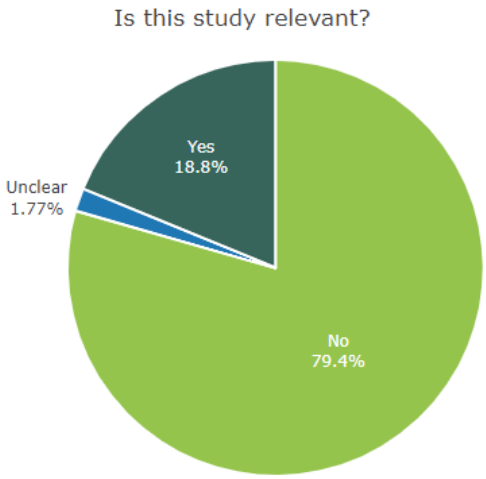


Figure 3. *SOLES provides a selection from the Pubmed and Embase indexed publications relevant to SPRINT.*

All pesticides were categories by research including effects on human health, ecosystem, effects in vitro models, effects on experimental animals and effects in observational studies on animals. The last category included field studies on animals including farm animals (Figure 4).

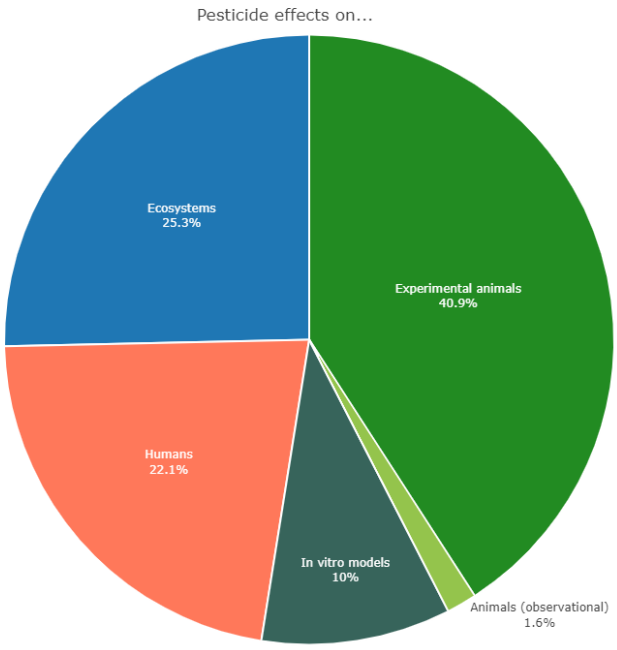


Figure 4. *Distribution of relevant publications by categories of 'pesticide effects on …'*

As show in **Figure 5**. most studies were published by authors from the US (23,247 studies) followed by China (21,993 studies). The next highest-ranking countries were from Europe: Germany (8,485), United Kingdom (8,186), France (7,316), Italy (6,244) and Spain (6,244). Canada and Brazil matched the contribution by European countries well with 6,777 and 5,813 studies, respectively.

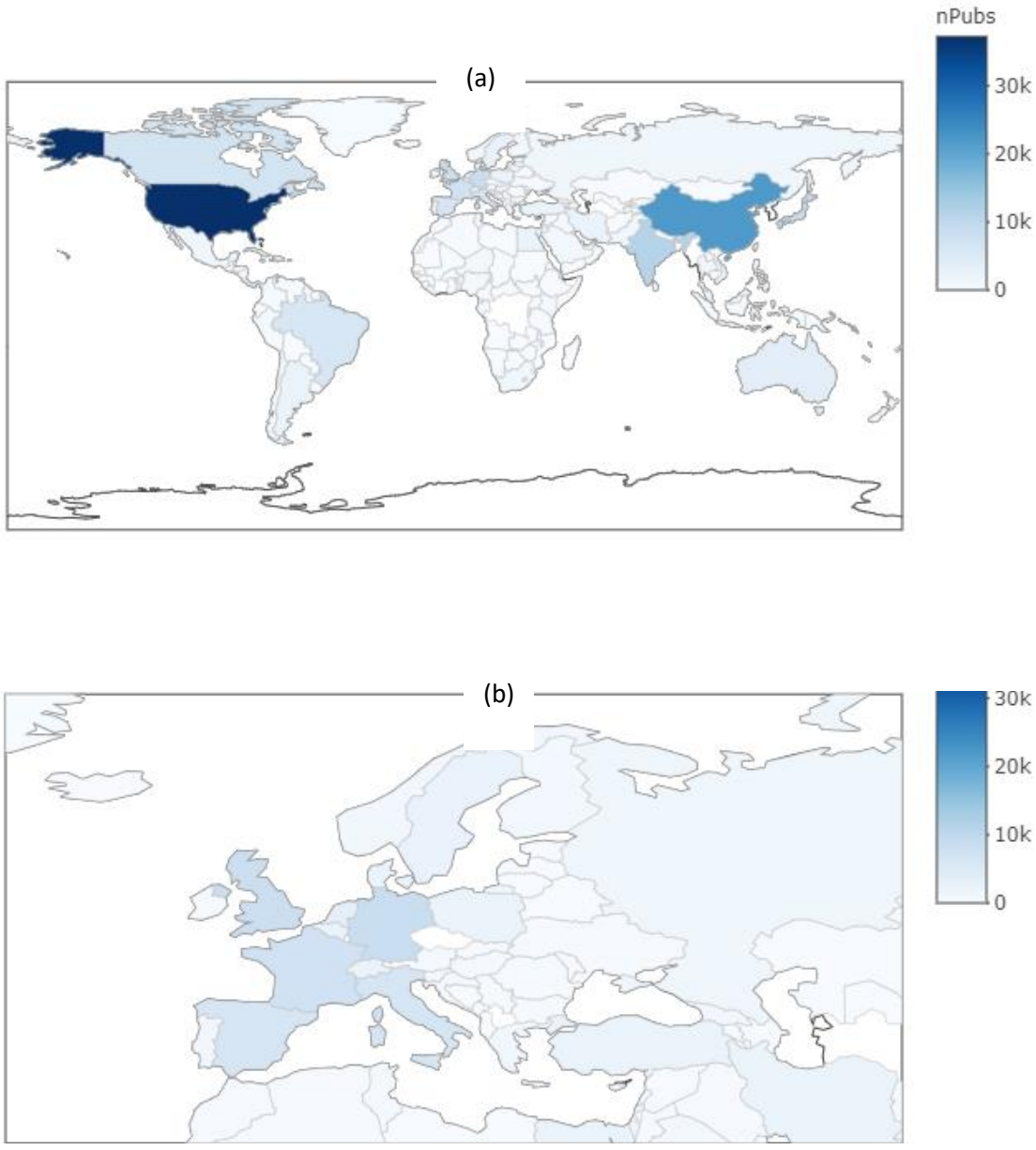


Figure 5. Worldwide map(a) and European map (b) of author location as indicator of the distribution across continents and countries. For information on the number of studies use the online tool entry 'Database summary' at https://camarades.shinyapps.io/SPRINT-SOLES/

The frequency of studies published over time shows a gradual development until 2010 and a more rapid increase of the interest over the past 10 years (**Figure 6**).

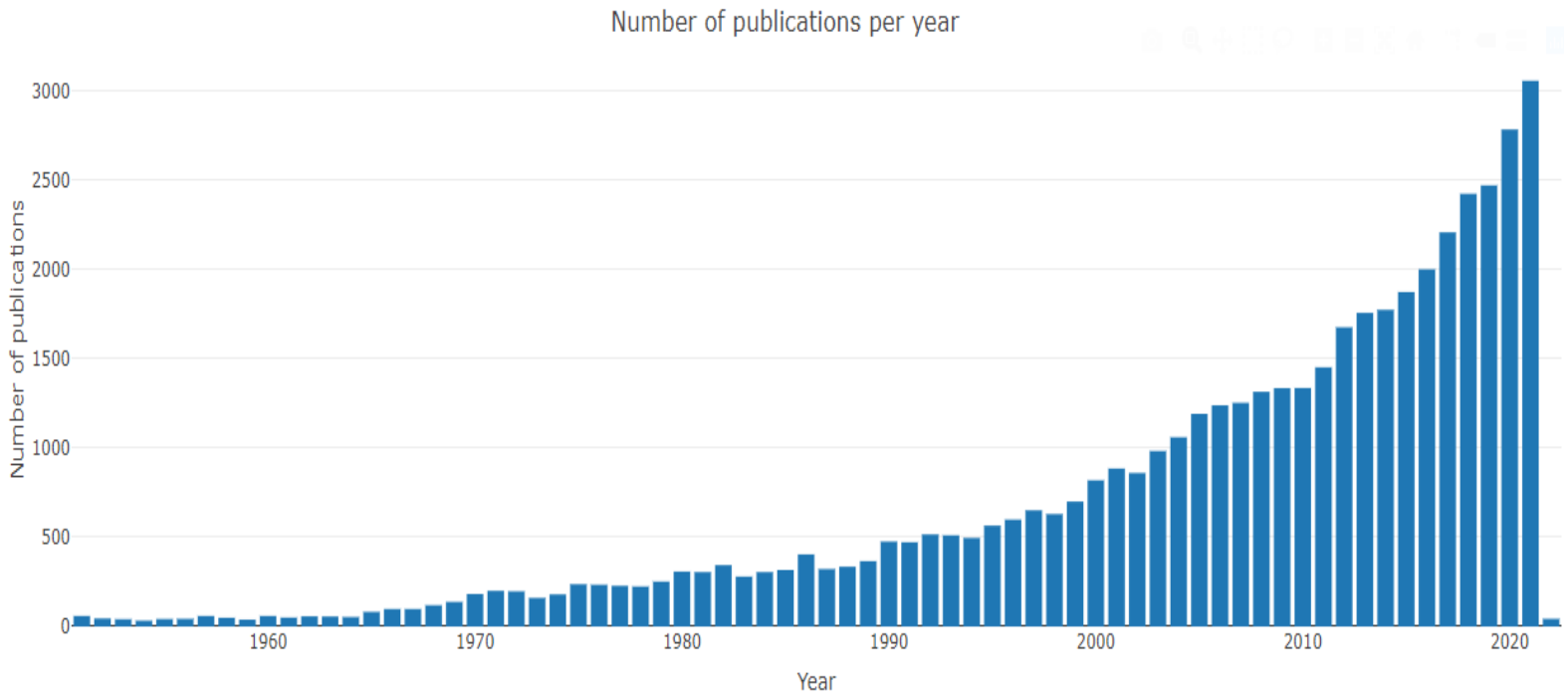


Figure 6. Development of the number of publications in the selection of relevant studies over time (N=48.567).

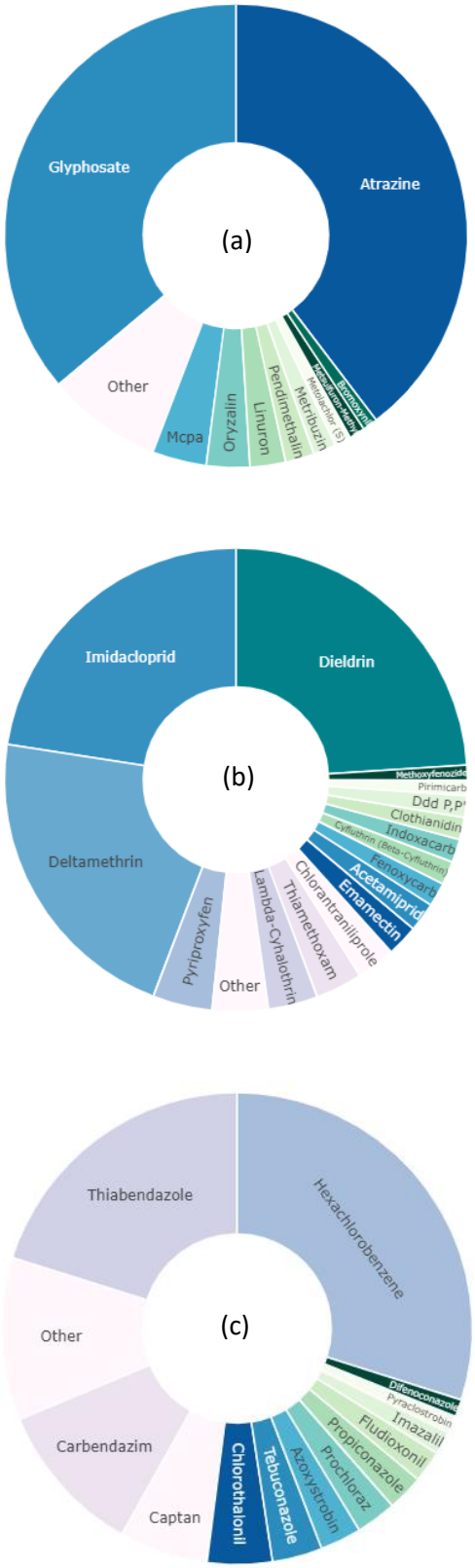


Figure 7. Plot showing the distribution of PPPs by application group: herbicides (a), insecticide (b) and fungicides (c). Online the PPP can be selected by group of application.

Figure 7 showed the qualitative distribution of studied PPPs by application group whereas **Figure 8** presents the number of publications within each group of application. Atrazine (2972 studies) and glyphosate (2,706 studies) are by far the most studied herbicides followed by 2-methyl-4-chloro phenoxy acetic acid (MCPA, 295 studies). Hexachlorobenzene (HCB) was the most reported fungicide (1,572 studies) followed by thiabendazole (1,059 studies) and carbendaim (557 studies) as shown in

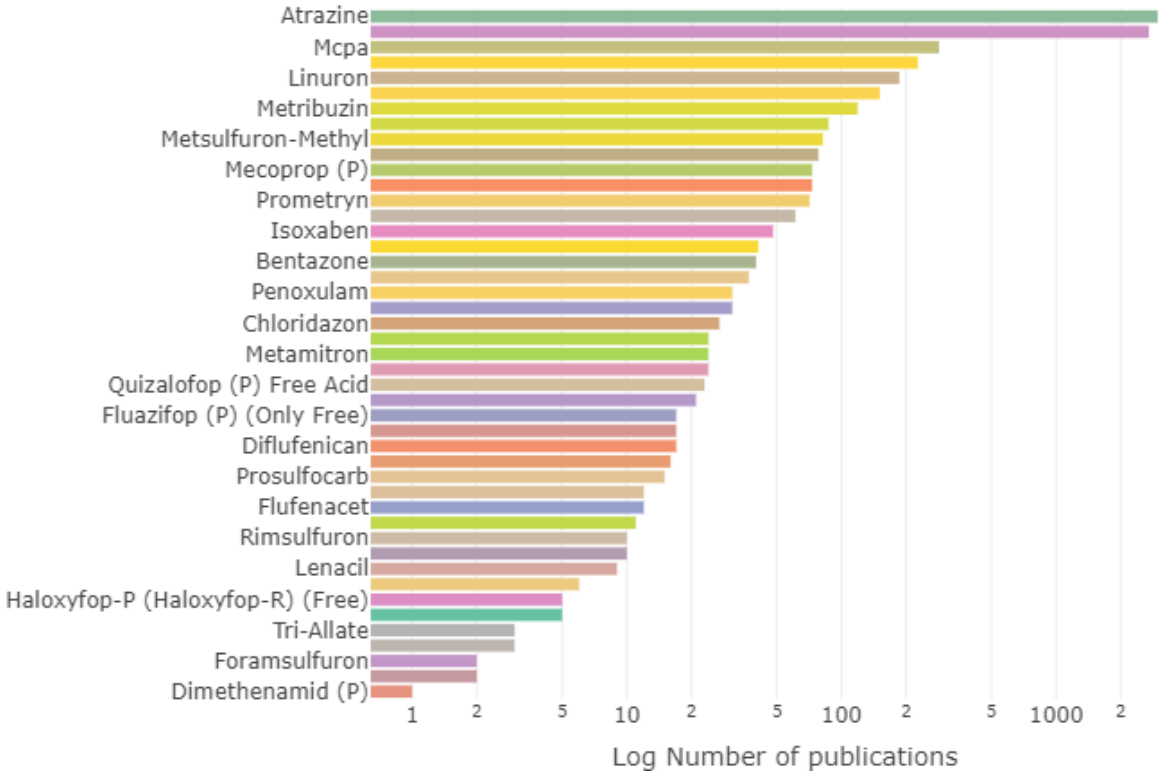


Figure 8. Frequency of publications reporting on at least one of the herbicides from the SPRINT selection.

Figure 9. For insecticides the most reported PPP was dieldrin (1,395 studies), closely followed by deltamethrin (1,295 studies) and imidacloprid (1,312 studies) **(Figure 10.)**

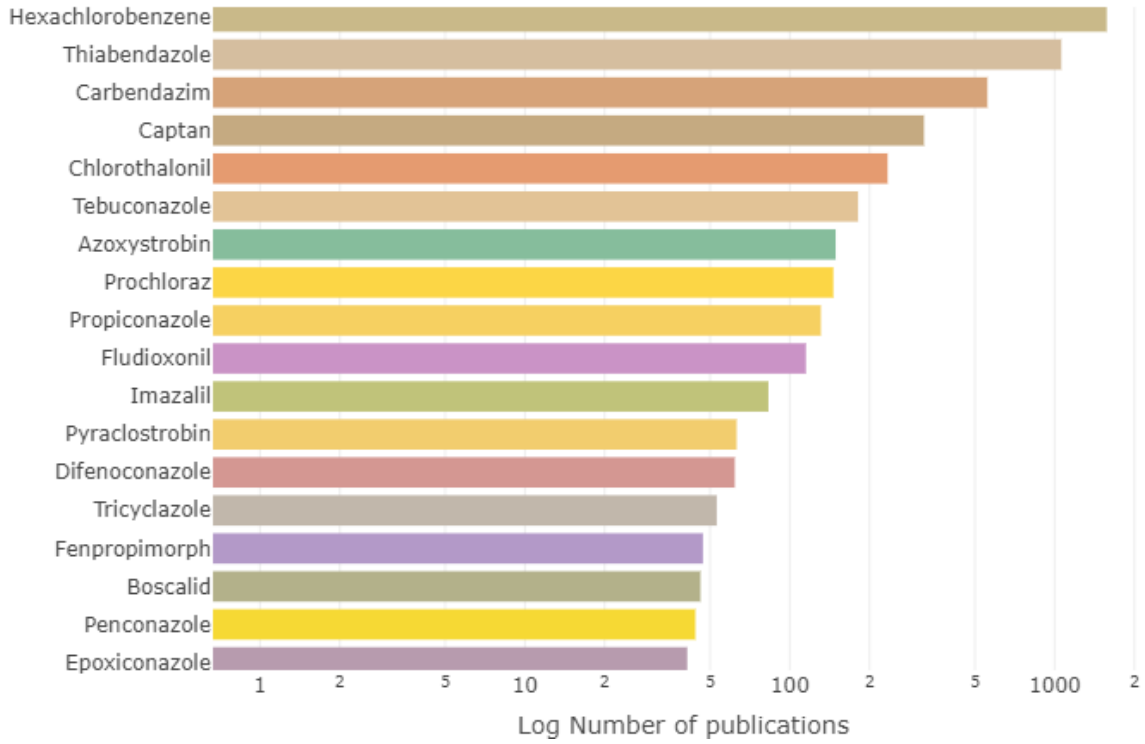


Figure 9. Frequency of publications reporting on at least one of the fungicides from the SPRINT selection.

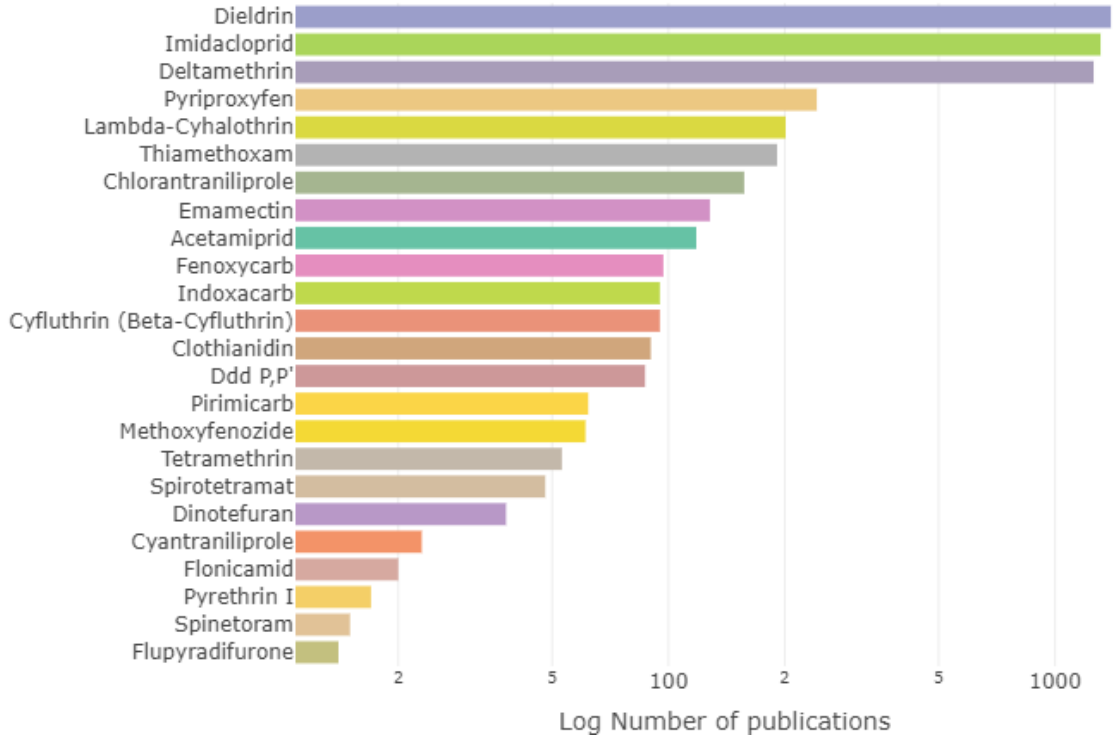


Figure 10. Frequency of publications reporting on at least one of the insecticides from the SPRINT selection.

In **Table 1** some of performance indicators of the machine learning are presented for the search in Pubmed and Embase. A highly sensitive search tries to retrieve all relevant documents by using a broad search. Search with a high specificity tries to target the citations that come close to the topic of interest. For the machine learning we have prioritized sensitivity over specificity, meaning that the probability of not retrieving citations that may be of interest is kept small. As it is difficult to maintain a good balance in our case, specificity was much smaller. This is shown by the high sensitivity for the overall result of the machine learning with a high sensitivity of 0.95 and a specificity of close to 0.50. For human health this trade-off was much better because identification of human-related citations is overall easier. For animals and ecosystem, the performance in specificity was much lower probably due difficulty to target specific species of interest by search term. In addition, for ecosystem health not only research at on the level of a species can be relevant but also research describing the effect of PPPs on the level of population dynamics, trophic levels and biodiversity. With a high sensitivity and much lower specificity there is still much manual work to sort out the relevant from the less- and non-relevant citations. To compensate for this, additional dedicated filters were developed for experimental animals and domestic animals as a proxy to farm animals. These filters can be activated in the 'Search database' function and are based on lists that contain the species of interest. In the case of domestic animals, animals not bred on European farms and pets were also captured. This needed some further manual selections to sort out the different uses of PPPs related to farms and farm animals (including disinfection treatment and use as biocide).

Table 1. Sensitivity and specificity characteristics for searches in Pubmed and Embase. [a]After machine learning for inclusion/exclusion (not matched to PPP but some will still be irrelevant for SPRINT). Numbers are based on searches performed on 20 November 2021.

Data-base	Category	Citations[b]	PPP-related[b]	Sensitivity	Specificity
Pubmed	Total after machine learning[a]	215,997	50.641	0.9526	0.4891
	Human	44,137	7,506	0.9643	0.9353
	Animal	200,218	44,484	0.9733	0.2338
	Ecosystem	178,471	44,044	1.000	0.4570
Embase	Total after machine learning[a]	27,989	5,985	0.9526	0.4891
	Human	25,098	986	0.9643	0.9353
	Animal	25,098	5,254	0.9733	0.2338
	Ecosystem	23,815	5,343	1.000	0.4570

1.4. References

- Tricco et al. PRISMA Extension for Scoping Reviews (PRISMA-ScR): Checklist and Explanation. Ann Intern Med. 2018 169:467-473.
- Morgan RL, Whaley P, Thayer KA, Schünemann HJ. Identifying the PECO: A framework for formulating good questions to explore the association of environmental and other exposures with health outcomes. Environ Int. 2018 Dec;121(Pt 1):1027-1031.

A. PPP effect on humans

A.1. Introduction and objectives

A.1.1. Objective

The objective of this section is to map relevant studies that report on the effects of PPPs on human health.

A.1.2. Research questions

The scope for this review is defined by specific pre-defined broad health-related categories of resilience and reproductive health reported in peer-reviewed scientific literature.

Each of these categories was subdivided in subcategories that can be defined in a dedicated search strategy. In addition, we searched for specific adverse health outcomes and diseases related to selected organs and organ systems.

1. What health outcomes are reported with respect to the main SPRINT categories resilience, reproductive health?
2. What health outcomes are reported in the resilience subcategories Immune system disease, gut microbiome and respiratory disease?
3. What health outcomes are reported in the reproductive health subcategories congenital malformations, prematurity/low birth weight, birth defect, still birth, male infertility, female infertility and time to pregnancy?
4. What diseases are reported in the SPRINT selection of organs and organ systems (lung, kidney, brain/mental, blood)?

A.2. Methods

A.2.1. Search strategy

The development of the SPRINT SOLES was described in section 1.2. The starting point was the use of the 'human' filter which provided a subset of 8,492 studies. For answering the research questions existing search strategies were used in part based on the thesaurus terms in Pubmed and also based on published protocols in an international register of systematic reviews PROSPERO.

A.2.2. PECO statement and selection of studies

Population - Humans from all genders, ages and ethnic backgrounds, including their offspring.

Note 1: specific populations of interest include: farmers and their families, non-farmer residents in the rural environment (also often referred to as 'neighbours') and the 'average' consumer.

Exposure - Exposures to human-made or natural products marketed and used as plant protection products (pesticides) today (SPRINT list of 200+ PPPs) or at any time in the past, their active ingredients their metabolites, degradation products, adjuvants and co-formulants as part of products, including mixed exposures.

Note 2: Exposure measured by environmental sampling of any environmental compartment including sinks and contaminations to describe fate and exposure. Second, all types of human biological media (body fluids, exhaled air and all other excreta like urine and feces) to reflect internal exposure.

Note 3: Exposures from all uses, professionally as well as private home use, due to residue of PPP from all sources and routes of uptake such as diet, indoor/outdoor air, soil, water, home dust, etc) by any exposure route, including oral, dermal or inhalation.

Note 4: Any type of use in an occupational/professional setting such as integrated pest management as well as private use indoor (home) or outdoor (garden).

Comparator – Low or lower exposed populations and subgroups such as alternative farming as no/low PPP use alternative. Self-reported exposures by consumers will be considered to reflect a baseline in exposure to PPPs. In biomonitoring studies often, the lowest tertile or quartile of a distribution of exposure biomarkers is used.

Note 5: we are also interested in designs where rural neighbours may also serve as comparator to farmers and their families (within conventional and organic farm systems)

Outcome – Any adverse health outcome (including effect biomarkers) related to toxicity mechanisms considered relevant to the impact of PPPs on human health

Note 6: For carcinogenicity we will use the IARC key characteristics that have been added in 2019 to the Preamble for hazard classification (see https://monographs.iarc.who.int/wp-content/uploads/2019/07/Preamble-2019.pdf).

An overview of the used search strategies dedicated to each of the predefined categories of human health outcomes and organs and organ systems is provided in Appendix 5. Some searches that yielded a small set of studies were combined (e.g. mental health was combined with mental disorders). For reproductive health we used a number of search strategies to capture the entire field. This resulted in some duplication due to the search terms used. We also retrieved studies that described multiple outcomes that fitted in the scope of different subcategories of reproductive and developmental health. In that case the study was used multiple times and endpoints were divided between the subcategories

A.2.3. Screening

The searches were divided among four experts who did the title-abstract screening and provided a 2- to 3-page template with characteristics of the included studies and a synopsis of the findings. Each expert provided a concluding statement (see Annex A-C).

Studies that were included provide research on:

- Any effect-related outcome indicating relevant changes in health status such as a disease or mortality on any level (individual/group/population/community)

Studies that excluded were studies that report on

- Environmental fate and exposure;
- Biotransformation/metabolism of active ingredients on cellular, organ or organism level;
- Biomarkers informing on exposure; and
- Purely theoretical simulation studies.

The reasons for not including studies were logged and a summary is provided in the flow diagram describing the search. More details are provided in Annex A.

A.3. Results

Below the PRISMA flow diagram (**Figure 11**) shows the selection process with numbers of studies included and excluded. Reasons for exclusion are provided for each species/endpoint in the scoping reports in Annex B.

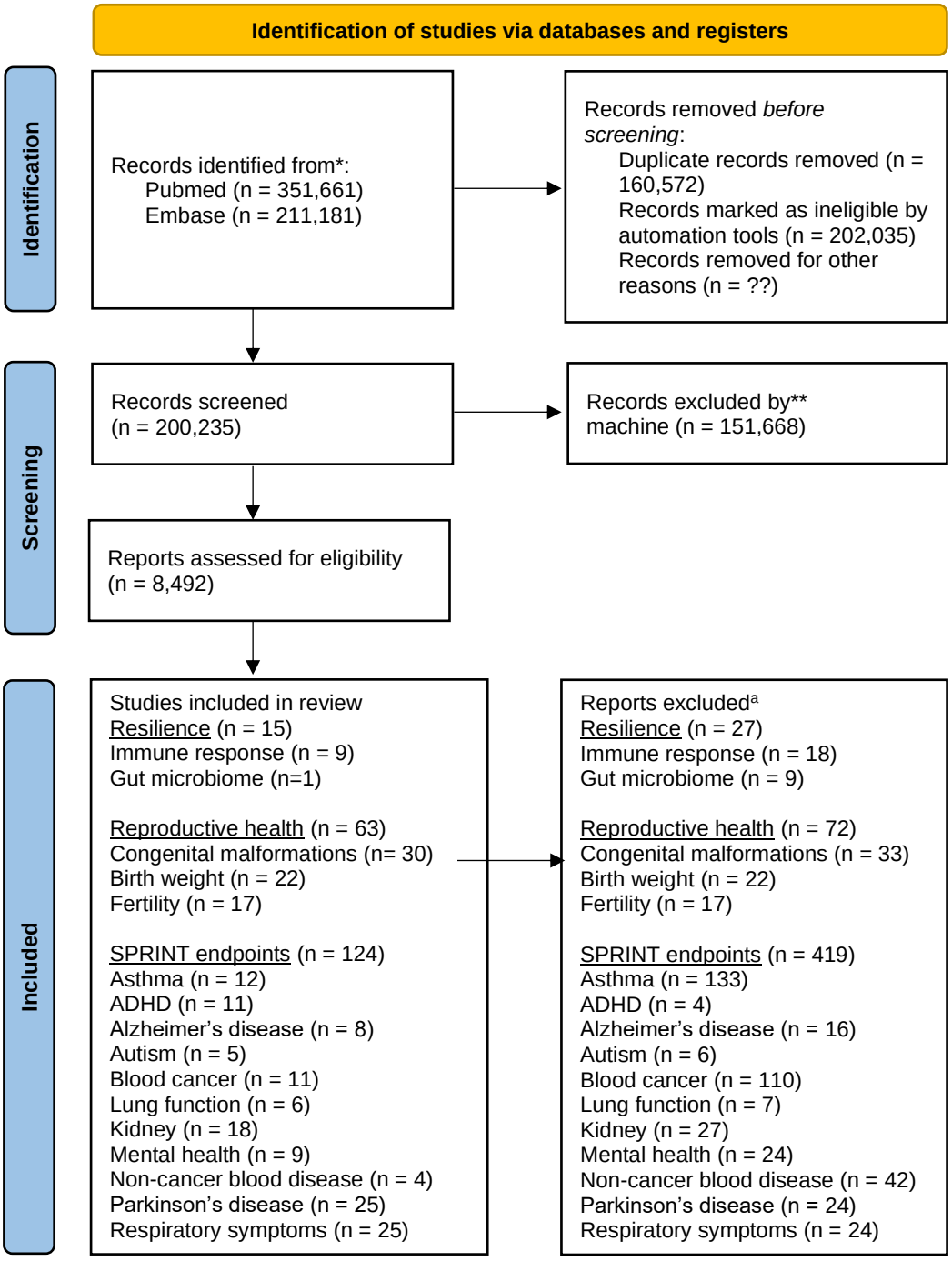


Figure 11. Flow diagram systematic scoping review of human health outcomes (Page et al., 2021).
[a]Reasons for excluding studies are specified in Annex A.

SPRINT has a focus on pre-specified health organs and organ systems: lungs, kidney and central nervous system. For this scoping review this is operationalized by study of respiratory symptoms, asthma and lung function, chronic kidney disease, and a cluster of neurodegenerative diseases: ADHD, Alzheimer's disease, Autism, mental disease/disorders and Parkinson's disease. Below the evidence for a role of PPP exposures in the onset of these health conditions is presented in more detail.

A.3.1. Immune response

Although the study set is limited to nine included studies, there are indications that maternal OCP levels in blood and breast milk are associated with different biomarkers of immunotoxicity in infant's development. Included studies have reported on pro-inflammatory indicators in auto-immune response but these effects need further confirmation because exposure was only based on questionnaire data by self-assessment. There is a lack on studies addressing current use pesticides.

A.3.2. Gut microbiome

Only one study from Norway described exposure to OCP and other persistent organic pollutants (POPs) and their relationship to gut microbiome in a mother-child cohort.
IN this study 24 POPs were analysed from breast milk and related to developing infant gut microbiome, more specifically to microbiome functionality, explaining up to 34% of variance in essential signalling molecules. Some effects were reported for polychlorinated biphenyls (PCBs), and per- and polyfluoroalkyl substances (PFASs but no associations were specifically involving OCP. Environmental toxicant exposure may influence infant gut microbial function during a critical developmental window and lead to a disruption of the microbial community that that influence life-long health. Future studies are needed to replicate these novel findings and investigate whether this has any impact on child health.

A.3.3. Reproductive health and developmental effects

A.3.3.1. Congenital malformations

All 24 included studies were published in the last 20 years. Twenty studies were published since 2012 and 16 over the past five years indicating that interest in pesticide exposures as a risk factor in congenital malformations is growing. Initially OCP were studied as part of a larger group of POPs. More recently attention was shifted to include also less resistant PPPs such as atrazine and glyphosate. Most studies were performed in the general population and only few involved worker's exposures. Most of the studies used quantitative exposure data from analyses of relevant biological media such as cord blood. Only few studies classified exposure in other less reliable ways. General indicators such as body weight, length and growth-related parameters were often reported. Most reported associations were reported with cryptorchidism, hypospadias and gastroschisis. Many studies have a focus on male or female offspring. Few studies addressed ratio of males to females. There are clear indication of PPPs having endocrine disruptive effects leading to feminization in male offspring and

masculinisation in female offspring. In recent anogenital distance and index in female offspring was suggested to be affected by OCP or glyphosate. Malformations is a difficult field for systematic review and meta-analysis due to the complexity of the definition of timepoint of exposure during the reproductive cycle and a very wide range of different endpoints to be studied in new-borns.

A.3.3.2 Birth weight

Of 22 included studies 17 were published in the past 10 years. As biometric parameters at birth are routinely collected and registered there are many opportunities to relate these outcomes in large mother-child cohorts to environmental exposure including pesticides. In total 16 studies reported body-weight related (mostly biometric parameters) at birth without any indication of development later in life. However, they may reflect intra uterine development during pregnancy and associations were observed with exposure during all trimesters. Studies of atrazine in drinking water in studies from the US and India suggest a small but consistent effect on a range of birth weight-related endpoints. Organochlorines, glyphosate, pyrethroids and neonicotinoids were also reported to be associated with birth weight anomalies. Exposure-response relationships were observed for some specific PPPs. It is not clear to what extent these associations are confounded by host or other environmental factors. Biometrics at birth represent a global indicator of a potential underlying adverse effect. Other endpoints observed later in the development of new-borns may lead to better understanding of the value of the observed effects. Further studies are need to understand the underlying causes of anomalies in body weight and other biometric indicators that are collected at birth and how they are related to perinatal exposure to pesticides. A potential role of paternal exposures in occupational settings needs more future studies.

A.3.3.3. Fertility

All 17 included studies were published in the past 20 years and covered mostly OCP and CUP in a wide range of study designs. Most studies reported on biomonitoring of OCP in blood and breast milk as predictor of fertility. There is substantial evidence for OCP as risk factor in reduced fertility supported by the analysis of OCP from semen fluid and follicle fluid in infertility clinics. Multiple studies implicated atrazine, chlorpyrifos, diazinon and pyrethroids as potential risk factors for reduced fertility. These findings were supported by urinary metabolite levels providing quantitative data and the possibility to study exposure-response relationships. Because of the increasing role of treatment of fertility in healthcare settings there is an opportunity for multi-center studies reporting on PPP residues in semen and follicular fluids and how these levels relate to fertility endpoints and effects on fertility in offspring in prospective mother-child cohorts.

A.3.4. Respiratory symptoms

Based on 25 studies on PPP and respiratory symptoms some evidence is provided for PPPs being able to cause changes in respiratory symptoms and possible infections. The evidence is

strongest for prenatal effects, where pesticide exposures are measured in blood or urine. There is an obvious lack of prospective studies among farmers and other adults using high quality exposure measures, for example exposure biomarkers.

A.3.4.1. Asthma

Over a period of 20 years six studies reported exposures of the general population related to the risk of asthma based on self-reported symptoms. In two studies exposure of dithiocarbamates estimated by urinary excretion of ETU was associated with self-reported wheezing. Two studies reported an increase of the risk of asthma in young children following exposure of the mother. Six studies described mostly self-reported exposures to multiple PPPs in male workers and observed associations with a wide range of PPPs, indicating that pesticide applications can be considered a risk for self-reported asthma symptoms. Overall, many studies reported exposure-response relationships based on biomonitoring which strengthens the suggestion that pesticides exposure may be considered a risk factor of asthma. An inherent weakness in asthma research is the difficulty to adjust for other host or environmental factors that may attenuate the reported risk estimates.

A.3.4.2. Lung function

Of the small number of six studies available, some studies relate to current exposures in Europe (France, Spain and UK). Exposures were based on analysis of body fluids in three studies. Lung functions were not reported as %-predicted and respiratory complaints were mostly self-reported. More studies are needed to assess potential effects of low environmental exposures to currently used pesticide mixtures specifically in young children.

A.3.5. Kidney

In the eighteen studies investigating PPPs and their potential effects on renal function, the observations are consistently suggesting an increased risk for chronic kidney disease among farmers (mainly in low-income countries), pesticide applicators and their wives and children living and working in agricultural communities with frequent exposures to pesticides. The quality of drinking water is a main suspected route of pesticide exposure associated with kidney disease, at least supported by Sri Lankan data. In addition to the included, two extensive meta-analyses on over 60 studies showed associations between the use of agrochemicals and chronic kidney disease of unknown etiology. From the current data it is not possible to propose a pesticide or class of pesticides specifically linked to kidney disease. The main factors that prevent to draw strong conclusions are: small sample sizes, single time point analyses, no determination of pesticide levels in biological samples, lack of age/gender or geographic location matched/unmatched controls, lack of renal biopsy information, the wide variety of agricultural compounds which may also contain toxic adjuvants or which may only become toxic after metabolization, etc. Future research should include longitudinal studies, toxicokinetics, RNA, DNA, protein analysis urine metabolomics, biomonitoring by analyses of blood, urine and renal tissue, profound inventory a persons' historical pesticide use, intervention studies with clean drinking water, consensus on which (renal) biomarkers

to include and how to measure them in a standardized way, etc. Clarification of the role of pesticides in human kidney disease can never come from epidemiological studies alone but needs to be complemented with in vivo and in vitro experiments.

A.3.6. Neurodegenerative disease

Neurotoxicity is one of the key-modes of action of OCP and CUP. Neurotoxicity is driving effects at high exposures in occupational setting and also one of the main mechanisms involved in the severe effects of intoxications. The effect of exposure at low concentrations is less well understood and is described for some key health effects and diseases occurring in early life and also in elderly. PPP related neurotoxicity and neurodevelopment as a result of pre- and perinatal and inter-uterine exposures during pregnancy are described in section 4.2 on reproductive health and developmental effects.

A.3.6.1. ADHD

All eleven included studies used analysis of maternal blood, cord blood or maternal urine for exposure classification. No studies attempted to include genetic factors. In a six studies different expressions of the exposure to organochlorines p,p' DDE, ß-HCH and HCB were reported to be associated with the risk of ADHD. The wide variety in exposure and outcome definitions make it difficult to draw any conclusion. CUP, more specifically reported as exposure to pyrethroids was consistently linked to an increased risk for ADHD in three studies. Chlorpyrifos was also reported to an associated with increased risk for ADHD. More and better designed population-based studies are needed to verify if an association between pesticide exposures and the risk of ADHD can be found.

A.3.6.2. Alzheimer's disease

Eight studies were included that reported on the role of pesticide exposure in the onset of Alzheimer's disease. Most studies that were available considered OCP exposure status based on blood levels. Five out of eight included studies were from only two research groups (in India and Canada). No studies were available for CUP. AD prevalence was reported in comparison to healthy controls and in one study with patients diagnosed with other neurodegenerative diseases including dementia and Parkinson's disease. Some studies also relating pesticides to outcomes of standard cognitive tests. Results are all from small study groups (in some cases nested in a cohort study) and show an inconsistent pattern of positive and negative findings related to HCB, dieldrin and DDT/DDE even at high exposures in one study in workers and in one study reporting on brain tissue analysis. Nevertheless, it was possible to find indications for and effect of gene environment interaction as shown for genetic polymorphisms. More studies are needed to further study if and to what extent CUP is a risk factor in AD.

A.3.6.3. Autism

In a small collection of five included studies associations of autism spectrum disorder and development delay were reported for a wide range of pesticides covering mainly environmental exposures. Only one study addressed self-reported home-use of pesticides. Exposure assessment was mostly based on level or frequency of detects in maternal or child's blood. Studies reporting on temporal trends and spatial patterns provided only weak evidence due to the many other factors not accounted for in the analysis. More studies are needed to clarify if there is a role for pesticide exposure in the risk of autism.

A.3.6.4. Mental health/disorders

Although the study set is limited to only nine included studies there are indications that blood levels of OCP might have an influence on depression in workers. One study also reported sleeping problems with the use of CUPs. Unspecified PPP involved in cases of poisoning were associated with a higher reported risk for mental health problems. This is a finding that raises the question of potential reverse causation. Prenatal exposures to OCP were associated with mental health and differences were reported between male and female offspring. Studies that used self-reported exposure and self-reported mental health effects reported also associations but these findings should be interpretated with caution. A registry-based study on glyphosate was not considered reliable to draw any conclusion due to a poor study design.

A.3.6.5. Parkinson's disease

Based on 21 included studies, Parkinson's disease (PD) has been studied in relation to a wide range of pesticides over a period of 15 years. Occupational studies have looked at groups of pesticides as well as individual pesticides and many different expressions of exposure levels and by exposure duration. In three occupational studies blood was analyzed to confirm exposures. However, most of the included studies based an association with the risk of PD on self-reported exposures. Overall, pesticide applications resulting in contaminations of environments compartments such as water bodies are implicated to have an influence on PD risk but the evidence varies from one to the other compound and is inconsistent for some of the much-used compounds. In residential settings the evidence was retrieved from a variety of environmental samples related to use at home, at a distance from applications on nearby farmland and also related to private water wells as well as ground water. Only three studies used biomarkers to study the relationship between exposure and disease in more detail looking at *ABCB1*, CYP2D6 and GSTP1 polymorphisms which may provide useful information on the mode of action but these findings need confirmation in follow-up studies. Only one study tried to link pesticides, glycoproteins in samples of brain tissue with Lewy pathology. Overall, the evidence for involvement of CUP in the onset of Parkinson's disease is inconsistent and more studies are needed with improved methods of exposure assessment, i.e. human biomonitoring.

A.3.7. Blood

Effects on blood have been subdivided by blood cancer and other non-cancer blood pathology.

A.3.7.1. Blood cancer

In the past 20 years, eleven included studies have reported on the association of pesticide exposure and different manifestations of lymphohematopoietic cancers (LHC). Non-Hodgkin lymphoma (NHL) was the most studied and associated with OCP exposures in eight studies. Other studies found indications of a potential role for pesticide exposure as a risk factor for one or more subtypes of LHC for: captan, dieldrin, metribuzin, chlordane/heptachlor, heptachlor epoxide and dieldrin. Other PPPs such as glyphosate and p,p'DDT and p,p'DDE have been reported not to be associated with LHC. More recent studies used blood-based data which improved confidence in exposure classification and resulted in reports of trends of risk with exposure intensity. Most included studies have addressed OCP. More studies are needed to explore potential associations of LHC with current use pesticides.

A.3.7.2. Non-cancer blood disease

Four included studies reported that in high exposure settings in workers and in the general populations non-cancer blood effects were related to pesticide exposure. Neutrophils and their function were studied in relation to immunological disorders, blood anaemia and blood clotting disorders. Two studies suggest a potential role for pesticides in infants (<5 y) which need confirmation in follow-up studies. It is unclear to what extent differences in risk between males and female are related to exposure level or may indicate a gender-related difference in susceptibility. Overall, associations between pesticide exposure and blood biomarkers are most often related to high exposures such as in cases reports of intoxications (not included here).

A.4. Discussion

The aim of this scoping review was to map available evidence and determine gaps in knowledge. This field is particularly challenging because of the many factors that may have an influence on the association between pesticide exposure and potential health effects. Host factors may be constitutional and acquired characteristics that may each attenuate the relationship between exposure and health effect. In addition to host factors there are many other factors that all may have an influence in the etiology of disease. Environmental factors include life-style factors and indoor and outdoor air quality. Compared to consumers, farmers and their families have additional exposures derived from organic dust and include mycotoxins and endotoxins as well as allergens that may give rise to high exposures (e.g. from grass, trees and animals). Farmers may also use their own wells for the supply of drinking water. Not all studies have been adjusted for these exposure and risk modifying factors during the analysis of their data.

Specifically related to the use of PPPs it is well known that in addition to the active ingredients, products may contain certain co-formulants to optimize crop protection performance. Especially co-formulants with surfactant properties may have its own toxicity or interact with the effects of pesticide active ingredients.

Many of aforementioned limitations can be resolved by improvement of study designs to include the collection of repeated samples from the same person e.g. during PPP use-period and PPP non-use periods. Recruiting controls with well-defined and characterized exposures, preferably matching the index populations for person- or environmental characteristics. In both groups should contextual data should be collected to be considered in the analysis for a reliable interpretation of the study outcome. Large cohorts with extended follow-up in sufficiently large populations provide such conditions as demonstrated by some successful cohorts from which the Agricultural Health Study (AHS) with more than 50,000 farmers and their families in the US and provided many publications that were cited for different health outcomes in the current report. Mother-child cohorts generate rich datasets often supported by PPP analysis of biological tissues such as breast milk, cord blood, macula, placenta or other tissues collected at birth. To definitely conclude on a putative etiological involvement for particular PPPs, future research should consider toxicokinetic modelling of biomonitoring outcomes, genotyping and phenotyping and complete exposure record and person's health status. Lastly, it should be noted that attribution to pesticides can never come from epidemiological studies alone, but needs to be complemented with in vivo and in vitro studies in controlled laboratory settings.

A.4.1. Knowledge gap analysis

The most important data gaps are related to study of current use pesticides and the use of methods of exposure assessment that provide reliable estimates of internal dose, e.g. biomarkers of exposure. For OCP these methods have provided strong evidence for observed associations between pesticide exposures and health effects in populations of worker and also in the general populations. Due to extensive body of evidence, there are indications of effects from maternal and paternal exposure after birth with potential effects later in life. Only few relevant studies were retrieved that covered immune response and only one relevant study on gut microbiome in humans. None of these studies indicated a clear and sufficiently clear link to pesticide exposures.
Further studies are required to address weaknesses and gaps in current study designs addressing the role of pesticide exposures in human health:

- Adverse effects observed at birth in early life that may persist until later in life. To find out about this follow-up over a long period of time is needed such as in prospective mother-child cohorts that are running in many countries in Europe.
- Changes observed in immune responses currently reflected in changed markers of signaling pathways. This requires the determination of such markers in population-based studies.
- There is a lack of understanding of mixture effects of PPPs on human health. The review showed that there are important gaps in all topics evaluated, and indicated where future research should have its focus.

A.5. Conclusions and recommendations

A.5.1. Conclusions

Effects of pesticides on humans are primarily based on population-based observational studies and hospital-based patient-control studies. Overall, they provide limited evidence of associations between pesticide exposures and some health endpoints in humans. For other health effects a role of pesticides exposures is inconclusive due to inconsistency and contradicting results.
The evidence from epidemiological studies is in part based on quantitative exposure data by use of advanced method such as human biomonitoring using a variety of body fluids. Such exposure-response relationships with groups or specific PPPs strengthen the confidence of an association. Some of the reported associated with adverse health effects persist also after adjustment for other known host and environmental factors. This is the case for organochlorine pesticides (OCP) because of the many large datasets that have been analysed. This is not the case for less- and non-persistent pesticides that are currently used (CUP). These PPPs are metabolized and so far in a very limited number of studies it shown that enzymes involved in these metabolic pathways have a significant influence on health risk.

Human studies often provide evidence suggestive of an association that need confirmation from studies in a controlled laboratory environment that often provides mechanistic data to support the biological plausibility. Different types of research can then be integrated in weighted-evidence evaluation.

A.5.2. Recommendations

Based on the appraisal of the retrieved and included body of evidence we have the following recommendations for future research:

- Increased use of human biomonitoring for quantitative and integrated assessment of exposures of workers and residents in epidemiological studies
- Based on the large body of evidence indicating a role for OCP in reproductive and developmental disease this strategy should become available to study the exposure to current use pesticides (CUP) in mother-child cohort to confirm the role of current use pesticides
- Use of improved study designs to further study associations between PPP exposures and certain health outcomes where the evidence base is still insufficient
- It is suggested to improve the research effort to complement observational studies populations of workers and residents with controlled experimental studies to match the probabilistic evidence with supporting evidence on the toxicity mechanism preferably using biomarkers that can also be applied in population-based studies to support those metabolic pathways involved in experimental findings with relevance to humans.
- Joint evaluation of the evidence derived from from human, animal and in vitro studies in a weighted evidence approach

- Based on 'post-market' studies in exposed populations of farmers, neighbours and consumers concerns for adverse health effects can be verified in experimental settings using exposures levels reflecting real life exposures of PPP mixtures.

A.6. References

For all human effects references of included and excluded studies is available online as SPRINT SOLES Projects

A.7. Annexes

Annex A describes the PPP effects of PPP on human health

B. PPP effects on animals

B.1. Introduction and objectives

The objective of this section is to map relevant studies that report on the effects of PPPs on animal health.

B.1.1. Research questions

The scope for this review is defined by specific pre-defined broad health-related categories of resilience and reproductive health reported in peer-reviewed scientific literature.

Each of these categories was subdivided in subcategories that can be defined in a dedicated search strategy. In addition, we searched for specific adverse health outcomes and animal diseases related to selected organs and organ systems.

B.2. Methods

B.2.1. Search strategy farm animals

Farm animals were captured as 'domestic animals' and included

B.2.2. Search strategy experimental animals

Experimental animals were captured as '

B.2.3. PECO statement and selection process

B.2.3.1. PECO statement

PECO is an approach to structure the search, formulate the search question and decide on eligibility of retrieved studies. It consists of the following components: population, exposure, comparator and outcome (Morgan et al., 2018)[3]. For the effects of PPP exposures on animals and their health impact this statement is provided below:

Population – Mammals of both sexes and their offspring, both in controlled experimental settings (of e.g. toxicity studies) as well as in less/uncontrolled settings in farms and including mammalian farm animals including cow, sheep, goat, chicken, cat

[3] Morgan RL, Whaley P, Thayer KA, Schünemann HJ. Identifying the PECO: A framework for formulating good questions to explore the association of environmental and other exposures with health outcomes. Environ Int. 2018 Dec;121(Pt 1):1027-1031.

Note 1: suggest to consider using the animal search filter[4]

Exposure - Exposures to human-made or natural products marketed and in any application as plant protection products (pesticides) today (SPRINT list of 200+ PPPs) or at any time in the past, their active ingredients their metabolites, degradation products, adjuvants and co-formulants as part of products, including mixed exposures.

Note 2: Any type of use in an occupational/professional setting such as integrated pest management as well as private use indoor (home) or outdoor (garden)

Note 3: Exposures are often monitored in environmental compartments (water, soil, air) as well as intermediate sinks and contaminations in pastures (drinking points, feed) and housing (stables, e.g. dust and manure) to describe external exposure. Second all types veterinary samples from farm animals and their products (e.g. dairy products and meat) analysed for PPP residues for product quality reasons.

Comparator - We will use livestock bred in organic farming as comparator to conventional farming. In controlled laboratory studies we will use a defined sham-exposed group as the comparator, realizing that it will be very difficult to reach zero exposure due to the ubiquitous occurrence of pesticides and their residues.

Outcome - Any health or health-related outcome (including effect biomarkers) considered relevant to the impact of PPP exposure on animal health.

B.2.3.2. Selection of studies

Studies that were included provide research on:

- Any effect-related outcome indicating relevant changes in health status such as a disease or mortality on any level (individual/group/population/community)

Studies that excluded were studies that report on

- Environmental fate and exposure;
- Biotransformation/metabolism of active ingredients on cellular, organ or organism level;
- Biomarkers informing on exposure; and
- Purely theoretical simulation studies.

The reasons for not including studies were logged and a summary is provided in the flow diagram describing the search. More details are provided in Annex A.

[4] van der Mierden S, Hooijmans CR, Tillema AH, Rehn S, Bleich A, Leenaars CH. Laboratory animals search filter for different literature databases: PubMed, Embase, Web of Science and PsycINFO. Lab Anim. 2021 Sep 24:236772211045485. doi: 10.1177/00236772211045485. Epub ahead of print. PMID: 34559023. Animal filters can be found at https://osf.io/q6uxs/

B.3. Results

B.3.1. Literature search

Below the PRISMA flow diagram (**Figure 12**) shows the selection process with numbers of studies included and excluded. Reasons for exclusion are provided for each species/endpoint in the scoping reports in Annex B.

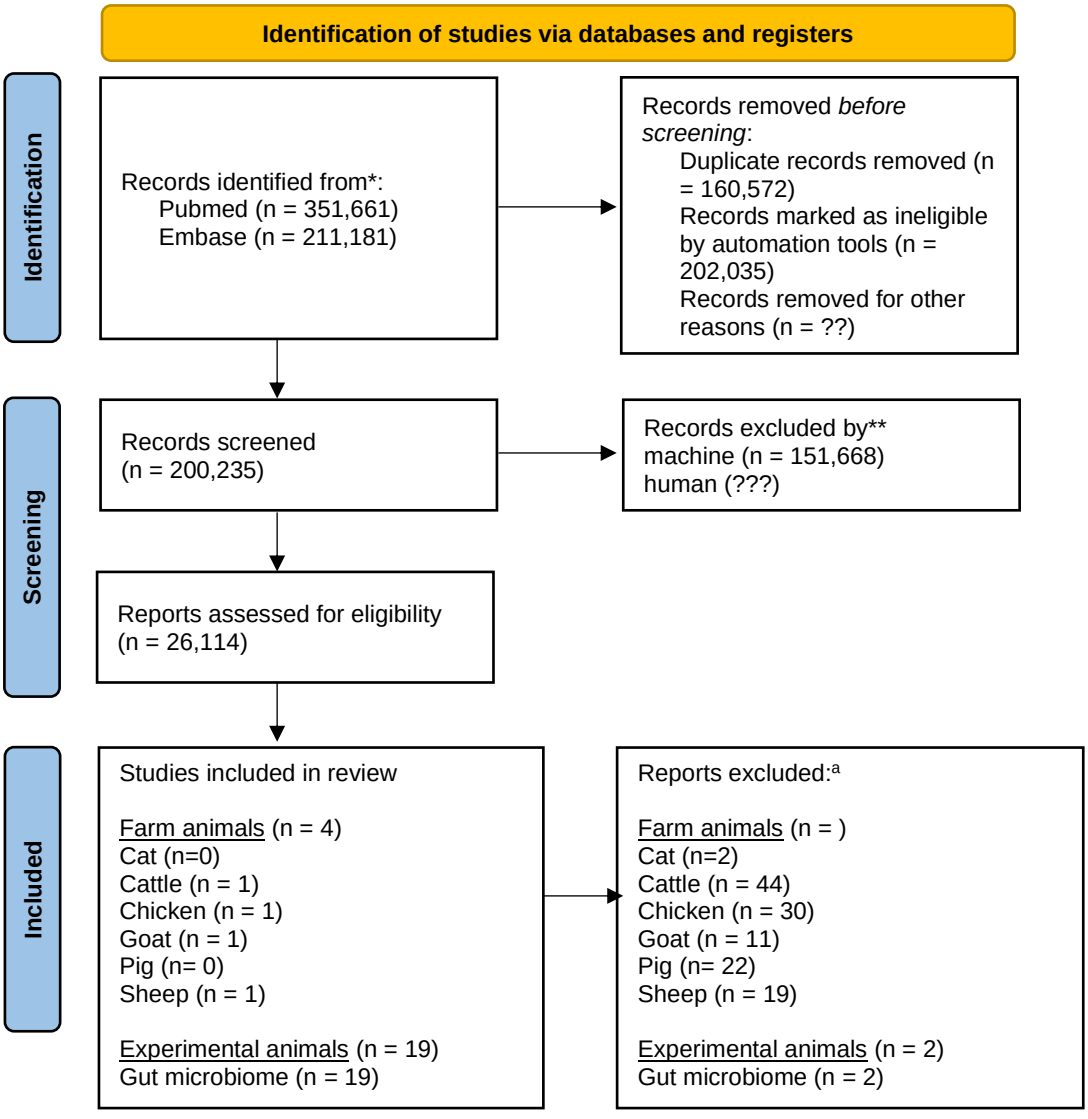


Figure 12. Flow diagram systematic scoping review of animal health outcomes (Page et al., 2021). [a]Reasons for excluding studies are specified in Annex B.

In this section we will discuss experimental animals and farm animals. Note that only for the SPRINT selection farm animals species a scoping review was provided as part of this deliverable.

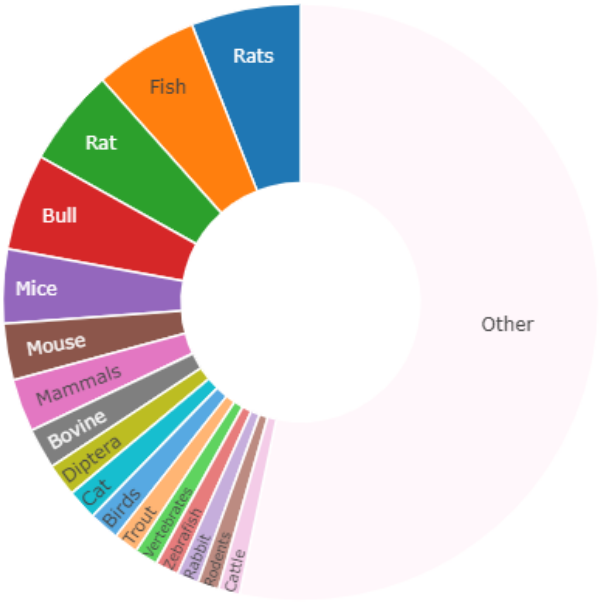


Figure 13. Distribution of studies by animal species.

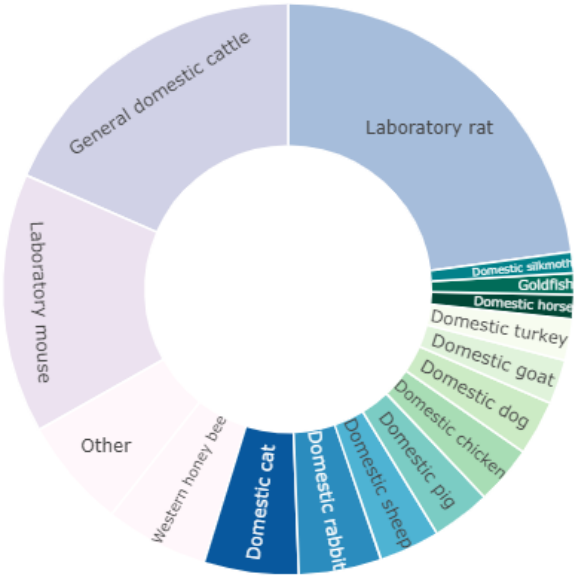


Figure 14. Distribution of domestic and laboratory animals by species.

B.3.1. Effects on gut microbiome in experimental animals

The nineteen included studies reported effects on the gut microbiome of experimental animals treated with 14 types of PPPs (including substances or their commercial formulations); only one study considered different substances combined in a cocktail formulation. The relevant literature is very recent. All 19 studies were published in the last 6 years. In particular, one study was published in 2015 and one in 2016; six were released in 2018, two in 2019, five in 2020 and four in 2021. Glyphosate and its commercial formulations were the main PPPs of interest in experimental animal studies, with nine of the included nineteen studies addressing the effects of these pesticides on microbiome. Only three studies addressed the connection between microbiome alteration and behavioural effects induced by pesticides and in all 3 studies the pesticide of interest was glyphosate. Overall, the studies showed clear effects of glyphosate and its formulations in altering microbiome in experimental studies, however studies on the microbiome effects of most pesticides and how they might affect behaviour are lacking.

B.3.2. Farm animals

Below the PPP effects on farm animals are discussed, e.g. by linking observed farm animal diseases to PPP residues from feed and/or from the indoor (stable) and outdoor environment.

B.3.2.1. Effects on cat disease and reproduction

No studies were included as none one retrieved study cat disease or one retrieved study on cat reproduction were linked to PPP exposures.

B.3.2.2. Effects on cattle disease and reproduction

No studies were included as none of the 15 retrieved studies on cattle disease linked to PPP exposures. For reproduction of the 29 retrieved studies one was included that described two different types of PPP uses: cyfluthrin ear tag and topical applications, or cyfluthrin ear tag, topical, premise spray and pyrethrin fog spray applications. The use of pyrethrin- and cyfluthrin-based insecticides, regardless of application, did not negatively affect reproductive parameters in beef bulls when administered over 18 weeks.

B.3.2.3. Effects on chicken disease, reproduction

No studies were included as none of the retrieved 5 studies on diseases in chicken was linked to PPP exposures. For reproduction of 26 retrieved studies one was included that described glyphosate residues broiler breeder diets. The average glyphosate residue level in five flocks of broiler breeders was 0.09 mg/kg, maximum was 0.19 and minimum was 0.004 mg/kg. Egg laying percent was not affected by residue level. Average hatchability was 79% (SD = 5.8%). A negative association between feed glyphosate residue level and egg hatchability ($P = 0.03$) was found. To further investigate potential effects of feed glyphosate residues on egg hatchability an experiment with glyphosate residues in feed over a range

covering the conditions in commercial settings and including feed free of residues is warranted.

B.3.2.4. Effects on goat disease and reproduction

No studies were included as none of the retrieved six studies on goat disease was linked to PPP exposures. For reproduction of six retrieved studies, one was included that described an experimental study of PPP use in Damascus-Alpine crossbred male goats. Goats received a standard diet or standard diet supplemented with 15 mg atrazine per kg BW for 6 months. Atrazine was reported to impair sperm morphology and sperm membrane integrity. Sperm fertilization competence was not measured.

B.3.2.5. Effects on pig disease and reproduction

No studies were included as none of the two studies on pig disease or 21 studies on pig reproduction were linked to PPP exposures.

B.3.2.6. Effects on sheep disease and reproduction

No studies were included as none of the retrieved thirteen studies on sheep disease was linked to PPP exposures. For reproduction of seven retrieved studies, one was included that described a study of Rambouillet rams (N=28) that were allocated to 3 treatment groups. Fenoxycarb were given via capsules at 10x or 20x the potential exposure levels in pasture or hay. The first was dosed for 60 days (at least one spermatogenic cycle). Ewes (N=40) were dosed for 20 days (at least one estrus cycle) and then allocated to ram treatment groups. No-exposure control was used. No treatment-related pattern of reproductive, physiologic, or behavioral abnormality was observed in any of the treatment groups.

B.4. Discussion

The aim of this scoping review is to map available evidence and determine gaps in knowledge.

B.4.1. Experimental animals

For this review we have focused on studies reported on gut microbiome because this field is not well covered by research in humans.

B.4.2. Domestic animals

In this study we only assessed PPP health effects on domestic/farm cats. Studies on the use of parasite control e.g. in flee-bands were excluded in this study. Although the cat has been much used in an experimental-animal models for neurotoxicity studies in an experimental

setting, no studies were retrieved that described adverse health effects from environmental exposures exposure through the food-chain (e.g. in cats as predators of farm rodents) or related to residues in cat food. Also, the PKB model developed by EFSA may yield some opportunities for assessment of potential health risks in domestic animals.

B.4.3. SPRINT selection of farm animals

Studies on PPP effects on farm animals are not well developed. Of the retrieved studies most reported on endpoints related to treatment of farm animals for parasite control (including vector control) in different species of farm animals (only) but did not report on health effects. Some studies addressed the control of infectious disease including zoonoses. Indirectly, parasite control and control of infectious diseases may have an effect on the animal health most often the substances are given as part of a treatment by a veterinary rather than an environmental or feed-based exposure. In pigs several experiments involving PPPs were reported: a study reported on feeding pigs with glyphosate-or dieldrin-spiked feed in an experimental setting and did not report on adverse health effects. Another study reported on exposure of piglets to neonicotinoids but no health outcomes were reported. In poultry one study reported on measurement and two studies on modeling of PPP residues in eggs.

B.5. Conclusions and recommendations

This part has been completed for farm animals and experimental animals only for gut microbiome studies.

B.5.1. Conclusions

Effects of PPPs on farm animals are obviously not a focus point, likely because emphasis is on economically important infectious diseases (e.g. mastitis) and performance-related diseases such as ketosis. However, PPPs seem to have attracted some attention regarding livestock reproduction. Gut microbiome studies in experimental animals and are considered promising models to study the role of microbiome changes in health effects of PPPs.

B.5.2. Recommendations

A full systematic review will likely not disclose additional important information. A systematic review on alterations in the gut microbiome will be feasible.

B.6. References

Links to specific citations can be found in the online SPRINT SOLES system using the domestic filter for selection of farm animals.

B.7. Annexes

Annex B describes the effects of PPP by animal species in more detail.

C. PPP effects on ecosystem

C.1. Introduction and objectives

C.1.1. Objective

The objective of this section is to map relevant studies that report on the effects of PPPs on ecosystem health.

C.1.2. Research questions

The scope for this review is defined by specific pre-defined broad health-related categories of resilience and reproductive health reported in peer-reviewed scientific literature.

Each of these categories was subdivided in subcategories that can be defined in a dedicated search strategy, e.g. aquatic ecosystem and terrestrial ecosystem.

C.2. Methods

C.2.1. Search strategy

C.2.1.1. Aquatic ecosystem

Search strings have been prepared for each species and endpoint of interest (see Annex C).

C.2.1.2. Terrestrial ecosystem

Search strings have been prepared for each species and endpoint of interest (see Annex C).

C.2.2. PECO statement

PECO is an approach to structure the search, formulate the search question and decide on eligibility of retrieved studies. It consists of the following components: population, exposure, comparator and outcome (Morgan et al., 2018)[5]. For the effects of PPP exposures on animals and their health impact this statement is provided below:

Population – Plant species targeted as crops and any species or ecological communities that pesticides are not targeting but that may by affected (non-target species).

[5] Morgan RL, Whaley P, Thayer KA, Schünemann HJ. Identifying the PECO: A framework for formulating good questions to explore the association of environmental and other exposures with health outcomes. Environ Int. 2018 Dec;121(Pt 1):1027-1031.

Note: vertebrate and invertebrate species and also any effects on microorganisms (bacterial, fungi) in environmental compartments (water, soil) including the development of microbial resistance.

Exposure - Exposures to human-made or natural products marketed and used as plant protection products (pesticides) today (SPRINT list of 200+ PPPs) or at any time in the past, their active ingredients their metabolites, degradation products, adjuvants and co-formulants as part of products, including mixed exposures. All types of internal exposure assessment by biomonitoring of non-target species in the framework of ecological or ecotoxicological research and routine monitoring surveys.

Comparator – Plants not treated with PPPs in integrated pest management or organic farming. Eco-systems close to organic farming systems will be the internal reference. Data from eco-systems not affected by agricultural systems are externa reference.

Outcome - Any health or health-related outcome (including effect biomarkers) leading to higher productivity of plant species as targets for pest control. Second, for ecosystem health the effects indicators of population dynamics of non-target species and biodiversity of an ecosystem, including the microbiome.

C.2.3. Search and selection of studies

We have prepared a list of dedicated search terms for the health outcomes of interest (see Appendix 7 for technical details.

C.2.3.1 Aquatic ecosystem

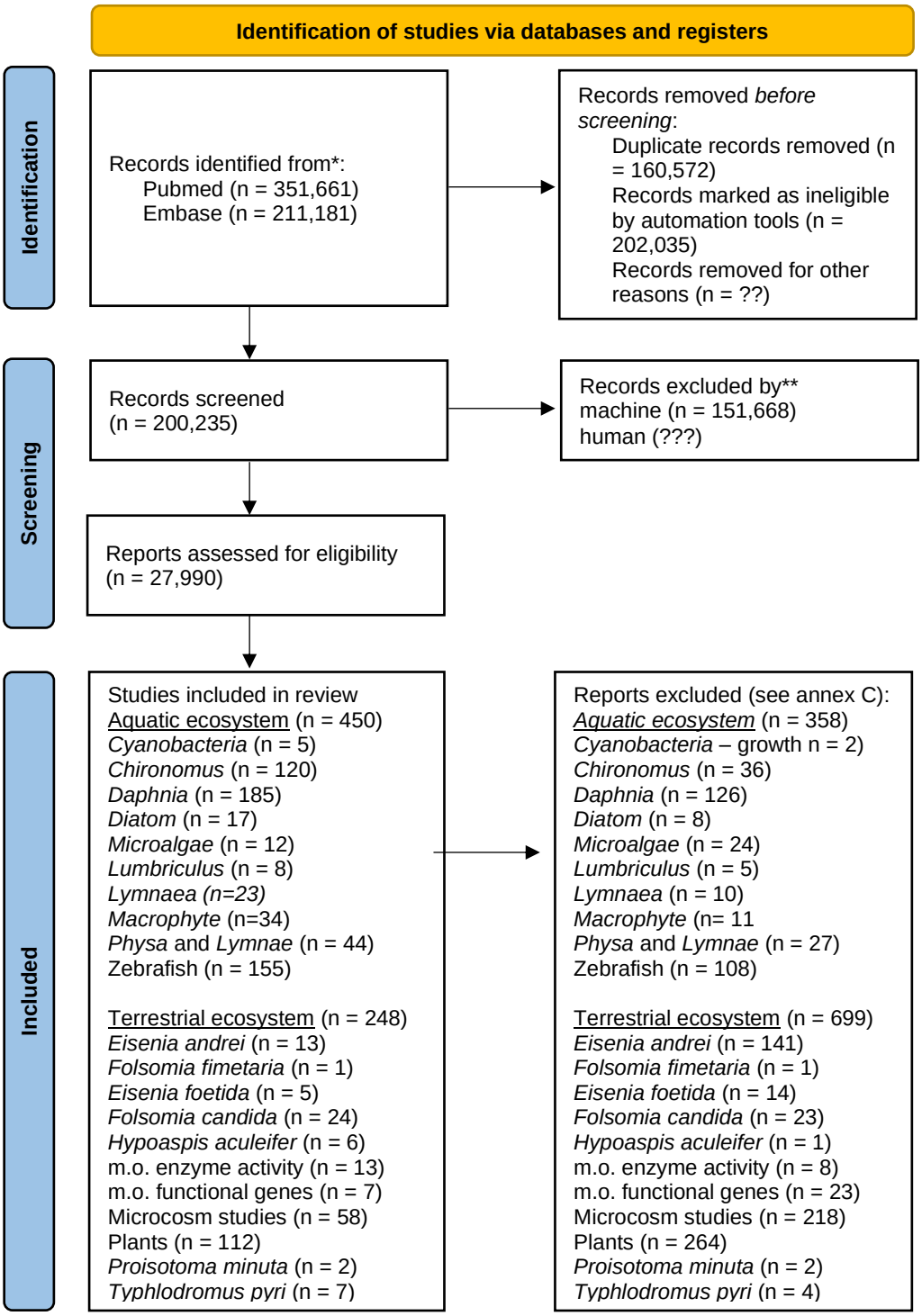
The search strategy is based on species and endpoint. For nine species search strategies were developed on survival, growth, life cycle and sub-individual level. The detailed search strings for all species – endpoint pairs have been specified in Annex C.

C.2.3.2. Terrestrial ecosystem

The search strategy is based on species and endpoint and for each search specified in Annex C.

C.3. Results

Below the PRISMA flow diagram (**Figure 15**) shows the selection process with numbers of studies included and excluded. Reasons for exclusion are provided for each species/endpoint in the scoping reports in Annex B.



.**Figure 15.** Flow diagram systematic scoping review of human health outcomes (Page et al., 2021).
[a]Reasons for excluding studies are specified in annex C.

C.3.1 Aquatic ecosystem

This section describes the effects of PPPs on aquatic ecosystems, including effects on organisms form distinct trophic and functional levels: microalgae (cyanobacteria, diatoms, green microalgae), macrophytes, invertebrates (*Chironomus*, *Daphnia*, *Lumbriculus*, *Physa, Lymnaea*), and one invertebrate species (zebrafish). The research in each species is divided by endpoint, e.g. effects on survival, growth, life cycle and sub-individual effects. More specific results can be found in Annex C.

C.3.1.1. Effects on Cyanobacteria growth

For cyanobacteria five studies were included. Herbicides, fungicides and insecticides can induce negative effects in cyanobacteria regarding growth and photosynthetic efficiency. Recovery of communities and cultures was often observed, which is relevant in the field context. Future research is needed addressing the interaction among stressors, especially considering natural stressors (e.g. temperature, light irradiation, salinity) and pesticide mixtures to better meet field conditions and improve prediction modeling.

C.3.1.2 Effects on Diatom growth

Seventeen included studies reported on toxic effects regarding growth, photosynthetic efficiency, pigment production and gene expression. These effects were documented for several herbicides (atrazine, s-metoalachlor, bentazone, hexazinone, isoproturon, alachlor, diuron and glyphosate). The data on atrazine and isoproturon highlight the recovery capacity of diatoms in the short term, and the data on s-metolachlor show that environmental concentrations are not likely to induce toxic effects. No negative effects were observed following exposure to the fungicide chlorthalonil or the insecticide endosulfan, but the insecticide imidacloprid affected diatom growth in a gender specific manner. Mixtures of pesticides and the combination of these with natural stressors were rarely addressed, yet these represent the most realistic conditions for pesticide exposure.

C.3.1.3. Effects on green microalgae growth

In twelve included studies effects of pesticides on microalgae were reported. While negative effects of herbicides are more often addressed, those were also found following exposure of green microalgae to fungicides but not to insecticides. The capacity of microalgae to recover from impairment induced by exposure was documented and should be taken into account in future studies for a more realistic risk assessment; the same applies to the modulation of pesticide effects by natural variables and other contaminants within complex natural matrices. More studies are needed to explain difference in results of effect of PPP between microcosm and field experiments. There is also a need to study PPP mixtures in this type of experiment set-up.

C.3.1.4. Effects on macrophyte growth

Thirty-four included studies reported on PPP effects on Macrophyte growth. Negative effects of pesticides to macrophytes have been documented regarding growth, photosynthetic efficiency, pigment production and even adverse molecular pathways (gene expression). However, these are generally mild, occurred at non-realistic concentrations (e.g. above protective thresholds or quantified concentrations in water) or are transient, with macrophytes recovering in the short term. The sensitivity to some herbicides (e.g. atrazine) was found to depend on ecological features of the test system, the interacting effect of natural stressors and the presence of other pesticides in the experimental treatment. Single-species tests apparently have satisfactory outcomes that do not differ significantly from those resulting from complex multi-species experiments, which suggests that future studies with macrophytes for risk assessment purposes can feasibly rely on simpler methodologies.

C.3.1.5. Effects on Chironomus

Chironomus riparius was the most used species, followed by *Chironomus t*entans. The most studied PPP were chlorpyrifos, imidacloprid and atrazine. The insecticide Terbufos showed the highest toxicity on *Chironomus tepperi*. The effect of PPP mixtures was evaluated mainly in the OP pesticides. Nanoatrazine showed to be highly toxic for life cycle parameters in *Chironomus sancticaroli* with mentum deformities. Xhlorantraniliprole was able to alter the development and growth in *Chironomus riparius*. Sub-organismal response was mainly focused on CYP-450 enzyme system, glutathione-S-transferase (GST) and catalase (CAT), as indicators of oxidative stress. Imidacloprid show to induce oxidative stress through CAT, malondialdehyde (MDA) or glutathione (GSH) alterations. Chlorantraniliprole and esfenvalerate also impair the oxidative stress markers GST, CAT or GSH of *Chironomus riparius*. In addition, expression of genes involved in detoxification and redox activity (ZnCUSOD) were modified, either when exposed to single compounds or to binary mixtures. A similar modification in the genes' expression was also reported for endosulfan. In conclusion, *Chironomus* has showed to be a suitable model to evaluate the effects of PPP. Oxidative stress is suggested to be the main toxicity mechanism in these assays. More studies are needed for a better understanding of the PPP effects on *Chironomus*.

C.3.1.6. Effects on Daphnia

The main studied species was *Daphnia magna*, followed by *Ceriodaphnia dubia* and *Daphnia pulex*. Differences between species have been observed. Parent compounds compared to their metabolites showed to be more toxic. Likewise, in general formulated products showed to be more noxious than single active ingredients. Deltamethrin and spinosad were reported to be the most toxic for survival endpoints and effects on feeding and development. Fenoxycarb stands out as an endocrine disruptor with observed effects on reproductive and developmental endpoints, for example, altering the sex male rate of *D. magna*. Effects at the sub-organismal response were studied in *D. magna*, mainly focusing the metabolomic alterations. Atrazine was one of the most studied PPP showing a very high toxicity with effects

on oxidative stress and energy metabolism. This was also confirmed by proteomics analysis glutathione measurements and vitellogenin expression related to endocrine and detoxification efficiency. Although many studies evaluated the effects of PPPs on *Daphnia*, few studies performed a global evaluation at the organism and sub-organism level. There is also a lack of studies on mixture toxicity of PPP on *Daphnia*.

C.3.1.7. Effects on Lumbriculus

Few studies evaluated the effects of plant protection products (PPPs) on *Lumbriculus* and the retrieved focused on imidacloprid or thiamethoxam. Imidacloprid revealed to be the most toxic PPP tested, being indicated as a very harmful compound to *Lumbriculus variegatus*. Other PPPs evaluated were esfenvalerate chlorpyrifos and dieldrin, with a low impact in this species. More studies using different mixture combinations are needed to better understand the toxicity of PPP combinations. There is a lack of molecular studies evaluating the effects at the sub-organism level. This can be overcome by focusing further studies on molecular responses and connect them to the observed effects at the organism level.

C.3.1.8. Effects on the aquatic snails

Diuron, atrazine and imidacloprid altered the *Physa's* growth and behavior. Highest toxicity was observed for azoxystrobin, followed by cypermethrin. Reproductive and developmental changes were observed for *Lymnaea stagnalis* when exposed to pyraclostrobin. Also, neonicotinoids induced changes in the metabolite profiles and polyamines revealed to be neurotoxic. *Physa* showed to be a suitable organism for assess PPP toxicity. Further studies should be carried out to better understand the aquatic snail's adaptation capability in continued PPPs exposures.

C.3.1.9. Effects on zebrafish survival

Given the large number of studies on the effects of pesticides on fish, this analysis was focused on zebrafish, assumed as a model organism in ecotoxicological studies. Survival studies have been reported over the past decade covering distinct PPP classes. Most of them have focused on individual PPPs and only a few studies tested mixture, mostly binary and ternary. In general, studies reported lethal effects on zebrafish, however, often did not specify whether the concentrations were environmentally relevant. Glyphosate was the most studied PPP, followed by atrazine, imidacloprid and by lambda-cyhalothrin. For most a.i. only a single study was found. Commercial formulations of PPP, as well as PPP metabolites, were punctually studied and therefore there is a gap that needs to be addressed. Also, important to stress that recent studies based on nanotechnology have shown promising reducing the impacts on aquatic organisms.

C.3.1.10. Effects on zebrafish growth

There are a huge number of studies evaluating the effects of plant protection products (PPPs) on Zebrafish (Danio rerio). Herbicides, fungicides and insecticides can induce negative effects

in zebrafish in distinct life stages (embryonic, larval, juvenile and adult stages) regarding growth, development, behavior (e.g. distance moved, exploration bottom-dwelling, time latency to enter the top zone and interaction with mirror) and malformations (that indicate general parameters such as body weight, length and growth-related parameters). Among the distinct PPPs, the herbicides, glyphosate, atrazine and the triazole fungicides as well as the prochloraz were the most studied. The data on these PPPs, also raise concern regarding the reproduction, fecundity, fertilization, sexual development, intersex and endocrine disrupting. However, some contrasting results were observed for the atrazine effects, from adversely to moderate or no effect on gonodal development. Most of the studies were focused on the individual toxicity of PPPs, with just a few (≅13%) assessing the combined effects of PPPs. These studies highlighted the potential increased toxicity that might be triggered by the simultaneous presence of several pesticides in the aquatic ecosystems entailing the synergistic responses.

C.3.1.11. Sub individual effects on zebrafish

A large number of studies evaluate the effects of distinct plant protection products (PPPs) classes, such as herbicides, fungicides, insecticides and fungicides, on Zebrafish (*Danio rerio*). In general, these PPPs can induce acute and sub-lethal toxicity effects on Zebrafish, altering the activities of the antioxidant enzymes, energy metabolism, exert neurotoxicity and lipoperoxidative damage and induced alteration in the gene expression levels such as, vitellogenin expression. Among the PPPs, the herbicides, glyphosate, atrazine and the insecticide, dieldrin were the most tested. The data regarding glyphosate and atrazine were not consistent among studies (negative effects vs. no effects). It is important to highlight that mixture of PPPs were rarely addressed (2) yet reporting synergistic effects (certain genes expression exerted greater variations upon exposure to mixtures compared to the individual compounds), emphasizing the need of considering mixtures of PPPs and their combination with natural stressors.

C.3.2. Terrestrial ecosystem

This section describes the effects of PPPs on terrestrial ecosystem by effects on microcosm and mesocosm studies, effects on plants and by effects on specific organisms: *Eisenia andrei*, *Fimetaria*, *Foetida*, *Folsomia*, *Hypoaspis*, *Proisotoma minuta* and *Typhodromu pyry*. Studies on soil microbiome are addressed by microbial enzyme activity and functional genes. More detailed descriptions of the sets of included studies and the effects of PPPs on different species can be found in Annex C. Studies on insects and vertebrate species are not included.

C.3.2.1. Effects on Enzyme activity of microorganisms

In thirteen included studies research on the effects of pesticides on enzyme activities of microorganisms were reported. Soil contaminants such as pesticides alter the enzyme activity, most often in a negative way by suppressing specific enzyme activities, with different magnitudes of the effect dependent on the kind of enzyme. The dosage plays an important role but also application at the field rate seems to induce an effect. Important

enzymes such as urease and phosphatases are already involved in almost every study, similarly β-glucosidase needs more studies. In combination with the enzymatic activity, it would be interesting to investigate linked aspect such as litter decomposition. The number of studies on multiple simultaneously applied pesticides is still limited, as is the research on the effect of repeated applications on enzyme activity.

C.3.2.2. Effects on functional genes of microorganisms

Seven studies were included for research on the role of functional genes for the effects of pesticides. This field of research is still relatively new and not much used, more studies need to be conducted to validate the use of functional genes as a proxy for microbial pesticide degradation. The available studies showed good correlations and potential for some of the functional genes and therefore more studies are needed to confirm these preliminary findings. Further knowledge about the behaviour of the functional genes is necessary for example to monitor how repeated applications change the gene expression or how it changes when a pesticide is reintroduced after a period of non-use.

C.3.2.3. Effects on microorganisms tested in microcosms

In this review 58 studies were included that used a microcosm or mesocosm approach which provides a scaled experimental setting to bridge controlled lab studies and field testing. This type of studies provides more realistic exposure conditions compared to lab studies. In approximately half of the studies microorganisms were assayed in isolation, whereas other studies used combined settings with other organisms. Carbendazim and imidacloprid were most studied and resulted in the lowest effect concentrations expressed as effect concentration corresponding to the concentration with an observed effect in 50% of the population (EC50). In a similar way for a lethal outcome LC50 is used. For imidacloprid the lowest reported LC50 was 4 g/ha and for carbendazim this value was 0.1 g/h (higher values were reported in other studies). Other PPPs studied showed effects on microorganisms in a micro or mesocosm study were the currently used herbicides glyphosate, atrazine, and conazoles fungicides and pyrimethanil. More studies are needed to explain difference in results of effect of PPP between microcosm and field experiments. There is also a need to study PPP mixtures in this type of experiment set-up.

C.3.2.4. Effects on plants

A hundred and twelve studies were reported covering the effects of PPPs on non-target terrestrial plants. Exposure concentrations in the range of recommended field dose were applied in many studies of soil exposure and/or foliar spray application. However, toxicity is normally not observed at these doses. In four lab studies, atrazine, difenoconazole, and metazachlor decreased plant growth when applied at field dose. Two field studies also observed effects on plant growth caused by glyphosate and pendimethalin at field dose. More studies are needed using mixture exposure to PPPs, and most studies did not follow any standardized toxicity test protocol.

C.3.2.5. Effects on Eisenia andrei

Thirteen papers were included for effects of PPPs on the earthworm E. andrei. The endpoints analysed were reproduction (number of juveniles, cocoon hatching and production), survival, bioaccumulation, DNA damage and biomarkers. The pesticides used were insecticides, herbicides, and fungicides, however, not many papers tested fungicides, being a weakness in this topic. Clothianidin was the most toxic compound, with LC50 and EC50 of 2.5 and 0.7 mg/kg, respectively. Two papers tested mixture toxicity using the following combinations: Metsulfuron-methyl + mineral oil, endosulfan + metolachlor and temephos + metolachlor.

C.3.2.6. Effects on Folsomia fimetaria

Only one study was selected for F. fimetaria, using the insecticides imidacloprid and thiacloprid. The endpoints analysed in this study were survival and reproduction. Since this is an important species (springtail with a sexual life cycle in contrast to the asexual Folsomia candida), that enables to test the genders, and infer the impact it might have on the population density in the field.

C.3.2.7. Effects on Eisenia foetida

Five studies were reported on PPP toxicity effects on E. foetida. The pesticides used were insecticides (deltamethrin, DDT, DDD, DDE, imidacloprid and RH-5849) and fungicides (triadimefon, difenoconazole, propiconazole and hexachlorobenzene), with no tests assessing toxicity of herbicides or others. The research field with this species also lacks on field studies and the use of reproduction and survival as endpoints.

C.3.2.8. Effects on Folsomia candida

Twenty and four studies were included, covering the effects of PPP on survival and reproduction. Most of the papers tested insecticides, and other PPP group studied were acaricides, fungicides, and herbicides. Clothianidin exposure was highly toxicity to F. candida, with LC50 and EC50 of 0.07 and 0.05 mg/kg, respectively. More studies are needed using different endpoints, other than survival and reproduction, such as multigeneration tests, decomposition tests, and bioaccumulation tests.

C.3.2.9. Effects on Hypoaspis aculeifer

Six studies were reported on *H. aculeifer,* using the insecticides deltamethrin, dimethoate, and abamectin, and the fungicide difenoconazole. The effects were observed in avoidance behaviour, reproduction, and survival. There is a lack on mixture toxicity studies and mesocosms studies with this species.

C.3.2.10. Effects on Proisotoma minuta

The selection is limited to only two studies with P. minuta. Clothianidin and metsulfuron-methyl exposure affected reproduction and survival. The toxicity of clothianidin was found at low exposure concentration, as observed for other soil invertebrates (EC50 of 0.1 mg/kg). There is a lot of potential for P. minuta testing, since this species is found on agricultural soils in temperate zones. The limited number of studies with this species is a weakness in the topic of terrestrial ecotoxicology of PPPs.

C.3.11. Effects on Typhlodromus pyri

In seven included studies research on the effects of pesticides on predatory mite T. pyry. The endpoints were related to reproduction, oviposition, avoidance, mortality, and reduction of population. There is a gap in the scientific literature concerning the impact of pesticides in T. pyri on female mortality and population dynamics. These endpoints have direct impacts on the food web, facilitating or not the appearance of pests, that otherwise would be biologically controlled.

C.4. Discussion

C.4.1. Aquatic ecosystem

Most of the retrieved studies indicate a clear relationship between the PPP exposure and the observed effects. All the searched biological groups, covering distinct trophic and functional levels, revealed to be affected by PPPs, either when exposed to individual compounds or combinations of PPPs.
Concerning the distinct aquatic algae and plants assessed, including green microalgae, diatoms, cyanobacteria and macrophytes, the studies revealed that they are most affected by herbicides, which is expected due to the PPP action mode. However, significant effects were also reported when producers were exposed to fungicides. Exposure to PPPs were able to produce a variety of effects, from growth inhibition to changes on photosynthetic efficiency, pigment production and gene expression. Recovery of producers after exposure was observed, which should be considered in future studies for a more realistic environmental risk assessment. Also, findings from single-species tests with macrophytes do not differ significantly from those resulting from multi-species experiments, suggesting that future studies for assessing risk of PPP can rely on simpler ecotoxicological methodologies.
In what regards the invertebrates, the diptera *Chironomus*, the crustacean *Daphnia*, the blackworm Lumbriculus and the aquatic snails *Physa* and *Lymnaea*, all of them were negatively impaired when exposed to PPPs, either to single compounds or to combinations of distinct PPPs. Among the several endpoints studied, effects at individual level (e.g. impairments on growth, development and life cycle), as well as at sub-individual level (with focus on the oxidative stress and gene expression) were reported. However, there is a recognized need to link effects at the molecular and biochemical levels with impacts at the individual level. Effects on the reproduction of the aquatic snail *Physa* were also found, emphasizing the importance of this species in the risk assessment of PPP.
In line with the microalgae, aquatic plants and invertebrates, effects of PPPs on the vertebrate zebrafish have also been reported at distinct life stages (embryonic, larval, juvenile, and adult stages). Effects on survival, growth, development, behavior, and malformations of zebrafish were clearly linked to PPP exposure. Likewise, studies have also revealed effects on reproduction, fecundity, fertilization, sexual development, intersex and endocrine disruption. Furthermore, sub-individual effects were also observed, including changes in the activity of the antioxidant enzymes, changes in the metabolism, neurotoxic effects and alteration in the gene expression.
Although only few studies have focused on the combined effects of PPPs on aquatic organisms, they stress the increased toxicity that can occur in real aquatic systems due to the presence of several PPPs. Also, important to stress that recent studies based on nanotechnology have shown promising reducing the impacts on aquatic organisms.

C.4.2. Terrestrial ecosystem

Based on the data presented in the selected studies, the effects of PPPs on soil organisms were observed in different endpoints.
The studies of PPP effects on microorganisms include the assessment of enzyme activity and functional genes. Most studies were conducted in the laboratory. The PPP exposures were associated with attenuation of different enzymatic activities related to both activating and deactivating pathways. The effects were depended on the exposure concentration and on the role of enzyme in

toxicity mechanisms. Studies on functional genes are promising additional methods to assess PPP effects on biodegradation in soils.

The microcosms studies covered mainly the effects of PPPs on microorganisms alone, and fewer studies assessed the effects of PPPs using multi-species approaches. The presence of plants and soil invertebrates in these designs highlighted the value of these organisms in affecting PPP fate in soils, and therefore, their toxicity on all species. There is an increasing interest in performing higher-tier experiments, however the number of studies is still low. While microcosm studies provide many advantages, this field has its limitations because it cannot match the complexity in real life and therefore cannot replace field experiments.

The studies of PPP effects on non-target terrestrial plants had a focus on plant growth and germination and reported less frequently on toxicity endpoints such as genotoxicity, pigment content and cell viability. Overall, effects on plants were extensively reported but there is a lack of field experiments. The effects of herbicides on terrestrial plants were more evident than other PPP groups.

Most of the effects reported in the search were related to survival of adults (LC50) and the number of juveniles produced (EC50). Other endpoints analyzed were related to biomarkers, such as acetyl cholinesterase concentration, and ecological aspects, such as decomposition measured by bait lamina. Exposure concentrations varied, being some related to the recommended application dose, others relating to the predicted environmental concentration, others developed by the researchers aiming to cause some effect on the species tested. Since there are different routes of exposure to soil invertebrates, different concentrations were tested depending on the type of exposure. Toxicity of insecticides, followed by fungicides and herbicides were the most reported, in this order. Most of the insecticides tested, e.g. neonicotinoids, were toxic to *Folsomia candida* and *Eisenia andrei* on low concentrations causing 50% mortality on adult populations. Herbicides, as glyphosates, did not present high toxicity to the tested species. Few papers used mixture toxicity, and when using it the preferred tested combination was herbicide and fungicide. Laboratory studies were preferred to field studies, mostly likely because EFSA only has on guideline for field testing with earthworms. More studies are needed on toxicity of mixture toxicity, in order to understand the impact these substances can cause to species in the field. Regarding the tested species, different tests such as multigeneration or mesocosm tests are relevant, but rarely performed. Concerning the testes species, despite the guidelines suggesting the use of species found in the field such as *Folsomia fimetaria* and *Proisotoma minuta*, the number of tests performed with these species were very low, being one for the first and 2 tests using the later. There is a need to develop methodologies using species that are found in the field in order to understand the impact of these PPP in the soil biota.

C.5. Conclusion

C.5.1. Aquatic ecosystem

Effects of pesticides on aquatic ecosystem are mostly based on ecotoxicological bioassays under controlled conditions. For all the species studied, there is a strength of evidence supporting connections between PPP exposure and effects on non-target aquatic species. Distinct endpoints were impaired, including lethal and sub-lethal effects at distinct levels of biological organization. Although there is a vast literature on the effects of pesticides on the aquatic ecosystem, there is a need for more research to understand the mechanisms involving pathways on sub-organism level and their consequences at the organismal level. Furthermore, more realistic ecotoxicological designs must be

considered in future studies, either considering the use of more complex approaches (e.g. multispecies assays) or the use of combinations of pesticides at environmental relevant concentrations.

It is recommended that future studies address several research gaps, including:

- Studies at sub-organismal level, namely at molecular and biochemical level, acting as early-warning signals of effects at higher levels of organization.
- Studies using more realistic conditions and scales, as the use of microcosmos, mesocosms or field studies, as well as the use of realistic concentrations.
- Studies with mixtures of PPPs and the combination of these with natural stressors (as temperature, light, salinity, etc) to gather a more realistic predictive capacity.

C.5.2. Terrestrial ecosystem

Based on the data presented in the selected studies, the effects of PPPs on soil organisms were observed in different endpoints. The studies of PPP effects in the terrestrial ecosystem mostly used controlled laboratory experiments. Field testing is much less practiced and required to fully appreciate the complexity of PPP impact in real life. For the different species and endpoints reported in the studies considered in this review, evidence was provided for PPP effects, especially on enzymatic activity in microbiota in controlled laboratory testing. Microcosm studies confirmed these effects and additionally suggested effects on plant growth inhibition, reproduction and community abundance in soil invertebrates. There is a lack of understanding of mixture effects of PPPs on terrestrial ecosystem.

Based on critical appraisal of the studies included in this review we have the following suggestions for future research:

- Increase of the use of microcosm approaches for better understanding of the full complexity of PPP impact in real life
- Increased use of biomarkers of PPP (de)toxicifcation pathways in laboratory experiments to study the toxicity mechanisms of PPP effects on biodegradation
- Study of PPP mixtures for a better understanding combined exposures on the terrestrial ecosystem.

C.6. References

Links to the used citations can be found in online in SPRINT SOLES by use of the ecosystem filter.

C.7. Annexes

Details on PPP effects in micro and mesocosms and on specific species can be found in Annex C2.

Disclaimer: This report is part of a project that has received funding by the European Union's Horizon 2020 research and innovation program under grant agreement number 862568.

Appendix 1: Examples of published systematic scoping reviews on pesticides

Lehmann DM, Camp AA. A systematic scoping review of the methodological approaches and effects of pesticide exposure on solitary bees. PLoS One. 2021 May 14;16(5):e0251197. doi: 10.1371/journal.pone.0251197. PMID: 33989308; PMCID: PMC8121328.

Teysseire R, Manangama G, Baldi I, Carles C, Brochard P, Bedos C, Delva F. Assessment of residential exposures to agricultural pesticides: A scoping review. PLoS One. 2020 Apr 28;15(4):e0232258. doi: 10.1371/journal.pone.0232258. PMID: 32343750; PMCID: PMC7188210.

Dereumeaux C, Fillol C, Quenel P, Denys S. Pesticide exposures for residents living close to agricultural lands: A review. Environ Int. 2020 Jan;134:105210. doi: 10.1016/j.envint.2019.105210. Epub 2019 Nov 16. PMID: 31739132.

Page MJ, McKenzie JE, Bossuyt PM, Boutron I, Hoffmann TC, Mulrow CD, et al. The PRISMA 2020 statement: an updated guideline for reporting systematic reviews. BMJ 2021;372:n71. doi: 10.1136/bmj.n71 For more information, visit: http://www.prisma-statement.org/

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Appendix 2: Proposal and outline protocol for a Systematic Online Living Evidence Summary (SOLES) of the effects of pesticides on human health

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Purpose

The SPRINT (Sustainable Plant Protection Transition) project is an EU Horizon 2020 project aiming to develop a global health risk assessment toolbox to assess impacts of plant protection products on the plants and animals in the environment, livestock and human health and to propose several transition pathways (https://sprint-h2020.eu/). A systematically derived scoping of existing knowledge has been proposed to 1) summarise published and otherwise available research evidence and 2) present it in a format which allows a richer understanding of existing knowledge and allows downloading of annotated bibliographies for further research.

SOLES: Systematic Online Living Evidence Summaries

Evidence is incremental, and new experimental findings offer the greatest value when considered in the context of other studies that have addressed the same or related research questions in different settings. Systematic reviews capture, summarise, and critically appraise the available evidence relevant to a pre-specified research question. They are considered the most effective method of reaching a rigorous understanding of the literature and using this understanding to inform decision-making. Unfortunately, traditional systematic reviews are often labour intensive and time consuming, meaning that findings are often outdated by the time of dissemination.

SOLES are interactive web applications which allow users to interact with a visual summary of the curated information, interrogate the dataset, and download relevant citations filtered by study characteristics of interest. An exemplar resource intended for use by stakeholders in COVID-19 research, including researchers working within the field or performing rapid or systematic reviews of COVID-19 literature is available at https://camarades.shinyapps.io/COVID-19-SOLES/ with the approach described in detail in 10.32384/jeahil17465. The approach is also being deployed in SOLES for Alzheimer's Disease (https://camarades.shinyapps.io/LivingEvidence_AD) and for drug selection for a repurposing trial in Motor Neuron Disease (https://camarades.shinyapps.io/MND-SOLES-CT/).

Inputs

For SPRINT-SOLES we will seek relevant information from 1) the published literature, through regular (possibly automated) searches of PubMed, BioRxiv and MedRxiv, augmented with less frequent searches of EMBASE and Web of Science and 2) toxicology databases such as EcoTox. We will develop 3 search strategies, intended to identify 1) human epidemiological studies reporting the effects of exposure; 2) *in vivo* studies seeking to elicit toxic effects (including transgenerational effects) in non-target animal species (i.e. non-pests) ; and 3) *in vitro* studies seeking to elicit toxic or mechanistic effects of toxicity in cell cultures derived from human, plant, farm animal, and non-target species. Our searches will be targeted at the 187 PPP active ingredients of interest previously determined by the SPRINT consortium. We will focus on evidence relevant for Europe, but envision separate sections for different continents.

Manual and automated screening

We will combine out search returns and de-duplicate the corpus using our inhouse Automated Systematic Search Deduplicator (10.1101/2021.05.04.442412). These will then be uploaded to a

project on the Systematic Review Facility SyRF (RRID:SCR_018907, description at 10.1136/ bmjos-2020-100103) where trained reviewers, including Edinburgh and Nijmegen CAMARADES team members, SPRINT members and collaborators and, potentially, reviewers crowd sourced through social media platforms, will annotate a sample of 1000 search returns as "not relevant"; "human epi study"; "in vivo study; "in vitro study", or a combination of these. We will ensure annotation quality by 1) providing reviewer training using an exemplar annotated article set using the Learn2SyRF platform, and 2) reviewing each record by 2 independent blinded assessors, with any disagreement reconciled by a third.
These 1000 annotation decisions will be used as a training set to train the machine (the EPPI Centre citation screening tool) to perform automated screening. It is unlikely that performance will be sufficient after the first training set, and so we will add further training sets until we achieve sensitivity (recall) of at least 95% with a specificity of at least 80% for each of the three annotation decisions. At each stage, we will identify those records with the greatest discrepancy between human [0,1] and machine [0 – 1] scores to establish whether this discrepancy might be due to human error (10.1186/s13643-019-0942-7), thus improving the fidelity of the dataset for further rounds of training.

PDF retrieval and annotation
We will retrieve full texts of included studies using an automated tool which aligns our institutional subscriptions to the CrossRef platform. We will retrieve xml versions where available, failing which pdf or html versions, and these will be converted to text files.
Annotation will be automated based on the frequency with which of terms of interest appear in the full texts f included articles. Using a dictionary of the 187 chemicals of interest we will use regular expressions in R to count the number of times each chemical is mentioned in each article, and label the article based to the most frequently mentioned chemical(s). Similarly, starting with a list of known outcomes of interest and supplemented with some human screening we will create a dictionary of outcome measures of interest and apply the same approach. Because a single mention of a chemical entity or an outcome measure does not necessarily mean that they are experimental components of that publication, we will establish thresholds for annotation based on the number of times mentioned, or the proximity to other terms of interest. We expect that these annotations will need to be performed on full text rather than title and abstract, because in similar reviews we have found title and abstract to be insufficient (maximum recall 87% for human title/ abstract screening, compared with 99% for human full text screening). For the in vivo strand we will use our existing tools to identify species, and we will label the corpus for reporting of measures to reduce risk of bias (10.1101/2021.06.04.447092).

Presentation
We will present a visual representation of these three literatures allowing interrogation by 2) single plant protection product; 2) combinations of plant protection products; 3) outcome measures reported; 4) species or cell type; and 5) Boolean combinations of these categorisations. At every stage of categorisation, we will provide the opportunity to download relevant citations, in a format that may either be imported to common reference manager tools or uploaded to SyRF for the extraction of outcome data for further annotation and meta-analysis.

Validation
The SOLES representation will not be perfect, and it is important for users to know what performance is being achieved. We will therefore conduct validation exercises and report the performance of each stage. Once this exceeds a pre-determined threshold, we will implement the "living" component of the SOLES, in which the search and analysis are updated on a monthly basis. We are currently exploring

the possibility of users being able to define their preferred trade-off between sensitivity and specificity[6], and would be happy to discuss this further.

Accessibility

SPRINT-SOLES will be freely available in the public domain. To support transparency and reproducibility, at key Deliverables (and at the request of SPRINT collaborators) a snapshot of the dataset will be taken, and made available on the University of Edinburgh digital repository DataShare, where it will be allocated a DOI.

[6] Some users will place a priority on maximizing retrieval (beyond that conventionally achieved by human reviewers), at the cost of reduced specificity. Others would rather accept a lower sensitivity for a "cleaner" dataset.

Appendix 3: PECO statements for SPRINT systematic scoping reviews

With the Population-Exposure-Comparator-Outcome (PECO statement) the focus of the systematic search and selection of the literature is defined. Due to the three scoping reviews to be delivered we will describe the three PECO statements for human, animal and non-target species separately. The PPP aspect in the definition of exposure is common to all three statements.

A. Human health

Population - Humans from all genders, ages and ethnic backgrounds, including their offspring in all geographical regions.

Note 1: specific populations of interest include: farmers and their families, non-farmer residents in the rural environment (also often referred to as 'neighbours') and the 'average' consumer.

Note 2: it is suggested to separate out populations in Europe or EU member states from populations in middle- and low-income countries and of other continents where the use and selection PPP is different.

Exposure - Exposures to human-made or natural chemical products marketed and used as plant protection products (pesticides) today (SPRINT list of 200+ PPPs) or at any time in the past, their active ingredients their metabolites, degradation products, adjuvants and co-formulants as part of products, including mixed exposures.

Note 3: Exposure measured by environmental sampling of any environmental compartment including sinks and contaminations to describe fate and exposure. Second, all types of human biological media (body fluids, exhaled air and all other excreta like urine and feces) to reflect internal exposure.

Note 4: Exposures from all uses, professionally as well as private home us, and also including any exposure known or unintended due to residue of PPP from all sources and routes of uptake such as diet, indoor/outdoor air, soil, water, home dust, etc) by any exposure route, including oral, dermal or inhalation also including administered PPP in a controlled healthy volunteer study setting.

Note 6: Any type of use in an occupational/professional setting such as integrated pest management as well as private use indoor (home) or outdoor (garden)

Comparator – Low or lower exposed populations and subgroups such as alternative farming as no/low PPP use alternative. Exposures in consumers will be considered to reflect a baseline in exposure to PPPs.

Note 6: we are also interested in designs where rural neighbours may also serve as comparator to farmers and their families (within conventional and organic farm systems)

Outcome - Any health outcome (including effect biomarkers) related to toxicity mechanisms considered relevant to the impact of PPPs on human health

Note 7: For carcinogenicity we will use For that we will use the IARC key characteristics that have been added in 2019 to the Preamble for hazard classification (see https://monographs.iarc.who.int/wp-content/uploads/2019/07/Preamble-2019.pdf).

B. Animals

Population – Mammals of both sexes and their offspring, both in controlled experimental settings (of e.g. toxicity studies) as well as in less/uncontrolled settings in farms and including mammalian farm animals including cow, sheep, goat, chicken, cat

Note 1: suggest to consider using the animal search filter[7]

Exposure - Exposures to human-made or natural products marketed and in any application as plant protection products (pesticides) today (SPRINT list of 200+ PPPs) or at any time in the past, their active ingredients their metabolites, degradation products, adjuvants and co-formulants as part of products, including mixed exposures.

Note 2: Any type of use in an occupational/professional setting such as integrated pest management as well as private use indoor (home) or outdoor (garden)

Note 3: Exposures are often monitored in environmental compartments (water, soil, air) as well as intermediate sinks and contaminations in pastures (drinking points, feed) and housing (stables, e.g. dust and manure) to describe external exposure. Second all types veterinary samples from farm animals and their products (e.g. diary products and meat) analysed for PPP residues for product quality reasons.

Comparator - We will use livestock bred in organic farming as comparator to conventional farming. In controlled laboratory studies we will use a defined sham-exposed group as the comparator, realizing that it will be very difficult to reach zero exposure due to the ubiquitous occurrence of pesticides and their residues.

Outcome - Any health or health-related outcome (including effect biomarkers) considered relevant to the impact of PPP exposure on animal health.

[7] van der Mierden S, Hooijmans CR, Tillema AH, Rehn S, Bleich A, Leenaars CH. Laboratory animals search filter for different literature databases: PubMed, Embase, Web of Science and PsycINFO. Lab Anim. 2021 Sep 24:236772211045485. doi: 10.1177/00236772211045485. Epub ahead of print. PMID: 34559023. Animal filters can be found at https://osf.io/q6uxs/

C. Ecosystem (Plants/Non-target species)

Population – Plant species targeted as crops and any species or ecological communities that pesticides are not targeting but that may by affected (non-target species).

Note: vertebrate and invertebrate species and also any effects on microorganisms (bacterial, fungi) in environmental compartments (water, soil) including the development of microbial resistance.

Exposure - Exposures to human-made or natural products marketed and used as plant protection products (pesticides) today (SPRINT list of 200+ PPPs) or at any time in the past, their active ingredients their metabolites, degradation products, adjuvants and co-formulants as part of products, including mixed exposures. All types of internal exposure assessment by biomonitoring of non-target species in the framework of ecological or ecotoxicological research and routine monitoring surveys.

Comparator – Plants not treated with PPPs in integrated pest management or organic farming. Eco-systems close to organic farming systems will be the internal reference. Data from eco-systems not affected by agricultural systems are externa reference.

Outcome - Any health or health-related outcome (including effect biomarkers) leading to higher productivity of plant species as targets for pest control. Second, for ecosystem health the effects indicators of population dynamics of non-target species and biodiversity of an ecosystem, including the microbiome.

Appendix 4: Existing and new indicators

A. Human (see Table 1.7 on page 21-22 of Part B of the Grant Agreement for more details)

EFSA bioassays (rodent): suggest to include ISO, OECD standard numbers and 'NTP' AND '13-weeks study' as search terms
In vitro: Human cell lines. Cytotoxicity, micronuclei test (ISO10993-3; OECD TG 487)
In vivo: Rats (Sprague-Dawley). Reproductive/developmental toxicity, target organ toxicity, haematological and hormonal changes, microbiome (13-week study, NTP 2011)
Search string: ISO, ISO10993-3, ISO 10993-3, ISO 10393, OECD Test Guideline, TG 487, TG487, 13-week study, 13 week study, NTP 2011, 90-days study, 90 days study; 90d study, 90 d study
Number of retrieved studies from the SPRINT SOLES database: 72

In vitro SPRINT indicators
In vitro SPRINT indicators: Human organoids/cell lines of kidney, colon ileum and airway. Transcriptomic and differential gene expression analyses, growth, viability and cell lineage differentiation.
Search string for in vitro: Human organoid*, kidney cell line*, colon ileum cell line, colon cell line, airway epithelial cell line, transcriptomic and differential gene expression essay, cell lineage differentiation essay
Number of retrieved studies from the SPRINT SOLES database: 2

In vivo SPRINT indicators
In vivo SPRINT indicators: Mouse Behavioural Assay. Behavioural tests in animal models of mood disorder; and subsequent changes in immune, endocrine and physiological systems. As determined from mouse model toxicity assay In vivo: Mouse Assay: Faecal Matter Transplant study. Behavioural tests (as above) in mice animals receiving faecal transplant from PPP-treated donors.
Search string for In vivo: mouse behavioural assay, behavioural*, mood disorder*, immune*, endocrine system, endocrine disruption, physiological system*, toxic*, faecal matter transplant, fecal matter transplant faecal transplant, fecal transplant, gut microbiome donor*
Number of retrieved studies from the SPRINT SOLES database: 332

B. Animal (see Table 1.7 on page 21-22 of Part B of the Grant Agreement for more details)

EFSA bioassays: n/a
SPRINT indicators
In vitro: Bovine intestinal organoids. Transcriptomics and differential gene expression analyses, growth, viability and cell lineage differentiation.
In vivo: Ross 308 chicken Health status, growth rate.

C. Ecosystem (see Table 1.6 on page 20-21 of Part B of the Grant Agreement for more details)

EFSA tests (terrestrial): suggest to include species and ISO, OECD and EFSA standard numbers as search terms

Soil microbial activity. Cmin, Nmin (*OECD 217, OECD 216, ISO 14238*).

- Collembola *(Folsomia candida, F. firmentaria).* Mortality, reproductivity *(ISO 2014, OECD 232*).
- Earthworms *(Eisenia fetida, E. andrei).* Mortality, reproduction, growth (*ISO 11268-1, OECD 207, ISO 11268-2, OECD 222*).
- Beneficial insects (*e.g. Aphidius rhopalosiphi, Typhlodromus pyri).*
- Mortality, reproductivity (Mead-Briggs et al. 2000, Blümel et al.2000).
- Terrestrial plants e.g. *Lactuca sativa, Zea mays, Triticum aestivum*).
- Root growth (*ISO 11269-1*); emergence & early growth (*ISO 11269-2, OECD 208*).
- Honey bees *(Apis mellifera).* Colony size, amount of food, amount of brood, mortality, gut microbiome

EFSA tests (aquatic): *suggest to include ISO, OECD and EFSA standard numbers as search terms*

- Freshwater algae (*Raphidocelis subcapitata*). Growth inhibition (OECD 201).
- Freshwater plant (*Lemna sp.)* Growth inhibition (OECD 221).
- Freshwater insects (*Chironomus sp*.). Larval survival and growth, number of adults emerged (OECD 218).
- Fish (*Oncorhynchus mykiss; Gambusia holbrooki*). Mortality, behavior, biomarkers (OECD 203, OECD 204)

SPRINT

Aquatic microcosm (producers, first and second consumers). Bioaccumulation, trophic transfer.

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Appendix 5: Definition of health outcomes (See Figure 1.1 on page 11 of Part B of the Grant Agreement)

Below Pubmed MESH terms are indicated to define outcomes for humans, animals and ecosystem health for literature searches. Note that the Pubmed MESH terms can be converted to EMBASE Emtree terms (see https://libguides.ru.nl/EmbaseEN/PubMed)

A. Human

Table 5.1 Medical Subject Headings available for use as search terms

SPRINT health outcome	Category	Existing MESH term (to be used as category for SOLES)	MESH Tree Structures to be used to retrieve literature items	Includes
Resilience	Immunity	Immune system disease	Pubmed: https://meshb.nlm.nih.gov/record/ui?ui=D007154	
	Microbiome	Brain gut axis	Pubmed: https://meshb.nlm.nih.gov/record/ui?ui=D000087502	
		Gastrointestinal microbiome	Pubmed: https://meshb.nlm.nih.gov/record/ui?ui=D000069196	
Reproductive health	Pregnancy	Pregnancy complication	Pubmed: https://meshb.nlm.nih.gov/record/ui?ui=D011248	Miscarriage
		Congenital abnormalities	Pubmed: https://meshb.nlm.nih.gov/record/ui?ui=D000013	
		Infant, newborn, diseases	Pubmed: https://meshb.nlm.nih.gov/record/ui?ui=D007232	
		Birth weight	Pubmed: https://meshb.nlm.nih.gov/record/ui?ui=D001724	
		Pregnancy outcome	Pubmed: https://meshb.nlm.nih.gov/record/ui?ui=D011256	
	Infertility Fertility	Male infertility	Pubmed: https://meshb.nlm.nih.gov/record/ui?ui=D007248	
		Female infertility	Pubmed: https://meshb.nlm.nih.gov/record/ui?ui=D007247	
		Time to pregnancy	Pubmed: https://meshb.nlm.nih.gov/record/ui?ui=D061685	
	Lactation	Lactation	Pubmed: https://meshb.nlm.nih.gov/record/ui?ui=D007774	
Diseases (SPRINT selection)	Lung	Respiratory tract disease	Pubmed: https://meshb.nlm.nih.gov/record/ui?ui=D012140	
	Kidney	Kidney	Pubmed: https://meshb.nlm.nih.gov/record/ui?ui=D007674	
	Brain/mental	Nervous system disease	Pubmed: https://meshb.nlm.nih.gov/record/ui?ui=D009422	Parkinson's disease
		Neurodevelopment-mental disorders	Pubmed https://meshb.nlm.nih.gov/record/ui?ui=D065886	ADHD
	Blood	Hematologic Diseases	Pubmed: https://meshb.nlm.nih.gov/record/ui?ui=D006402	Cancers

Table 5.2. Existing registered and/or published search strategies. The searches were conducted from a subset of 8492 human studies related to SPRINT PPPs.

SPRINT health outcome	Category	Health outcome	Registered and/or published search strategies*
Resilience	Immunity	Immune response	Luca Bernier, Giacomo Maffioletti, Andrea Polli. Effects of aerobic exercise on the immune system: a systematic review. PROSPERO 2018 CRD42018081512 Available from: https://www.crd.york.ac.uk/prospero/display_record.php?ID=CRD42018081512 Search string: immune system, immune function, immune cells, immunosenescence, immune response, immunological parameters
	Micro-biome	Gut microbiome	Hill L, Popov J, Figueiredo M, Caputi V, Hartung E, Moshkovich M, Pai N. Protocol for a systematic review on the role of the gut microbiome in paediatric neurological disorders. Acta Neuropsychiatr. 2021 Aug;33(4):211-216. doi: 10.1017/neu.2021.8. Epub 2021 Apr 5. PMID: 33818352. Available from: https://www.cambridge.org/core/journals/acta-neuropsychiatrica/article/protocol-for-a-systematic-review-on-the-role-of-the-gut-microbiome-in-paediatric-neurological-disorders/85BE70A1FC3029AC405B5840DEC78F93 (accessed 25-01-2022) Search string: microbiome, microbiota, gut microbiome, fecal microbiota, faecal microbiota
		Respiratory system	RICHA RAO. Systematic review comparing microbiota in upper versus lower respiratory tract in children during health and respiratory disease.. PROSPERO 2020 CRD42020202115 Available from: https://www.crd.york.ac.uk/prospero/display_record.php?ID=CRD42020202115 (accessed 25-01-2022) Search string: organism, microorganism, bacteria, virus, fungus

Reproductive health	Pregnancy	Prematurity/low birth weight	Carlos Renato Moreira-Maia, Adelar Pedro Franz, Gul Unsel Bolat, Himmi Bolat, Alicia Matijasevich, Iná dos Santos, Rita de Cássia Silveira, Renato Soibelmann Procianoy, Luis Augusto Rohde. ADHD and prematurity or low birth weight: a systematic review and meta-analysis. PROSPERO 2016 CRD42016049421 Available from: https://www.crd.york.ac.uk/prospero/display_record.php?ID=CRD42016049421 Search string: premature birth, low birth weight, very low birth weight, extremely low birth weight, preterm birth, very preterm birth
		Congenital malformations	Available from: https://fn.bmj.com/content/fetalneonatal/early/2021/06/09/archdischild-2021-322158/DC1/embed/inline-supplementary-material-1.pdf?download=true (accessed 25-01-2022) Fasna Naseer, Shibi T.D, Antriya Annie Tom, Meby Susan Mathew, Madhavan Namboothiri. G, Mintu Mathew. Systematic review on birth defect due to parental exposure to pesticides from various sources. PROSPERO 2021 CRD42021292988 Available from: https://www.crd.york.ac.uk/prospero/display_record.php?ID=CRD42021292988 (accessed 25-01-2022) Search string: Congenital Abnormalities, congenital abnormality, congenital, Deformity, congenital deformities, congenital defect, congenital defect, birth defect, congenital anomality, congenital anomilities, Esophageal Atresia, esophageal atresia, oesophageal atresia, Gastroschisis, gastroschis, congenital fissure of the Abdominal Cavity, Hernia, Umbilical, congenital, exomphalos, omphalocele, Hirschsprung Disease, hirschsprung disease, congenital megacolon, hirschsprung's disease, hirschsprungs disease, aganglionic, megacolon, Rectosigmoid Colon Aganglionosis, Rectosigmoid Aganglionosis, Congenital Intestinal Aganglionosis, Colonic Aganglionosis, Total Colonic Aganglionosis, Anorectal Malformations, Anorectal Malformation, Anorectal Anomal, Anorectal Atresia, Anorectal Stenos, Anus Imperforate, imperforate anus, anal atresi Short Bowel Syndrome, Short Bowel Syndrome, intestinal failure, pediatric intestinal failure, paediatric intestinal failure, Intestinal Atresia, Congenital Intestinal Atresia, Apple Peel Syndrome, Apple-Peel Intestinal Atresia, Jejunal Atresia, Apple Peel Small Bowel Syndrome, Familial Apple Peel Jejunal, Atresia, anogenital distance
		Still birth	Tamara Escanuela Sanchez, Molly Byrne, Keelin O'Donoghue, Sarah Meaney, Karen Matvienko-Sikar. A protocol for a systematic review of behaviour change techniques used in the context of stillbirth prevention. PROSPERO 2021 CRD42021264914 Available from: https://www.crd.york.ac.uk/prospero/display_record.php?ID=CRD42021264914 (accessed 25-01-

			2022); Search strategy available from: https://www.crd.york.ac.uk/PROSPEROFILES/264914_STRATEGY_20210629.pdf Search string: stillbirth, fetal death, foetal death, intrauterine death, perinatal death
	Infertility Fertility	Fertility	Albert Salas-Huetos, Marc Yeste, Marc Llavanera i Bruguera, Ariadna Delgado Bermudez. Identifying fertility biomarkers in human semen: a clinical approach through -OMICS to male infertility diagnosis. PROSPERO 2020 CRD42020176417 Available from: https://www.crd.york.ac.uk/prospero/display_record.php?ID=CRD42020176417 (accessed 25-01-2022) Search string: fertility, infertility, subfertility, infertility, male infertility, male fertility, alteration, abnormality, anomaly, fertilization, fecundability, pregnancy, miscarriage, implantation rate, dysfunction, azoospermia, asthenozoospermia, oligozoospermia, teratozoospermia, in vitro fertilization, intracytoplasmic sperm injection, assisted reproductive technologies, time to pregnancy
	Lactation	Lactation	Maria Luisa Imaz, Merce Torra, Dolors Soy, Luisa Garcia, Rocio Martin-Santos. Clinical lactation studies of lithium: a systematic review. PROSPERO 2019 CRD42019120928 Available from: https://www.crd.york.ac.uk/prospero/display_record.php?ID=CRD42019120928 (accessed 25-01-2022) Search string: lactation, breastfeeding, postpartum period, puerperium, neonates, nursing infants
Diseases (SPRINT selection)	Lung	Lung function	Jate Ratanachina, Sara De Matteis, Paul Cullinan, Peter Burney. The association between pesticide exposure and lung function: a systematic review. PROSPERO 2017 CRD42017078131 Available from: https://www.crd.york.ac.uk/prospero/display_record.php?ID=CRD42017078131 (accessed 25-01-2022) Search string: pulmonary function, respiratory function, lung function, spirometry, spirometer, FEV1, FVC, FEV1/FVC, forced expiratory volume, vital capacity, Tiffeneau-Pinelli index, PEEF, PEF, MEF, Peak Expiratory flow, Peak Expiration Flow, Peak Flow Rate, Oximetry, provocation test, Bronchial challenge, Bronchial hyperresponsiveness, DLCO, TLCO, Diffusing capacity
		Respiratory health effects	Farokhi A, Heederik D, Smit LAM. Respiratory health effects of exposure to low levels of airborne endotoxin - a systematic review. Environ Health. 2018 Feb 8;17(1):14. doi: 10.1186/s12940-018-0360-7. PMID: 29422043; PMCID: PMC5806377. https://ehjournal.biomedcentral.com/articles/10.1186/s12940-018-0360-7 (accessed 25-01-2022);

			Available from: https://ehjournal.biomedcentral.com/articles/10.1186/s12940-018-0360-7#Sec29 (accessed 25-01-2022) Search string: Respiratory symptoms, Asthma, Wheeze, Cough, Dyspnoea, Chest tightness, Lung function, Spirometry, Pulmonary function, FEV1, PEF, FVC, Forced expiratory volume, Forced vital capacity, Spirometer
		Asthma	Lam J, Koustas E, Sutton P, Padula AM, Cabana MD, Vesterinen H, Griffiths C, Dickie M, Daniels N, Whitaker E, Woodruff TJ. Exposure to formaldehyde and asthma outcomes: A systematic review, meta-analysis, and economic assessment. PLoS One. 2021 Mar 31;16(3):e0248258. doi: 10.1371/journal.pone.0248258. PMID: 33788856; PMCID: PMC8011796. Publication available from https://journals.plos.org/plosone/article?id=10.1371/journal.pone.0248258#sec041 Protocol available from: https://prhe.ucsf.edu/sites/g/files/tkssra341/f/Formaldehyde%20protocol%20FINAL%20UPLOADED%20TO%20PROSPERO%202016-05-03.pdf Search string: asthma, reactive airway, reactive airways, airway inflammation, wheeze, wheezes, wheezing, dyspnea, lung function test, spirometry, lung function, lung functions, respiratory function, respiratory functions, pulmonary function, pulmonary functions, bronchus hyperreactivity, bronchial hyperreactivity, bronchial hyper-reactivity, bronchial hypersensitivity, bronchial hyper-sensitivity, bronchospasm, bronchospasm, bronchial spasm, bronchial spasms, airway resistance, airway obstruction, airway obstruction, airway, resistance, bronchoconstriction, bronchial constriction, bronchial constrictions, respiratory health, reactive airway disease
		Respiratory tract microbiome	RICHA RAO. Systematic review comparing microbiota in upper versus lower respiratory tract in children during health and respiratory disease. PROSPERO 2020 CRD42020202115 Available from: https://www.crd.york.ac.uk/prospero/display_record.php?ID=CRD42020202115 (accessed 25-01-2022) Search string: organism, microorganism, bacteria, virus, fungus AND child AND respiratory, lung, nasopharyngeal, oropharyngeal, throat, nose
	Kidney	Chronic kidney disease	YAGAHIRA CASTRO-SESQUEN, John Kwagyan, Vimal K. Derebail, S. Mehdi Nouraie, Santos Saraf, Marina Jerebtsova. A systematic review and meta-analysis of chronic kidney disease in adults with sickle cell trait. PROSPERO 2021 CRD42021275274 Available from:

			https://www.crd.york.ac.uk/prospero/display_record.php?ID=CRD42021275274 (accessed 25-01-2022) Search strategy online available at: https://www.crd.york.ac.uk/PROSPEROFILES/275274_STRATEGY_20211120.pdf (accessed 25-01-2022) Search string: glomerulus filtration rate, glomerular filtration rate, glomerulus filtration rate, kidney gfr, kidney glomerulus filtration rate, proteinuria, albuminuria, albumin, kidney disease*, kidney disorder*, kidney pathology, nephropath*, renal disease, renal disorder, chronic kidney disease, chronic kidney failure, renal insufficiency, chronic kidney disease, chronic renal disease, chronic kidney failure, chronic renal failure, end stage renal disease, end stage kidney disease (39 studies) Additional search terms from personal communication with Benjamin Vervaet: chronic interstitial nephritis, -interstitial nephritis, glomerulosclerosis, tubulointerstitial fibrosis, tubulointerstitial infiltration, Interstitial Fibrosis Tubular Atrophy, IFTA, solute wasting, polyuria, anuria, dysuria, nocturia (2 case reports)
	Brain/ mental	ADHD	Jack Hollingdale, Ozge Kilic, Jennifer Kahle, Nicole Sylver, Susan Young, Ben Greer, Ayse Sakalli-Kani, Kelly Cocallis. A comparison of ADHD in females and males: a systematic review. PROSPERO 2020 CRD42020103830 Available from: https://www.crd.york.ac.uk/prospero/display_record.php?ID=CRD42020103830 Carlos Renato Moreira-Maia, Adelar Pedro Franz, Gul Unsel Bolat, Himmi Bolat, Alicia Matijasevich, Iná dos Santos, Rita de Cássia Silveira, Renato Soibelmann Procianoy, Luis Augusto Rohde. ADHD and prematurity or low birth weight: a systematic review and meta-analysis. PROSPERO 2016 CRD42016049421 Available from: https://www.crd.york.ac.uk/prospero/display_record.php?ID=CRD42016049421 Search string: attention deficit hyperactivity disorder, ADHD, attention disorder, hyperactivity disorder, impulsivity, inattent
		Alzheimer disease	Rayo Akande, Yizhou Yu. A meta-analysis on the association of vitamin B, D, and E levels with the progression and risk of Alzheimer's disease. PROSPERO 2021 CRD42021275439 Available from: https://www.crd.york.ac.uk/prospero/display_record.php?ID=CRD42021275439 (accessed 14-02-2022) Search available from: https://www.crd.york.ac.uk/PROSPEROFILES/274232_STRATEGY_20210818.pdf (accessed 14-02-2022) Search string: alzheimer*, alzheimer disease

		Autism	Anna Bertoletti, Simone Dal Bosco, Marina Camassola, Caroline Kuyven, Marina Valle, Rafaella Menezes, Isabella Mata. Association between premature exposure to agricultural pesticides and the occurrence of autism spectrum disorder: a systematic review. PROSPERO 2020 CRD42020204842 Available from: https://www.crd.york.ac.uk/prospero/display_record.php?ID=CRD42020204842 (Accessed 25-01-2021) Search string: autistic disorder, autism spectrum disorder, asperger syndrome, autism, autistic, ASD
		Neurocognitive and neurobehavioral	Paola Viglietti, Alexandra Goldberg. The impacts of chronic postnatal exposure to current use agricultural pesticides on child and adolescent neurocognitive and neurobehavioural outcomes: A systematic review. PROSPERO 2021 CRD42021258519 Available from: https://www.crd.york.ac.uk/prospero/display_record.php?ID=CRD42021258519 (accessed 25-01-2022) Available from: https://www.crd.york.ac.uk/PROSPEROFILES/258519_STRATEGY_20210601.pdf (accessed 25-01-2022) Search string: neurocogniti*, neurocognitive delay*, neurocognitive deficit*, cognitive delay*, cognitive deficit*, cognitive dysfunction*, neurobehaviour*, neurobehavior*, neurobehavioural delay*, neurobehavioral delay*, neurobehavioural deficit*, neurobehavioral deficit*, behavioural delay*, behavioral delay*, behavioural deficit*, behavioral deficit*, neurodevelopmental delay*, neurodevelopmental deficit*, neurodevelopmental disorder*, executive function*, dysexecutive, dysexecutive function*, dysexecutive syndrome*, executive dysfunction*, neurotoxic*, neurotoxicity syndrome*
		Parkinson's Disease	Vaccari C, El Dib R, de Camargo JLV. Paraquat and Parkinson's disease: a systematic review protocol according to the OHAT approach for hazard identification. Syst Rev. 2017 May 15;6(1):98. doi: 10.1186/s13643-017-0491-x. PMID: 28506248; PMCID: PMC5433017. Available from: https://systematicreviewsjournal.biomedcentral.com/articles/10.1186/s13643-017-0491-x (accessed 25-01-2022) Search string: Idiopathic Parkinson's Disease, Lewy Body Parkinson Disease, Lewy Body Parkinson's Disease, Primary Parkinsonism, Idiopathic Parkinson Disease, Parkinson's Disease, Parkinson Disease, Parkinson Patients, Parkinson Patient, Parkinson's Patients, Parkinson's Patient, Paralysis Agitans
		Mental disorders	Sonja Orlovska-Waast, Sophie Wiben Brix, Ole Köhler-Forsberg, Daniel Kondziella, Jesper Krogh, Michael Eriksen Benros. Cerebrospinal fluid (CSF) immune-related alterations in severe mental

			disorders: a systematic review and meta-analysis. PROSPERO 2017 CRD42017058938 Available from: https://www.crd.york.ac.uk/prospero/display_record.php?ID=CRD42017058938 (accessed 25-01-2022) Search string: psychosis, psychotic syndrome, psychotic, psychotic symptoms, schizophrenia, schizophreniform disorder, schizoaffective, depression, major depressive disorder, bipolar disorder
		Mental health	Blazej Cieslik, Justyna Mazurek, Sebastian Rutkowski, Pawel Kiper, Andrea Turolla, Joanna Szczepanska-Gieracha. A review of reviews of virtual reality application in mental health. PROSPERO 2020 CRD42020136632 Available from: https://www.crd.york.ac.uk/prospero/display_record.php?ID=CRD42020136632 Fear, phobias, agoraphobia, aviophobia, claustrophobia, panic, social phobia, anxiety/phobias, eating disorder, depression, mental disorder, developmental disability, psychosis, schizophrenia, mental health, burn pain, phantom limb pain
	Blood	Blood diseases	Search string (based on MESH terms): anemia, blood coagulation, blood Platelet*, bone marrow, erythroblastosis, hemoglobinopathy* hemorrhagic, leukocyte*, Methemoglobinemia*, pancytopenia, polycythemia, sulfhemoglobinemia, thrombophilia
		Blood cancer	van Dyk M, Boylan C, Michelet R, Mc Laughlin AM, Kichenadasse G, May N, Ziesenitz V, Van Den Anker JN, Groenland SL, Huitema ADR, Steeghs N, Mikus G, Kloft C, Tapp H. Plasma concentration guided dosing of drugs used for the treatment of childhood leukaemias: protocol for a systematic review. BMJ Open. 2022 Jan 3;12(1):e053308. doi: 10.1136/bmjopen-2021-053308. PMID: 34980620; PMCID: PMC8724759. Available from: https://bmjopen.bmj.com/content/bmjopen/12/1/e053308.full.pdf?with-ds=yes Search string: #1 (leukemia, leukaemia.*, cancer.*, neoplas.*, metasta.*, malignan.*, myeloma.*, oncolog.*, Hodgkin, NHL, polycythemia Vera) (445) #2 (blood, hemic, hematologic.*, haematologic.*) (746) #1 and #2 (121)

For searches in PROSPERO the following filters were used. SS=search strategy: SS AND (Epidemiologic OR Prevention OR Systematic Review OR Meta-Analysis OR IPD OR Methodology OR Review of reviews OR Qualitative synthesis)

B. Animal

Resilience outcomes: gut microbiome, immunological response

Reproductive outcomes: Time to or required number of inseminations to pregnancy, birth weight, growth rate, malformation at birth/miscarriage/number of offspring

Disease: gastrointestinal illness, mastitis (cow), deficiency in immunological systems

SPRINT health outcome	Category	Existing MESH term (to be used as category for SOLES)	MESH Tree Structures to be used to retrieve literature items	Includes
Resilience	Resilience	Immune system disease	Pubmed: https://meshb.nlm.nih.gov/record/ui?ui=D007154	
		Body weight	Pubmed: https://meshb.nlm.nih.gov/record/ui?ui=D001835	Weight gain/loss
Reproduction	Fecundity	Fertility	Pubmed: https://meshb.nlm.nih.gov/record/ui?ui=D005298	
Disease	Livestock	Livestock	Pubmed: https://meshb.nlm.nih.gov/record/ui?ui=D058751	Cattle, sheep, horses, poultry
	Cat	Cat diseases	Pubmed: https://meshb.nlm.nih.gov/record/ui?ui=D002371	
	Chicken	Poultry diseases	Pubmed: https://meshb.nlm.nih.gov/record/ui?ui=D011201	
	Cow	Cattle diseases	Pubmed: https://meshb.nlm.nih.gov/record/ui?ui=D002418	
	Goat	Goat diseases	Pubmed: https://meshb.nlm.nih.gov/record/ui?ui=D015511	
	Pig	Swine diseases	Pubmed: https://meshb.nlm.nih.gov/record/ui?ui=D013553	
	Sheep	Sheep diseases	Pubmed: https://meshb.nlm.nih.gov/record/ui?ui=D012757	

C. Plant/Ecosystem

SPRINT health outcome	Category	Existing MESH term (to be used as category for SOLES)	MESH Tree Structures to be used to retrieve literature items	Includes
Resilience	Aquatic ecosystem	Aquatic organisms	Pubmed: https://meshb.nlm.nih.gov/record/ui?ui=D059001	
	Microbiome	Microbiota	Pubmed: https://meshb.nlm.nih.gov/record/ui?ui=D064307	Aquatic, soil
		Aquatic microbiology	Pubmed: https://meshb.nlm.nih.gov/record/ui?ui=D014871	Aquatic ecology
	Ecosystem	Biodiversity	Pubmed: https://meshb.nlm.nih.gov/record/ui?ui=D044822	
	Freshwater	Freshwater biology	Pubmed: https://meshb.nlm.nih.gov/record/ui?ui=D056145	
	Green algae	Chlorophyta	Pubmed: https://meshb.nlm.nih.gov/record/ui?ui=D000460	
	Terrestrial ecosystem	Soil microbiology	Pubmed: https://meshb.nlm.nih.gov/record/ui?ui=D012988	
	Earthworms	Oligochaeta	Pubmed: https://meshb.nlm.nih.gov/record/ui?ui=D009835	Lumbricus and Eisenia
	Insects	Insecta	Pubmed: https://meshb.nlm.nih.gov/record/ui?ui=D007313	Bees
	Bats	Chiroptera	Pubmed: https://meshb.nlm.nih.gov/record/ui?ui=D002685	
	Plants	Biomass (vegetation)	Pubmed: https://meshb.nlm.nih.gov/record/ui?ui=D018533	
	Plant resilience	Plant immunity	Pubmed: https://meshb.nlm.nih.gov/record/ui?ui=D057865	Plant disease resistance
Productive outcome	Fecundity	Fertility	Pubmed: https://meshb.nlm.nih.gov/record/ui?ui=D005298	
	Infertility	Plant infertility	Pubmed: https://meshb.nlm.nih.gov/record/ui?ui=D051479	
	Growth	Plant growth regulators	Pubmed: https://meshb.nlm.nih.gov/record/ui?ui=D010937	
	Root growth	Plant roots	Pubmed: https://meshb.nlm.nih.gov/record/ui?ui=D018517	
Disease	Plant disease	Plant diseases	Pubmed: https://meshb.nlm.nih.gov/record/ui?ui=D010935	
	Fish disease	Fish diseases	Pubmed: https://meshb.nlm.nih.gov/record/ui?ui=D005393	
	Endangered animals	Endangered species	Pubmed: https://meshb.nlm.nih.gov/record/ui?ui=D056727	Plants

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Appendix 6: Authorship according to SPRINT policy

Table 1. Experts involved in annotation of citations to generating the machine learning algorithm and in preparation of scoping reports.

Name	Description of contribution	Review	User of outcome in WP	Participating in training the machine	Role in write-up
Nelson Abrantes	Lead reviewer aquatic ecosystem: amphibians and fish	C	4	+	M
Isabel Campos	Aquatic ecosystem: multispecies and trophic transfer	C	5	+	C
Claudia de Lima e Silva	Terrestrial ecosystem: soil invertebrates such as Collembola, mites, earthworm and others)	C	-	-	C
Peter Fantke	Human health outcome (to be specified)	A, B, C	6	+	
Vera Felix da Graca Silva	Soil eco-system	C	2, 9	+	-
Ana Gonzalez	Aquatic ecosystem: aquatic arthropods, annelids and molluscs	C	-	-	C
Eoin Gunnigle	Experimental animals: mouse studies	A	4	+	C
Paula Harkes	Soil microbiome	C	2	+	-
Jakub Hofman	Terrestrial ecosystem: plants, trophic transfer, wildlife (vertebrates)	C	5	+	-
Esperanza Huerta	Soil invertebrates	C	2	+	-
Kayode Jegede	Terrestrial ecosystem: soil invertebrates such as Collembola, mites, earthworm and others)	C	-	-	-
Chrow Khurshid	Human health outcome to be specified	C	2	+	-

Frank Langevelde	Lead terrestrial ecosystem: bees and beneficial arthropods	C	2	+	-
Philipp Mäder	Soil microbiome	C	2	+	C
Daniele Mandrioli	Experimental animals: rat studies	B	2, 4	-	C
Paula Mayer-Gruner	Soil microbiome	C	2	+	C
Joana Pereira	Aquatic ecosystem: microalgae and freshwater aquatic plants	C	-	-	C
Daria Sgargi	Experimental animals: rat studies	B	2, 4	+	C
Paul Scheepers	Lead reviewer human health/reproductive health/ neurodegenerative disease/blood	A	3, 4, 5	+	M
Vivi Schlünssen	Human health: respiratory system	A	2	+	C
Paula da Silva Tourinho	Lead Soil ecosystem: plants	C	2	+	M
Martin Tang Sørensen	Lead reviewer animal health/farm animal diseases (cow, chicken, goat, sheep)	B	2	+	M
Benjamin Vervaet	Human health: kidney	A	4	+	C

M = main author; C = co-author

Table 2. Development of the SPRINT SOLES.

Name	Field of expertise	Review	User of outcome in WP	Participating in training the machine	Role in write-up
Kaitlyn Hair	SOLES team	A, B, C	-	+	-
Malcolm Macleod	SOLES team	A, B, C	-	+	-
Kim Wever	SOLES team	A, B, C	-	+	-

Appendix 7: Search strings for PubMed and EMBASE

- Removed insect repellent
- Incorporated all suggestions by Paul, Hans and Alice (see emails)
- Checked all OR's and field codes in PubMed
- Added alternative spellings from Pubmed translated search (search details) to EMBASE and SCOPUS
- In EMTREE, individual compound names (also ones not on our list) fall under broader EMTREE terms such as pesticide. Therefore these broader terms are not exploded.
- EMTREE has numerous synonyms for each compound (up to 50 per compound!). I have not entered these back into Pubmed or SCOPUS, because in Pubmed I count on supplementary concept indexing terms to catch these, and in SCOPUS there are already half a million hits, which seems rather noisy…

PubMed	19-10-2021	351.003 hits (reviews removed)
#1	Pesticides	Pesticides[Mesh] OR Agrochemicals[mesh:noexp] OR Pest Control[Mesh] OR Herbicide Resistance[Mesh] OR Insecticide Resistance[Mesh] OR Fumigation[Mesh] OR pest control[tiab] OR insect control[tiab] OR mite control[tiab] OR rodent control[tiab] OR weed control[tiab] OR pesticide[tiab] OR pesticides[tiab] OR pesticidal[tiab] OR agrochemical[tiab] OR agrochemicals[tiab] OR agrichemical[tiab] OR agrichemicals[tiab] OR agricultural chemical[tiab] OR agricultural chemicals[tiab] OR algicide[tiab] OR algicides[tiab] OR algicidal[tiab] OR algaecide[tiab] OR algaecides[tiab] OR algaecidal[tiab] OR acaricide[tiab] OR acaricides[tiab] OR acaricidal[tiab] OR biocide[tiab] OR biocides[tiab] OR biocidal[tiab] OR chemosterilant[tiab] OR chemosterilants[tiab] OR chemosterilization[tiab] OR chemosterilizing[tiab] OR defoliant[tiab] OR defoliants[tiab] OR defoliate[tiab] OR defoliated[tiab] OR defoliating[tiab] OR defoliation[tiab] OR defoliations[tiab] OR defoliator[tiab] OR defoliators[tiab] OR fumigant[tiab] OR fumigants[tiab] OR fumigate[tiab] OR fumigated[tiab] OR fumigating[tiab] OR fumigation[tiab] OR fumigations[tiab] OR fumigators[tiab] OR fungicide[tiab] OR fungicides[tiab] OR fungicidal[tiab] OR herbicide[tiab] OR herbicides[tiab] OR herbicidal[tiab] OR insecticide[tiab] OR insecticides[tiab] OR insecticidal[tiab] OR ixodicide[tiab] OR ixodicides[tiab] OR ixodicidal[tiab] OR larvicide[tiab] OR larvicides[tiab] OR larvicidal[tiab] OR miticide[tiab] OR miticides[tiab] OR miticidal[tiab] OR molluscacide[tiab] OR molluscacides[tiab] OR molluscacidal[tiab] OR molluscicidal[tiab] OR nematicide[tiab] OR nematicides[tiab] OR nematicidal[tiab] OR phytocide[tiab] OR phytocides[tiab] OR phytocidal[tiab] OR rodenticide[tiab] OR rodenticides[tiab] OR rodenticidal[tiab] OR muricide[tiab] OR muricides[tiab] OR muricidal[tiab]

#2	Individual compounds	2, 4-D[all fields] OR Acetamiprid[all fields] OR Aclonifen[all fields] OR Ametoctradin[all fields] OR Atrazine[all fields] OR Azadirachtin[all fields] OR Azoxystrobin[all fields] OR Bentazone[all fields] OR Bifenthrin[all fields] OR Bixafen[all fields] OR Boscalid[all fields] OR Bromoxynil[all fields] OR Captan[all fields] OR Carbamate[all fields] OR Carbendazim[all fields] OR Carfentrazone[all fields] OR Chlorantraniliprole[all fields] OR Chloridazon[all fields] OR Chlorothalonil[all fields] OR Chlorotoluron[all fields] OR Chlorpropham[all fields] OR Chlorpyrifos[all fields] OR Clomazone[all fields] OR Clothianidin[all fields] OR Cyantraniliprole[all fields] OR Cyflufenamid[all fields] OR Cyfluthrin[all fields] OR Cymoxanil[all fields] OR Cypermethrin[all fields] OR Cyproconazole[all fields] OR Cyprodinil[all fields] OR DDD[all fields] OR DDE[all fields] OR DDT[all fields] OR Deltamethrin[all fields] OR Dicamba[all fields] OR Dicloran[all fields] OR Dieldrin[all fields] OR Difenoconazole[all fields] OR Diflufenican[all fields] OR Dimethenamid[all fields] OR Dimethoate[all fields] OR Dimethomorph[all fields] OR Dimoxystrobin[all fields] OR Dinotefuran[all fields] OR Diuron[all fields] OR Emamectin[all fields] OR Epoxiconazole[all fields] OR Esfenvalerate[all fields] OR Ethofumesate[all fields] OR Famoxadone[all fields] OR Fenbuconazole[all fields] OR Fenhexamid[all fields] OR Fenoxycarb[all fields] OR Fenpropidin[all fields] OR Fenpropimorph[all fields] OR Fenvalerate[all fields] OR Fipronil[all fields] OR Flazasulfuron[all fields] OR Flonicamid[all fields] OR Florasulam[all fields] OR Fluazifop[all fields] OR Fluazinam[all fields] OR Fludioxonil[all fields] OR Flufenacet[all fields] OR Flumioxazin[all fields] OR Fluopicolide[all fields] OR Fluopyram[all fields] OR Fluoxastrobin[all fields] OR Flupyradifurone[all fields] OR Fluroxypyr[all fields] OR Flusilazole[all fields] OR Flutolanil[all fields] OR Fluxapyroxad[all fields] OR Folpet [all fields] OR Foramsulfuron[all fields] OR Haloxyfop[all fields] OR Hexachlorobenzene[all fields] OR Imazalil[all fields] OR Imidacloprid[all fields] OR Indoxacarb[all fields] OR Iprovalicarb[all fields] OR Isoproturon[all fields] OR Isoxaben[all fields] OR Isoxaflutole[all fields] OR Kresoxim[all fields] OR Lambda-Cyhalothrin[all fields] OR Lenacil[all fields] OR Lindane[all fields] OR Linuron[all fields] OR Mandipropamid[all fields] OR MCPA[all fields] OR Mecoprop[all fields] OR Meptyldinocap[all fields] OR Metalaxyl[all fields] OR Metamitron[all fields] OR Metazachlor[all fields] OR Metconazole[all fields] OR Methabenzthiazuron[all fields] OR Methiocarb[all fields] OR Methoxyfenozide[all fields] OR Metobromuron[all fields] OR Metolachlor[all fields] OR Metrafenone[all fields] OR Metribuzin[all fields] OR Metsulfuron-methyl[all fields] OR Myclobutanil[all fields] OR Napropamide[all fields] OR Neonicotinoid[all fields] OR Nicosulfuron[all fields] OR Organochlorine[all fields] OR Organophosphorus[all fields] OR Organophosphate[all fields] OR Oryzalin[all fields] OR Oxadixyl[all fields] OR Oxyfluorfen[all fields] OR Penconazole[all fields] OR Pencycuron[all fields] OR Pendimethalin[all fields] OR Penoxsulam[all fields] OR Permethrin[all fields] OR Phosmet[all fields] OR Phoxim[all fields] OR Piperonyl butoxide[all fields] OR Pirimicarb[all fields] OR Pirimiphos-methyl[all fields] OR Prochloraz[all fields] OR Prometryn[all fields] OR Propamocarb[all fields] OR Propaquizafop[all fields] OR Propiconazole[all fields] OR Propoxur[all fields] OR Propyzamide[all fields] OR Prosulfocarb[all fields] OR Prothioconazole[all fields] OR Pymetrozine[all fields] OR Pyraclostrobin[all fields] OR Pyraflufen-ethyl[all fields] OR Pyrethrin I[all fields] OR Pyrethrin II[all fields] OR Pyrethroids[all fields] OR Pyrimethanil[all fields] OR Pyriofenone[all fields] OR Pyriproxyfen[all fields] OR Pyroxsulam[all

		fields] OR Quinoxyfen[all fields] OR Quizalofop[all fields] OR Rimsulfuron[all fields] OR Sedaxane[all fields] OR Spinetoram[all fields] OR Spinosad[all fields] OR Spinoxynad[all fields] OR Spinosyn A[all fields] OR Spinosyn D[all fields] OR Spirotetramat[all fields] OR Spiroxamine[all fields] OR Tau-Fluvalinate[all fields] OR Tebuconazole[all fields] OR Terbuthylazine[all fields] OR Terbutryn[all fields] OR Tetraconazole[all fields] OR Tetramethrin[all fields] OR Thiabendazole[all fields] OR Thiacloprid[all fields] OR Thiamethoxam[all fields] OR Thiencarbazone-methyl[all fields] OR Thiophanate-methyl[all fields] OR Tolylfluanid[all fields] OR Dimethylamino sulfotoluidid[all fields] OR Tri-allate[all fields] OR Tricyclazole[all fields] OR Trifloxystrobin[all fields] OR CGA 321113[all fields] OR Zoxamide[all fields]
#3	Pesticides OR Individual compounds	#1 OR #2
#4	Remove reviews	#3 NOT review[ptyp]

EMBASE	09-11-2021	223.024 (non-relevant publication types removed)
#1	Pesticides	Pesticide/ OR Biocide/ OR Carbamate pesticide/ OR Carbamate insecticide/ OR Chemosterilant/ OR Fumigant/ OR Fungicide/ OR Herbicide/ OR Insecticide/ OR Organochlorine insecticide/ OR Organophosphate insecticide/ OR Larvicidal agent/ OR Molluscacide/ OR Organochlorine pesticide/ OR Organophosphate pesticide/ OR Rodenticide/ OR Muricide/ OR exp Chemical pest control/ OR Insect control/ OR Mite control/ OR Rodent control/ OR Weed control/ OR exp Pesticide Resistance/ OR (pest control OR insect control OR mite control OR rodent control OR weed control OR pesticide OR pesticides OR pesticidal OR agrochemical OR agrochemicals OR agrichemical OR agrichemicals OR agricultural chemical OR agricultural chemicals OR algicide OR algicides OR algicidal OR algaecide OR algaecides OR algaecidal OR acaricide OR acaricides OR acaricidal OR biocide OR biocides OR biocidal OR chemosterilant OR chemosterilants OR chemosterilization OR chemosterilizing OR defoliant OR defoliants OR defoliate OR defoliated OR defoliating OR defoliation OR defoliations OR defoliator OR defoliators OR fumigant OR fumigants OR fumigate OR fumigated OR fumigating OR fumigation OR fumigations OR fumigators OR fungicide OR fungicides OR fungicidal OR herbicide OR herbicides OR herbicidal OR insecticide OR insecticides OR insecticidal OR ixodicide OR ixodicides OR ixodicidal OR larvicide OR larvicides OR larvicidal OR miticide OR miticides OR miticidal OR molluscacide OR molluscacides OR molluscacidal OR molluscicidal OR nematicide OR nematicides OR nematicidal OR phytocide OR phytocides OR phytocidal OR rodenticide OR rodenticides OR rodenticidal OR muricide OR muricides OR muricidal).tw.
#2	Individual compounds	(2, 4-D OR Acetamiprid OR Aclonifen OR Ametoctradin OR Atrazin OR Atrazine OR Azadirachtin OR Azadirachtins OR Azoxystrobin OR Bentazone OR Bifenthrin OR Bixafen OR Boscalid OR Bromoxynil OR Captan OR Carbamate OR Carbamates OR Carbamic OR Carbamation OR Carbendazim OR Carbendazime OR Carfentrazone OR Chlorantraniliprole OR Chlorantranilipole OR Chloridazon OR Chlorothalonil OR Chlorotoluron OR Chlortoluron OR Chlorpropham OR

		Chlorpyrifos OR Clomazone OR Clothianidin OR Cyantraniliprole OR Cyflufenamid OR Cyfluthrin OR Cymoxanil OR Cypermethrin OR Cypermethrine OR Cyproconazole OR Cyprodinil OR DDD OR DDE OR DDT OR Deltamethrin OR Dicamba OR Dicloran OR Dieldrin OR Difenoconazole OR Diflufenican OR Dimethenamid OR Dimethoate OR Dimethomorph OR Dimoxystrobin OR Dinotefuran OR Diuron OR Emamectin OR Epoxiconazole OR Esfenvalerate OR Ethofumesate OR Famoxadone OR Fenbuconazole OR Fenhexamid OR Fenoxycarb OR Fenpropidin OR Fenpropidine OR Fenpropimorph OR Fenvalerate OR Esfenvalerate OR Ethofumesate OR Fipronil OR Flazasulfuron OR Flonicamid OR Florasulam OR Fluazifop OR Fluazinam OR Fludioxonil OR Flufenacet OR Foe 5043 OR Flumioxazin OR Fluopicolide OR Fluopyram OR Fluoxastrobin OR Flupyradifurone OR Fluroxypyr OR Flusilazole OR Flutolanil OR Fluxapyroxad OR Folpet OR Foramsulfuron OR Haloxyfop OR Hexachlorobenzene OR Hexachlorobenzen OR Hexachlorobenzes OR Imazalil OR Enilconazole OR Imidacloprid OR Indoxacarb OR Iprovalicarb OR Isoproturon OR Isoxaben OR Isoxaflutole OR Kresoxim OR Lambda-Cyhalothrin OR Lenacil OR Lindane OR Lindan OR hexachlorocyclohexane OR Linuron OR Mandipropamid OR MCPA OR Mecoprop OR Meptyldinocap OR Metalaxyl OR Metamitron OR Metazachlor OR Metconazole OR Methabenzthiazuron OR Methiocarb OR Methoxyfenozide OR Metobromuron OR Metolachlor OR Metrafenone OR Metribuzin OR Metsulfuron-methyl OR Myclobutanil OR Systhane OR Napropamide OR Napropamid OR Devrinol OR Neonicotinoid OR Neonicotinoids OR Nicosulfuron OR Organochlorine OR Chlorinated hydrocarbons OR Organochlorines OR Organochlorinated OR Organophosphorus OR Organophosphate OR Organophosphates OR Oryzalin OR Oxadixyl OR Oxyfluorfen OR Oxyfluorofen OR Penconazole OR Penconazol OR Pencycuron OR Pendimethalin OR Penoxsulam OR Permethrin OR Permethrine OR Phosmet OR Phoxim OR Piperonyl butoxide OR Pirimicarb OR Pirimiphos-methyl OR Prochloraz OR Prometryn OR Prometryne OR Propamocarb OR Propaquizafop OR Propiconazole OR Propoxur OR Propyzamide OR Prosulfocarb OR Prothioconazole OR Pymetrozine OR Pyraclostrobin OR Pyrachlostrobin OR Pyraflufen-ethyl OR Pyrazon OR Pyrethrin I OR Pyrethrin II OR Pyrethroids OR Pyrethroides OR Pyrethroid OR Pyrethrins OR Pyrethrin OR Pyrimethanil OR Pyriofenone OR Pyriproxyfen OR Pyroxsulam OR Quinoxyfen OR Quizalofop OR Rimsulfuron OR Sedaxane OR Spinetoram OR Spinosad OR Spinoxynad OR Spinosyn A OR Spinosyn D OR Spirotetramat OR Spiroxamine OR Tau-Fluvalinate OR Tebuconazole OR Tebuconazol OR Terbuthylazine OR Terbutylazine OR Terbutryn OR Terbutryne OR Tetrachloroisophthalonitrile OR Tetraconazole OR Tetramethrin OR Thiabendazole OR Thiabendazol OR Thiacloprid OR Thiamethoxam OR Thiencarbazone-methyl OR Thiophanate-methyl OR Tolylfluanid OR Dimethylamino sulfotoluidid OR Tri-allate OR Tricyclazole OR Trifloxystrobin OR CGA 321113 OR Zoxamide).af.
#3	Pesticides OR Individual compounds	#1 OR #2

#4	Remove reviews etc.	3 AND (article OR article in press OR conference paper).pt.

SCOPUS	09-11-2021	504.101 (filter on Document Type: Article)
#1	Pesticides	TITLE-ABS-KEY(pest control) OR TITLE-ABS-KEY(insect control) OR TITLE-ABS-KEY(mite control) OR TITLE-ABS-KEY(rodent control) OR TITLE-ABS-KEY(weed control) OR TITLE-ABS-KEY(pesticide) OR TITLE-ABS-KEY(pesticides) OR TITLE-ABS-KEY(pesticidal) OR TITLE-ABS-KEY(agrochemical) OR TITLE-ABS-KEY(agrochemicals) OR TITLE-ABS-KEY(agrichemical) OR TITLE-ABS-KEY(agrichemicals) OR TITLE-ABS-KEY(agricultural chemical) OR TITLE-ABS-KEY(agricultural chemicals) OR TITLE-ABS-KEY(algicide) OR TITLE-ABS-KEY(algicides) OR TITLE-ABS-KEY(algicidal) OR TITLE-ABS-KEY(algaecide) OR TITLE-ABS-KEY(algaecides) OR TITLE-ABS-KEY(algaecidal) OR TITLE-ABS-KEY(acaricide) OR TITLE-ABS-KEY(acaricides) OR TITLE-ABS-KEY(acaricidal) OR TITLE-ABS-KEY(biocide) OR TITLE-ABS-KEY(biocides) OR TITLE-ABS-KEY(biocidal) OR TITLE-ABS-KEY(chemosterilant) OR TITLE-ABS-KEY(chemosterilants) OR TITLE-ABS-KEY(chemosterilization) OR TITLE-ABS-KEY(chemosterilizing) OR TITLE-ABS-KEY(defoliant) OR TITLE-ABS-KEY(defoliants) OR TITLE-ABS-KEY(defoliate) OR TITLE-ABS-KEY(defoliated) OR TITLE-ABS-KEY(defoliating) OR TITLE-ABS-KEY(defoliation) OR TITLE-ABS-KEY(defoliations) OR TITLE-ABS-KEY(defoliator) OR TITLE-ABS-KEY(defoliators) OR TITLE-ABS-KEY(fumigant) OR TITLE-ABS-KEY(fumigants) OR TITLE-ABS-KEY(fumigate) OR TITLE-ABS-KEY(fumigated) OR TITLE-ABS-KEY(fumigating) OR TITLE-ABS-KEY(fumigation) OR TITLE-ABS-KEY(fumigations) OR TITLE-ABS-KEY(fumigators) OR TITLE-ABS-KEY(fungicide) OR TITLE-ABS-KEY(fungicides) OR TITLE-ABS-KEY(fungicidal) OR TITLE-ABS-KEY(herbicide) OR TITLE-ABS-KEY(herbicides) OR TITLE-ABS-KEY(herbicidal) OR TITLE-ABS-KEY(insecticide) OR TITLE-ABS-KEY(insecticides) OR TITLE-ABS-KEY(insecticidal) OR TITLE-ABS-KEY(ixodicide) OR TITLE-ABS-KEY(ixodicides) OR TITLE-ABS-KEY(ixodicidal) OR TITLE-ABS-KEY(larvicide) OR TITLE-ABS-KEY(larvicides) OR TITLE-ABS-KEY(larvicidal) OR TITLE-ABS-KEY(miticide) OR TITLE-ABS-KEY(miticides) OR TITLE-ABS-KEY(miticidal) OR TITLE-ABS-KEY(molluscacide) OR TITLE-ABS-KEY(molluscacides) OR TITLE-ABS-KEY(molluscacidal) OR TITLE-ABS-KEY(molluscicidal) OR TITLE-ABS-KEY(nematicide) OR TITLE-ABS-KEY(nematicides) OR TITLE-ABS-KEY(nematicidal) OR TITLE-ABS-KEY(phytocide) OR TITLE-ABS-KEY(phytocides) OR TITLE-ABS-KEY(phytocidal) OR TITLE-ABS-KEY(rodenticide) OR TITLE-ABS-KEY(rodenticides) OR TITLE-ABS-KEY(rodenticidal) OR TITLE-ABS-KEY(muricide) OR TITLE-ABS-KEY(muricides) OR TITLE-ABS-KEY(muricidal)
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		KEY(Chlorantraniliprole) OR TITLE-ABS-KEY(Chlorantranilipole) OR TITLE-ABS-KEY(Chloridazon) OR TITLE-ABS-KEY(Chlorothalonil) OR TITLE-ABS-KEY(Chlorotoluron) OR TITLE-ABS-KEY(Chlortoluron) OR TITLE-ABS-KEY(Chlorpropham) OR TITLE-ABS-KEY(Chlorpyrifos) OR TITLE-ABS-KEY(Clomazone) OR TITLE-ABS-KEY(Clothianidin) OR TITLE-ABS-KEY(Cyantraniliprole) OR TITLE-ABS-KEY(Cyflufenamid) OR TITLE-ABS-KEY(Cyfluthrin) OR TITLE-ABS-KEY(Cymoxanil) OR TITLE-ABS-KEY(Cypermethrin) OR TITLE-ABS-KEY(Cypermethrine) OR TITLE-ABS-KEY(Cyproconazole) OR TITLE-ABS-KEY(Cyprodinil) OR TITLE-ABS-KEY(DDD) OR TITLE-ABS-KEY(DDE) OR TITLE-ABS-KEY(DDT) OR TITLE-ABS-KEY(Deltamethrin) OR TITLE-ABS-KEY(Dicamba) OR TITLE-ABS-KEY(Dicloran) OR TITLE-ABS-KEY(Dieldrin) OR TITLE-ABS-KEY(Difenoconazole) OR TITLE-ABS-KEY(Diflufenican) OR TITLE-ABS-KEY(Dimethenamid) OR TITLE-ABS-KEY(Dimethoate) OR TITLE-ABS-KEY(Dimethomorph) OR TITLE-ABS-KEY(Dimoxystrobin) OR TITLE-ABS-KEY(Dinotefuran) OR TITLE-ABS-KEY(Diuron) OR TITLE-ABS-KEY(Emamectin) OR TITLE-ABS-KEY(Epoxiconazole) OR TITLE-ABS-KEY(Esfenvalerate) OR TITLE-ABS-KEY(Ethofumesate) OR TITLE-ABS-KEY(Famoxadone) OR TITLE-ABS-KEY(Fenbuconazole) OR TITLE-ABS-KEY(Fenhexamid) OR TITLE-ABS-KEY(Fenoxycarb) OR TITLE-ABS-KEY(Fenpropidin) OR TITLE-ABS-KEY(Fenpropidine) OR TITLE-ABS-KEY(Fenpropimorph) OR TITLE-ABS-KEY(Fenvalerate) OR TITLE-ABS-KEY(Esfenvalerate) OR TITLE-ABS-KEY(Ethofumesate) OR TITLE-ABS-KEY(Fipronil) OR TITLE-ABS-KEY(Flazasulfuron) OR TITLE-ABS-KEY(Flonicamid) OR TITLE-ABS-KEY(Florasulam) OR TITLE-ABS-KEY(Fluazifop) OR TITLE-ABS-KEY(Fluazinam) OR TITLE-ABS-KEY(Fludioxonil) OR TITLE-ABS-KEY(Flufenacet) OR TITLE-ABS-KEY(Foe 5043) OR TITLE-ABS-KEY(Flumioxazin) OR TITLE-ABS-KEY(Fluopicolide) OR TITLE-ABS-KEY(Fluopyram) OR TITLE-ABS-KEY(Fluoxastrobin) OR TITLE-ABS-KEY(Flupyradifurone) OR TITLE-ABS-KEY(Fluroxypyr) OR TITLE-ABS-KEY(Flusilazole) OR TITLE-ABS-KEY(Flutolanil) OR TITLE-ABS-KEY(Fluxapyroxad) OR TITLE-ABS-KEY(Folpet) OR TITLE-ABS-KEY(Foramsulfuron) OR TITLE-ABS-KEY(Haloxyfop) OR TITLE-ABS-KEY(Hexachlorobenzene) OR TITLE-ABS-KEY(Hexachlorobenzen) OR TITLE-ABS-KEY(Hexachlorobenzes) OR TITLE-ABS-KEY(Imazalil) OR TITLE-ABS-KEY(Enilconazole) OR TITLE-ABS-KEY(Imidacloprid) OR TITLE-ABS-KEY(Indoxacarb) OR TITLE-ABS-KEY(Iprovalicarb) OR TITLE-ABS-KEY(Isoproturon) OR TITLE-ABS-KEY(Isoxaben) OR TITLE-ABS-KEY(Isoxaflutole) OR TITLE-ABS-KEY(Kresoxim) OR TITLE-ABS-KEY(Lambda-Cyhalothrin) OR TITLE-ABS-KEY(Lenacil) OR TITLE-ABS-KEY(Lindane) OR TITLE-ABS-KEY(Lindan) OR TITLE-ABS-KEY(hexachlorocyclohexane) OR TITLE-ABS-KEY(Linuron) OR TITLE-ABS-KEY(Mandipropamid) OR TITLE-ABS-KEY(MCPA) OR TITLE-ABS-KEY(Mecoprop) OR TITLE-ABS-KEY(Meptyldinocap) OR TITLE-ABS-KEY(Metalaxyl) OR TITLE-ABS-KEY(Metamitron) OR TITLE-ABS-KEY(Metazachlor) OR TITLE-ABS-KEY(Metconazole) OR TITLE-ABS-KEY(Methabenzthiazuron) OR TITLE-ABS-KEY(Methiocarb) OR TITLE-ABS-KEY(Methoxyfenozide) OR TITLE-ABS-KEY(Metobromuron) OR TITLE-ABS-KEY(Metolachlor) OR TITLE-ABS-KEY(Metrafenone) OR TITLE-ABS-KEY(Metribuzin) OR TITLE-ABS-KEY(Metsulfuron-methyl) OR TITLE-ABS-KEY(Myclobutanil) OR TITLE-ABS-KEY(Systhane) OR TITLE-ABS-KEY(Napropamide) OR TITLE-ABS-KEY(Napropamid) OR TITLE-ABS-KEY(Devrinol) OR TITLE-ABS-KEY(Neonicotinoid) OR TITLE-ABS-KEY(Neonicotinoids) OR TITLE-ABS-KEY(Nicosulfuron) OR TITLE-ABS-KEY(Organochlorine) OR TITLE-ABS-KEY(Chlorinated hydrocarbons) OR TITLE-ABS-KEY(Organochlorines) OR TITLE-ABS-KEY(Organochlorinated) OR TITLE-ABS-KEY(Organophosphorus) OR TITLE-ABS-KEY(Organophosphate) OR TITLE-ABS-KEY(Organophosphates) OR TITLE-

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#3	Pesticides OR individual compounds	#1 OR #2
#4	Remove reviews etc.	filter on Document Type: Article in the left-hand sidebar

Disclaimer: This report is part of a project that has received funding by the European Union's Horizon 2020 research and innovation program under grant agreement number 862568.

ANNEX A: Human

Resilience

Immune system disease

Gut microbiome

Reproductive health

Congenital malformations

Birth weight

Fertility

SPRINT endpoints

Asthma

ADHD

Alzheimer's disease

Autism

Blood cancer

Lung function

Immune response

Kidney

Mental health

Non-cancer blood disease

Parkinson's disease

Immune response

Author name		Paul Scheepers PhD
Topic description (see Table)		Immune response
Search	Used filter 1:	human
	Used filter 2:	n/a
	Complete search strategy	immune system, immune function, immune cells, immunosenescence, immune response, immunological parameters
Selection	Total before/after TiAb screening	27/9
	Reason for exclusion 1	Non-primary study (N=5)
	Reason for exclusion 2	Non-human study (N=5)
	Reason for exclusion 3	Other outcomes (N=5)
	Reason for exclusion 5	Exposure/biomarker study (N=3)
	Reason for exclusion 6	Case reports (N=0)
Description	Type of research	Most studies used a case-control design. Some were nested in a prospective cohort
	Population	Six studies assessed PPP exposures of the general population and six assessed immune response in an occupational setting.
	Exposure	Exposure assessment was most often based on questionnaire data and additionally also using analysis of breast milk or blood
	Comparator	Self-reported no/never use in exposure assessment by questionnaire. In biomonitoring the lowest quartile or tertile values of an exposure biomarker are often used.
	Outcome	Immune response as indicated by pro-inflammatory markers, whit blood cell count or immunoglobulins
	Gender	Most studies considered both genders and one occupational study reported a difference in immune response with a higher response in female farmers.
Recommendation	Future needs	The number of studies that could be included for this endpoint is low.
	Full systematic review	Number of eligible studies is too low.
	Your interest	Not applicable
References	Link to included studies	The collection of articles and screening result can be viewed here

Over the past 27 years 10 studies were included: 4 studies were reported on outcomes from Canada, and one from each of the following countries: Australia, Czech Republic, Egypt, Germany and Japan. Below effects on indicators of immune response are reported based on statistical significance as reported by the authors. When authors reported no associations, this is also mentioned

Studies reporting on effects based on self-reported exposures

Rosenberg et al. (1999) reported on self-reported occupational and environmental exposure of 322 study participants in Canada to PPPs based on questionnaire data. Antinuclear antibodies (ANA) were associated with lifetime exposures to carbamates, pyrethroids, phenoxy acetic acid herbicides and with the sum of the following OCPs: alanine, chlordane, dieldrin, endrin, heptachlor and lindane. ANA levels were not associated with reported exposures to DDT. Kruse and co-workers (2005) also performed a study using

questionnaires and observed an association of DDE with white blood cell (WBC) counts and the following immunoglobulins: IgE, IgG and IgA. Hexachlorobenzene was inversely associated with IgM. Gamma HCH was associated with a rise in NK cells (CD56+). DDE was associated with consistently lower eosinophilic granula. Richter studied occupational exposures to organochlorines in air in Czech Republic and observed an association of hexachlorobenzene (HCB) inhalation exposure with immunoglobulin levels and protein changes. They also observed a depression of antibody response, nutrient condition and nicotinism to be associated with HCB. Rosenberg and co-workers (200&0 studied bromoxynil in serum from 98 female and 114 male cereal farmers in Canada. Exposure was also based on self-reported use. An inverse relation with the level of anti-nuclear antibodies (ANA) was observed in female but not male farmers after adjustment for age. for other endpoints no difference by gender were observed No associations were observed for 2,4 dichloro-phenoxy acetic acid, bromoxynil applied in winter time. Exposure did not have a significant effect on allergy.

Studies reporting on effects based on biomonitoring

Dewailly and co-workers (2000) performed a study on OCPs in mothers and infants at 3, 7, and 12M in an Inuit community in Canada. Otitis media was not different between breastfed infants were compared to 73 bottle fed infants. Third compared to 1st quartile blood levels p,p'DDE and HCB of the infants were associated with Otitis media with ORs of 1.87 and 1.49, respectively. Nagayama and co-workers (2007) studied OCP levels in breast milk and in 101 infants of 10 months age. HCE was associated with an increase in the following CD8+, CD3+, CD4+/CD8+ T-cell ratio. DDT in breastmilk was associated with CD16+ T lymphocytes. For dieldrin no effects were observed. Schaalen and co-workers studied 180 newborns in Egypt and related immune response to the following OCPs in breastmilk: alpha and beta-endosulfan, aldrin, endrin, dieldrin, hexachlorobenzene, DDT and 7-chloroepoxide. Effects were observed on TNFα, IL-10 and WBC count of the breastfed infants. Bilrah et al.(2003) studied 47 infants with a menu rich in fish with 65 infants from an urban environment as a reference group. Blood hexachlorobenzene and p,p'DDE were different for aforementioned groups and associated with reduced TNFα levels.

Conclusion:

Although the study set is limited to ten included studies, there are indications that maternal OCP levels in blood and breast milk are associated with different biomarkers of immunotoxicity in infant's development. Other studies have reported on pro-inflammatory indicators in auto-immune response but these effects need further confirmation in biomonitoring studies because exposure was only reported from questionnaire data by self-assessment. There is a lack on studies addressing current use pesticides.

Gut microbiome

Author name		Eoin Gunnigle, PhD
Topic description (see Table)		Gut Microbiome
Search	Used filter 1:	Human
	Used filter 2:	n/a
	Complete search strategy	Gut Microbiome, Gut, Microbiome, Gut Health
Selection	Number of studies before/after TiAb screening	10/1
	Reason for exclusion 1 (N)	Non-human (4)
	Reason for exclusion 2 (N)	Non-primary study (5)
Description	Type of research	Environmental toxicological study (N=1)
	Population	Study was performed on mother-child pairs.
	Exposure	Maternal exposure to environmental toxicants (N=1)
	Comparator	Exposure to multiple environmental toxicants that are globally present in breastmilk and associated with gut microbiome composition and function among infants at 1 month.
	Outcome	A wide range of endpoints was used to indicate exposure rates including microbiome disruption (16S rRNA sequencing), Short-Chain Fatty Acid concentrations and covariates. 1-month questionnaires used.
	Gender	Maternal exposure was used.
Recommen-dation	Future needs	Unclear whether these potential toxicant-induced alterations have implications for child health, and this needs studying both in this cohort and in countries with higher contamination.
	Full systematic review	No
	Your interest	Not applicable
References	CSV-format and Endnote-format	Gut microbiome.csv
Studies	Selection of included studies	Available online in SPRINT SOLES

This study was performed in 2019 in Norway. Below the results of this study are presented. Associations are all based on results reported as statistically significant. If no associations were observed this is also indicated.

Early disruption of the microbial community may influence life-long health. Environmental toxicants can contaminate breast milk and the developing infant gut microbiome is directly exposed. This study investigated whether environmental toxicants in breastmilk affect the composition and function of the infant gut microbiome at 1 month. They measured environmental toxicants in breastmilk, fecal short-chain fatty acids (SCFAs), and gut microbial composition from 16S rRNA gene amplicon sequencing using samples from 267 mother-child pairs in the Norwegian Microbiota Cohort (NoMIC). They tested 28 chemical exposures: polychlorinated biphenyls (PCBs), polybrominated flame retardants (PBDEs), per- and polyfluoroalkyl substances (PFASs), and organochlorine pesticides. They assessed chemical exposure and alpha diversity/SCFAs using elastic net regression modeling and generalized linear models, adjusting for confounders, and variation in beta diversity (UniFrac), taxa abundance (ANCOM), and predicted metagenomes (PiCRUSt) in low, medium, and high exposed groups.

Results

PBDE-28 and the surfactant perfluorooctanesulfonic acid (PFOS) were associated with less microbiome diversity. Some sub-OTUs of Lactobacillus, an important genus in early life, were lower in abundance in samples from infants with relatively "high" (> 80th percentile) vs. "low" (< 20th percentile) toxicant exposure in this cohort. Moreover, breast milk toxicants were associated with microbiome functionality, explaining up to 34% of variance in acetic and propionic SCFAs, essential signalling molecules. Per one standard deviation of exposure, PBDE-28 was associated with less propionic acid (− 24% [95% CI − 35% to − 14%] relative to the mean), and PCB-209 with less acetic acid (− 15% [95% CI − 29% to − 0.4%]). Conversely, PFOA and dioxin-like PCB-167 were associated with 61% (95% CI 35% to 87%) and 22% (95% CI 8% to 35%) more propionic and acetic acid, respectively.

Conclusions

Environmental toxicant exposure may influence infant gut microbial function during a critical developmental window. Future studies are needed to replicate these novel findings and investigate whether this has any impact on child health.

Congenital malformations

Author name		Paul Scheepers PhD
Topic description (see Table)		Congenital malformations
Search	Used filter 1:	Human
	Used filter 2:	n/a
	Complete search strategy	Congenital Abnormalities, congenital abnormality, congenital, Deformity, congenital deformities, congenital defect, congenital defect, birth defect, congenital anomality, congenital anomilities, Esophageal Atresia, esophageal atresia, oesophageal atresia, Gastroschisis, gastroschis, congenital fissure of the Abdominal Cavity, Hernia, Umbilical, congenital, exomphalos, omphalocele, Hirschsprung Disease, hirschsprung disease, congenital megacolon, hirschsprung's disease, hirschsprungs disease, aganglionic, megacolon, Rectosigmoid Colon Aganglionosis, Rectosigmoid Aganglionosis, Congenital Intestinal Aganglionosis, Colonic Aganglionosis, Total Colonic Aganglionosis, Anorectal Malformations, Anorectal Malformation, Anorectal Anomal, Anorectal Atresia, Anorectal Stenos, Anus Imperforate, imperforate anus, anal atresi Short Bowel Syndrome, Short Bowel Syndrome, intestinal failure, pediatric intestinal failure, paediatric intestinal failure, Intestinal Atresia, Congenital Intestinal Atresia, Apple Peel Syndrome, Apple-Peel Intestinal Atresia, Jejunal Atresia, Apple Peel Small Bowel Syndrome, Familial Apple Peel Jejunal, Atresia, anogenital distance
Selection	Number of studies before/after TiAb screening	63/30
	Reason for exclusion 1 (N)	Other health outcome reported (3)
	Reason for exclusion 2 (N)	Not PPP-related (4)
	Reason for exclusion 3 (N)	Exposure/kinetic/biomarker study (19)
	Reason for exclusion 4 (N)	Non-primary study (1)
	Reason for exclusion 5 (N)	Case reports (0)
	Reason for exclusion 6 (N)	Non-human (6)
	Reason for exclusion 7 (N)	Language (0)
Description	Type of research	Case-control studies (N=26)
	Population	25 studies in general population; 3 studies in occupational setting
	Exposure	Exposure by biomonitoring (N=26), by location (N=2), by maternal exposure (24), by parental exposure (2)

	Comparator	With verified exposure status using biomonitoring (N=21), by residential location (N=2) or by self-reported exposure status (N=3)
	Outcome	15 specific malformations; some studies reported on aggregated malformations or defects at birth (N=5) or still birth related to a severe malformation (N=1)
	Gender	Exposure of the mother was considered in most studies; In occupational setting the father's exposure was reported (N=1); gender of the new-born was selectively reported and often related to genetical malformation; for non-genetical malformations many studies did not specify gender.
Recommen-dation	Future needs	Further confirmation of evidence to rule out spurious findings that are not supported by causality
	Full systematic review	For some specific health outcomes related to organochlorine pesticides and atrazine the number of studies warrants a systematic evaluation of the evidence.
	Your interest	Depends on availability of recently published systematic reviews
References	CSV-format and Endnote-format	Congenital malformations.csv
Studies	Selection of included studies	Study selection is available here

All 24 included studies were published in the last 20 years. Twenty studies were published since 2012 and 16 over the past five years.
Seventeen of the included studies were performed in large birth cohorts in the US (one in Hawaii). Five studies were conducted in Europe (Denmark France, Spain, Sweden) and four from China. The other studies were from other countries (Brazil, Hawaii, Kyrgyzstan).
The most reported malformations were hypospadias (4), birth weight (4), gastroschisis (3), Cryptorchidism (3), neurocognitive/auditory defects (2), anogenital distance (2), head circumference (2), cardiac defects (2). holoprosencephaly (1), atopic dermatitis (1), stenosis (1), Some studies grouped malformations at birth (3).

Below the results of the different studies are presented. Associations are all based on results reported as statistically significant. If no associations were observed this is also indicated.

<u>Organochlorine pesticides</u>
Most studies report on p,p'DDT, p,p'DDE, hexachlorobenzene (HCB), ß-hexachlorohexane (ß-HCH) together with other persistent organic pollutants (POPs) in cord blood levels and their association with malformations at birth. Significant effects on length, body weight, malformations and stillbirth in a study of organochlorine levels in placenta material reported by Toichuev et al. (2016). Effects on head circumference, bodyweight and length at birth were recently described to be more pronounced in female offspring (FO) as compared to male offspring (MO) (Wang et al, 2022). These authors also reported on a threshold in umbilical cord blood for HCB and the occurrence of birth defects. Changes in anogenital distances (AGD) as function of exposure were studied in Spain (Garcia-Villarion et al., 2020) and observed feminizing effects in MO and masculinizing effects in FO. Exposure to 2,4-D was linked to auditory processing determined 6, 9

and 12 M after birth (Silver et al., 2019). Schweinemachers (2003) reported on severe malformations after still birth (autopsy). Infant death was significantly associated with exposure. Specific malformations associated to 2,4-D and MCPA exposure were: heart or non-cardiac/muscoskeletal disorder, circularly/respiratory disorder. In a study in Denmark 8 organochlorine pesticide blood levels showed a trend for *trans*-chlordane in mother milk with the risk of cryptorchidism in MO. In a Swedish study no significant association was observed for this endpoint (Axelsson et al., 2020). Another study reported on the lack of finding an association with atopic dermatitis 7 months after birth (Ochinai et al. 2019). Metolachlor and dicloran exposures were linked to reduced birth weight and abdominal malformations (Barr et al., 2010), vinclozolin and acetochlor exposure and birthweight (Wickerham et al., 2012) and phenyl urea and methyl-4-chlorophenoxy acetic acid (4-CPA) in colostrum and hypospadias (Haraux et al. 2018) and 2,4-D and MCPA were observed to be a risk in still birth (Schweinmacher, 2003). In France the sum of fifteen organochlorine PPPs analysed in colostrum were linked to an increased risk of cryptorchidism (Brucker-Davis et al 2008). For the same endpoint Damgaard et al. (2006) reported to be associated to exposure to eight organochlorine pesticides in mother milk. In analysis of the single compounds only trans chlordane could be linked to the risk of cryptorchidism.

Atrazine

This herbicide is much used in the US and resulted in widespread contamination of surface water with penetration into drinking water aquifers. Most studies related exposure of the mother during pregnancy. Atrazine exposure was not observed to be associated with eight different cardiac subtypes (Kim, 2017). Winston and co-workers (2016a) observed a small effect on hypospadias in MO and determined thresholds for an increased risk for water concentration and water consumption. Agopian et al. (2013a) observed a statistically significant positive trend between atrazine intake and the risk of stenosis in offspring (M/F distribution not reported). Atrazine in drinking water and gastroschisis was observed to be significant in birth five birth defects (Winchester et al., 2009) and specifically with gastroschisis (Waller et al., 2010) also reported specifically for mothers aged above 25 years (Agopian et al., 2013b). In a third study by Agopian et al (2013c) medium atrazine levels were compared to low-levels in drinking water and reported to be associated to the sum of three malformations in male offspring (hypospadias, cryptorchidism and small penis). Other adverse birth outcomes were reported by Chevrier et al., 2011 and included a negative association of atrazine in urine with growth, head circumference and a positive association with congenital malformations at birth.

Glyphosate

Three studies reported on glyphosate. This herbicide was associated with aggregated birth defects in 695 families where the father was applicant of herbicides (Garry et al., 2002). In the 1,532 children in these families, severe malformations were more frequent in female offspring (FO) compared to male offspring (MO). The sex ratio in offspring was opposite F/M ratio 1.75). A recent pilot-study reported on glyphosate and AMPA in urine in a pregnancy cohort anogenital distance (AGD) was determined in FO and MO (Lesseur et al., 2021). Statistically significant associations with urinary excreted glyphosate above group median were reported to be associated with increased AGD in FO. (not significant after adjustment for infant size and age at AGD exam). In MO no association was detected. Glyphosate was also associated with a cardiac defect (atrial septal defect) in FO (Rappazzo et al., 2018).

Other substances

Urinary pyrethroid metabolites in urine were associated with increased body weight and length and decreased for small for gestational age (SGA) and premature birth (Xu et al., 2013). In the

Dominican Republic permethrin levels in air and blood were both associated with cognitive neurodevelopment (based on two different tests) determined 36M after birth (Horton et al. 2011). Rappazzo and co-workers (2018) reported an association between pendimethalin but not paraquat with an increased risk of hypospadias in MO in residents living < 500m from farmland where these PPPs were applied. Wohlfahrt and co-workers (2012) reported on 94 female workers of which 59 were exposed to pesticides and 35 were not. Malformation in male offspring was assessed at 6-11 y. A trend was observed with overall small genitals. High exposures were associated with 2.4.7% smaller testes and 9.4% decreased penile length.

Unspecified pesticide exposures

Unspecified self-reported exposures to pesticides were associated with an increased risk for Holoprosencephaly (HPE) in resident living close to farmland (Addissie et al., 2020). Winston et al., (2016b) reported a higher prevalence of hypospadias in the population residing in an intensive use area for pesticides compared to the prevalence in an area less intensive use in Hawaii. Five birth defects were reported to be increased related to self-reported exposure as 'any' vs. 'no' exposure to pesticides. In France self-reported paternal activities as 'farming' and use of 'pesticides' in were related to congenital malformations in children of <5 y (Ueker et al. 2016).

Conclusion

All 24 included studies were published in the last 20 years. Twenty studies were published since 2012 and 16 over the past five years indicating that interest in pesticide exposures as a risk factor in congenital malformations is growing. Initially organochlorines were studied as part of a larger group of POPs. More recently attention was shifted to include also less resistant PPPs such as atrazine and glyphosate. Most studies were performed in the general population and only few involving worker's exposures. Most of the studies used quantitative exposure data from analyses of relevant biological media such as cord blood. Only few studies classified exposure in other less reliable ways. There are several malformations that indicate general indicators such as body weight, length and growth-related parameters. Most associations are with cryptorchidism, hypospadias and gastroschisis. Many studies have a focus on MO or FO. Few studies addressed ratio of F/M. There are clear indication of PPPs having endocrine disruptive effects leading to feminization in MO and masculinisation in FO. In recent anogenital distance and index in female offspring was suggested to be affected by organochlorine and glyphosate. Malformations is a difficult field for systematic review and meta-analysis due to the complexity of the definition of timepoint of exposure during the reproductive cycle and the many different endpoints that can be studied in new-borns.

Birth weight

Author name		Paul Scheepers PhD
Topic description (see Table)		Birth weight
Search	Used filter 1:	human
	Used filter 2:	n/a
	Complete search strategy	premature birth, low birth weight, very low birth weight, extremely low birth weight, preterm birth, very preterm birth
Selection	Total before/after TiAb screening	24/22
	Reason for exclusion 1	Non-primary study (N = 1)
	Reason for exclusion 2	Other outcomes (N = 1)
Description	Type of research	Most studies used a case-control design and were nested in a prospective mother-child cohort. Studies on residues in drinking water studies considering distance to agricultural fields used an ecologic design
	Population	Twenty studies assessed PPP exposures of the general population and one studied female farmers.
	Exposure	Exposure assessment was most often analysed maternal blood, urine or cord blood.
	Comparator	In biomonitoring the lowest tertile or quartile values of an exposure biomarker are often used. In drinking wate studies intakes were calculated based on water analysis
	Outcome	Used abbreviations for biometric endpoints were BL = body length FTD = full term delivery GA = gestational age GD = gestational duration HC = head circumference IUGR = intra uterine growth rate LBW = low birth weight PMB = premature birth POG = period of gestation PTB = preterm birth PTD = preterm delivery SGA = small for gestational age SGL = short gestational length VLBW = very low birth weight VPTB = very preterm birth WGA = weight at gestational age
	Gender	All studies reported on exposure of females who gave birth. Most studies considered both genders but the endpoint was not disaggregated by sex except for one study where the risk for LBW in female offspring was higher than in male offspring (Ling et al., 2018).

Recommendation	Future needs	Support exposure assessment by use of biomonitoring. Adjustment of risk estimates for contributions from genetic predisposition and co-exposures to other known risk factors
	Full systematic review	The current evidence relates to many different PPPs. The atrazine studies may qualify for a systematic review and meta-analysis
	Your interest	Not applicable
References	Link to included studies	The collection of articles and screening result can be viewed here

Over the past 24 years 22 were included: 9 from the US, 3 from India, 2 from Switzerland, 2 from France, and one study from each of the following countries: Australia, Kyrgyzstan and Porto Rico. In 5 studies it remained unclear in which country the data were collected. Below the effect on birthweight are summarized (see above a list of the used abbreviations for reported outcomes).
Below the results of the different studies are presented. Associations are all based on results reported as statistically significant. If no associations were observed this is also indicated.

Occupational exposures (N=1)
Sathyanarayana et al 2010 studied exposures 27 PPPs in 2,246 female farmers in 1993-1997 in the US. Ever use of carbaryl was linked to reduced body weight at birth by 81g. This finding was adjusted from maternal BMI and smoking.

Exposures to atrazine in the general population (N=9)
Munger and co-workers (1997) used an ecological design to assess the risk of pesticide exposure in 13 communities in the US in 1986/7. An increased risk for IUGR was observed for atrazine, metolachlor and cyanzinc. This association persisted after adjustment for smoking and social-economic factors. Durand and co-workers (2015) studied maternal atrazine intake from drinking water in 3,510 children and observed a weak association with prematurity specifically an association of the use period corresponding to the third trimester of pregnancy. No associations were observed for LBW and SGA. Other adverse birth outcomes were reported by Chevrier et al., 2011 and included a negative association of atrazine in urine with IUGR and HC. Rinsky and co-workers (2012) studied PTB and how this was related to high vs. low atrazine intake from drinking water. After adjustment for maternal age, race, ethnic background and smoking an association was observed. Albouy-Llaty and co-workers (2016) compared high to low concentrations of Atrazine exposure from drinking water during the 2nd trimester of the pregnancy in 13,654 mothers and observed an association with PTB of their offspring adjusted for body weight of the mother. Almberg et al., (2017) studied Atrazine in drinking water using an ecological design involving 134,258 singleton live births and observed low atrazine exposure to be associated with PTD, VPTD and LBW but not SGA or PMB. Almberg and co-worker (2018) performed another similar study in 14,445 live singleton births and observed LBW to be associated with atrazine exposure during the 1st and 2nd trimester of pregnancy. No associations were found for SGA, VLBW, PTB and VPTB (see list of abbreviations on previous page). Atrazine in drinking water was also studied in 24,154 births in India by Ochoa-Acuna and co-workers. Consumption of drinking water with atrazine concentrations >0.1µg/L/<0.1µg/L during the 3rd trimester of the pregnancy was associated with an increase of SGA prevalence by 17-19%. No association with PTD was observed.

Exposure to organochlorine pesticides in the general population (N=6)
Kanjani and -co-workers studied organochlorine pesticides (OCP) in breastmilk of 815 women and concluded that this exposure indicator was not associated with LBW, SGA, miscarriage, stillbirths and HC. They only found a borderline association between oxychlordane and PMB. Mustafa and co-workers (2013) studied organochlorine pesticides (OCP) in cord blood in 156 PTB and 151 FTB. Blood alpha-and gamma-

HCH and p,p'DDE were associated with the risk of PTB. For POG gene-environmental interactions were found, a protective effect of CYP1A1m2 (Aa/aa) and GSTM1 null. Basterrechea et al. (2014) studied HCB in blood of 1,568 Spanish mothers. No associations were observed on any of the following indicators of intra uterine development of gestation duration: LBW, BL, GA, and SGA. Tyagi and co-workers (2015) studied OCP in blood and compared 50 cases with control mother child couples and observed α-HCH to be associated with LBW and POG. Dieldrin had a negative effect on placenta weight. Blood p,p'DDE and ß-HCH were associated with decreased POG. In a study in Kyrgyzstan, Toichueve et al. (2018) studied OCP in placenta in mother from cotton production, urban and rural regions. They reported the concentration of total OCP in placenta tissue to be associated with for LBW and PTD. Dwivedi et al. (2021) studied OCP in 221 mother-child pairs from India and observed effects of dieldrin and aldrin in cord blood on PTB, LBW and the prevalence of cesarean deliveries compared to spontaneous deliveries.

Exposures to current used pesticides in the general population (N=6)

Ling and co-workers (2018) studied residential exposure to 17 PPPs in homes <2km from agricultural land. Exposures during the 1st and 2nd trimester of the pregnancy were associated with a 3-7% increase of the risk for 24,693 pre-term births (PTB) specifically for all pesticides (including organophosphorus pesticides, pyrethroids and carbamates) an for combined use of two or more PPPs (including glyphosate, paraquat and imidacloprid). The risk was higher for female compared to male offspring. No effects on 4,412 cases of LBW were observed except with exposure to myclobutanil and possibly also the category of pyrethroids. Lesseur et al., (2012) studied the association of maternal urinary glyphosate and AMPA in 164 Child-mother couples of which 69 were PTB and 94 FTB. Associations were observed for SGL in spontaneous delivery but not with <37w PTB in a binary analysis. Silver et al (2021) also studied maternal urinary excretion of glyphosate and AMPA collected during the 26th week of pregnancy and observed higher urinary levels to be associated with increased risk of PTB. Ding and co-workers (2015) studied how maternal urinary pyrethroids in 454 mothers translated to the risk of birth weight anomalies in Swiss children. The sum of 3-PBA, cis- and trans-DCCA was associated with LBW but had no observed effect on BL, HC and GD. Chikawa and co-workers (2019) studied urinary excretion of neonicotinoid in 57 mother-child pairs admitted in the hospital for intensive neonatal care in Japan. They observed an exposure related effect on VLBW, SGA for N-desmethyl-acetamiprid (DMAP, a metabolite of acetamiprid) and observed and increase of small gestational age relative to appropriate gestational age with maternal DMAP levels. Xu and co-workers (2020) studied how urinary pyrethroids metabolites (3PBA, DBCA and 4F3PBA) were associated with LBW, GA but not for SGA and PMB.

Conclusion:

Of 22 included studies 17 were published in the past 10 years. As biometric parameters at birth are routinely collected and registered there are many opportunities to relate these outcomes in large mother-child cohorts to environmental exposure including pesticides. There are 16 outcomes measured (mostly biometric parameters) and they only provide a snap-shot without informing development later in life. However, they may reflect intra uterine development during pregnancy and associations were observed with exposure during all trimesters. Studies atrazine in drinking water in studies from the US and India suggest a small but consistent effect for a range of birth weight-related endpoints. Organochlorines, glyphosate, pyrethroids and neonicotinoids were also reported to be associated with birth weight anomalies. Exposure-response relationships were observed for some specific PPPs. It is not clear to what extent these associations are confounded by other host or environmental factors. Biometrics at birth represent a global indicator of a potential underlying adverse effect. Other endpoints observed later in the development of the new-born will lead to better understanding of the value of the observed effects. Further studies are need to understand the underlying causes of anomalies in body weight and other biometric indicators that are collected at birth and how they are related to perinatal exposure to pesticides. A potential role of paternal exposres in occupational settings needs more future studies.

Fertility

Author name		Paul Scheepers, PhD
Topic description (see Table)		Fertility
Search	Used filter 1:	Human
	Used filter 2:	n/a
	Complete search strategy	fertility, infertility, subfertility, infertility, male infertility, male fertility, alteration, abnormality, anomaly, fertilization, fecundability, pregnancy, miscarriage, implantation rate, dysfunction, azoospermia, asthenozoospermia, oligozoospermia, teratozoospermia, in vitro fertilization, intracytoplasmic sperm injection, assisted reproductive technologies, time to pregnancy
Selection	Number of studies before/after TiAb screening	407/17
	Reason for exclusion 1 (N)	Other health outcome (104)
	Reason for exclusion 2 (N)	Exposure studies (109)
	Reason for exclusion 3 (N)	Not PPP-related (24)
	Reason for exclusion 4 (N)	Biomarker study (18)
	Reason for exclusion 5 (N)	Non-primary study (3)
	Reason for exclusion 6 (N)	Case reports (16)
	Reason for exclusion 7 (N)	Non-human (6)
Description	Type of research	Case-control studies (N=13) and ecological studies (n=3) and registry-based studies (N=1)
	Population	All 17 studies were performed in the general population. Four studies recruited participants for case-control in fertility clinics.
	Exposure	Exposure by biomonitoring or clinical lab results (N=14) and in 3 studies by self-assessment using questionnaire data
	Comparator	With verified exposure status using biomonitoring using the level in the lowest tertile or quartile as a reference (N=14). Other studies used questionnaire data to define controls
	Outcome	A wide range of endpoints was used to indicate subfertility and infertility including malformation of male genitals and some clinical lab results used in infertility treatment,
	Gender	Maternal exposure and paternal exposure were both used. In offspring male fertility based on semen quality parameters was more often reported than female fertility. Two studies reported on fertility of couples.
Recommen-dation	Future needs	Because of the increasing role of treatment of fertility in healthcare settings there is an opportunity for multi-center studies reporting on

		PPP residues in semen and follicular fluids and how they relate to fertility endpoints and effects on fertility in offspring.
	Full systematic review	Relevant literature on fertility is difficult to select because of the large number of non-relevant studies retrieved. It is also difficult to cluster points for meta-analysis
	Your interest	Would be too challenging
References	CSV-format and Endnote-format	Ferility.csv
Studies	Selection of included studies	Study selection is available here

All 17 studies included studies were published in the last 19 years. Eight studies were reported from the US, two from Denmark and other studies were performed in the following countries: Australia, Colombia, Germany, Pakistan, South Africa, Switzerland, Taiwan and Tanzania.

Below the results of the different studies are presented. Associations are all based on results reported as statistically significant. If no associations were observed this is also indicated.

Organochlorine pesticides (OCP)

Polsky and co-workers compared exposures of persistent organic pollutants in blood samples from 101 cases with erectile dysfunction and 234 matched controls. Effects were reported for PCBs but no effects on erectile dysfunction were observed for two OCPs (oxychlordane and trans-nonachlor). Weiss and co-workers (2006) compared serum, follicular fluid or seminal plasma for OCP levels in 89 couples from Germany and 21 couples from Tanzania. In German couples, serum concentrations of DDT+DDE were high and associated with a lower pregnancy rate compared to couples from Tanzania. No effect on semen quality was observed. Mahalingaiah et al, (2012) studied OCP in blood of 720 patients of a clinic for in vitro fertilisation (IVF). The highest blood HCB (4th quartile) was associated with a risk of failed implantation. This was supported by a dose-response trend. No link with blood DDT/DDE level was observed. Mumford et al (2015) reported on OCP in blood in 501 males. Comparing 4th to 1st quartiles blood levels were not related semen quality where flame retardants (PBDEs) suggested an effect on reduced semen quality. Kristensen and co-workers (2016) studied prenatal exposure to OCPs in 170 users and non-users of contraceptives at the age of 20y. In 43 non-users the blood level of DDE in 4th to 1st quartile was associated with a 28% lower follicle number. Those women in the high and medium group for blood HCB had a 30% and 28% decrease in follicle number compared to the reference group, respectively. Maternal blood HCB was inversely associated with free androgen index amongst non-users of hormonal contraceptives (N=73). No associations were found in contraceptive users. Al-Hussaini and co-workers (2018) studied 8 OCPS in a population of 300 infertile couples recruited in a fertility clinic. Exposure to lindane, DDT, chlorpyriphos and diazinon estimated by analysis of follicle fluids were associate with lower retrieval of fertilization cleavage and lindane DDT, diazinon, chlorpyrifos, p,p'DDE and HCB were associated with a lower implantation rate. Chlorpyrifos, pentachlor and cyfluthrin were associated with early embryo cleavage rates. Gallo et al. 2018 studied OCP in blood of 169 non-users of hormonal contraceptives in the US and observed blood gamma-HCH to be 25.6 times more likely to result in infertility treatments. Chen and co-workers (2018) analysed OCP in breast milk of 68 Taiwanese mothers and studied fertility of male offspring. Increased OCPs were explained from dietary habits and that o,p'DDE and p,p'DDE was associated with higher infertility in offspring compared to controls with lower blood OCP values. Amir et al., (2021) performed a biomonitoring study in Pakistan that indicated that OCP in blood is a better biomarker than OCP in hair or urine to study effects on fertility in males.

They observed an association blood total OCP with a higher risk of defective sperm parameters and of blood HCB with reduced sperm motility. Swan and co-workers (2003) collected semen and collected urine for analysis of pesticides in a group of 86 males in the US who were defined cases with low values for semen concentration, percentage and sperm motility) and compared to controls who had normal values. Cases where 30 times more likely to have high alachlor, 16.7 times more likely to have high diazinon and 11.3 times more likely to have atrazine than men with low levels of these pesticides. 2,4-D and metolachlor where associated with poor semen quality in some analyses. No effect on sperm motility and morphology was observed for DEET malathion and only a weak association for a urinary metabolite of chlorpyrifos and chlorpyrifos methyl (3,5,6-trichloro-2-pyrinidol, TCPY). In 2006 Swan and co-workers reported on sperm motility in 25 infertile study persons admitted to a fertility clinic (cases) in the US and compared these with 493 patients with normal fertility (controls). Cases were more likely than controls to have high exposure to alachlor (OR=30) or atrazine (OR=11) or diazinon (OR=16) as indicated by analysis of urine. For eight other current use pesticides no associations were found. Meeker and co-workers (2004) reported a weak association was observed for TCPY in semen with sperm concentration and motility in 272 males in the US.

Current use pesticides

. Meeker and co-workers (2008) recruited 207 male patients from an infertility clinic, analysed pyrethroid metabolites in urine: and observed an inverse exposure-response relationship between urinary excretion of trans-DCCA and sperm motility. In the highest quartile the motility was reduced by 15.5%. trans-DCCA and 3-PBA were associated with reduced sperm motility and morphology. 3-PBA and cis-DCCA excretion was association with increased sperm DNA damage. In another study on pyrethroid use Whitworth and co-workers (2015) studied self-reported residential use assessed by questionnaire in 420 females adjusted for age, BMI education and parity. Indoor spraying of dichlorodiphenyltrichloroethane was associated with lower anti Müllerian hormones that is used as clinical indicator for fertility in women. Sanin et al. (2009) performed an ecological study in 5 regions in the US involving 2,592 women and used self-reported questionnaire data for exposure classification. The highest time to pregnancy (TTP) was observed in a region with a 30-y history of glyphosate use in the sugar cane production and was much higher compared to a glyphosate low-use region. Agopian and co-workers (2013) studied atrazine exposure in the US in 16.433 male cases from the Texas Birth Defects Registry. Medium compared to low atrazine exposure taken from the US Geographical Survey was associated to malformations including risk for small penis.

Unspecified pesticide exposures

Istvan and co-workers reported on 1,737 female workers were classified for exposure to pesticides by use of a job exposure matrix. Maternal pesticide exposure resulted in a higher risk for reduced semen volume and low semen count in male offspring at the age of 18-22 y. Wohlfahrt et al., (2012) reported on a study in females who were exposed to CUP at work at work. After dividing the group in an exposed (n=59) and unexposed controls (n-35) group based on the blood PPP levels the sons were assessed at 6-11y. Pesticide exposure status was linked to penile length with a trend for smaller genitals with higher exposures. The highest exposures to pesticides were associated with 24.7% smaller testes and 9.4 reduced penile length in comparison to controls.

Conclusion

All 17 included studies were published in the past 20 years and covered mostly OCP and CUP in a wide range of study designs. Most studies reported on biomonitoring of OCP in blood and breast

milk as predictor of fertility. There is substantial evidence for OCP as risk factor in reduced fertility. Specifically, the analysis of OCPs from semen fluid and follicle fluid in infertility clinics indicate a potential role for OCP in fertility. Multiple studies implicated atrazine, chlorpyrifos, diazinon and pyrethroids as potential risk factors for reduced fertility. These findings were supported by urinary metabolites levels providing quantitative data and the possibility to study exposure-response relationships. Because of the increasing role of treatment of fertility in healthcare settings there is an opportunity for multi-center studies reporting on PPP residues in semen and follicular fluids and how they relate to fertility endpoints and effects on fertility in offspring in prospective mother-child cohorts.

Asthma

Author name		Paul Scheepers PhD
Topic description (see Table)		Asthma
Search	Used filter 1:	human
	Used filter 2:	n/a
	Complete search strategy	asthma, reactive airway, reactive airways, airway inflammation, wheeze, wheezes, wheezing, dyspnea, lung function test, spirometry, lung function, lung functions, respiratory function, respiratory functions, pulmonary function, pulmonary functions, bronchus hyperreactivity, bronchial hyperreactivity, bronchial hyper-reactivity, bronchial hypersensitivity, bronchial hyper-sensitivity, bronchospasm, bronchospasm, bronchial spasm, bronchial spasms, airway resistance, airway obstruction, airway obstruction, airway, resistance, bronchoconstriction, bronchial constriction, bronchial constrictions, respiratory health, reactive airway disease
Selection	Total before/after TiAb screening	145/12
	Reason for exclusion 1	Non-primary study (N=3)
	Reason for exclusion 2	Non-human study (N=7)
	Reason for exclusion 3	Other outcomes (N=82)
	Reason for exclusion 4	No outcome reported (N=4)
	Reason for exclusion 5	Exposure/biomarker study (N=16)
	Reason for exclusion 6	Case reports (N=21)
Description	Type of research	Most studies used a case-control design. Some were nested in a prospective cohort
	Population	Six studies assessed PPP exposures of the general population and six assessed asthma in an occupational setting.
	Exposure	Exposure assessment was most often based on questionnaire data and additionally also using biomonitoring.
	Comparator	Self-reported no/never use in exposure assessment by questionnaire. In biomonitoring the lowest quartile or tertile values of an exposure biomarker are often used.
	Outcome	Based on asthma self-reported symptoms using a standardized questionnaire
	Gender	Most studies considered both genders. Two studies only reported on females (as part of a mother child cohort.
Recommendation	Future needs	Support exposure assessment by use of biomonitoring. Adjust met of risk estimates for contributions from genetic predisposition and co-exposures to other known risk factors
	Full systematic review	The current evidence relates to many different PPPs. The atrazine studies may qualify for a systematic review and meta-analysis
	Your interest	Not applicable
References	Link to included studies	The collection of articles and screening result can be viewed here

Over the past 20 years 12 were included: 6 studies were conducted in the US, one in Costa Rica and one in Japan. In Europe the only study was reported from Spain. For 2 studies the study it was unclear where to data were collected. Below the results of the different studies are presented. Associations are all based on results reported as statistically significant. If no associations were observed this is also indicated.

Occupational exposures: Liang and co-workers (2014) studied exposures to 36 PPPs as self-reported in the past 12 months by 923 workers. The risk for asthma exacerbations was decreased for use of glyphosate and paraquat. These results were adjusted for hay fever. For allergic asthma a twofold increased risk of asthma was observed for pendimethalin and tenfold increased risk following self-reported use of aldicarb in the past year. These results were adjusted for smoking, family history of asthma and education. Hoppin and co-workers (2002) studied 20,468 workers of 16-88y of the Agricultural Health Study (AHS) for exposures to 40 current use pesticides (CUP). Self-reported complaints of wheeze were associated with paraquat, parathion, malathion, chlorpyrifos and thiocarbamate (S-ethyl-dipropyl thiocarbamate (EPTC). For Chlorpyrifos, EPTC, paraquat and parathion exposure-response trends were observed. Of herbicides atrazine and alachlor but not 2,4-D were associated with wheeze. Atrazine had a significant dose-response trend for use >20 d/y. Reported work with crops or animals did not change these results. In second a study by Hoppin et al. 2006 16 herbicides and OP pesticides were studied in a small group of 36 applicators with self-reported exposures in 1993-1997 as part of the AHS. Wheeze was associated with chlorimuron, dichlorvos, and for workers who applied chlorpyrifos > 40 d/y. In a third study Hoppin et al. 2009 studied self-reported use by male workers on 48 PPPs in the AHS cohort. The following PPPs were associated with an increased risk of allergic asthma and exposure response relationships were observed for: coumaphos, heptachlor, parathion, tetrachloroethylene/carbon-disulphide and ethylene bromide. The risk of non-allergic asthma was reported to be weakly associated with applications of DDT. The authors adjusted the risk estimates for a contribution of farm activities. Hoppin and co-workers (2017) assessed the use of 78 PPPs reported by 22,134 male workers in the EHS related to wheeze. For 19 PPPs an increased risk of allergic wheeze was observed, 21 PPP were associated with non-allergic wheeze and 11 PPPs with both types of asthma. Exposure-response relationships were found for 2,4 D, glyphosate, permethrin carbaryl and warfarin. Gascon and co-workers studied prenatal exposure to p,p' DDE and HCB in blood of 2,882 mothers. Asthma was evaluated in their children at 12-14 months and suggested an increased risk for wheeze based on comparison of the 3rd to the 1st quartile of blood levels for p,p' DDE but not for HCB. Cha and co-workers studied paraquat exposure in 2,882 workers and found no associations. Mwanga and co-workers (2016) reported on OP pesticides, pyrethroid in urine of 211 female workers. The exposures were associated with asthma symptoms only for dimethyl phosphate.

Exposures in the general population: Miyake and co-workers (2014) reported no increased risk of asthma in children associated with ß-HCH, HCB and p,p'DDE in breast milk. Liu and co-workers (2012) studied exposure during the 3rd trimester of pregnancy to piperonylbutoxide (PBA) and permethrin in air. Inhalation exposure was associated with dry cough in children at the age of 5-6. Sunyer et al., (2005) studied DDE in cord blood of Spanish mothers and reported on asthma complaints in their children (N=405). Based on the ratio of the 4th/1st quartile DDE blood levels an OR was reported of 2.63. No interaction was found with atopic constitution (based on blood IgE). In Costa Rica ethylene thiourea (ETU) in urine was measured as a biomarker of exposure to dithiocarbamates during pregnancy by Hoppin et al. (2020). The risk was related to exposure as the ratio of urinary ETU (4th/1st quartile) in offspring at 10-19 months which resulted in an OR for asthma of 2.35 (not significant) and showed a protective effect for wheezing (OR = 0.50). Raherison and co-workers (2019) studied the effect of environmental pesticide exposures in school children in a wine vineyard region in France. During the application season 56 pesticides were measured in outdoor air samples. Most (90%) were fungicides (mainly folpet and dithiocarbamates) and 10 % consisted of insecticides. In a subgroup of 96 children urine ETU was found to be associated with self-reported asthma and rhinitis symptoms based on questionnaire data.

Conclusion: Over a period of 20 years six studies studied exposures of the general population related to the risk of asthma based on self-reported asthma symptoms. In two studies exposure of dithiocarbamates estimated by urinary excretion of ETU was associated with self-reported wheezing. Two studies reported an increase of the risk of asthma in young children following exposure of the mother. One study reported a risk for increased organochlorine levels in breastmilk. The other six studies described mostly self-reported exposures to multiple PPPs in male workers and observed associations with a wide range of PPPs, indicating that pesticide applications can be considered a risk for self-reported asthma symptoms. Overall, many studies reported exposure-response relationships based on biomonitoring which strengthens the suggestion that pesticides exposure may be considered a determinant of asthma. An inherent weakness in asthma research is the difficulty to adjust for other host or environmental factors that may attenuate the reported risk estimates.

ADHD

Author name		Paul Scheepers PhD
Topic description (see Table)		ADHD and related outcomes
Search	Used filter 1:	human
	Used filter 2:	n/a
	Complete search strategy	attention deficit hyperactivity disorder, ADHD, attention disorder, hyperactivity disorder, impulsivity, inattent
Selection	Total number before/after TiAb screening	15/11
	Reason for exclusion 1	Non-primary study (N=1)
	Reason for exclusion 2	Non-human study (N=1)
	Reason for exclusion 3	No outcome reported (N=1)
Description	Type of research	Most studies used a case-control design nested in a mother-child cohort
	Population	All studies were related to the general population and looked ad perinatal development of the child. One study also considered autism and had a focus on young children
	Exposure	Most studies used maternal exposure and used maternal blood, cord blood or mother milk for PPP analyses. One study looked at spatial and a second study at a trend in time
	Comparator	Biomonitoring studies used 1st tertile, compared by frequency of detects at or higher than LOD or used P90 of a low-exposed control group as a reference.
	Outcome	ADHD and also reported were specific learning disorder (SLD), hyperactive impulsive symptoms (HIS), learning disorder (LD)
	Gender	Only one study reported on outcome by gender and reported a difference
Recommendation	Future needs	The number and size of studies is small and the outcome definition is not well developed. Gene-environment interactions are not studied which is a shortcoming due to indication of genetic risk factors.
	Full systematic review	No
	Your interest	Not applicable
References	CSV-format and Endnote-format	The collection of articles and screening result can be viewed here
Four studies were conducted in the US and one in Mexico. Other studies were from Europe (two from Denmark, two from Greece, one from Norway and one from Spain). Of the remaining two studies the geographical location was not clear. In the overview below all reported changes (increased or decreased risks in ADHD or related endpoints) were reported as statistically significant. If not, we included the finding as not association with ADHD or related endpoints.		

Organochlorines (n=6)

Makris et al., 2019 studied DDT and HCI in serum of mothers giving birth to 114 children in Greece and reported an association of increased blood level of ß-HCH, total-HCH and p,p' DDE were associated with an increased risk for autism spectrum disease (ASD, n= 39 cases) but not with the risk of less frequent cases of ADHD (n = 21) or SLD (n= 32). In a second study also Greece Krykiklaki et al., (2016) reported on maternal serum levels of HCB and DDE during the 1st trimester of pregnancy. At age of 4 y, children were tested in the McCarthy Scales of Children's Ability for executive function, perceptual performance and general cognitive function. Elevated maternal HCB in serum was associated with decreased scores in this test but no effect of DDE was observed. Forns et al. 2018 analysed p,p' DDE and HCB in cord bloods from 4,437 mother. Children diagnosed for ADHD (n = 226) were assessed at 3, 6, 12 and 24 months. No associations between aforementioned pesticides were observed at any of these endpoints in early development. Lenters and co-workers analysed p,p' DDE, HCB and ß-HCH in breast milk of 1,199 mothers in Norway. Increased ß-HCH-levels in breast milk were associated with an increased risk of ADHD in children at the age of 13 y (OR=1.75) and was higher in girls than boys. The exposure during lactation to p,p' DDE and HCB indicated a protective effect on ADHD with an OR of 0.64. In a second study from Denmark, Strøm et al. (2014) reported on POP blood levels, including p,p' DDE, and HCB, in a prospective cohort of 965 mothers during week 30 of pregnancy. After a very long follow-up time. at the age of 20y, the risk for ADHD was increased two-fold but no association with behavioural and affective disorders or with scholastic achievement was observed. In Spain Ribas-Fitó and co-workers (2017) reported on maternal HCB in blood collected at birth and increased risk of teacher-diagnosed ADHD and poor social competence in offspring at the age of 4 y.

Pyrethroids (n=3)

3-Phenoxybenzoic acid (PBA) in urine is a metabolite of different pyrethroids such as permethrin, cypermethrin and deltametrin and much used to study exposure related to health outcomes. All studies used this as a urinary biomarker for exposure to pyrethroids. Wagner-Schuman and co-workers (2015) performed a study in 678 children of 8-15y diagnosed with HIS as part of the NHANES study (https://www.cdc.gov/nchs/nhanes/index.htm) in the US. The frequency of detects of 3-Phenoxybenzoic acid (PBA) in urine is a metabolite of pyrethroids such as permethrin, cypermethrin and deltametrin) and related to ADHD with an OR of 2.42 (95%CI 1.06-5.57). An exposure-dependent increase of the risk of HIS by 50% was found with each 10 µg/L increase of urinary PBA excretion. Quirós-Alcalá et al (2014) also used the NHANES data to study urinary PBA, cis, and trans-DCCA (cis/trans-(2,2-dichlorovinyl)-2,2-dimethylcyclopropane-1-carboxylic acid) and how this was related to parent-reported cases of ADHD and LD in children of 6-15 y. Increased urinary PBA was associated with LD, ADHD and the sum of LD+ADHD. cis/trans-DCCA was not associated with ADHD or LD. Dalsager and co-workers (2019) determined pyrethroids in maternal urine from 1,207 mothers. In children of 2-4 y each doubling in maternal 3-PBA concentration translated to 3% higher ADHD score and 13% higher odds of having an ADHD score above P90. For both PBA and TCPY in urine concentrations above the group median were associated with a higher ADHD score.

Organophosphor compounds

Forten-Mexburry et al. 2014 studied 187 mother-child pairs. Exposure to 3,4,6-trichoro-2-pyridinol (TCPY) and chlorpyrifos and chlorpyrifos methyl was determined by urine analysis. Among children of 5-16 y, ADHD in boys was associated with 3rd vs. 1st tertial and in girls this association was found with the 2nd vs. 1st tertile of urinary TCPY. Dalsager and co-workers (2019)

also determined the urinary excretion of TCPY in maternal urine from 1,207 mothers. TCPY levels above the group median were associated with a a higher score in a test for ADHD.

Glyphosate

Fluegge et al. (2015) studied sales data on glyphosate use related to ADHD from hospital records in the US. This approach is not suitable to study the association between glyphosate and ADHD or any other outcome.

Conclusion:

All eleven included studies used analysis of maternal blood, cord blood or maternal urine for exposure classification. No studies attempted to include genetic factors. In a six studies different expressions of the exposure to organochlorines p,p' DDE, ß-HCH and HCB were reported to be associated ADHD. The wide variety in exposure and outcome definitions make it difficult to draw a firm conclusion. Unspecified pyrethroids were consistently linked to an increased risk for ADHD in three studies. Chlorpyrifos showed was reported to be associated with an increased risk for ADHD. More and better designed population-based studies are needed to clarify the association between pesticide exposures and ADHD.

Alzheimer's disease

Author name		Paul Scheepers, PhD
Topic description (see Table)		Alzheimer's disease (AD)
Search	Used filter 1:	Human
	Used filter 2:	n/a
	Complete search strategy	Alzheimer disease, Alzheimer's disease, Alzheimer, Lewy body, dementia, cognitive decline
Selection	Number of studies before/after TiAb screening	24/8
	Reason for exclusion 1 (N)	Other health outcome reported (3)
	Reason for exclusion 2 (N)	Not PPP-related (2)
	Reason for exclusion 3 (N)	Exposure/kinetic/biomarker study (5)
	Reason for exclusion 4 (N)	Non-primary study (3)
	Reason for exclusion 5 (N)	Case reports (1)
	Reason for exclusion 6 (N)	Non-human (2)
Description	Type of research	Case-control studies (N=6)
	Population	Studies in general population (N=8);
	Exposure	Exposure by blood analysis (N=6), brain tissue (N=1), unclear (N=1)
	Comparator	With verified exposure status using biomonitoring or tissue analysis (N=7), patients with other non-Alzeimer neurodegenerative disease like Parkinson's disease (N=1)
	Outcome	AD (N=19); AD and PD (N=1)
	Gender	Most studies did not report the gender distribution in the population. Some studies matched by sex (N=2). One study reported not gender-related difference in the risk of AD
Recommen-dation	Future needs	The number of studies with sufficient power is small and results have been inconsistent. All studies described exposures to organochlorines. So far no studies addressed currently used PPPs
	Full systematic review	The number of studies is too low
	Your interest	n/a
References	CSV-format and Endnote-format	Alzheimer disease.csv
Studies	Selection of included studies	Study selection is available here

Study selection

All 8 included studies were published in 2012-2019 (no studies retrieved in 2020-2022).
Three studies were performed by the same group of researchers in North-India), two studies were published by the same research group and related to the Canadian Study of Health and Aging.
One study was from Europe (United Kingdom) An additional small study was performed in Costa Rica. Most studies reported specifically on Alzheimer's (AD).One study reported on both AD and Parkinson's disease.
In the overview below all reported changes (increased or decreased risks in or related endpoints) were reported as statistically significant. If not, we included the finding as not association with AD or related endpoints.

Specific in occupational settings

Steenland also reported on the MMSE in a group of workers pre-selected based on a Tremor-at-rest in 89 workers. These study subjects had higher dieldrin blood levels than a reference (not clear). They reported a weak association the outcome of the Mini Mental State Exam (MMSE) the provides a test score for cognitive capability (p-trend = 0.10). A weak association was also observed for o=p,p'DDT (p-trend = 0.09) but not for p,p'DDE.

Residential settings:

In a hospital-based study organochlorine pesticide (OCP) levels in substantia nigra in brain tissue from patients with AD, Parkinsons' disease (PD) or cortical Lewy body dementia (CLBD) were compared to brain tissue from controls (health or disease status not reported) (Corrigan et al 2010). Gamma-HCH was approx. tenfold higher in the group PD+AD vs. brain tissue from controls. Dieldrin was higher in PD vs. AD and also in PD vs. controls. p,p'DDE was only observed to be higher in PD vs. brain tissue from CLBD patients (not controls).

Singh and co-workers reported on three small studies based on the same population in north of India. One studied serum cholesterol, ß-HCH and dieldrin to be higher in AD patients compared to controls (resp. 1.16x, 11.38x and 10.45x). In a gene-environmental interaction analysis APOE genotype was significantly positively associated with OCP and AD (Singh et al., 2012). In a second study an statistically significant increase blood concentration of ß-HCH, dieldrin and p,p'-DDE was reported in AD patients vs. controls (Singh et al., 2013). In a third study Singh et al. performed an age-matched comparison between AD and controls and observed an increase risk for AD for ß-HCH, dieldrin (also for copper) and reported attenuation of this relationship with GSTP1*B (p=0.009) GST1*C (p=0.11). Interaction with CYP2D6 was also studied but results were not reported but, in the conclusion, it was stated that both GSTP1 and CYP2D6 polymorphisms interact with AD prevalence (Singh et al., 2014). Two Canadian studies were preformed as part of the Canadian Health and Aging study in patients aged 65+ years. Nested in this cohort-study two case-control studies were reported by Medehouenou and co-workers (2014, 2019). The first study reported that OCPb blood were not associated with the risk for AD. Hexachlorobenzene (HCB) and p,p'DDT were negatively associated with AD prevalence. In a second study published in 2019 Medehouenou and co-workers reported not finding an association between AD and PCB and OCP exposures based on blood levels. p,p'DDE blood where not associated with AD but with a the MMSE test score (a non-AD type of dementia). In a study by Bhagyashree (2019) AD was compared with controls matched by age. On the association with exposure to OCP no data were presented in the abstract (access to full text was not available).

Conclusion:

Eight studies were included that reported on the role of pesticide exposure in the onset of Alzheimer's disease. Most studies that were available considered OCP exposures status based on blood levels. Five out of eight included studies were from only two groups (in India and Canada). No studies were available for currently use pesticides. AD prevalence was reported in comparison to healthy controls and in one study with patients diagnosed with other neurodegenerative diseases including dementia and Parkinson's disease. Some studies also related pesticides to outcomes of standard cognitive tests. Results are all from small study groups (in some cases nested in a cohort study) and show an inconsistent pattern of positive and negative findings related to HCB, dieldrin and DDT/DDE even at high exposures in one study in workers and in one study reporting on brain tissue analysis. Nevertheless, it was possible to find indications for and effect of gene environment interaction as shown for APEOE, GSTP1 and CYP2D6 polymorphisms. More studies are needed to further if and to what extent OCP is a risk factor in AD.

Autism

Author name		Paul Scheepers PhD
Topic description (see Table)		Autism spectrum disorder
Search	Used filter 1:	human
	Used filter 2:	n/a
	Complete search strategy	Search string: autistic disorder, autism spectrum disorder, asperger syndrome, autism, autistic, ASD
Selection	Total number before/after TiAb screening	10/5
	Reason for exclusion 1	Non-primary study (N=3)
	Reason for exclusion 2	No outcome reported (N=2)
Description	Type of research	Most studies were conducted as a case-control study nested in a mother-child cohort
	Population	All studies were related to the general population and looked ad perinatal development of the child. One study also considered ADHD and had a focus on young children (1 study)
	Exposure	Most studies used maternal exposure and used maternal blood analysis for during pregnancy. One study looked at spatial and a second study at a trend in time
	Comparator	Biomonitoring studies used 1st tertile, compared by frequency of detects at or higher than LOD or used P90 of a low-exposed control group as a reference.
	Outcome	ASD diagnosis, intellectual disability
	Gender	Most studies did not report on outcome by gender or discussed differences in risk by gender
Recommendation	Future needs	The number and size of studies is small.
	Full systematic review	No
	Your interest	Not applicable
References	CSV-format and Endnote-format	The collection of articles and screening result can be viewed here

Four studies were performed in the US, one in Finland and one in Greece. Five studies reported on specific PPPs and two studies looked at either spatial or temporal differences. Below the results of the different studies are presented. Associations are all based on results reported by the study authors as statistically significant. If no associations were observed this is also indicated.

Scheslack-Postava (2013) reported on POPS, DDT, DDE and HCB in maternal serum collected from 75 cases and 75 controls from the Finnish Prenatal Study of Autism. Using the 90th percentile of the control values as comparator ORs were reported for PCBs (1.91) and for DDE (1.78). Temporal trends from 1970 to 2005 were reported for the US by Nevison and co-workers (2014) to be decreasing for organochlorines and increasing for glyphosate. Spatial differences were reported in two studies: Shelton et al (2012) reported on exposures during pregnancy at different (<1.25, 1.25-1.50 and 1.50-1.75 km) distances of homes from agricultural applications. ASD was associated with exposure before conception to pyrethroids, during the 2ne trimester with chlorpyrifos and during the 3rd trimester to organophosphorus (OP) pesticides. Developmental

delay was reported to be associated with exposure to carbamates but without a specific period of vulnerability. Von Ehrenstein et al. (2019) used GIS to study exposures at early age (0-1 years) of residents <2 km from farmland. From 11 pesticides reported a positive association was observed for glyphosate, chlorpyriphos, diazinon, malathion, ivermectin and permethrin related to an increased risk of ASD and for exposure to glyphosate, chlorpyriphos, diazinon, permethrin, methyl bromide and myclobutanil on the risk of developmental delay (DD). Keil and co-workers studied self-reported use of PPP in a household setting for flea treatment in pets. Imidacloprid was associated with an increased risk for ASD (R=1.3) and also specifically during a susceptibility window during pregnancy (OR=2.0). Markris reported on DDT and HCH-isomers in blood serum in 114 children with an age ranging 6-13 y. Inn 39 children with the diagnosis ASD total HCH isomers and specifically ß-HCH were two-fold higher compared to control subjects. The frequency of detects for heptachlor epoxide and p,p'DDT levels were lower in the ASD group compared to references.

Conclusion:
In a small collection of five included studies associations of autism spectrum disorder and development delay were reported for a wide range of pesticides covering both environmental exposures. Only one study addressed self-reported home-use. Exposure assessment was mostly based on level or frequency of detects in maternal or child's blood. Studies reporting on temporal trends and spatial patterns provided only weak evidence due to the many other factors not accounted for in the analysis. More studies are needed to clarify the role of pesticide exposure in autism.

Blood cancer

Author name		Paul Scheepers, PhD
Topic description (see Table)		Blood cancer
Search	Used filter 1:	Human
	Used filter 2:	n/a
	Complete search strategy	#1 (leukemia, leukaemia.*, cancer.*, neoplas.*, metasta.*, malignan.*, myeloma.*, oncolog.*, Hodgkin, NHL, polycythemia Vera) (445) #2 (blood, hemic, hematologic.*, haematologic.*) (746) #1 and #2 (121)
Selection	Number of studies before/after TiAb screening	121/11
	Reason for exclusion 1 (N)	Other outome reported (63)
	Reason for exclusion 2 (N)	Not PPP-related (12)
	Reason for exclusion 3 (N)	Exposure study (21)
	Reason for exclusion 4 (N)	Non-primary study (2)
	Reason for exclusion 5 (N)	Case report (1)
	Reason for exclusion 6 (N)	Non-human (1)
Description	Type of research	8 case-control designs, 2 cohort studies, 1 case-control study nested in a cohort
	Population	8 in general population and 3 in workers populations
	Exposure	6 studies used self-reported qualitative (ever/never exposure) information on specific PPP active ingredients. More recently published studies used quantitative exposure information, most often serum levels of persistent organic pollutants including organochlorine pesticides (p,p'DDE, beta-HCH and hexachlorobenzene were most often reported and sometimes combined with other POPs like PCBs
	Comparator	8 studies used an internal and 3 an external reference. Most studies defined cut-offs within the study group and compared 'ever' to 'never' exposed by Odds ratio (OR). In 5 studies an external reference was used. When using exposure based on quantitative information (e.g. blood levels) the outcome as Odd ratio (OR) comparing e.g. third with first tertile.
	Outcome	Primarily malignancies of blood and lymphatic system (Lymphohematopoietic cancers) and most studies reported on NHL (or subtype)
	Gender	Studies in the general populations were gender-balanced and also considered gender in the analysis. In some occupational studies only males were

		included in the analysis due the low number of females involved in e.g. pesticide applicaitons.
Recommen-dation	Future needs	Quantitative exposure information, preferably by analysis of parent PPPs or their metabolites also of PPPs not in the group of organochlorines
	Full systematic review	To be considered for NHL
	Your interest	Not applicable
References	CSV-format and Endnote-format	Blood cancer.txt and Blood cancer.csv
Studies	Selection of included studies	Study selection is available here

All eleven included studies were published in the past two decades. Most were case-control studies with small to very small numbers of cases. Two cohort studies and one case-control study (nested in a cohort) were all from the Agricultural Health Study (AHS) study based in a large population of farmers and their spouses in the US. Other studies reported on populations in the US, Canada, Singapore, China and Europe (Norway, Sweden, Germany, France and Greece). Studies reported on both genders but in occupational studies number of included females was low and often not included in the analysis.

Below the results of the different studies are presented. Associations are all based on results reported as statistically significant. If no associations were observed this is also indicated.

Most included studies reported on malignancies or pre-malignant stages related to lymphohematopoietic cancers (LHC). Non-Hodgkin lymphoma (NHL) was most studied (with 8 publications reporting on NHL or subtypes) followed by multiple myeloma (MM), chronic lymphatic lymphoma (CLL), acute myeloid lymphoma (AML) and myelodysplastic syndrome (MDS). Significant positive associations with exposure to organo chlorine pesticides (OCP) were reported in one cohort and 7 case control studies reporting on p,p'DDT/p,p' DDE, dieldrin, hexachlorocyclohexane (HCH) and/or hexachlorobenzene (HCB) in relation to NHL (Hardell et al, 2001; Nördstrom et al., 2000, Cocco et al., 2009, Spinelly et al., 2007; Delancey et al., 2009; Presutti et al., 2016; Bassig et al., 2019 and 2020) and for carbaryl, captan and DDT with MM (Greenburg et al., 2008). Professional applications of captan were positively associated with different types of blood cancers in the American AHS cohort (Greenburg et al., 2008). Professional use of dieldrin, chlorothalonil and carbon tetrachloride/carbon disulphide was associated with monoclonal gammopathy of undetermined significance (MGUS, a pre-stage of MM) (Landgren et al. 2009). Delancey and co-workers reported on positive association of occupational exposure to metribuzin with LHC, leukaemia and NHL. Bassig and co-workers (2019; 2020) suggested positive associations including significant positive trends with blood levels related to chlordane/heptachlor, heptachlor epoxide and dieldrin and AML. MSD was associated in a small study with unspecified pesticide exposures in Greece (Avgerinou et al., 2017). The results are inconsistent for DDT and NHL as associations were not confirmed in comparatively high exposed populations in Singapore and China (Bassig et al., 2020). Other substances that were not or only weakly associated to cancer included glyphosate and p,p'DDT/p,p'DDE (Avgerinou et al., 2017; Bassig et al., 2019). In conclusion, many different subtypes of blood cancers have been studied with multiple positive associations mostly in case-control studies relying on exposure classification on self-reported data or registries. More recently studies used blood-based data which improved confidence in exposure classification and resulted in reports of trends of risk with exposure intensity. Adjustments for sex and age were applied in all studies. Exposure duration and latency were not often considered. Currently these studies report on persistent PPPs (organochlorines) and in the future it would be useful to extend this to new generation PPPs that are less persistent.

The first reports have been published but results should be reproduced to increase confidence in the findings. NHL (with subtypes) is the type of blood cancer that is most associated with pesticide exposures in both occupational and general populations and could be considered for a full systematic review.

Consclusion

In the past 20 years, eleven included studies have reported on the association of pesticide exposure and different manifestations of lymphohematopoietic cancers (LHC). Non-Hodgkin lymphoma (NHL) was most studied and associated with organochlorine pesticide exposures in eight studies. Other studies found indications of a potential role as risk factor for one or more subtypes of LHC for: captan, dieldrin, metribuzin, chlordane/heptachlor, heptachlor epoxide and dieldrin. Other PPPs such as glyphosate and pp'DDT and p,p'DDE have been reported not to be associated with LHC. More recent studies used blood-based data which improved confidence in exposure classification and resulted in reports of trends of risk with exposure intensity. Most included studies have addressed OCPs. More studies are needed to explore potential associations of LHC with current use pesticides

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Lung function

Author name		Vivi Schlünssen, PhD
Topic description (see Table)		Lung function
Search	Used filter 1:	human
	Used filter 2:	n/a
	Complete search strategy	pulmonary function, respiratory function, lung function, spirometry, spirometer, FEV1, FVC, FEV1/FVC, forced expiratory volume, vital capacity, Tiffeneau-Pinelli index, PEEF, PEF, MEF, Peak Expiratory flow, Peak Expiration Flow, Peak Flow Rate, Oximetry, provocation test, Bronchial challenge, Bronchial hyperresponsiveness, DLCO, TLCO, Diffusing capacity https://www.crd.york.ac.uk/prospero/display_record.php?ID=CRD42017078131
Selection	Total number before/after TiAb screening	14/6
	Reason for exclusion 1	Out of scope (2)
	Reason for exclusion 2	Not exposure to PPPs (1)
	Reason for exclusion 3	wrong or no outcome (5)
Description	Type of research	epidemiological studies, one human provocation study
	Population	Workers, general population
	Exposure	To PPP residues in working or general environment
	Comparator	no or low exposed compared to (higher) exposed
	Outcome	(change in) Lung function (FEV1, FVC) among workers or offspring (after prenatal exposure)
	Gender	Information on differences in risk by gender was not provided in most studies. Only one study stated that the risk was equal for males and females
Recommendation	Future needs	More focus on high quality exposure assessment for specific PPPs. Increase the use of biomarkers of exposure. A need for more prospective epidemiological studies on PPP and lung function (changes)
	Full systematic review	No
	Your interest	Not applicable
References	CSV-format and Endnote-format	The collection of articles and sreening result can be viewed here
The 6 included papers consist of one review on occupational pesticide exposure and respiratory health, 2 cross sectional epidemiological studies on paraquat exposure among farmers and lung function, 2 studies on prenatal exposure (POPS/organochlorine) and lung function among offspring, and one specific bronchial provocation study (flax containing glyphosate) and lung		

function changes. Most included studies were on unspecific PPP and based on questionnaire information.
Below the results of the different studies are presented. Associations are all based on results reported as statistically significant. If no associations were observed this is also indicated.

The review (Ye at al., 2013) found suggestive evidence for a link between occupational pesticide exposure and COPD and provided strong evidence for an association with bronchitis or asthma. An abnormal (obstructive) lung function is a hallmark of COPD.
Both studies on paraquat exposure among farmers (Cha et al., 2012, Dias-Criollo et al., 2020) support an association between paraquat and obstructive or restrictive lung function patterns, where paraquat exposure was assessed based on questionnaires (Cha 2012) or analysis of spot urine samples (Dias-Criollo et al., 2020). Cha and co-workers observed an increase of the risk with exposure duration expressed as number of paraquat application years. This was supported by the In an double-blinded intervention study by Jamison and co-workers, 1986), a decrease in lung function (FEV1) was observed after inhalation of glyphosate containing dust significantly different from the reference condition consisting of inhalation of dust without glyphosate. This study supported a role of glyphosate in acute bronchial obstruction in a simulated work environment.
Two studies reported on a role of prenatal PPP exposures as a risk factor in obstructive lung function patterns in their offspring: in a study in Spain by Abellan and co-workers (2019) measured organochlorine compounds in cord blood (including DDT, DDE, HCB), and observed a decrease in lung function in children at the age of 4 and 7 years, respectively. For DDE exposure a in both FEV1 and FEV1/FVC was observed for both age groups. HBC exposure was associated with a decline in FEV1 and FVC but only in 7-year-old children. In the second study in Denmark, Hansen et al. (2015) analysed maternal blood sample in gestational week 30 for POPs, including DDE and HCB. Lung function was measured in the 20-year-old offspring. Based on a comparison of the highest to the lowest exposure level (by tertiles) resulted in an OR of 2-3 for both HCB and p,p'DDE. Bombardelli and co-workers (2021) studied 31 rural workers of 25-45 y in Brazil. Exposure to pesticides was self-reported and found to be associated with effect on respiratory muscle strength and tidal volume. No information was provided on specific pesticides or on other exposures that may also have had an influence on the outcomes studied. Raherison and co-workers (2019) studied the effect of environmental pesticide exposures in school children in a wine vineyard region in France. During the application season 56 pesticides were measured in outdoor air samples. Most (90%) were fungicides (mainly folpet and dithiocarbamates) and 10 % consisted of insecticides. In a subgroup of 96 children urine was analysed for ethylenethiourea (ETU derived from dithiocarbamates by metabolism). Related to air levels no effects on lung function were observed. ETU in urine was found to be associated with self-reported asthma and rhinitis symptoms based on questionnaire data.

Conclusion: Of the small number of studies available, some studies relate to current exposures in Europe (France, Spain and UK). Exposures were based on analysis of body fluids in three studies. Lung functions were not reported as %-predicted and respiratory complaints were mostly self-reported. More studies are needed to assess potential effects of low environmental exposures to currently used pesticide mixtures specifically in young children.

Immune response

Author name		Paul Scheepers PhD
Topic description (see Table)		Immune response
Search	Used filter 1:	human
	Used filter 2:	n/a
	Complete search strategy	immune system, immune function, immune cells, immunosenescence, immune response, immunological parameters
Selection	Total before/after TiAb screening	27/9
	Reason for exclusion 1	Non-primary study (N=5)
	Reason for exclusion 2	Non-human study (N=5)
	Reason for exclusion 3	Other outcomes (N=5)
	Reason for exclusion 5	Exposure/biomarker study (N=3)
	Reason for exclusion 6	Case reports (N=0)
Description	Type of research	Most studies used a case-control design. Some were nested in a prospective cohort
	Population	Six studies assessed PPP exposures of the general population and six assessed immune response in an occupational setting.
	Exposure	Exposure assessment was most often based on questionnaire data and additionally also using analysis of breast milk or blood
	Comparator	Self-reported no/never use in exposure assessment by questionnaire. In biomonitoring the lowest quartile or tertile values of an exposure biomarker are often used.
	Outcome	Immune response as indicated by pro-inflammatory markers, whit blood cell count or immunoglobulins
	Gender	Most studies considered both genders and one occupational study reported a difference in immune response with a higher response in female farmers.
Recommendation	Future needs	The number of studies that could be included for this endpoint is low.
	Full systematic review	Number of eligible studies is too low.
	Your interest	Not applicable
References	Link to included studies	The collection of articles and screening result can be viewed here

Over the past 27 years 10 studies were included: 4 studies were reported on outcomes from Canada, and one from each of the following countries: Australia, Czech Republic, Egypt, Germany and Japan. Below effects on indicators of immune response are reported based on statistical significance as reported by the authors. When authors reported no associations, this is also mentioned

Studies reporting on effects based on self-reported exposures

Rosenberg et al. (1999) reported on self-reported occupational and environmental exposure of 322 study participants in Canada to PPPs based on questionnaire data. Antinuclear antibodies (ANA) were associated with lifetime exposures to carbamates, pyrethroids, phenoxy acetic acid herbicides and with the sum of the following OCPs: alanine, chlordane, dieldrin, endrin, heptachlor and lindane. ANA levels were not associated with reported exposures to DDT. Kruse and co-workers (2005) also performed a study using

questionnaires and observed an association of DDE with white blood cell (WBC) counts and the following immunoglobulins: IgE, IgG and IgA. Hexachlorobenzene was inversely associated with IgM. Gamma HCH was associated with a rise in NK cells (CD56+). DDE was associated with consistently lower eosinophilic granula. Richter studied occupational exposures to organochlorines in air in Czech Republic and observed an association of hexachlorobenzene (HCB) inhalation exposure with immunoglobulin levels and protein changes. They also observed a depression of antibody response, nutrient condition and nicotinism to be associated with HCB. Rosenberg and co-workers (200&0 studied bromoxynil in serum from 98 female and 114 male cereal farmers in Canada. Exposure was also based on self-reported use. An inverse relation with the level of anti-nuclear antibodies (ANA) was observed in female but not male farmers after adjustment for age. for other endpoints no difference by gender were observed No associations were observed for 2,4 dichloro-phenoxy acetic acid, bromoxynil applied in winter time. Exposure did not have a significant effect on allergy.

Studies reporting on effects based on biomonitoring

Dewailly and co-workers (2000) performed a study on OCPs in mothers and infants at 3, 7, and 12M in an Inuit community in Canada. Otitis media was not different between breastfed infants were compared to 73 bottle fed infants. Third compared to 1st quartile blood levels p,p'DDE and HCB of the infants were associated with Otitis media with ORs of 1.87 and 1.49, respectively. Nagayama and co-workers (2007) studied OCP levels in breast milk and in 101 infants of 10 months age. HCE was associated with an increase in the following CD8+, CD3+, CD4+/CD8+ T-cell ratio. DDT in breastmilk was associated with CD16+ T lymphocytes. For dieldrin no effects were observed. Schaalen and co-workers studied 180 newborns in Egypt and related immune response to the following OCPs in breastmilk: alpha and beta-endosulfan, aldrin, endrin, dieldrin, hexachlorobenzene, DDT and 7-chloroepoxide. Effects were observed on TNFα, IL-10 and WBC count of the breastfed infants. Bilrah et al.(2003) studied 47 infants with a menu rich in fish with 65 infants from an urban environment as a reference group. Blood hexachlorobenzene and p,p'DDE were different for aforementioned groups and associated with reduced TNFα levels.

Conclusion:

Although the study set is limited to ten included studies, there are indications that maternal OCP levels in blood and breast milk are associated with different biomarkers of immunotoxicity in infant's development. Other studies have reported on pro-inflammatory indicators in auto-immune response but these effects need further confirmation in biomonitoring studies because exposure was only reported from questionnaire data by self-assessment. There is a lack on studies addressing current use pesticides.

Kidney

Author name		Benjamin Vervaet, PhD
Topic description (see Table)		Chronic Kidney disease
Search	Used filter 1:	Human
	Used filter 2:	n/a
	Complete search strategy	Glomerular filtration rate, GFR, kidney gfr, kidney glomerular filtration rate, proteinuria, albuminuria, albumin, kidney disease*, kidney disorder*, kidney pathology, nephropath*, renal disease, renal disorder, chronic kidney disease, chronic kidney failure, renal insufficiency, chronic kidney disease, chronic renal disease, chronic kidney failure, chronic renal failure, end stage renal disease, end stage kidney disease, acute kidney injury, renal injury
Selection	Number of studies before/after TiAb screening	45/18
	Reason for exclusion 1 (n)	Case reports on parasites/zoonosis (4)
	Reason for exclusion 2 (n)	Other health outcomes (6)
	Reason for exclusion 3 (n)	Human self-harm/(un)intentional misuse (11)
	Reason for exclusion 4 (n)	Review without PPP-link (2)
	Reason for exclusion 5 (n)	Technical methods paper (1)
	Reason for exclusion 6 (n)	Case report, PPP's not studied (1)
	Reason for exclusion 6 (n)	Non-human (2)
Description	Type of research	Case-control studies (n=13) Observational studies (n=4) Registry-based studies (n=1)
	Population	Farmers: 5 studies Chronic kidney disease (CKD) patients: 3 studies CKD with unknown etiology (CKDu) patients (potentially linked to PPP's): 5 studies General population: 2 studies Pesticide applicators: 3 studies
	Exposure	Blood or urine samples (n=11) Application usage data (n=1) Self-administered questionnaires (n=3) Exposure intensity index (n=1) Assumed exposures by occupation (n=2)
	Comparator	-Initial PPP exposure statuses to define control groups were verified by questionnaires for occupation (7), living in CKDu endemic/non-endemic areas (4), drink water source (2), identified pesticide use (n=3), or no prior verification (n=2). -From these only two studies applied highest tertile as comparator vs non-use (retrieved from questionnaire assessed exposure). -Actual exposures were determined by the means described under topic above "Exposure".

	Outcome	Most studies aimed to correlate PPP concentrations (or exposure risks) to presence/absence of kidney disease. The quality with which kidney disease/injury as well as PPP exposures were assessed was highly variable.
	Gender	Most studies include male and female, with overrepresentation by males. The latter is inherent to kidney disease in this context.
Recommen-dation	Future needs	Despite epidemiological associations, evidence for pesticides as cause for renal disease remains circumstantial. Need for studies on human renal (and other) tissue: 1) in situ pesticide identification/quantification of persons at low/high risk vs controls. 2) Obtaining insight in human kidney (blood/urine) molecular signalling pathways and metabolomics in persons at low/high risk, 3) gene-environment interactions (e.g. epigenetics) and 4) longitudinal follow-up of patients at risk vs controls. Link this information to precisely and prospectively monitored exposures or to retrospective exposure estimates via questionnaires. Such exposome analyses should not be restricted to pesticides, such that the relative contribution of (particular) pesticides to the risk of kidney disease can be assessed.
	Full systematic review	Would be relevant to put together the current pesticide/kidney observations in humans, but no solid etiological conclusions can be drawn due to disperse data, inconsistency in use of renal functional parameters, different samples (blood, urine, not kidney so far!), different pesticides under study, etc. Overall, pesticides as a group are currently suspected to cause kidney disease, yet the identity and mechanism of action of the culprit(s) remains obscure.
	Your interest	Given the above, the main message can only be to point out to the (renal) knowledge gaps and propose strategies to address them (e.g combining human epidemiological and environmental exposure data, with clinical and molecular data, with data of systematic in vivo/in vitro research). Such a text would look more like the introduction of a (huge) grant proposal, than a review. Nonetheless, I'm open to any discussion on how to potentially approach this.
References	CSV-format and Endnote-format	Kidney.csv
Studies	Selection of included studies	SPRINT SOLES online

Seventeen out of 18 studies were published in the last 10 years, with half of those in the last 3 years. Most studies are from Asia (Sri Lanka (6), India (2), Thailand (2), China (1)) and the US (4). Two studies from Central America (Nicaragua, Mexico) and 1 from Europe (Czech Republic).

The selected studies can roughly be divided in two categories. Those that show an association between pesticides and renal function (n=14), and those that do not (n=4).
Association (n=14): Jayasumana (2x), Lebov (2x), Richer, Siddarth, Jayatilake, Ghosh, Mueangkhiao, Zhang, Abdul, Shearer, López-Gálvez, Taira et al.
No association (n=4): Aroonvilairat, Grice, Smpokou, Pry.

Among the selected studies 4 were considered to be the stronger. They all demonstrated an association between pesticide use/exposure

1) Jayasuma et al. (2015) conducted a study in Sri Lanka on 125 CKDu patients (CKDu is a form of chronic kidney disease of unknown etiology; pesticides are a potential culprit) versus 180 controls. The highest risk for CKDu was observed among participants who drank well water (OR 2.52 95% CI 1.12-5.70) and had history of drinking water from an abandoned well (OR 5.43 95% CI 2.88-10.26) and spray glyphosate (OR 5.12 95% CI 2.33-11.26) as a pesticide. Water analysis showed significantly higher amount of hardness electrical conductivity and glyphosate levels in abandoned wells. Surface water from reservoirs in the endemic area also showed contamination with glyphosate but at a much lower level. This work also hypothesized a toxic co-action between metals and pesticides.
2) Lebov et al. (2016) evaluated the association between exposure to 39 specific pesticides (self-questionnaire) in 320 end-stage renal disease (ESRD) patients identified in a prospective cohort study of licensed pesticide applicators (55.580) in the US. Positive exposure-response trends were observed for the herbicides alachlor, atrazine, metolachlor, paraquat, and pendimethalin, and the insecticide permethrin. More than one medical visit due to pesticide use (HR=2.13; 95% CI 1.17 to 3.89) and hospitalisation due to pesticide use (HR=3.05; 95% CI 1.67 to 5.58) were significantly associated with ESRD. These observations support an association between ESRD and chronic exposure to specific pesticides.
3) Lebov et al. (2015) investigated the relationships between ESRD among wives of licensed pesticide applicators (N=31,142). They identified 98 ESRD cases and among those who did apply pesticides, the rate of ESRD was significantly elevated for those who reported the highest (vs. lowest) cumulative general pesticide use (HR: 4.22; 95% CI: 1.26, 14.20). Among wives who never applied pesticides, ESRD was associated with husbands' ever use of paraquat (HR=1.99; 95% CI: 1.14, 3.47) and butylate (HR=1.71; 95% CI: 1.00, 2.95), with a positive exposure-response pattern for husband's cumulative use of these pesticides. These observations support that ESRD may be associated with direct and/or indirect exposure to pesticides among farm women.
4) Jayasumana et al. (2015) performed a case-control study in Sri Lanka on patients with CKDu and 2 control groups, one from an area where CKDu patients live and one from an area where CKDu is not prevalent. Creatinine adjusted values of urinary glyphosate was significantly higher when compared to non-endemic controls. The highest urinary glyphosate concentration was recorded in CKDu patients (range 61.0-195.1 µg/g creatinine).

A short summary of the other studies in the selection

Richter et al. investigated a group of persons with occupational exposure to hexachlorobenzene (HCB) from 1983-1990. The changes in HCB serum levels versus kidney function before and after technical adjustments to lower HCB exposure were assessed. Blood levels of HCB decreased over time and kidney functions also changed (although not specified in the abstract if there was improvement; article itself is in Czech). Siddarth et al. designed a case-control study to evaluate the relationship of the blood organochlorine pesticides (OCPs) level with the estimated glomerular

filtration rate (eGFR) and oxidative stress (OS) in chronic kidney disease (CKD) patients. Higher levels of pesticides (hexachlorocyclohexane, endosulfan, aldrin, pp'-DDE) were found in patients with chronic kidney disease (CKD) and aldrin and pp'-DDE correlated negatively with eGFR. Oxidative stress correlated with total pesticide residues in CKD patients. Jayatilake et al. performed a case-control study in Sri Lanka to determine the prevalence of and risk factors for CKDu. They measured urinary pesticides (2,4-D, Pentachlorophenol, 3,5,6-trichloropyridinol, *p*-nitrophenol, 1-naphthol, 2-naphthol, Glyphosate, AMPA) and found them to be above reference levels (specified in full text, but origin of reference levels unclear) in 31.6% of those with CKDu. Aroonvilairat et al. compared 64 Thai orchid farmers (heavily exposed to pesticides) versus 60 controls. Pesticide use was verified by questionnaire. This yielded use of: Cypermethrin, Methomyl, Abamectin, Chlorpyrifos, EPN, Carbosulfan, Omethoate, Captan, Mancozeb, Carbendazim, Glyphosate, Paraquat. No effect on kidney function (serum creatinine and Blood Urea Nitrogen) was observed. Grice et al. observed no clear association between persistent pesticides (Hexachlorobenzene, β-hexachlorocyclohexane, Oxychlordane, Trans-nonachlor, PP_DDE, OP_DDT, PP_DDT) in blood and ESRD development in a diabetic kidney disease case-control study. Ghosh et al. performed a case-control study with CKDu patients and measured OCPs (α-HCH, β-HCH, γ-HCH, Aldrin, Dieldrin, α-endosulfan, β-endosulfan, pp'-DDT, pp'-DDE) in CKDu, healthy control and CKD patients of known etiology. The levels of 4 of these pesticides (γ-HCH, aldrin, β-endosulfan, and *p*, *p*'-DDE) significantly correlated negatively with the estimated glomerular filtration rate (eGFR) in CKDu. Two of them (γ-HCH and *p*, *p*'-DDE) significantly and negatively correlated with eGFR in CKD. Smpokou et al. analysed 12 pesticides or their metabolites (2,4-dichlorophenoxyacetic acid, 3-phenoxybenzoic acid, 4-fluoro-3-phenoxybenzoic acid, chloro-3,3,3-trifluoro-1-propen-1-yl-2,2-dimethylcyclopropanecarboxylic acid, cis/trans 3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropane carboxylic acid, ethylenethiourea, glyphosate, 4-chloro-2-methylphenoxy acetic acid, 3-hydroxy-pyrimetanil, 5-hydroxytiabendazole, hydroxy-tebuconazole and 3,5,6-trichloro-2-pyridinol) and two mycotoxins (ochratoxin A (OTA) and citrinin (CIT)) in urine samples from 350 young adults from a rural Nicaraguan community at risk of developing CKDu. No correlation with urinary pesticides and loss of renal function was observed. Mueangkhiao et al. performed an observational study in Thai farmers and found an association between urinary kidney injury markers and pesticide exposure intensity index. Interrogation by questionnaire revealed use of Glyphosate, Paraquat, Oxyfluorfen and SpinosynI). Zhang et al. identified several pesticide residues in serum of 44 patients with diabetic kidney disease (DKD) and not in non-DKD controls, and suggested to use them a biomarker for early detection of kidney disease. A total of 11 candidate metabolites were discovered to be significantly different between DKD and non-DKD groups. They are Hexadecanoic Acid (C16:0), Linolelaidic Acid (C18:2N6T), Linoleic Acid (C18:2N6C), Trans-4-Hydroxy-L-Proline, 6-Aminocaproic Acid, L-Dihydroorotic Acid, 6-Methylmercaptopurine, Piperidine, Azoxystrobin Acid, Lysopc 20:4, and Cuminaldehyde. Among these metabolites, the most significant effect was observed for Linolelaidic Acid (C18:2N6T). Abdul et al. examined the expression of urinary paraquat, glyphosate and biomarkers of renal injury (Kim1, NGAL and B2M) among Sri Lankan farmers in regions with versus without CKDu. They noted higher urinary herbicide levels among farmers in endemic areas, which is potentially linked to the observed decline in some parameters of renal function/injury. Pry et al. surveyed 56 CKDu cases and 54 controls in Sri Lanka by using a questionnaire. This study revealed no correlation with self-reported pesticide use (Carbosulfan, Carbofuran, Curateer, Glyphosate, MCPA, DPA, Metamifop) and the risk for development of CKDu. Shearer et al. evaluated the associations of pesticide use (n=41; Table2 from https://www.sciencedirect.com/science/article/pii/S0013935121005703?via%3Dihub) with measured kidney function among 1,545 male pesticide applicators in the Biomarkers of Exposure and Effect in Agriculture (BEEA) study, a subcohort in the Agricultural Health Study in the US. Pendimethalin and atrazine may be associated with decreased altered kidney function in this cohort. Use of several other pesticides was associated with higher eGFR (malathion and methyl

bromide). Malathion and methyl bromide, as well as diazinon, were associated with reduced odds of CKD. López-Gálvez et al. longitudinally assessed kidney function of seasonal farm workers both in conventional farming and organic farming versus office workers in Mexico. Although the degree of pesticide (unspecified insecticides) exposure was not measured, this study showed increased risk when working in agricultural environment. Taira et al. conducted a field-based descriptive study in Sri Lanka and quantified two renal tubular biomarkers (Cystatin-C and L-FABP), seven neonicotinoids (DMAP, Dinotefuran, Thiamethoxam, Clothianidin, Thiacloprid, Imidacloprid, Acetamiprid, Nitenpyram) and a metabolite N-desmethyl-acetamiprid in urine samples from 15 CKD patients, 15 family members, and 62 neighbours. They concluded that urinary neonicotinoids may be one of the potential risk factors for renal tubular dysfunction.

Conclusion

Throughout the studies investigating PPPs and their potential effects on renal function, the observations are consistent that the risk for kidney disease is increased among farmers (mainly in low-income countries), pesticide applicators and their wives and children living and working in agricultural communities with frequent exposures to pesticides. The quality of drinking water is a main suspected route of pesticide exposure associated with kidney disease, at least supported by Sri Lankan data. In addition to the here selected manuscripts, 2 extensive meta-analyses on over 60 studies showed associations between the use of agrochemicals with chronic kidney disease of unknown etiology (Chapman et al, Valcke et al; refs below).

From the current data it is not possible to propose a pesticide or class of pesticides specifically linked to kidney disease. The main factors that prevent to draw strong conclusions are: small sample sizes, single time point analyses (e.g. chronic kidney disease can only be established by 2 measurements at least 3 months apart), no determination of pesticide levels in biological samples, lack of age/gender or geographic location matched/unmatched controls, lack of renal biopsy information, the wide variety of agricultural compounds which may also contain toxic adjuvants or which may only become toxic after metabolization, etc.

To definitely conclude on a putative etiological involvement for particular PPPs, future research should include: longitudinal studies, toxicological pharmacokinetics, molecular analysis (RNA, DNA, protein) and unbiased chemical and metabolic "omics" analyses in biological samples (blood, urine and renal tissue!), profound inventory on a persons' historical pesticide use (questionnaires, local databases on pesticide use), intervention studies with clean drinking water, consensus on which (renal) biomarkers to include and how to measure them in a standardized way, etc. Lastly, it should be noted that dissection of the role of pesticides (incl. identification of putative culprits) in human kidney disease can never come from epidemiological studies alone, but needs to be complemented with in vivo and in vitro experiments.

Additional references outside the current scoping

-Chapman E. *et al.* Risk factors for chronic kidney disease of non-traditional causes: a systematic review. *Rev. Panam. Salud Pública* 43, 1 (2019).

-Valcke, M., Levasseur, M.-E. E., Soares da Silva, A. & Wesseling, C. Pesticide exposures and chronic kidney disease of unknown etiology: an epidemiologic review. *Environ. Heal.* 16, 49 (2017).

Mental health

Author name		Paul Scheepers PhD
Topic description (see Table)		Mental health
Search	Used filter 1:	Human
	Used filter 2:	n/a
	Complete search strategy	psychosis, psychotic syndrome, psychotic, psychotic symptoms, schizophrenia, schizophreniform disorder, schizoaffective, depression, major depressive disorder, bipolar disorder
Selection	Total before/after TiAb screening	33/9
	Reason for exclusion 1	Non-primary study (N=1)
	Reason for exclusion 2	Non-human study (N=1)
	Reason for exclusion 3	Other outcomes (N=3)
	Reason for exclusion 5	Exposure/biomarker study (N=3)
	Reason for exclusion 6	Case reports related to auto intoxications (N=16)
Description	Type of research	Most studies used a case-control design.
	Population	Eight studies assessed PPP exposures of the general population and one study reported on workers.
	Exposure	Exposure assessment was most often based on analysis of breast milk or blood. Three studies used only self-reported (ever/never) exposures based on questionnaire data and one study used distance of the home to agricultural land as a proxy for exposure to glyphosate.
	Comparator	In biomonitoring the lowest quartile or tertile values of an exposure biomarker was used. Qualitative data were based on self-reported assessments of ever/never exposures
	Outcome	Depression was most studied
	Gender	Most studies considered both genders. One study only looked at females. One study reported differences between male and female offspring following maternal exposure
Recommendation	Future needs	The number of studies that was included for this endpoint is low.
	Full systematic review	Number of eligible studies is too low.
	Your interest	Not applicable
References	Link to included studies	The collection of articles and screening result can be viewed here

Over the past 10 years 9 studies were included: 4 studies were reported on outcomes from the US. One from each of the following countries: Belgium, Colombia, Denmark, Uganda and United Kingdom.

Below effects on indicators of mental health are reported based on statistical significance as reported by the authors. When authors reported no associations, this is also mentioned

Studies reporting on effects based on self-reported exposures

Umbach and co-workers (2014) collected data by telephone interviews on application of 50 PPPs in a group of 1,702 farmers in the US who were all professional users. The risk of depression was elevated with

an OR ranging from 1.1 to 1.9 for aluphosphide, ethylenedibromide, 2,4,5-trhcicholorphenoxyacetic acid dieldrin, diazinon, malathion and parathion.
Fuhrimann and co-workers (2021) studied 253 female workers in small-scale farms in Uganda for occupational pesticide applications. Exposures to 2,4-D, glyphosate, mancozeb, OP pesticides, carbamates and pyrethroids were reported. Sleeping problems were compared to persons that did not apply pesticides. Self-reported exposures to mancozeb was associated with an increased risk for sleep problems in the past year and past week. Glyphosate exposure was only associated with sleeping problems in the past week. Imidacloprid was associated with 'some protective associations' related to mental health. Hyland et al. (2021) performed a registry-based study in the US comparing residents living < 1 km from agricultural land with families living further away. Prenatal exposure of mothers was related to youth-reported depression and maternal-reported internalizing behavior and anxiety. It was suggested that glyphosate use increased the risk twofold. Beard and co-workers (2013) studied self-reported 'ever' vs. 'never' use in 16, 893 female users of 50 PPP in the US and also included cases of poisoning. After adjustment for age, they reported that moderate exposures were not linked to depression but high exposures such as in poisoning are.

Studies reporting on effects based on biomonitoring
Strøm and co-workers 2014 studied exposures to OCPs in blood in 965 Danish mothers-child pairs as part of a cohort study. Exposures to OCP were associated with an increased risk of depression in children. Paz-y-Mino et al (2011) studied 182 patients in Colombia for the risk of social fear and suggested a relationship with glyphosate in blood. Vieiera and co-workers (2021) performed a study of maternal exposure and their 6,788 children in the US. Prenatal PPP exposure was estimated by modelling. OCP exposure was associated with an increased risk of hyperactivity and mixed PPP exposures were associated with an increased risk for symptoms of depression. Vermeir and co-workers (2021) studied HCB and p,p'DDE in cord blood of 206 mother child pairs as part of the Flemish Environmental Health Study (FLESH). At the age of 3 y their reported an association between OCP exposure and slower development of language comprehension for OCP except for p,p'DDE. p,p'DDE exposures were associated with diminished masculine play behavior in boys. Jeddy et al., (2018) studied HCB, ßHCH, p,p'DDT and p,p'DDE in maternal serum of 400 toddlers of 15-18M in the UK. The HCB levels of the highest to the lowest tertile were associated with an increased risk for vocabulary comprehension at 15M and intelligibility scores at 38M. No associations were reported for ßHCH and p,p'DDE and inconsistent results for pp'DDT. With cord blood levels of p,p'DDT there were associated with an increased risks of verbal comprehension scores at 15M but a protective effect in daughters for the risk of depression.

Conclusion:
Although the study set is limited to only nine included studies, indications that blood levels of OCP have an influence on depression in workers. One study also reported sleeping problems with the use of CUPs. Unspecified PPP use also related to poisoning was associated with a higher reported risk for mental health problems which is a finding that raises the question of potential reverse causation. Prenatal exposures to OCP were associated with mental health and differences were reported between male and female offspring. Studies that used self-reported exposure and self-reported mental health effects reported also associations but these findings should be interpretated with caution. A registry-based study on glyphosate was not considered reliable to draw any conclusion due to a poor study design.

Non-cancer blood disease

Author name		Paul Scheepers, PhD
Topic description (see Table)		Non-cancer blood disease
Search	Used filter 1:	Human
	Used filter 2:	n/a
	Complete search strategy	anemia, blood coagulation, blood Platelet*, bone marrow, erythroblastosis, hemoglobinopathy* hemorrhagic, leukocyte*, Methemoglobinemia*, pancytopenia, polycythemia, sulfhemoglobinemia, thrombophilia
Selection	Number of studies before/after TiAb screening	46/4
	Reason for exclusion 1 (N)	Other outcome reported (4)
	Reason for exclusion 2 (N)	Not PPP-related (0)
	Reason for exclusion 3 (N)	Exposure study (5)
	Reason for exclusion 4 (N)	Non-primary study (2)
	Reason for exclusion 5 (N)	Case reports (21)
	Reason for exclusion 6 (N)	Non-human (6)
	Reason for exclusion 7 (N)	Language (4)
Description	Type of research	Case-control studies (N=3) and ecological studies (2)
	Population	5 studies in general population; 1 study in occupational setting
	Exposure	Exposure by biomonitoring (N=3) and worker exposure by dust analysis (N=1)
	Comparator	With verified exposure status using biomonitoring (N=3) or environmental monitoring (N=1)
	Outcome	Most blood-based outcomes where immune-system related. Two studies specifically reported anaemia in young children.
	Gender	Occupational considered both genders. In one study a higher risk was reported for males compared to females in two subgroups related to different outcomes. Studies in general population did not report on gender differences.
Recommen-dation	Future needs	Blood-based disease is difficult to frame. Combining this category to immune system related disorders would be recommend
	Full systematic review	Not useful
	Your interest	Not applicable
References	CSV-format and Endnote-format	Blood cancer.txt and Blood cancer.csv
Studies	Selection of included studies	Study selection is available here
Four included studies were based on populations recruited in Benin, Egypt and Poland. For one study the geographical location was unclear.		

Below the results of the different studies are presented. Associations are all based on results reported as statistically significant. If no associations were observed this is also indicated.

Two studies reported on anaemia in young children. Topkonnon and co-workers (2014) reported blood anaemia as secondary outcome (side effect) in infants (age 6-30M) evaluating the efficacy of deltamethrin-treated nets efficacy in malaria prevention in Benin. A difference in blood anaemia was observed between infants from villages with more and less resistance of *Plasmodium falciparum* but a possible association with exposure to deltamethrin remained unclear. A second study reported on altered bleeding tendencies in 360 infants of (10-24M) from chlorinated pesticide intoxicated mother in Egypt (Schallan et al., 2012). Pesticide residues of hexachlorocyclohexane, DDT, hepta-chloroepoxide, alpha and beta endosulfan, aldrin, endrin and dieldrin were measured in maternal mother milk and in serum of infants. Blood biomarkers related to blood clotting/coagulation were reported and suggested organochlorine inducted immunotoxicity in infants suggesting a risk of exposure during lactation or across the placenta. Two studies reported on occupational exposures. Queiroz et al., (1997) studied respiratory burst and chemotaxis of polymorphonuclear leukocytes from 51 male and female workers with exposure to hexachlorobenzene and reported significantly reduced neutrophil functions but these effects were not related to exposure duration. In a study among workers in industrial production of pesticides in Poland, Sliwiński and co-workers (1991) reported on exposures of carbamate, triazine compounds, cupric oxychloride, captan, lindane and carboxine. Effects were reported on neutrophil counts and chemotaxis. Note that the authors also reported on lung function and lung emphysema. Effects were more pronounced in males compared to females but it is unclear to what extent this is explained by differences in exposure levels between males and females.

Conclusion
Four included studies reported that in high exposure settings in workers and in the general populations non-cancer blood effects were related to pesticide exposure. Neutrophils and their function were studied in relation to immunological disorders, blood anaemia and blood clotting disorders. Two studies suggest a potential role for pesticides in infants <5 y) which need confirmation follow-up studies. It is unclear to what extent differences between males and female are related to exposure level or may indicate a gender-related difference in susceptibility. Overall, associations between pesticide exposure and blood biomarkers have been related to high exposures such as in cases reports of intoxications (not included here).

Parkinson's Disease

Author name		Paul Scheepers, PhD
Topic description (see Table)		Parkinson's Disease (PD)
Search	Used filter 1:	Human
	Used filter 2:	n/a
	Complete search strategy	Idiopathic Parkinson's Disease, Lewy Body Parkinson Disease, Lewy Body Parkinson's Disease, Primary Parkinsonism, Idiopathic Parkinson Disease, Parkinson's Disease, Parkinson Disease, Parkinson Patients, Parkinson Patient, Parkinson's Patients, Parkinson's Patient, Paralysis Agitans
Selection	Number of studies before/after TiAb screening	49/25
	Reason for exclusion 1 (N)	Other health outcome reported (3)
	Reason for exclusion 2 (N)	Not PPP-related (4)
	Reason for exclusion 3 (N)	Exposure/kinetic/biomarker study (3)
	Reason for exclusion 4 (N)	Non-primary study (7)
	Reason for exclusion 5 (N)	Case reports (5)
	Reason for exclusion 6 (N)	Non-human (6)
Description	Type of research	Case-control studies (N=14)
	Population	Studies in general population (N=16); Studies in occupational setting (N=4) and study in both settings (N=1)
	Exposure	Exposure by biomonitoring (N=3), by location (N=2), by concentration in well water N=1) and ground water (N=1); qualitative e.g. by questionnaire (N=8)
	Comparator	With verified exposure status using biomonitoring or tissue analysis (N=4), by residential location (N=3), by other means e.g., self-reported exposure status (N=14)
	Outcome	Patients (N=19); PD patients and Alzheimer patients (N=1); Lewy pathology (N=1)
	Gender	Most studies did not report the gender distribution in the population studied. Some studies adjusted reported risk estimates for sex by matching or post-hoc correction. One study only included males and one occupational study reported the risk by gender
Recommen-dation	Future needs	Further confirmation of association with specific PPPs and rule out spurious findings not supported by causality. Three studies reported on PPP exposure and specific susceptibility and/or effect biomarkers that inform PD aetiology and/or pathology. More studies of this type would strengthen the understanding of how pesticides are involved in the onset of Parkinson's disease.
	Full systematic review	For some specific health outcomes (e.g. organochlorine pesticides and atrazine) the number

		of studies warrants an evaluation of the evidence in full systematic review.
	Your interest	Depends on availability of other recently published systematic reviews
References	CSV-format and Endnote-format	Parkinsons disease.csv
Studies	Selection of included studies	Study selection is available here

All 25 included studies were published in 2006-2020 (no studies retrieved in 2021/2022). There was a steady increase with 30% of all studies published in 2019 (N=3) and 2020 (N=4).
Thirteen of the included studies were performed US (one form Hawaii) and five in Europe (3 from France, and form Finland, Greece each one study) Other studies were from Bolivia, India, Israel and Pakistan. Most studies reported specifically on Parkinson's disease (PD). One study reported on both PD and Alzheimer's disease. Below effects on PD are reported based on statistical significance as reported by the authors of included studiies. When authors reported no associations, this is also mentioned.

Specific in occupational settings
Some specific pesticides were positively associated with PD:
Aldrin (Tufail et al., 2020)
2,4,5,-T (Schrestha et al., 2020)
2,4-dichlorophenoxy acetic acid (Tanner et al., 2009)
Benomyl (Schrestha et al., 2020;)
Dichlorvos (Shrestha et al., 2020)
Permethrin specifically related to use in farm animals (Shrestha et al., 2020)
Terbufos, trifurabalin (Schrestha et al., 2020)

Residential settings:
a. Some specific pesticides were positively associated with PD:
Alachlor in groundwater (James et al., 2015)
Atrazine in ground water (James et al., 2015)
Benomyl (Rhodes et al., 2013)
2,4-DD (Hugh-Jones et al., 2020)
Chlorpyrifos (Hugh-Jones et al., 2020; Gatto et al., 2009)
Cyazine (Rhodes et., 2013)
Dieldrin (Rhodes et., 2013)
p,p'DDE (Rhodes et al., 2013; Dardiotis et al., 2020)
Endosulfan (Rhodes et., 2013)
Hexachlorobenzene (HCB) in plasma (Dardiotis et al., 2020)
ß-HCH in human serum (Richardson et al., 2011, 2009)
Metam (Rhodes et., 2013)
Methomyl in private well water (Gatto et al., 2009)
Paraquat (Brouwer et al., 2017)
Propargite in private well water (Gatto et al., 2009; Rhodes et al., 2013)
Triflumizal, Ziram (Rhodes et., 2013)
Simazinei ground water (James et al., 2015)

b. Some specific pesticides were not associated with PD
2,4,5-TP (Schrestha et al., 2020)
Atrazine (Caballero et al., 2019; Narayan et al., 2017)

Benomyl (Brouwer et al., 2017)
Diazinon (Caballero et al., 2019; Gatto et al, 2009; Shrestha et al., 2020)
Dieldrin in human serum collected in 1968-1972 (Weisskopf, 2010)
Dimethoate (Gatto et al., 2009)\
Lindane (Brouwer et al., 2017)
Maneb (Brouwer et al., 2017)
Paraquat (Brouwer, et al., 2017; Hugh-Jones et al., 2020; Gatto et al., 2009)

c. Association unclear/not reported
Paraquat and picloram (Calvo-Trujillo et al., 2019)

Unspecified pesticide exposures

In a large study by Tufail and co-workers in Pakistan reported on self-reported exposure status as 'working at a farm (y/n)' and 'working with pesticides'. Both were used as a proxy for exposures and were associated with an increased risk for PD. This study reported a negative association with active smoking status also reported by Tanner et al., 2009; Weisskopf et al., 2014). In a very small study ^{18}F-DOPA Positron emission tomography (PET) was applied to compare 13 PD patients with pesticide exposures from Israel to 13 PD patients with no known exposure from Austria. No differences were observed and no pesticide specific pathology was observed (Djaldetti et al, 2019). An increased risk of PD was observed to be associated with self-reported use of pesticides (Tanner et al., 2019), unspecified insecticides (Elbaz et al., 2009), unspecified amines (Calvo-Trujillo et al., 2019), ever/never use of OP-pesticides or carbamate pesticides (Narayan et al., 2017) and ever/never use and cumulative lifetime use of unspecified organochlorin pesticides (Duthell, 2010). Narayan et al., (2017) observed chemical-resistant glove use in pesticides applications as a risk factor in PD and interpreted this as an indication that gloves may not provide the protection expected. Hugh-Jones et al. 2020 observed an increase of PD since the use of glyphosate-resistant crops, observed higher prevalence related to commercial wood production, pastures, rice and sugar cane farming and aeras that used aquifer recharge as a source for drinking water. Brouwer et al. (2017) used a spatio-temporal model to estimate residential exposure to 157 pesticides, based on agricultural crops around the residential address in a study in the Netherlands. Homes <100m from the application were considered relevant.
Results: A total of 352 PD cases and 607 hospital-based controls were included. No significant associations with PD were found for paraquat, maneb, lindane and benomyl. In a hypothesis generating analysis increased risk of PD was found for 21 pesticides, mainly used on cereals and potatoes. Results were suggestive for an association between bulb cultivation and PD. Increased risk of PD was observed for exposure to a cluster of pesticides (in rotating crops). Shrestha et al., (2019) reported an association of PD risk with intensity weight life time exposure expressed in days of pesticide use. Caballero et al., 2018 observed an increased PD risk in rural inhabitants living <1000m from cropland. Moisan and co-workers observed farm density, insecticide to be positively associated with PD prevalence in a study France. This association persisted to be significant after adjustment for age, gender. In the same study working in permanent crops such as fruits were also associated with increased prevalence of PD. Tanner et al. (2009) reported an increased risk for pesticides use (y/n) and for a collection of 8 pesticides in a multicentre study in the US. In this study an association was observed specifically for 2,4-dichlorophenoxyacetic acid and the risk of PD. Elbaz reported on male workers in France and observed insecticide and organochlorin applications to be a risk factor in PD. In a large population with 83 PD cases in 79,557 workers with pesticide exposures in 1993-1997 in the US cumulative days of use was associated with an increased PD prevalence and also with some specific pesticides used 'more than half the time' was also associated with higher PD prevalence when comparing the highest

quartile with the lowest quartile of use frequency. However, no association was observed with overall pesticide use.

Biomarker studies informing involvement of pesticides in Lewy pathology and PD aetiology

Ross and co-workers (2019) reported on Lewy pathology studied in brain tissue from 705 patients from Hawaii analysed for heptachlor epoxide (HCE), hexachlorobenzene (HCB) and α-chlordane. and observed an increase of prevalence from 16.2% to 30.1% with unspecified pesticide exposure status and an interaction was observed with specific proteins that are thought to be relevant to Lewy pathology and PD: hematoxylin/eosin staining and α-synuclein, a neuronal protein that regulates neurotransmitter release by selective synaptic trafficking. An association of these biomarkers with HCE was observed and persisted after adjustment for HCB and α-chlordane but the association was not observed for HCB and α-chlordane when tested separately.

In a group of 282 workers with exposure to OP and carbamate pesticides in 1974-1999 two variants of the *ABCB1* gene was determined and were jointly associated with an increased risk of PD. This gene is involved in encoding p-glycoprotein and a xenobiotics transporter. Duthell (2010) also reported on to *ABCB1* polymorphisms (exon 21 and G2677[A,T]; exon 26 C3435T both associated with altered glycoprotein function) and observed only G2667[A,T] to increase the risk PD of cumulative lifetime exposure to unspecified organochlorin pesticides in farmers in France. In India, Singh and co-workers (2014) studied C2D6*4 and GSTP1 genotype to contribute to an increased risk of PD in exposures to ß-HCH, dieldrin and DDE. Rhodes et al., (2013) studied the contribution of SKP1 as an ubiquitin-proteosome system (UPS) inhibitor and found an interaction with exposure to benomyl, cyanazine, dieldrin, endosulfan, metam, propargite, triflumidate and ziram.

Conclusion:

Based on 21 included studies, Parkinson's disease (PD) has been studied in relation to a wide range of pesticides over a period of 15 years. In occupational studies have looked at groups of pesticides as well as individual pesticides and many different expressions of exposure level and in occupational settings also by exposure duration. In three occupation studies blood was analyzed to confirm exposures. Most of the findings suggested an association between self-reported exposures and the risk of PD. Overall pesticide applications resulting in contaminations of environments compartments such as water bodies are implicated to have an influence on PD risk but the evidence varies from one to the other compound and is inconsistent for some much-used compounds. In residential settings the evidence was retrieved from a variety of environmental samples related to use at home, at a distance from applications on nearby farmland and also related to private water wells as well as ground water. Very low number of studies use biomarkers to study the relationship between exposure and disease in more detail looking at *ABCB1* polymorphism, CYP2D6, GSTP1 which may provide useful information on the mode of action but these studies need confirmation in follow-up. Only one study tried to link pesticides, glycoproteins in samples of brain tissue with Lewy pathology.

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ANNEX B: Farm Animals

Farm animals

Cat

Cattle

Chicken

Goats

Pig

Sheep

Experimental animals

Gut microbiome

Cat

Author name		Martin T. Sørensen, PhD
Topic description (see Table)		Cat - reproduction
Search	Used filter 1:	Animals, Domestic animals
	Used filter 2:	Domestic cat
	Complete search strategy	(cat, cats, felis, feline, kitten, kittens) AND [estrus, oestrus, estrous, anestrus, anoestrus, anestrous, semen quality]
	Total number before TiAb screening	1/0
	Reason for exclusion 1 (N)	Study was on rats (1)
	Type of research	See above
	Population	Not applicable
Description	Population	Not applicable
	Exposure	Not applicable
	Comparator	Not applicable
	Outcome	Study not relevant to cat reproduction
Recommendation	Future needs	??
	Full systematic review	No
	Your interest	Not applicable
References		Not applicable
No relevant results to report		

Cow

Author name		Martin T. Sørensen, PhD
Topic description (see Table)		Cattle – Reproduction and production
Search	Used filter 1:	Animals, Domestic animals
	Used filter 2:	European cattle or taurine cattle, general domestic cattle
	Complete search strategy	(cattle, bovine, cow, cows, calf, calves, heifer, heifers, bull, bulls) AND [below replacement fertility, differential fertility, fecundability, fecundity, fertility determinants, fertility incentives, fertility preferences, fertility, below replacement, marital fertility, natural fertility, subfecundity, world fertility survey, reproductive sterility, sterility, reproductive, sub-fertility, subfertility, calving interval, calving intervals, calving to first AI, calving to conception, non-return, calving rates at first AI, inseminations per pregnancy, heat detection, semen quality, daily gain, average daily gain, adg, weight loss, milk yield, my, milk production]
	Total number before/after TiAb screening	29/1
	Reason for exclusion (N)	studies were on treatment for various parasites with end-points primarily related to parasite control (11)
	Reason for exclusion (N)	in vitro studies (6)
	Reason for exclusion (N)	studies were on feeding with glyphosate-resistant plants products; however no measurements on feed glyphosate content (4)
	Reason for exclusion (N)	studies on whales (3)
	Reason for exclusion (N)	study on soil fertility (1)
	Reason for exclusion (N)	study on human (1)
	Reason for exclusion (N)	review (1)
	Reason for exclusion (N)	Effect of fungicide treatment of feed crop on silage quality and feeding efficiency (1)
	Type of research	See above
Description of selected research	Population	Bulls of beef breeds (N=28)
	Exposure	i) Cyfluthrin ear tag and topical applications, or ii) cyfluthrin ear tag, topical, premise spray and pyrethrin fog spray applications
	Comparator	No exposure
	Outcome	The use of pyrethrin- and cyfluthrin-based insecticides, regardless of application, did not negatively affect reproductive parameters in beef bulls when administered over 18 weeks.

Recommendation	Future needs	n/a
	Full systematic review	No
	Your interest	Not applicable
References	Doi	https://doi.org/10.1111/rda.12729

Chicken

Author name		Martin T. Sørensen, PhD
Topic description (see Table)		Poultry – reproduction and production
Search	Used filter 1:	Animals, Domestic animals
	Used filter 2:	Domestic chicken
	Complete search strategy	(poultry, chicken, chickens, avian, hen, hens, laying hen.*, broiler, broilers) AND [hatch.*, semen quality, daily gain, average daily gain, adg, weight loss, egg production, egg yield]
	Total number before/after TiAb screening	26/1
	Reason for exclusion 1 (N)	Studies with experimentally treating poultry with PPPs (insecticides or glyphosate) (4)
	Reason for exclusion 2 (N)	Studies on pests and pest control (5)
	Reason for exclusion 3 (N)	Study on falcon or heron population size (2)
	Reason for exclusion 4 (N)	Study of presence of PPP in eggs (1)
	Reason for exclusion 5 (N)	Studies of injection of PPPs into eggs (6)
	Reason for exclusion 6 (N)	Study on simulating PPPs in eggs (2)
	Reason for exclusion 7 (N)	Study of insecticide resistance (1)
	Reason for exclusion 8 (N)	Study on feeding glyphosate-resistant corn to laying hens; however no measurements on feed glyphosate content (1)
	Reason for exclusion 9 (N)	Review (1)
	Reason for exclusion 10 (N)	Methods study (1)
	Reason for exclusion 11 (N)	Study on effects of blood levels of PPPs on egg incubation temperature in Artic kittiwake (1)
	Type of research	See above
Description of selected research	Population	Broiler layers (N = 5 flocks)
	Exposure	Glyphosate residues in broiler breeder diets
	Comparator	Not applicable
	Outcome	The average glyphosate residue level was 0.09 mg/kg, maximum was 0.19 and minimum was 0.004 mg/kg. Egg laying percent was not affected by residue level. Average hatchability was 79% (SD = 5.8%). A negative association between feed glyphosate residue level and egg hatchability (P = 0.03) was found.
Recommendation	Future needs	An experiment with glyphosate residues in feed over a range covering the conditions in commercial settings and including feed free of residues is warranted to further investigate potential effects of feed glyphosate residues on egg hatchability.
	Full systematic review	No
	Your interest	Not applicable
References	doi	https://doi.org/10.1038/s41598-021-98962-1

Goat

Author name		Martin T. Sørensen, PhD
Topic description (see Table)		Goat – Reproduction and production
Search	Used filter 1:	Animals, Domestic animals
	Used filter 2:	Domestic goat
	Complete search strategy	(goat, goats, buck, kid, kids) AND [below replacement fertility, differential fertility, fecundability, fecundity, fertility determinants, fertility incentives, fertility preferences, fertility, below replacement, marital fertility, natural fertility, subfecundity, world fertility survey, reproductive sterility, sterility, reproductive, sub-fertility, subfertility, estrus, oestrus, estrous, anestrus, anoestrus, anestrous, semen quality, daily gain, average daily gain, adg, weight loss, milk yield, my, milk production]
	Total number before/after TiAb screening	6/1
	Reason for exclusion 1 (N)	study on parasite control (2)
	Reason for exclusion 2 (N)	in vitro studies on antral follicles or testicular germ cells (2)
	Reason for exclusion 3 (N)	PPP residues in milk (1)
	Type of research	See above
Description of selected research	Population	Damascus-Alpine crossbred male goats
	Exposure	Standard diet (N=5) or standard diet supplemented with 15 mg Atrazine per kg BW (N=3) for 6 months.
	Comparator	No exposure control
	Outcome	Atrazine impaired sperm morphology and sperm membrane integrity. Sperm fertilization competence was not measured.
Recommendation	Future needs	??
	Full systematic review	No
	Your interest	Not applicable
References	Doi	https://doi.org/10.1016/j.chemosphere.2019.124858

Pig

Author name		Martin T. Sørensen, PhD
Topic description (see Table)		Pig – Reproduction and production
Search	Used filter 1:	Animals, Domestic animals
	Used filter 2:	Domestic pig
	Complete search strategy	(pig, pigs, porcine, boar, sow, sows, swine, piglet, piglets) AND [below replacement fertility, differential fertility, fecundability, fecundity, fertility determinants, fertility incentives, fertility preferences, fertility, below replacement, marital fertility, natural fertility, subfecundity, world fertility survey, reproductive sterility, sterility, reproductive, sub-fertility, subfertility, estrus, oestrus, estrous, anestrus, anoestrus, anestrous, non-return, wean to estrus, wean to oestrus, wean to estrous, wei, AI, inseminations per pregnancy, heat detection, semen quality, daily gain, average daily gain, adg, weight loss]
	Total number before/after TiAb screening	21/0
	Reason for exclusion 1 (N)	in vitro studies (8)
	Reason for exclusion 2 (N)	Study on feeding glyphosate-resistant plants products to pigs; however no measurements on feed glyphosate content (1)
	Reason for exclusion 3 (N)	study were on feeding pigs with glyphosate-or dieldrin-spiked feed; measurement on blood biomarkers (2)
	Reason for exclusion 4 (N)	studies on guinea pigs (3)
	Reason for exclusion 5 (N)	study with neonicotinoid in pigs experimentally infected with PRRSV (1)
	Reason for exclusion 6 (N)	studies were on experimental treatment of gilts with atrazine (4)
	Reason for exclusion 7 (N)	Study of pyrethroid-resistant (RR) mosquito (1)
	Reason for exclusion 8 (N)	studies were on percent infected goats and pigs after insecticide treatment (1)
	Type of research	See above
Description of selected research	Population	Not applicable
	Exposure	Not applicable
	Comparator	Not applicable
	Outcome	Studies not relevant to pig reprod. and prod.
Recommendation	Future needs	??
	Full systematic review	No
	Your interest	Not applicable
References		Not applicable
No relevant results to report		

Sheep

Author name		Martin T. Sørensen, PhD
Topic description (see Table)		Sheep – Reproduction and production
Search	Used filter 1:	Animals, Domestic animals
	Used filter 2:	Domestic sheep
	Complete search strategy	(sheep, ewe, ewes, lamb, lambs, ram, rams, mutton) AND [below replacement fertility, differential fertility, fecundability, fecundity, fertility determinants, fertility incentives, fertility preferences, fertility, below replacement, marital fertility, natural fertility, subfecundity, world fertility survey, reproductive sterility, sterility, reproductive, sub-fertility, subfertility, estrus, oestrus, estrous, anestrus, anoestrus, anestrous, semen quality, daily gain, average daily gain, adg, weight loss, milk yield, my, milk production]
	Total number before/after Abstract screening	7/1
	Reason for exclusion (N)	study on parasite control (1)
	Reason for exclusion (N)	in vitro studies on semen (2)
	Reason for exclusion (N)	study on rat (1)
	Reason for exclusion (N)	Study of neonatal exposure of lamb fetuses to glyphosate based herbicide. Subsequent measures of e.g. uterine cell proliferation and gene expression, however no measures of reproduction or production per se (1)
	Reason for exclusion (N)	Study with s.c. injections of glyphosate based herbicide to prepubertal ewe lambs and subsequent measures of e.g. uterine cell proliferation and ovary gene expression, however no measures of reproduction or production per se (1)
	Type of research	See above
Description of selected research	Population	Rambouillet rams (N=28) were allocated to 3 treatment groups and dosed for 60 days (at least one spermatogenic cycle). Rambouillet ewes (N=40) were dosed for 20 days (at least one estrus cycle) and then allocated to ram treatment groups.
	Exposure	i) Fenoxycarb at 10x the potential exposure levels in pasture or hay and ii) Fenoxycarb at 20x the potential exposure levels in pasture or hay. Fenoxycarb were given via capsules.
	Comparator	No exposure control

	Outcome	No treatment-related pattern of reproductive, physiologic, or behavioral abnormality was observed in Rambouillet sheep treated with fenoxycarb.
Recommendation	Future needs	??
	Full systematic review	No
	Your interest	Not applicable
References	Doi	https://doi.org/10.1111/rda.12729

Gut microbiome

Author name		Dr. Daniele Mandrioli, PhD; Daria Sgargi, PhD
Topic description (see Table)		Microbiome effects of pesticides on Experimental animals
Search	Used filter 1:	Animals
	Used filter 2:	Experimental animals
	Complete search strategy	rodent.*, rat, rats, Sprague-Dawley, Sprague Dawley, mouse, mice, hamster, hamsters, guinea pig, guinea pigs (linked by OR) AND faecal matter transplant, fecal matter transplant, faecal transplant, fecal transplant, gut microbiome donor*, microbiome (linked by OR)
Selection	Number of studies before/after TiAb screening	21/19
	Reason for exclusion 1	Wrong study design (N=2)
Description	Type of research	Experimental in vivo studies covering possible effects of pesticides on microbiome. Chronich (N= 18) and Subchronic (N= 2) toxicity studies
	Population	Rodents usually involved in experimental research, in particular mice (N= 11) and rats (N=8) exposed during the prenatal period (N=6) or in adult life (N= 13). Studies included only male animals (N= 12) or both sexes (N= 7).
	Exposure	PPP and their metabolites administered in feed, drinking water or using gavage. 14 pesticides were tested in total, alone (N= 14), chemical and formulation (N=4), or as cocktail (N=1)
	Comparator	Control groups of untreated comparable animals
	Outcome	Any effects on microbiome or microbiota of urine and faeces
Recommendation	Future needs	Studies on microbiome effects of PPPs other than glyphosate. Studies on PPPs behavioural effects also in relation with microbiome alterations
	Full systematic review	No
	Your interest	Systematic review perhaps feasible only for glyphosate and microbiome alterations
References	CSV-format and Endnote-format	Citations_EXPERIMENTAL- MICROBIOME.csv
Studies	Selection of included studies	Study selection is available here
Text proposal (100-300 words): 19 studies were considered eligible after Title and Abstract screening. The included studies reported effects on the gut microbiome of experimental animals treated with 14 types of PPPs (including substances or their commercial formulations); only one study considered different substances combined in a cocktail formulation.		

The relevant literature is very recent, all 19 studies were published in the last 6 years. In particular, 1 study was published in 2015 and 1 in 2016; 6 were released in 2018, 2 in 2019, 5 in 2020 and 4 in 2021. Glyphosate and its commercial formulations were the main PPPs of interest in experimental animal studies, with 9/19 studies addressing the effects of these pesticides on microbiome (Hu et al 2021, Mesnage et al 2021, Pu et al 2020, Tang et al 2020, Dechartres et al 2019, Aitbali et al 2018, Lozano 2018, Mao et al 2018, Nielsen et al 2018). Only 3 studies addressed the connection between microbiome alteration and behavioural effects induced by pesticides and in all 3 studies the pesticide of interest was glyphosate (Pu et al 2020, Dechartres et al 2019, Aitbali et al 2018). Overall the studies showed a clear effects of glyphosate and its formulations in altering microbiome in experimental studies, however studies on the microbiome effects of most pesticides and how they might affect behaviour are lacking.

Disclaimer: This report is part of a project that has received funding by the European Union's Horizon 2020 research and innovation program under grant agreement number 862568.

ANNEX C: Ecosystem

Aquatic ecosystem

Cyanobacteria

Chironomus

Daphnia

Diatom

Microalgae

Lumbriculus

Lymnaea

Macrophyte

Physa and *Lymnae*

Zebrafish

Terrestrial ecosystem

Eisenia and *Andrei*

Fimetaria

Foetida

Folsomia

Hypohaspis

Microorganism enzyme activity

Microorganism functional genes

Microcosm studies

Plants

Proisotoma minuta

Typhodromu pyry

Cyanobacteria growth

Author name		Joana Luísa Pereira
Topic description (see Table)		*Cyanobacteria* growth
Search	Used filter 1:	Ecosystem
	Used filter 2:	n/a
	Complete search strategy	#1 [cyano*] AND [grow* OR biomass OR yield] #2 [Anabaena] AND [grow* OR biomass OR yield]
Selection	Total number before/after TiAb screening	#1 – 2/0 #2 – 5/5
	Reason for exclusion 1	#1 - No effects of pesticides reported (n = 2)
Description	Type of research	Experimental studies (single species; multi-species enclosures)
	Population	Laboratory cultures + one study using natural communities in enclosures
	Exposure	To pesticides (active ingredient or commercial formulation)
	Comparator	Non contaminated medium used as control
	Outcome	Decreased growth rate, photosynthesis impairment, decreased chlorophyll production, decreased carbohydrate content, population decline
Recommendation	Future needs	Gap analysis: lack of studies entailing combination of PPP residues; lack of studies using natural samples of contaminated waters.
	Full systematic review	The association of the term "blue-green" to "cyano*" should be made as cyanobacteria have been named for long as blue-green algae. This would possibly improve the search outcomes. For a more complete systematic review. Still, a search for other cyanobacteria genus should be likely necessary to better cover the literature available as there are studies with cyanobacteria that are not retrieved by either "cyano*" or "Anabaena" but rather by searching for "microalga*". For example, when searching rather for diatoms, there is at least one study where the word "cyanobacterium" is in the abstract (DOI 10.1016/0269-7491(87)90195-3) and this study was not captured in the present search.
	Your interest	n/a
References	CSV-format and Endnote-format	Env_microalgae.xlsx #1 – Tab "SEARCH cyano" #2 – Tab "SEARCH Anabaena"

The two references found when generally searching for cyanobacteria (#1) date from 1998 and 2004, and none reported on toxic effects of pesticides in cyanobacteria. The replacement of the term "cyano*" by "Anabaena" (a common cyanobacteria gender used in toxicity tests and recommended for the purpose by the OECD guideline no. 201 for the Freshwater Alga and Cyanobacteria, Growth Inhibition Test) in the search thread surprisingly returned more hits (5) dating within 1991-2012, none corresponding to those retrieved with search #1.
In all of these five studies, growth (based on e.g. cell density, chlorophyll) or photosynthesis (e.g. based on fluorometric measurements or antioxidant enzymes) impairment were mostly focused, and one addressed combined effects, not with other pesticides but with UV-B (Chen et al. 2012). One study reports on a microcosm experiment (zooplankton, macroinvertebrates, phytoplankton, macrophytes, microbial communities) with the fungicide metiram – negative effects were noticed in cyanobacteria communities but ecological recovery was achieved within 8 weeks after treatment (Lin et al. 2012). Recovery was also found following single-species exposure to atrazine within hours following exposure (Brain et al., 2012). The remaining two studies report on negative effects of herbicides (atrazine, hexazinone, isoproturon) and other pesticides (lindane, pentachlorophenol, methyl parathion) in Anabaena, with the notable evidence that herbicides are not always more toxic to Anabaena sp. than other pesticides (Mostafa and Helling, 2002).

Conclusion: For cyanobacteria five studies were included. Herbicides, fungicides and insecticides can induce negative effects in cyanobacteria regarding growth and photosynthetic efficiency. Recovery of communities and cultures was often observed, which is relevant in the field context. Future research is needed addressing the interaction among stressors, especially considering natural stressors (e.g. temperature, light irradiation, salinity) and pesticide mixtures to better meet field conditions and gather more realistic predictive capacity.

Chironomus survival

Author name		Ana Gonzalez, PhD
Topic description (see Table)		Chironomus survival
Search	Used filter 1:	Ecosystem
	Used filter 2:	n/a
	Complete search strategy	Chironomus AND (mortal* OR lethal* OR acute* OR survival*)
Selection	Total number before/after TiAb screening	77/63
	Reason for exclusion 1	not a PPP study (1)
	Reason for exclusion 2	Not species of interest (2)
	Reason for exclusion 3	Other outcome (7)
	Reason for exclusion 4	Methodology study (4)
Description	Type of research	laboratory studies (61), field studies (2)
	Population	Laboratory cultures
	Exposure	To individual or combined PPP (active ingredient or commercial formulation)
	Comparator	Non contaminated medium/sediment used as control
	Outcome	Health-related outcome (effects on survival)
Recommendation	Future needs	Gap analysis: take account that in some cases NOEC, LOEC and acute toxicity show different results despite being included one in other
	Full systematic review	yes
	Your interest	Not applicable
References	CSV-format and Endnote-format	File names used
Studies	Selection of included studies	Chironomus.csv

The 63 included studies were published in 1995 - 2021. The genus Chironomus consists of various species. In the studies evaluating mortality and survival, the majority used *Chironomus riparius* (24), 17 used *C. tentans* and 9 used *C. dilutus* as study organisms. Other species from this genus were used only in some specific studies. In general, most studies use PPPs in individual exposure, highlighting the use of chlorpyrifos (CPF) (8), imidacloprid (8) and atrazine (8). In general, imidacloprid showed greater toxicity, mainly in a mixture. However, atrazine decreased the toxicity of OPs such as CPF in a mixture (Belden and Lydy 2000). Twelve studies evaluated the impact of mixtures mainly atrazine and OPs, imidacloprid and OPs or herbicides. Only one study evaluated the effects of polycyclic musk in *C. riparius* (Artiola-Garicano et al., 2003). Most studies evaluated the impact of PPPs by artificially polluting water. However, 6 studies used naturally or artificially polluted sediments (Ding et al., 2011; Werner et al., 2004; Xu et al., 2007). In line with climate change, the effect of temperature on PPPs toxicity was assayed in two studies (Tasson and Schulz 2012: Willming et al., 2013). The highest toxicity was observed in *C. tepperi* exposed to terbufos with a 96h LC50 = 2.13 µg/L (Chong et al., 2010). Moreover, one work studied the effect of esfenvalerate on *C. tentans* through a mesocosm experiment (Boulding et al., 2004).

Chironomus life cycle

Author name		Ana Gonzalez, PhD
Topic description (see Table)		Chironomus aquatic toxicity (life cycle)
Search	Used filter 1:	Ecosystem
	Used filter 2:	n/a
	Complete search strategy	Chironomus AND (growth* OR life cycle* OR development* OR feeding*,OR emergence*)
Selection	Total number before/after TiAb screening	65/44
	Reason for exclusion 1	not related to PPP (1)
	Reason for exclusion 2	not endpoint related (17)
	Reason for exclusion 3	Methodology study (3)
Description	Type of research	laboratory studies (42), field studies (2)
	Population	Laboratory cultures
	Exposure	To individual or combined PPP (active ingredient or commercial formulation)
	Comparator	Non contaminated medium/sediment used as control
	Outcome	Health-related outcome (effects on life cycle and feeding)
Recommendation	Future needs	n/a
	Full systematic review	yes
	Your interest	Not applicable
References	CSV-format and Endnote-format	[provide file name]
Studies	Selection of included studies	(Excel sheet Chironomus references life cycle)

The 44 included studies were published between 1997 and 2021. Three studies related the theoretical and lab/field organisms' response to the aim of improving the future guidelines and studies for example Brock et al., 2016. Concerning the PPPs employed in general were tested as single compounds although some studies evaluated the toxicity of the mixture (Maloney et al., 2018; imidacloprid +OPs) or as Hasenbein et al., 2015 highlighting the need to link the use of lethal and sublethal effects. In general, studies were focused mainly on insecticides such as chlorpyrifos, imidacloprid, esfenvalerate or thiamethoxam with some exceptions such as the fungicide tebuconazole (Pimentão et al., 2020). In general, the reviewed studies analyze effects on growth and emergence rate mainly on *Chironomus riparius* (Azevedo-Pereira et al., 2011; Ferreira-Junior et al., 2017). Only three studies focus on ecological studies through mesocosm (Bouldin et al., 2004) and microcosm systems (Bolyard et al., 2017, Moreira-Santos et al., 2004). Nanoatrazine results are highly toxic for *C. sancticaroli* even at low concentrations of 2 µg/L with mentum deformities (de Albuquerque et al., 2016) or chlorantraniliprole for *C. riparius* altering the development and growth at 2 µg/L, even modulating the ecological interactions (Rodrigues et al., 2018).

Chironomus sub-individual

Author name		Ana Gonzalez, PhD
Topic description (see Table)		Chironomus (sub-individual)
Search	Used filter 1:	Ecosystem
	Used filter 2:	n/a
	Complete search strategy	Chironomus AND (molecular response*, OR biochemical*, OR omics*, OR gene expression*, OR metabolomics*, OR transcriptomics*, OR proteomics*
Selection	Total number before/after TiAb screening	14/13
	Reason for exclusion 1	not species of interest (1)
Description	Type of research	laboratory studies (13)
	Population	Laboratory cultures
	Exposure	To individual or combined PPP (active ingredient or commercial formulation)
	Comparator	Non contaminated medium/sediment used as control
	Outcome	Health-related outcome (effects on molecular response, biochemical, omics, gene expression, metabolomics, transcriptomics, proteomics)
Recommendation	Future needs	n/a
	Full systematic review	yes
	Your interest	Not applicable
References	CSV-format and Endnote-format	File names used
Studies	Selection of included studies	(Excel sheet Chironomus references molecular)

The 13 included studies were published between 2004 and 2021. Three studies used *Chironomus tentans*, 2 *C. dilutus*, 1 for *C. sancticaroli* and 7 for the *C. riparius* as the main representative for OECD toxicity test in aquatic toxicology. For *C. tentans* atrazine was studied in acute exposure showing induction on Cytochrome P450 enzymes (Londoño et al., 2004), decreased haemoglobin (Anderson et al., 2008) and showed common toxicity pathways alteration with chlorpyrifos (Tang et al., 2018). Imidacloprid altered the oxidative stress through catalase (CAT), malondialdehyde (MDA) or glutathione (GSH) enzymes alteration and gene expression modulation on detox and redox genes (ZnCUSOD) in single or binary mixtures (Wei et al., 2020, 2021). Atrazine modulated the acetyilcholinesterase, erythroblastosis virus transcription factor (ETS) and glutation-S-transferase (GST) on *C. sancticaroli* after acute exposure (de Albuquerque et al., 2021). Finally, diverse PPPs were studied in *C. riparius* with thiamethoxam the less toxic compound (Saraiva et al., 2017), followed by spinosad and indoxacarb with altered GST and ETS and even globin protein modulation (Monteiro et al., 2019, 2020). Chlorantraniliprole and esfenvalerate decreased GST, CAT or GSH related to oxidative stress (Rodrigues et al., 2015 a,b) and altered gene expression joined to enzymatic activity was observed by endosulfan (Muñiz-González et al.., 2021).

Daphnia survival

Author name		Ana Gonzalez, PhD
Topic description (see Table)		Daphnia (survival)
Search	Used filter 1:	Ecosystem
	Used filter 2:	
	Complete search strategy	Daphnia AND (mortal*, OR lethal*, OR acute*, OR survival*)
Selection	Total number before/after TiAb screening	227/185
	Reason for exclusion 1	not endpoint (19)
	Reason for exclusion 2	Methodology study (16)
	Reason for exclusion 3	not Daphnia related (4)
	Reason for exclusion 4	not PPP related (2)
	Reason for exclusion 5	not aquatic study (1)
Description	Type of research	laboratory studies (170), field studies (3), combined (10), reviews (2)
	Population	183 laboratory grown organisms, 1 virtual laboratory, 2 references data
	Exposure	174 studies employed artificial polluted water, 10 natural polluted water, 1 sediment polluted
	Comparator	Non contaminated medium used as control
	Outcome	Health-related outcome (effects on survival)
Recommendation	Future needs	
	Full systematic review	Due to the large number of studies retrieved it is difficult to select and deeply analyse the literature.
	Your interest	Not applicable
References	CSV-format and Endnote-format	Daphnia_survival.csv

The 185 included studies were published between 1995 and 2021. In these studies the following species were used: in 153 studies (*Daphnia magna*), 5 studes (*Ceriodaphnia dubia*), 5 studies (*D. carinata*), 3 studies (*D. pulex*), 2 studies (*D. galeata*), 2 studies (*D. longispina*), 2 studies (*D. pulicaria*), 1 study (*D. spinulata*, *D. similis*, *D. exulis*). In addition some studies (10) combined different species to check the differential sensitivity for PPPs, 3 studies used *D. longispina* that showed to be more sensitive than *D. magna* (Vidal et al., 2012) or *C. dubia* compared to *D. magna* after sulfosate exposure (Reno et al., 2018). Some studies compared the PPP and its degradation products as Lavtižar et al. 2015 with strong Chlorantraniliprole toxicity (LC50 48h= 9.4 µg/L) than its products. Moreover, active ingredients concerning the commercial formulation of pesticides were evaluated, showing greater toxicity in formulation format (Cuhra et al., 2013). Stereoselectivity was another endpoint in some studies (Duan et al., 2018; Li et al., 2019). 151 studies addressed acute exposure, 19 studied chronic/subchronic exposure and 15 combine both designs. Most of the studies used single exposures of PPP to evaluate toxicity, highlighting the incipient need for studies on mixtures. Of the 185 studies, 141 studied single exposure as opposed to 28 with mixtures and 16 combining both. Binary mixtures were analyzed on *D. magna* analyzing theoretical and laboratory data (Loureiro et al., 2010; Paulaki et al., 2011). Synergetic effects were observed by de Perre et al. (2017) or on ternary mixtures in *C. dubia* (Barata et al., 2012). To sum up the most toxic compounds for survival in Daphnia were deltamethrin EC50 48h= 0.06 µg/L (Shen et al., 2012), Spinosad EC50

48h= 4.1 µg/L (Santos et al., 2019). Finally, it has been observed how both physical factors such as temperature (Willming et al., 2013) or UV irradiation (Lasalle et al., 2015), as well as the interaction with other compounds or nanomaterials (Felten et al., 2020), modulate pesticide toxicity.

Daphnia life cycle

Author name		Ana Gonzalez, PhD
Topic description (see Table)		Daphnia (life cycle)
Search	Used filter 1:	Ecosystem
	Used filter 2:	n/a
	Complete search strategy	Daphnia AND (growth* OR life cycle* OR development* OR feeding* OR emergence* OR reproduction*)
Selection	Total number before/after TiAb screening	202/118
	Reason for exclusion 1	Not endpoint (56)
	Reason for exclusion 2	Methodology study (21)
	Reason for exclusion 3	Not PPPs related/aquatic (4)
	Reason for exclusion 4	Not Daphnia (3)
Description	Type of research	laboratory studies (106), combined field-lab study (5), combined (7)
	Population	113 studies employed organisms grown in the laboratory, 5 study with field organisms
	Exposure	113 studies employed artificial polluted water, 5 employed field polluted water
	Comparator	Non contaminated medium used as control
	Outcome	Health-related outcome (effects on growth, life cycle, development, feeding, emergence)
Recommendation	Future needs	Stugesst to study locomotor activity and address the influence of temperature and UV irradiation
	Full systematic review	Due to the large number of studies retrieved it is difficult to select and deeply analyse the literature.
	Your interest	Not applicable
References	CSV-format and Endnote-format	Daphnia_life_cycle.csv
Studies	Selection of included studies	

The 118 included studies were published between 1981 and 2021. Of these studies 95 used (*Daphnia magna*), 4 studies (*Ceriodaphnia dubia*), 3 studies (*D. carinata*), 5 studies (*D. pulex*), 1 study (*D. galeata*), 1 study (*D. longispina*), 1 study (*D. exulis*). In addition, some studies (8) combined different species to check the differential sensitivity for PPPs (Ishimota et al., 2020). In general, *D. magna* showed less sensitivity than other species for example *D. longispina*. According to the type of exposures 58 studies employed acute, 46 Chronic and 14 combined both studies. Few studies evaluated the feeding effect on Daphnia after PPP exposure (12), with carbendazim and combined to UV irradiation (Ribeiro et al., 2011) or combining metals and pesticides (Lopes et al., 2005), notably the alterations by pyrethroids on *D. magna* (Barata et al., 2006) or spinosad was able to modulate the feeding and development in a laboratory and field setting by microcosms (Duchet et al., 2008). Much different nature PPP was employed being notable the use of fenoxycarb, imidacloprid, spinosad or atrazine (Duchet et al., 2008; Hosmer et al., 1998; Pestana et al., 2010; Stoecklel et al., 2008). In most studies, they jointly evaluated the effects on the life cycle after chronic exposure, observing alterations in development, growth or reproduction for example

on *Cerodaphnia dubia* (Roses et al., 2002). Among the PPPs, the role of fenoxycarb stands out as an endocrine disruptor and with an analogy with the juvenile hormone receptor, modulating both the correct development and reproduction, for example, altering the sex male rate in *D. magna* (Oda et al., 2007). Only one study showed altered locomotor activity (Hussain et al., 2020) opening a new evaluation way for PPPs effects on Daphnia. George et al., 2003 employed atmospheric samples to check the high PPPs values on air samples leading to strong alterations at the reproductive level in *D. magna* demonstrating the high contamination of PPP in all environmental compartments. 3 pesticides in single exposure notable the role of imidacloprid modulating embryo development and hatching (Qui et al., 2018). Single exposure mostly used whereas sixteen studies tested mixtures (Oropesa et al., 2016). Hassold & Backhaus (2014) tested fungicides combied and 3 studies combined both exposures. Finally, different studies evaluate the contribution of physical factors such as temperature or UV irradiation was addressed (Ribeiro et al., 2011; Seeland et al., 2012), suggesting enhancement of toxicity of pesticides, and emphasizing the impact of climate change on the PPP toxicity. Diet is also considered an important factor both in field and in laboratory settings, at the expense of the number of organisms per tank (Ieromina et al., 2014).

***Daphnia* sub-individual**

Author name		Ana Gonzalez, PhD
Topic description (see Table)		Daphnia sub-individual
Search	Used filter 1:	Ecosystem
	Used filter 2:	n/a
	Complete search strategy	Daphnia AND (molecular response* OR biochemical* OR omics* OR gene expression* OR metabolomics* OR transcriptomics* OR proteomics*)
Selection	Total number before/after TiAb screening	34/28
	Reason for exclusion 1	not endpoint (4)
	Reason for exclusion 2	Methodology study (2)
Description	Type of research	laboratory studies (28)
	Population	All the studies employed organisms grown in the laboratory
	Exposure	27 studies employed artificial polluted water, 1 natural polluted water sample
	Comparator	Non contaminated medium used as control
	Outcome	Health-related outcome (effects on molecular response, biochemical, omics, gene expression, metabolomics, transcriptomics, proteomics)
Recommendation	Future needs	More studies are required to improve the insight of mode of action of PPP toxicity
	Full systematic review	Yes
	Your interest	Not applicable
References	CSV-format and Endnote-format	Daphnia_sub_individual.csv
Studies	Selection of included studies	

The 28 included studies were published between 2007 and 2021, unlike previous endpoints of which results were reported from 1985 or 1993. This is explained by the novelty of omics in the field of aquatic toxicology studies. All studies used *D. magna,* except for Simpson et al., 2017 using *D. pulicaria* genotypes for sensitive comparative. Only one study employed natural polluted water samples being the most detected bentazone, focused on *in situ* bioassays altering CAT and GST activity on *D. magna* (Barata et al., 2007). Among the omics variants, the most used were metabolomics with (5) and transcriptomics (5). Of the 28 studies, 25 evaluated acute exposure and 3 chronic and all of them evaluated effects in water but not sediments. Concerning the mixture, only 2 studies evaluated mixture effects and 2 studies reported on both single and mixture. Other studies used only single exposure. Atrazine was employed in 5 works showing strong toxicity altering oxidative stress and energy metabolism (Le et al., 2017) by proteomics analysis or altering glutathione and vitellogenin expression related to endocrine and detoxification responses (Hannas et al. 2011; Schmidt et al., 2017). Fenoxycarb showed endocrine disruption (Toyota et al., 2017). Finally, some studies tested imidacloprid altering gene expression (Pfaff et al., 2021) or enzyme activity (Jemec et al., 2007). In general, pesticides have an effect on the response of the sub-organism mainly through the activation and modulation of oxidative stress pathways (Cui et al., 2017), detoxification pathways (Aksakal, 2020) or effects on the endocrine system (Toyota et al., 2014).

Diatom growth

Author name		Joana Luísa Pereira, PhD
Topic description (see Table)		*Diatom* growth
Search	Used filter 1:	Ecosystem
	Used filter 2:	n/a
	Complete searchstrategy	#1 [diatom*] AND [grow* OR biomass OR yield] #2 [Navicula] AND [grow* OR biomass OR yield]
Selection	Total number before/after TiAb screening	#1 – 21/13 #2 – 4/4
	Reason for exclusion 1	#1 – antifouling activity focused (n = 1)
	Reason for exclusion 2	#1 – addressing recovery or tolerance acquisition not toxic effects (n = 2)
	Reason for exclusion 3	#1 – coral reefs focused (thus symbiotic algae with many confounding factors); too forced link to agricultural pollution? (n = 1)
	Reason for exclusion 4	#1 – no details on pesticides addressed in Ab (n = 1)
	Reason for exclusion 5	#1 no diatoms mentioned (n = 1)
	Reason for exclusion 6	#1 focus on other organisms (diatoms as a diet) (n=1)
	Reason for exclusion 7	#1 Not focused on effects (n=1)
Description	Type of research	Experimental studies (single species; multi-species enclosures)
	Population	Laboratory cultures + colonized substrates; communities collected in situ; in situ enclosures (1)
	Exposure	To pesticides - active ingredient or commercial formulation (1)
	Comparator	Non contaminated medium used as control
	Outcome	Decreased growth rate, photosynthesis impairment, decreased pigment production, community structure changes, increased tolerance/resistance
Recommendation	Future needs	Gap analysis: single-species studies are scarce and limited to a few compounds; there is apparently a large range of sensitivity of diatoms depending on the species and this needs to be better characterized to define consistently sensitive species for risk assessment. Resilience of communities should be more systematically addressed
	Full systematic review	Possibly not as search #2 highlighted one study where diatoms were used that was not captured by search #1. Often diatoms are named as algae and this is possibly a constraint of searches based on the keyword "diatom". No solution for the problem is apparent as it is not suitable to run a search using each diatom genus as a keyword. The recommendation is to complete the search by looking at "microalga*" or "alga*) search terms. Finally, there are several studies with diatoms and pesticides missing from the collection considering

		previous knowledge of the field (e.g. those testing diatom communities by Ben Kefford's team).
	Your interest	n/a
References	CSV-format and Endnote-format	Env_microalgae.xlsx #1 – Tab "SEARCH diatom" #2 – Tab "SEARCH Navicula"

References found with search thread #1 were published between 1984 and 2021, while those retrieved with search #2 were published between 2008 and 2021 (3 out of 4 remaining after TiAb screening were captured by search #1.

The most studied pesticide was atrazine. Effects of atrazine include community shifts towards diatom-dominated assemblages in the long-and the short-term indicating an increased tolerance to atrazine of diatoms compared to other microalgae (Hamilton et al. 1987; Downing et al. 2004); inhibition in growth and photosynthesis (Magnusson et al. 2008) in *Navicula* sp., but transient, with full recovery in hours after exposure (Brain et al. 2012); increased functional and compositional resistance of communities but decreased resilience depending on biodiversity magnitude of tested communities (Baert et al. 2016). S-metoalachlor was also tested often under single-species and multi-species test designs encompassing different test periods. Noticeable effects of s-metoalachlor include decreased cell density and pigment production by field-collected communities (Debenest et al. 2009); additivity when dosed as a mixture within a commercial formulation with terbuthylazine plus copper regarding negative effects on growth and fatty acid profiles (Filimonova et al. 2018). Roubeix et al. (2012) added that no effects of s-metolachlor are recognizable in exposures to environmentally relevant concentrations, regardless tests are made with a single species, a population of different strains of such species or a multi-specific community, as well as that acetochlor (metabolite) was clearly more toxic than the parent compound regardless the level of biological diversity tested. Other PPP tested in single-species or multi-species assays were the fungicide chlorothalonil and the insecticide endosulfan, both well tolerated by diatoms compared to other algae species (Downing et al. 2004); the herbicides bentazon and hexazinone, with negative effects in growth and/or on photosynthetic efficiency (Macedo et al. 2008; Magnusson et al. 2008); the herbicide isoproturon that negatively affected communities collected in-situ regarding cell density and chlorophyll concentration after 3 days of exposure, yet recovery was observed in the following 3 days, specially by facultative heterotrophs (Debenest et al. 2009); the herbicide norflurazon, its primary metabolite and the fungicide tebuconazone tested singly and as a ternary mixture during 48 h, with negative effects in photosynthetic efficiency noted for the metabolite and the mixture; the herbicide alachlor with natural biofilms from different sites denoting negative effects in chlorophyll production and compositional changes difficult to interpret as directly resulting from exposure (Paule et al. 2015); the herbicide diuron that impaired photosynthetic activity (Magnusson et al. 2008; Moisset et al. 2015) and related gene expression in three diatom species differentially (Moisset et al. 2015); the herbicide glyphosate and the insecticide imidacloprid that showed differential effects in terms of gender-specific growth within communities collected at different sites following short exposure (Vidal et al. 2021). Finally, the influence of natural variables in the response of diatom communities was highlighted by Larras et al. (2014), constraining the applicability of protective thresholds established on the basis of risk assessment tools.

Conclusion: Seventeen included studies reported on toxic effects regarding growth, photosynthetic efficiency, pigment production and gene expression. These effects were documented for several herbicides (atrazine, s-metoalachlor, bentazone, hexazinone, isoproturon, alachlor, diuron and glyphosate). The data on atrazine and isoproturon highlight the recovery capacity of diatoms in the short term, and the data on s-metolachlor show that environmental concentrations are not likely

to induce toxic effects. No negative effects were observed following exposure to the fungicide chlorthalonil or the insecticide endosulfan, but the insecticide imidacloprid affected diatom growth in a gender specific manner. Mixtures of pesticides and the combination of these with natural stressors were rarely addressed, yet these represent the most realistic conditions for pesticide exposure.

Microalgae growth

Author name		Joana Luísa Pereira, PhD
Topic description (see Table)		Green microalgae
Search	Used filter 1:	Ecosystem
	Used filter 2:	n/a
	Complete searchstrategy	[vulgaris OR subcapitata OR selenastrum] AND [grow* OR biomass OR yield]
Selection	Total number before/after TiAb screening	36/12
	Reason for exclusion 1	Not focusing on algae, but rather in indirect effects of herbivores, crops, fungi - related to species names (n=20)
	Reason for exclusion 2	Not focusing on effects but rather on capacities (as a biosensor or as a bioremediation agent) (n=3)
	Reason for exclusion 3	No effects quantified in the abstract; focused on alternative test methods and parameters (correlations emphasized) (n=1)
Description	Type of research	Experimental studies (single species) denoting toxic effects of PPPs on standard algae species
	Population	Laboratory cultures
	Exposure	To pesticides, static or pulse exposure with or without recovery assessment
	Comparator	Non contaminated medium/sediment used as control
	Outcome	Decreased growth rate, biomass yield, chlorophyll production, photosynthetic efficiency
Recommendation	Future needs	Gap analysis: limited range of PPPs tested suggests that more characterization must be done. However, this picture should rather result from a limited performance of the tool (see next item).
	Full systematic review	Many studies with these species are available that were not captured by the search (e.g. studies from our own team)
	Your interest	n/a
References	CSV-format and Endnote-format	Env_microalgae.xlsx Tab "SEARCH green microalgae"

Only 12 studies were published between 1991 and 2019. Atrazine was the most frequent pesticide studied in 7 references. Herbicides were the most tested pesticides, which is logically related to the mode-of-action that is likely to affect algae such as plants. Still, fungicides (carbendazim tricyclazole and flusilazole) were also tested in a study where contaminated water from a conventional rice farm (with herbicide and fungicide application) was compared with the same matrix collected from an organic rice farm, the former impairing the growth of *Pseudokirchneriella subcapitata*, *Desmodesmus subcapitatus* and *Chlorella vulgaris* (Suárez-Serrano et al. 2010); conversely, photosynthetic efficiency was found to be insensitive to the insecticides methyl parathion, carbofuran and malathion (Choi et al. 2012). Exposure to the PPPs invariably led to negative effects on growth, biomass yield, chlorophyll production, photosynthetic efficiency and/or 14C uptake.

However, studies using pulse exposures generally found that microalgae can rapidly recover with a short period (Brain et al. 2012; Baxter et al. 2013; Weber et al. 2019) and one considers the possibility of adaptation to pulse exposure in the long-term (Weber et al. 2019). Two studies examined the interference of factors such as light intensity, temperature and nutrients in PPP toxicity to microalgae. Temperature can modulate toxic effects but, in a PPP-specific way: while increased atrazine toxicity was found when temperature decreases (Baxter et al. 2016), thiobencarb toxicity increases rather with increased temperature (Tasmin et al. 2018).
Other studies captured are not particularly informative. In one the abstract refers only to concentrations of glyphosate tolerated (vague) by green microalgae (Shaker et al. 2018); other compares expired with non-expired counterpart products (15 pesticides), indicating generally that some expired formulations are more toxic to *P. subcapitata* and remarking that careful disposal of expired products should be better considered (Satyavani et al. 2012); and another is dedicated to the correlation among different parameters (uptake and biomass yield; uptake and sensitivity) in toxicity tests with microalgae using atrazine as a model.

Conclusion: In twelve included studies effects of pesticides on microalgae were reported. While negative effects of herbicides are more often addressed, those were also found following exposure of green microalgae to fungicides but not to insecticides. The capacity of microalgae to recover from impairment induced by exposure was documented and should be taken into account in future studies for a more realistic risk assessment; the same applies to the modulation of pesticide effects by natural variables and other contaminants within complex natural matrices.

Lumbriculus survival

Author name		Ana Gonzalez, PhD
Topic description (see Table)		Lumbriculus survival
Search	Used filter 1:	Ecosystem
	Used filter 2:	n/a
	Complete search strategy	Lumbriculus AND (mortality*, lethal*, survival*, NOEC*, acute toxicity*)
Selection	Total number before/after TiAb screening	11/8
	Reason for exclusion 1	Not related to PPP (1)
	Reason for exclusion 2	Not related to endpoint (2)
Description	Type of research	Experimental studies
	Population	Laboratory cultures
	Exposure	To individual or combined PPP (active ingredient or commercial formulation)
	Comparator	Non contaminated medium used as control
	Outcome	Health-related outcome (effects on survival)
Recommendation	Future needs	n/a
	Full systematic review	yes
	Your interest	Not applicable
References	CSV-format and Endnote-format	lumbriculus_survival.csv
Studies	Selection of included studies	

All 8 studies selected were published during the last twenty-two years (1993-2020), all the works employed *Lumbriculus variegatus* as standardized species in this genus. The exposure time was mainly 24h-96h, or 10 days being Acute/subChronic assessment with some chronic 28d exposure. In general, all the studies employed single exposure being the most toxic imidacloprid reaching an LC50 96h= 6.2 µg/L (Alexander et al., 2007). The rest of the works evaluated chlorpyrifos, esfenvalerate, thiamethoxam although showing lower toxicity. Only one work was focused on mixture toxicity employing imidacloprid insecticide and the fungicide tebuconazole although not essential impacts were observed by mixture the imidacloprid was very toxic with LC50= 2.5 to 177 µg/L (Raby et al., 2019).

Lumbriculus life cycle

Author name		Ana Gonzalez, PhD
Topic description (see Table)		Lumbriculus life cycle
Search	Used filter 1:	Ecosystem
	Used filter 2:	n/a
	Complete search strategy	Lumbriculus AND (growth* OR life cycle* OR development* OR feeding* OR emergence* OR reproduction*)
Selection	Total number before/after TiAb screening	7/5
	Reason for exclusion 1	not related to PPP (1)
	Reason for exclusion 2	Not related to endpoint (1)
Description	Type of research	Experimental studies
	Population	Laboratory cultures
	Exposure	To individual or combined PPP (active ingredient or commercial formulation)
	Comparator	Non contaminated medium used as control
	Outcome	Health-related outcome (mortality, lethality, survival, NOEC, acute toxicity)
Recommendation	Future needs	n/a
	Full systematic review	yes
	Your interest	Not applicable
References	CSV-format and Endnote-format	lumbriculus_life_cycle.csv
Studies	Selection of included studies	n/a
All the 5 studies selected were published during the last twenty-two years (2007-2017), all the works employed *Lumbriculus variegatus* as standardized species in this genus. The exposure time was mainly subChronic/chronic assessment with some chronic 7, 10 or 28d exposure. Moreover, all the studies employed single exposure being the most toxic imidacloprid altering the growth and the egestion (Alexander et al., 2007; Sardo et al., 2010).		

Lymnaea survival

Author name		Ana Gonzalez, PhD
Topic description (see Table)		*Lymnaea* survival
Search	Used filter 1:	Ecosystem
	Used filter 2:	n/a
	Complete search strategy	(Lymnaea) AND (mortal* OR lethal* OR acute* OR survival*) Note: some studies were not retrieved (e.g. https://doi.org/10.1007/bf00197476) because the survival is not included in the title
Selection	Total number before/after TiAb screening	15/9
	Reason for exclusion 1	Other outcome (4)
	Reason for exclusion 2	Methodology study (2)
	Reason for exclusion 3	Not species of interest(1)
Description	Type of research	Laboratory studies (7) and 1 combined lab and field study
	Population	All the studies employed organisms grown in the laboratory
	Exposure	8 studies employed artificial polluted water
	Comparator	Non contaminated medium was used as control
	Outcome	Effects on survival
Recommendation	Future needs	More studies on mixture effects are needed
	Full systematic review	Too few studies available
	Your interest	Not applicable
References	CSV-format and Endnote-format	Lymnaea_survival.csv
Studies	Selection of included studies	

The 5 included studies cover the period 1993 – 2021. Four studies used *Lymnaea stagnalis,* 3 *L. acuminata* and 1 *L. luteola.* Compared to *Physa acuta* Lymnaea species seem to be more sensitive to pesticides exposure. Survival response was evaluated for acute (24-96h) and subchronic/ chronic (7-21d) test exposure, 4 studies for each. Two studies evaluated the PPPs effects employing microcosm exclusively (Van den Brink et al., 2000) or comparing to laboratory conditions by atrazine exposure (Jacqueline et al., 2009), in the last case no effects were observed on lab studies. However on microcosms seasonal survival was detected highlighting the importance of comparing responses between the field and laboratory setting. Regarding the PPPs evaluated the one that showed the greatest toxicity was azoxystrobin LC50 96h = 0.79 mg/L (Ali et al., 2021) followed by cypermethrin LC50 24h was 10.39 mg/L (Singh & Singh 2009). Only one study focused on mixture toxicity combining plant-derived molluscoid with piperonyl butoxide in *L. acuminata* with decreased survival joined to altered reproduction parameters (Singh et al., 2005).

Lymnaea life cycle

Author name		Ana Gonzalez, PhD
Topic description (see Table)		*Lymnaea* life cycle
Search	Used filter 1:	ecosystem
	Used filter 2:	n/a
	Complete search strategy	Lymnaea AND (growth* OR life cycle* OR development* OR feeding* OR emergence*)
Selection	Total number before/after TiAb screening	13/9
	Reason for exclusion 1	Not related endpoint (1)
	Reason for exclusion 2	Methodology study (2)
	Reason for exclusion 3	No related organism (1)
Description	Type of research	Laboratory studies (9)
	Population	All the studies employed organisms grown in the laboratory
	Exposure	8 studies employed artificial polluted water and 1 of them was run in a mesocosm
	Comparator	Non contaminated medium used as control
	Outcome	Health-related outcome (effects on growth, life cycle, development, feeding, emergence, reproduction)
Recommendation	Future needs	n/a
	Full systematic review	yes
	Your interest	Not applicable
References	CSV-format and Endnote-format	Lymnaea_life_cycle.csv
Studies	Selection of included studies	

The 9 included studies cover the period 1992 – 2018. Six 6 studies used *Lymnaea stagnalis,* 2 *L. acuminata* and 1 *L. palustris.* Compared to *Physa acuta* Lymnaea species seem to be more sensitive to pesticides exposure. Five studies evaluated the reproduction after different PPPs exposure, with altered egg production (Pressing 1993; Woin & Brönmark 1992), fecundity (Singh & Singh 2000), or combined studies for growth and reproduction (Baturo et al., 1995: Fider et al., 2016; Reategui-Zine & Salice 2018). Only one study evaluated the effects on *L. palustris* through mesocosm studies showing strong effects of hexachlorobenzene compared to atrazine on growth and eggs production (Baturo et al., 1995), besides feeding inhibition was observed on *L. stagnalis* by pyraclostrobin (Fidder et al., 2016).

Lymnaea molecular

Author name		Ana Gonzalez, PhD
Topic description (see Table)		*Lymnaea* molecular
Search	Used filter 1:	Ecosystem
	Used filter 2:	n/a
	Complete search strategy	Lymnaea AND (molecular response* OR biochemical* OR omics* OR gene expression* OR metabolomics* OR transcriptomics* OR proteomics*)
Selection	Total number before/after TiAb screening	5/5
Description	Type of research	laboratory studies (5)
	Population	All the studies employed organisms grown in the laboratory
	Exposure	4 studies employed artificial polluted water, 1 natural polluted water sample
	Comparator	Non contaminated medium used as control
	Outcome	Health-related outcome (effects on molecular response, biochemical, omics, gene expression, metabolomics, transcriptomics, proteomics)
Recommendation	Future needs	n/a
	Full systematic review	yes
	Your interest	Not applicable
References	CSV-format and Endnote-format	Lymnaea_molecular.csv
Studies	Selection of included studies	
The 5 studies analyzed cover the periods between 1995 and 2020. Baturo et al., 1995 seems the first approach to the metabolomics studies observing alteration on glycogen expression. Later on, Tufi et al., 2015 evaluated the imidacloprid effects on *L. stagnalis* with modulation on metabolites for polyamines related to neurotoxicity and decreased fatty aids. One study focused on mixture toxicity using field water naturally polluted with altered neonicotinoids pathways in *L. stagnalis* (Tufi et al., 2016). On mesocosm, no effects on xenobiotics were detected after hexachlorobenzene or atrazine exposure (Baturo & Lagadic 1996).		

Macrophyte growth

Author name		Joana Luísa Pereira
Topic description (see Table)		*Macrophyte* grow
Search	Used filter 1:	Ecosystem
	Used filter 2:	n/a
	Complete search strategy	#1 [macrophyte OR freshwater plant] AND [grow* OR biomass] #2 [macrophyte OR freshwater plant OR Lemna OR duckweed OR Myriophyllum] AND [grow* OR biomass]
Selection	Total number before/after TiAb screening	#1 – 19/14 #2 – 26/20
	Reason for exclusion 1	#2 - Not regarding freshwater ecosystems (n = 1)
	Reason for exclusion 2	#1 and #2 – no info on effects in macrophytes or not focused on macrophytes (n = 4)
	Reason for exclusion 3	#1 and #2 – focused on remediation capacities, not on effects (n = 1)
Description	Type of research	Experimental studies (single species; multi-species microcosms, mesocosms)
	Population	Laboratory cultures (33) + field-collected cultures (1)
	Exposure	To pesticides (active ingredient or commercial formulation) single or in mixtures; under different incubation conditions (nutrients, light, humic acids)
	Comparator	Non contaminated medium used as control
	Outcome	Decreased growth rate, photosynthesis impairment, decreased chlorophyll production, community composition changes, differential gene expression
Recommendation	Future needs	Consider including recovery periods in experiments; addressing effects of non-herbicidal PPPs, which are rarely addressed but are apparently meaningful.
	Full systematic review	Search for the general terms as in #1 clearly leads to the missing of relevant references. When one adds genera of interest to the search (#2), the coverage of the literature is larger. It remains unclear whether the addition of other genera to the search thread would reinforce this view. Several studies were not retrieved by the search, including several from our team that include the term "Lemna" in the Abstract
	Your interest	n/a
References	CSV-format and Endnote-format	Env_macrophytes.xlsx Tab "SEARCH macrophytes"

The outcome of search #1 was fully included in the outcome of search #2, thus the analysis was made over this latter only. Relevant publications were found dating from 1994 to 2021. Atrazine is the most frequently tested pesticide, especially when studies address issues that go beyond the simple assessment of sensitivity. For example, no ecological synergism was found when atrazine was dosed with the insecticide esfenvalerate, which was partially related to functional redundancy of the macrophytes community in outdoor mesocosms (Fairchild et al. 1994); no differences were found between plant systems in the negative effects of atrazine in macrophytes tested in outdoor mesocosms (McGregor et al. 2008); full recovery from atrazine exposure was found for *Lemna sp.* and Myriophyllum aquaticum, highlighting the importance of verifying long-term toxicity entailing recovery periods, realistic pulse exposures and/or considering sediment-bound contaminants for a more accurate assessment of the hazardous potential of pesticides to macrophytes (Mohammad et al. 2010; Teodorović et al. 2012; King et al. 2016; Mihajlović et al. 2019); predictability of effects of atrazine dosed singly and jointly with isoproturon and alachlor in simple communities from single-species laboratory assays was confirmed (Coutris et al. 2011); sensitivity to atrazine was found to be dependent on available photosynthetically active radiation (Brain et al. 2012), on the origin of the tested macrophytes (field populations bearing an history of exposure vs. laboratory cultures; Dalton et al. 2013); specific and generic molecular pathways of impairment (gene expression) were found after exposure of Potamogeton crispus and Myriophyllum spicatum to atrazine (Qu et al. 2021). Other herbicides, such as linuron, metazachlor, diuron, glyphosate or isoproturon, were shown to induce negative effects on macrophytes regarding growth, biomass, photosynthesis efficiency and/or pigment concentration (Van den Brink et al. 1997; Mohr et al. 2007; Burns et al. 2015; Sikorski et al. 2019; Varga et al. 2020), but either rapid recovery capacity or the adequacy of currently implemented safety benchmarks were generally highlighted. The protective benchmarks derived from complex mesocosms studies was compared to that from single-species standard tests with macrophytes considering 14 herbicides and the authors concluded that there are no appreciable differences, which facilitates regulatory assessment (van Wijngaarden and Arts 2018). Outdoor mesoscosms tests did not confirm negative effects in macrophytes (biomass or species composition of communities) of exposure to realistic concentrations (edge-of-field) of metribuzin (Fairchild and Sappington 2002), and the trophic status of ecosystems seems to influence the response of macrophytes to pesticide mixtures (Wendt-Rasch 2004). In exposures to mixtures of herbicides, fungicides and insecticides, the decline of Elodea nuttallii biomass was noticed, but this negative effect was caused by one of the herbicides present in the mixture (van Wijngaarden et al. 2004). However, negative effects of PPPs other than herbicides in macrophytes were already confirmed, namely Ceratophyllum demersum biomass decline provoked by the insecticide fenoxycarb (Sha et al. 2021).

Conclusion: Thirty-four included studies reported on PPP effects on *Macrophyte* growth. Negative effects of pesticides to macrophytes have been documented regarding growth, photosynthetic efficiency, pigment production and even adverse molecular pathways (gene expression). However, these are generally mild, occurred at non-realistic concentrations (e.g. above protective thresholds or quantified concentrations in water) or are transient, with macrophytes recovering in the short term. The sensitivity to some herbicides (e.g. atrazine) was found to depend on ecological features of the test system, the interacting effect of natural stressors and the presence of other pesticides in the experimental treatment. Single-species tests apparently have satisfactory outcomes that do not differ significantly from those resulting from complex multi-species experiments, which suggests that future studies with macrophytes for risk assessment purposes can feasibly rely on simpler methodologies.

Physa survival

Author name		Ana Gonzalez, PhD
Topic description (see Table)		*Physa* survival
Search	Used filter 1:	Ecosystem
	Used filter 2:	n/a
	Complete search strategy	(Physa OR Aquatic snail) AND (mortal* OR lethal* OR acute* OR survival*)
Selection	Total number before/after TiAb screening	6/5
	Reason for exclusion 1	Duplicate (1)
Description	Type of research	Experimental studies
	Population	Laboratory cultures
	Exposure	To individual or combined PPP (active ingredient or commercial formulation)
	Comparator	Non contaminated medium used as control
	Outcome	Health-related outcome (effects on survival).
Recommendation	Future needs	Gap analysis: the following articles 10.1038/s41598-020-64554-8 with *Physella acuta* was not captured by SOLES tool
	Full systematic review	yes
	Your interest	Not applicable
References	CSV-format and Endnote-format	Physa_survival.csv
Studies	Selection of included studies	Study selection is available here

The 5 included studies cover the period 998-2016, 3 studies employed *Physa acuta* and the other 2 *P. gyrina*. Regarding the PPPs analyzed all the studies except (Nebeker et al., 1998) using diuron, evaluated the effects of atrazine. In general, the studies were focused on acute or chronic exposure, through water toxicity assays, mainly using environmental realistic concentrations except for Gustafson et al. (2015). Due to the high resistance of this organism, showing low effects on its survival, it is interesting and necessary to perform new studies with various PPPs in simple exposure and/or binary mixtures. To establish this species as a study organism with its own OECD tests on a par with the better known Lymnaea.

Physa life cycle

Author name		Ana Gonzalez, PhD
Topic description (see Table)		*Physa* life cycle
Search	Used filter 1:	Ecosystem
	Used filter 2:	n/a
	Complete search strategy	(Physa) AND (growth* OR life cycle* development* OR feeding* OR emergence*)
Selection	Total number before/after TiAb screening	5/4
	Reason for exclusion 1	Duplicate (1)
Description	Type of research	Laboratory studies (3), field-lab study (1)
	Population	4 studies employed organisms grown in the laboratory, 1 study with field organisms
	Exposure	3 studies employed artificial polluted water, 1 employed field polluted water
	Comparator	Non contaminated medium used as control
	Outcome	Health-related outcome (effects on growth, life cycle, development, feeding, emergence)
Recommendation	Future needs	n/a
	Full systematic review	yes
	Your interest	Not applicable
References	CSV-format and Endnote-format	Physa_lifecycle.csv
Studies	Selection of included studies	Study selection is available here
The 4 included studies analyzed cover the period 1998 - 2017. Three studies employed *Physa acuta* and the 1 *P. gyrina*. Regarding the PPPs, the studies were (2) for atrazine, (1) for diuron and (1) for imidacloprid effects. Only one study used field water polluted samples and macroinvertebrates organisms from the field to establish the imidacloprid effects on natural systems observing a density increase (Pereira et al., 2017). The studies reported altered growth (Gustafson et al., 2015; Nebeker et al., 1998) and modulated behaviour (Roses et al., 1999).		

Zebrafish survival

Author name		Nelson Abrantes, PhD
Topic description (see Table)		Zebrafish survival
Search	Used filter 1:	Ecosystem
	Used filter 2:	n/a
	Complete search strategy	(Zebrafish OR Danio rerio) AND (survival OR mortal* OR lethal* OR immobilization OR acute)
Selection	Number of studies before/after TiAb screening	150/68
	Reason for exclusion 1	Not endpoint related (72)
	Reason for exclusion 2	Not PPP related (2)
	Reason for exclusion 3	No details on endpoints studied (8)
Description	Type of research	Experimental studies
	Population	Laboratory cultures
	Exposure	To individual or combined PPP (active ingredient or commercial formulation)
	Comparator	Non contaminated medium used as control
	Outcome	Health-related outcome (effects on survival)
Recommen-dation	Future needs	Lack of studies entailing combination of PPP; lack of studies on PPP metabolites; lack of studies on commercial formulations (potentially more toxic than the a.i); lack of studies testing realistic concentrations.
	Full systematic review	Due to the large number of studies retrieved it is considered difficult to select and deeply analyse the literature.
	Your interest	Not applicable
References	CSV-format and Endnote-format	zebrafish_survival.csv

Included studies (68 references) were published in the last decade (2010-2020). A total of 52 different active ingredients (a.i.) were studied, which, in general, were able to affect the survival of zebrafish in distinct life stages (embryonic, larval, juvenile and adult stages). Among the distinct PPP classes, fungicides (22 a.i.) and insecticides (20 a.i.) were the most tested PPPs and only 10 studies focused on herbicides. Nevertheless, the herbicide glyphosate was the most frequent PPP studied (7 studies), followed by the herbicide atrazine and the insecticide imidacloprid (6 studies each) and by the insecticide lambda-cyhalothrin (5 studies). Then, for most of the a.i. only one single study was found.

Most of the retrieved studies focused on individual toxicity of PPP (59), with just 11 studies assessing the combined effects of PPPs, using distinct combinations. Concerning the studies on mixtures, most of them reported synergistic effects (7), which emphasizes the importance of consider combinations of PPPs and more complex scenarios in predictive risk assessment. Only two studies observed antagonistic effects: Shao et al (2019) using an artificial mixture of triclosan, carbamazepine, diazinon and diuron, the most hazardous compounds found in the rivers Danube and Rhine, found a strong antagonistic relationship among them; Chang et al (2020), investigating the combined toxicity of the insecticide imidacloprid, the herbicide acetochlor and the fungicide

tebuconazole found that the exhibition of synergistic or antagonistic effects was depended on the tested ratios among the three PPPs.
Concerning glyphosate, one of the most common used herbicides, de Brito et al (2019) found that glyphosate and AMPA (the main metabolite of glyphosate) caused no acute toxic effect while Atanor 48 (a commercial formulation of glyphosate) and its major constituent, the surfactant polyethoxylated tallow amine (POEA), induced significant lethal effects in zebrafish (76.5 mg/L and 5.49 mg/L, respectively). Fiorino et al (2018) showed that the significantly highest cumulative mortality was observed only when zebrafish is exposed to 50 mg/l of glyphosate. Atrazine, also a commonly used pesticide worldwide, showed a higher lethal toxic effect on zebrafish compared to glyphosate (LC50 = 29.06 mg/L). The toxic effects of atrazine were also evaluated after photocatalytic degradation using nano-sized Mn-doped TiO2 (Ozmen et al, 2015). The results showed that Mn-doped TiO2 nanoparticles did not cause significant lethality, but nonfiltered samples caused lethality in zebrafish. Furthermore Mn-doping of TiO2 increased the photocatalytic degradation capability of nanoparticles and it successfully degraded AZT but their degradation caused an increase of the lethal effects on both fish embryos.

Recent studies using nanotechnology to improve pesticide formulations have shown promise regarding the pesticide use and their mobility in soil, thus allowing to improve agricultural practices and therefore reducing the impacts in the environment. Zhang et al (2020) observed that the median lethal concentration (LC50) value of nano-enabled azoxystrobin was significantly lower than the conventional form of azoxystrobin. In the same line, Wang et al (2018) found that microencapsulated pyraclostrobin compared to free pyraclostrobin could dramatically improve its photostability under ultraviolet light irradiation. Moreover, authors observed lower acute toxicity against zebra fish on the first day and gradually similar toxicity over time. Thus, this controllable release of pesticides has promising applications contributing to a more sustainable plant protection.

Conclusion:
Survival studies have been reported over the past decade covering distinct PPP classes. Most of them have focused on individual PPPs and only a few studies tested mixture, mostly binary and ternary. In general, studies reported lethal effects on zebrafish, however, often did not specify whether the concentrations were environmentally relevant. Glyphosate was the most studied PPP, followed by atrazine, imidacloprid and by lambda-cyhalothrin. For most a.i. only a single study was found. Commercial formulations of PPP, as well as PPP metabolites, were punctually studied and therefore there is a gap that needs to be addressed. Also, important to stress that recent studies based on nanotechnology have shown promising reducing the impacts on aquatic organisms.

Zebrafish growth and behaviour

Author name		Isabel Campos, PhD
Topic description (see Table)		Zebrafish growth
Search	Used filter 1:	Ecosystem
	Used filter 2:	n/a
	Complete search strategy	(Zebrafish OR Danio rerio) AND (biomass OR growth*OR develop* behave*)
Selection	Number of studies before/after TiAb screening	57/40
	Reason for exclusion 1	Not endpoint related (10)
	Reason for exclusion 2	No details on the PPP address in abstract (2)
	Reason for exclusion 3	Not PPP-related (3)
	Reason for exclusion 4	Not focused on PPP effects, but on the effect of benoxacor, a herbicide safener (1)
	Reason for exclusion 5	Not focusing on effects but rather on the development of a monitoring tool (1)
Description	Type of research	Experimental studies
	Population	Laboratory cultures
	Exposure	To individual or combined PPP (active ingredient or commercial formulation)
	Comparator	Non-contaminated medium used as control
	Outcome	Effects on biomass, growth, development and behaviour
Recommen-dation	Future needs	The number of studies focus on the effects of PPP mixtures is very small to assess the possibility of their synergetic responses (or no) on aquatic on communities (5 studies). So, there is a lack of studies entailing combination of PPP and/or PPP residues. Most of the studies are related with PPP effects on the development and behaviour of Zebrafish which are, mainly assess by molecular tools. So, in this search, there is a lack of studies assessing these parameters by other tools (measurements of length, body weight, among others). And the word development (joined to the search strategy) is not related to development parameters (growth, biomass); lack of studies using natural samples of contaminated water.
	Full systematic review	Due to the large number of studies retrieved it is considered difficult to select and deeply analyse the literature.
	Your interest	Not applicable
References	CSV-format and Endnote-format	Zebrafish_growth.csv
All 40 studies included studies were published in the last 15 years with the exception of one, that was published in 1990 (Braunbeck et al., 1990). Five studies reported the effects of mixtures of PPP in Zebrafish (Perez et al., 2013; Perre et al., 2017; Wang et al., 2018; Wang et al., 2021; Alla et al.,		

2021) while the others 37, entailed the individual toxicity of PPP. A huge number of studies is focused on the use of molecular tools to assess the sublethal effects (mainly in terms of development and behaviour) of PPP in Zebrafish.

Mixture of PPP (n=5)

Perez et al., 2013, studied the synergistic effects (development, behaviour and mortality) caused by atrazine and terbuthylazine (s-triazine) and chlorpyrifos, testing single exposure and binary combination of the s-triazine and the chlorpyrifos. Swimming behaviour, morphological abnormalities and mortality were tested, as well as the inhibition of acetylcholinesterase (AChE) activity (to evaluate the mode of action and toxicity of the chlorpyris in the presence of the s-triazine PPP). Binary mixtures elicited synergistic responses on the swimming behaviour of zebrafish larvae and a correlation between impairment of swimming behaviour of the larvae and inhibition of AChE activity. Furthermore, also Perre et al., 2017 found synergism in fish exposed to the combination of phostebupirim and cyfluthrin. Sublethal and lethal concentrations to Danio rerio (zebrafish) were up to 7 times lower for the mixture than in concurrently run individual compound exposures. The single (iprodione, pyrimethanil, pyraclostrobin and acetamiprid) and joint toxicity for these four PPP was also assessed by Wang et al., 2018, using traditional (lethal) and molecular (transcriptional responses) endpoints. In terms of toxicities, in general, synergistic responses were observed from all binary mixtures of iprodione in combination with pyrimethanil or acetamiprid and ternary mixtures of iprodione+pyraclostrobin in combination with pyrimethanil or acetamiprid. Futhermore, the expressions of P53 Tnf TRŒ≤ Tsh and Cyp19a exhibited greater changes upon exposure to combined pesticides compared with individual pesticides. Finally, Wang et al., 2021, Assessed the combined effects of thiophanate-methyl and fenvalerate on embryonic zebrafish (Danio rerio), and found that mixture of thiophanate-methyl and fenvalerate exhibited a synergetic response [96-h LC (50)] to zebrafish embryos. They concluded that mixtures of these PPP produced higher toxicity towards zebrafish compared to the result of testing each PPP separately. Alla et al., 2021 developed a bioassay for detecting estrogenic and/or anti-androgenic activity (e.g. insecticides) with the goal to evaluate complex mixtures of uncharacterized contaminants in water samples. Zebrafish were exposed during 24-h to endocrine disrupting chemicals (including pesticides, medicine, etc.). However, the abstract is omitted about the mixture effects (they are not quantified in the abstract) in the swimming behavior of fishes. It just refers the single compounds.

Glyphosate (n=4)

Four studies reported on glyphosate. Although no apparent changes were noted in general morphology of exposed and nonexposed ovaries of zebrafish (Danio rerio) to glyphosate, this herbicide was associated with significant increase in diameter of oocytes and an increased in the expression of SF-3 in the oocytes, which can raise concern for reproduction in female fish (Armiliato et al., 2014). For instance, Rodrigues et al., evaluated the acute toxicity and genotoxicity of glyphosate-based herbicides on zebrafish (Danio rerio) and observed that they are phytotoxic and induce toxic effects on zebrafish early life stages. In the evaluation of the effects of this herbicide on the early development of zebrafish, Zhang et al., found that a delay occurred in the epiboly process and body length eye and head area were reduced at concentrations higher (> 10 mg/L). Fiorino et al., 2018, also assess the effects of glyphosate on early life stages observed hatching stimulation in embryos of zebra fish exposed to the highest concentration of glyphosate.

Atrazine (n=4)

Some contrasting results (from adversely to moderate or no effect on gonodal development) have been published over the last decade regarding the potential effects of atrazine (ATZ) on aquatic species. For instance, Corvi et al., 2012, assess the effects of ATZ exposure on sexual development

and differentiation of zebrafish (Danio rerio) and concluded that long-term exposure to ATZ at or above environmentally relevant concentrations does not significantly impact zebrafish gonadal development or sexual differentiation. Contrary, exposure to ATZ can be potentially teratogenic (egg coagulation, growth retardation, edema formation, hatching success, scoliosis) and genotoxic (DNA tail moments) and can cause oxidative stress in zebrafish embryos (Adeyemi et al., 2015). Walker et al., 2018 also found that exposure of zebrafish to ATZ resulted in significant differences in craniofacial cartilage elements, led to decreased survival and increased heart rates, delayed vertebrae mineralization, gross craniofacial defects and decreased hatching rates (depending on the exposure concentration). Paternal ATZ exposure resulted in alterations across a variety of behavioral traits (distance moved, exploration bottom-dwelling, time latency to enter the top zone and interaction with a mirror) (Jia et al., 2021).

Prochloraz (n=4)
A life cycle exposure of zebrafish to this fungicide led to an increased proportion of males and also to an alteration of intersex and stages of the gonads (Kinnberg et al., 2007). Similar results were also obtained by Baumann et al., 2015, where the investigations of the gonads of zebrafish revealed persistent effects on sexual differentiation, sex ratio was skewed towards males and significantly more intersex individuals.

Triazole (n=4)
Triazole fungicides are widely used and have been detected in water ecosystems, in concentrations that may affect functions of aquatic organisms. Teng et al., 2017 observed that female bioaccumulate more difenoconazole than males, growth of the liver and ovary were inhibited in females and that male fish growth was promoted. Li et al., 2019 also observed effects in exposures to tebuconazole, leading to a diminished fecundity of zebrafish. As for the penconazole, it provokes endocrine disrupting effect on zebrafish and has gender-specific endocrine effects in zebrafish (Jia et al., 2021). Propiconazole can decrease growth, fecundity and fertilization (Teng et al., 2020) decrease survival, induced hypopigmentation and alter locomotor activity of zebrafish, although these sub-lethal responses were observed at concentrations above what is typically detected in the environment (Sounder et al., 2019).

Other PPP
Indoxacarb was associated with significant reduction on the hatching rate of embryos of zebrafish (30 ppm) and survival of adults (14 days) (Rammohan et L., 2019). Behavioral changes and hepatic steatosis in conjunction with a reduction in fertility was observed in zebrafish exposed to Lindane (gamma-hexachlorocyclohexane) (Braunbeck et al., 1990). The herbicide cyhalofop-butyl had significant negative impacts on zebrafish at different life stages, spontaneous movements and hatching rates (Cao et al., 2016). Exposure of zebrafish to clomazone and its two formulations caused mortality, morphological changes, decreased spontaneous movements, underdeveloped embryos and interfered with the growth of zebrafish embryos (Stevanovic et al., 2017). Metolachlor may disrupt transcripts associated with swim bladder formation and morphology, which can affect larval zebrafish activity (Yang et al., 2021). The fungicide Fluxapyroxad induced abnormal spontaneous movement, malformations and decreased heartbeat, hatching percentage and body length of the embryos of zebrafish (Lin et al., 2021). Pyriproxyfen impaired effects on early developmental stages of zebrafish at higher concentration (Maharajan et al., 2018). Synthetic organic insecticides including pyrethroids (deltamethrin (DM), acephate (AP) and thiamethoxam (TM)) led to different toxic effects on embryos of zebrafish (Liu et al., 2018). For instance, while DM exposure led to embryo development delay and a significant increase in embryo mortality at 24 and 48h and DM and AP decreased embryo chorion surface tension, TM did not show significant

developmental toxicity. The phenylurea herbicide Linuron impairs oxidative respiration and exerts neurotoxicity effects during early development of zebrafish (Maharaj et al., 2020). The insecticide Deltamethrin impaired the development of swim bladder which is a key organ for motion (Wu et al., 2020). Awoyemi et al., 2020 assessed the effects of two pyrethroids against embryo-larval zebrafish and concluded disparity in the mechanistic effects across the pyrethroids types. The pyrethroid insecticide bifenthrin influenced the neurodevelopment processes in zebrafish (Frank et al., 2018). Strobilurin fungicides, such as azoxystrobin and pyraclostrobin, lead to diminished growth and movement (by the decreased mitochondrial function) in zebrafish (Kumar et al., 2020) as well as delays in sexual development and alter reproduction (Cao et al., 2019) in exposed to Azoxystrobin. Toxic effects induced by kresoxim-methyl strobilurin fungicides during larval development were observed by Jiang et al., 2019.

Conclusion:
There are a huge number of studies evaluating the effects of plant protection products (PPPs) on Zebrafish (*Danio rerio*). Herbicides, fungicides and insecticides can induce negative effects in zebrafish in distinct life stages (embryonic, larval, juvenile and adult stages) regarding growth, development, behavior (e.g. distance moved, exploration bottom-dwelling, time latency to enter the top zone and interaction with mirror) and malformations (that indicate general parameters such as body weight, length and growth-related parameters). Among the distinct PPPs, the herbicides, glyphosate, atrazine and the triazole fungicides as well as the prochloraz were the most studied. The data on these PPPs, also raise concern regarding the reproduction, fecundity, fertilization, sexual development, intersex and endocrine disrupting. However, some contrasting results were observed for the atrazine effects, from adversely to moderate or no effect on gonodal development. Most of the studies were focused on the individual toxicity of PPPs, with just a few (≅13%) assessing the combined effects of PPPs. These studies highlighted the potential increased toxicity that might be triggered by the simultaneous presence of several pesticides in the aquatic ecosystems entailing the synergistic responses.

Zebrafish sub-individual

Author name		Isabel Campos, PhD
Topic description (see Table)		Zebrafish sub individual
Search	Used filter 1:	Ecosystem
	Used filter 2:	Zebra fish OR Danio rerio
	Complete search strategy	(Zebrafish OR Danio rerio) AND (molecular response*OR biochemical*OR omics OR gene expression OR metabolomics OR transcriptomics OR proteomics)
Selection	Number of studies before/after TiAb screening	56/47
	Reason for exclusion 1	Non-primary study (1)
	Reason for exclusion 2	No details on the PPP address in abstract (5)
	Reason for exclusion 3	Not focus on effects (2)
	Reason for exclusion 4	No effects of the individual pesticide quantified in the abstract (1)
Description	Type of research	Experimental studies
	Population	Laboratory cultures
	Exposure	To individual or combined PPP (active ingredient or commercial formulation)
	Comparator	Non contaminated medium used as control
	Outcome	Health-related outcome (Effects on molecular response, biochemical, omics, gene expression, metabolomics, transcriptomics, proteomics)
Recommen-dation	Future needs	The number of studies focus on the effects of PPP mixtures is small and the same applies to the effects of biological PPP (just one study).
	Full systematic review	Due to the large number of studies retrieved it is difficult to select and deeply analyse the literature.
	Your interest	Not applicable
References	CSV-format and Endnote-format	Zebra fish_mol.csv

Included studies (47 references) were published in the last two decades (2009-2021). In general, the studies were focused on individual toxicity of PPP, with the exception of Sposito et al., 2018 (imidacloprid, 2-hydroxy-atrazine, tebuthiuron and atrazine) that evaluate the effects of the mixtures and found changes in the expression of Zebra fish target genes (cyp1a, hsp70, among others), and Shen et al., 2020 (malathion, chlorpyris and lambda-cyhalothrin) that compared the individual vs. mixture toxicities, for studying synergistic effects. Authors found that the certain gene expressions exerted greater variations upon exposure (Zebra fish) to mixtures compared with their individual compounds. The most studied PPP were glyphosate (6), atrazine (5) and dieldrin (4). Biochemical responses to glyphosate effects were not consistent, since some authors found alterations on oxidative stress through ROS, enzymes activities of alanine aminotransferase and aspartate aminotransferase or protein, glucose, glycogen and triglyceride levels (Velasques et al., 2016; Panetto et al., 2019), while others did not found alterations (Lopes et al., 2017). Regarding gene expression, authors (Velasques et al., 2016; Panetto et al., 2019; Lopes et al., 2017; Pereira et al., 2018; Moreira et al., 2019; Moraes et al., 2020) are more consistent and observed a reduction

in some enzymes (i.e., sod2, gst, gpx, AChE). Effects of atrazine and dieldrin on Zebra fish (liver, brain) include alterations on antioxidant enzymes activities (i.e., SOD, CAT), neurotoxicity (AChE), in addition to GSH and MDA content, mRNA levels for the genes encoding these oxidant proteins up-regulated significantly, gene expression (Jin et a., 2010; Wang et al., 2015; Sposito et al., 2018) and histological effects (Jin et al., 2012). In terms of neurotoxicity, and using transcriptomic analysis, Horzmann et al., 2021, did not observed significant difference in genome methylation or brain size between zebrafish exposure to non-contaminated (control) and contaminated with atrazine. The study of Jin et al., 2020 seems to be the first to report transcriptional effects of atrazine on zebrafish. PPPs that were also studied include organochlorine pesticides (ethoxychlor, endosulfan, heptachlor, metolachlor) and it was found to induced alteration in the gene expression levels of the biomarker vtg1 after 96 h of exposure (Chow et al., 2013), impairment of neurotransmission and energy production and induction of steroidogenesis, induced GST, inhibit ChE and LDH, and reduced LPO levels among others (Quintaneiro et al., 2017). Diverse PPP were studied in zebrafish such as Linuron, imidacloprid (Yadav et al., 2020; Guerra et al., 2021), diuron, triazoles pesticides (difenoconazole, tebuconazole, epoxiconazole, hexaconazole and penconazole: Yu et al., 2015; Altenhofen et al., 2017; Wang et al., 2017; Teng et al., 2018, 2018; Jia et al., 2019, 2020), metalaxyl, Chlothalonil, Quizalofop, linuron, ametryn, acetamiprid, diazinon, pyriproxyfen, boscalid, azoxystrobin, flutolanil, chlorothalonil and dinotefuran. In general, these PPP induced acute and sublethal toxicity effects on zebrafish, decreasing enzymes related to oxidative stress, altered gene expression joined to enzymatic activity.

It is important to highlight PPP studies with zebrafish exposure to a more recent class of insecticide, spirocyclic tetramic acids (spirodiclofen, spiromesifen and spirotetramat) (Wu et al., 2018; Zhang et al., 2019). These authors reported effects on enzymes activities (CAT, SOD ACC, LPL), fatty acid synthesis (FAS), mRNA levels of the genes related to lipid metabolism.

Conclusion:

A large number of studies evaluate the effects of distinct plant protection products (PPPs) classes, such as herbicides, fungicides, insecticides and fungicides, on Zebrafish (*Danio rerio*). In general, these PPPs can induce acute and sub-lethal toxicity effects on Zebrafish, altering the activities of the antioxidant enzymes, energy metabolism, exert neurotoxicit and lipoperoxidative damage and induced alteration in the gene expression levels such as, vitellogenin expression. Among the PPPs, the herbicides, glyphosate, atrazine and the insecticide, dieldrin were the most tested. The data regarding glyphosate and atrazine were not consistent among studies (negative effects vs. no effects). It is important to highlight that mixture of PPPs were rarely addressed (2) yet reporting synergistic effects (certain genes expression exerted greater variations upon exposure to mixtures compared to the individual compounds), emphasizing the need of considering mixtures of PPPs and their combination with natural stressor (complex scenarios) in predictive risk assessment.

Eisenia* and *Andrei

Author name		Claudia de Lima e Silva, PhD
Topic description (see Table)		Terrestrial Ecosystem
Search	Used filter 1:	ecosystem
	Used filter 2:	none
	Complete search strategy	andrei; soil (species *Eisenia foetida/fetida – Anellida; earthworm,* which is one of the model species for testing) eisenia; soil (genre of the species *Eisenia foetida/fetida – Anellida; earthworm,* which is one of the model species for testing) Observation: the search with the keywords – andrei; soil lead to 100% of the findings already included in he search using the keywords – eisenia; soil
Selection	Total number before/after TiAb screening	N=154/13
	Reason for exclusion 1	Different topic, e.g. vermicomposting (N=10)
	Reason for exclusion 2	Not enough data on abstract (please check Comparator) (N=10)
	Reason for exclusion 3	Feeding experiments (N=1)
	Reason for exclusion 4	Already added to the list from a different keyword search (Folsomia candida, Hypoaspis aculeifer)
Description	Type of research	Field studies and experimental (lab study),Insecticide, Fungicide, Herbicide, Acaricide Bactericide, Larvicide, Molluscide, Rodenticide, different endpoints
	Population	andrei; soil eisenia; soil
	Exposure	Exposure was quantified in days in soil or filter paper. The quantification of the exposure (endpoints) are on Outcome row.
	Comparator	Availability of information abstract, since this is the piece of information that we are analysing. Therefore if the abstract doesn't provide the following: endpoint analysed, outcome, concentrations of the pesticides and concentrations that caused effect on the studied species, type of substrate, name of the species and not only the taxonomic group, duration of exposure (e.g.), the paper is excluded, since without this information is not possible to be sure that the authors followed standardized (not all the times guidelined) procedures when performing the test. All the tests performed in laboratory using soil invertebrates use control as a comparator.

		Filed tests are each designed individually, and most of the times, the comparator is related to data present on the scientific literature.
	Outcome	bait lamina reproduction/survival toxicity bioaccumulation reproduction cocoon hatching cocoon production survival DNA damage GSH AChE, CES, CAT, GST MXR avoidance lethality and reproduction
Recommendation	Future needs	Use of ecological endpoints such as bait lamina, burrowing and others
	Full systematic review	Yes, based on SOLES database
	Your interest	I have worked since 2015 with earthworms
References	CSV-format and Endnote-format	Csv-format

Text proposal (100-300 words):
Only one paper (Niemeyer J. C.de Santo F. B.Guerra N.Ricardo Filho A. M.Pech T. M., 2018) performed a multi species testing with *E. andrei.* The most tested endpoint was survival and reproduction.
None of the papers performed a field test with the species. This is comprehensive since this species is no found on natural soils. In order to have more realism on tests performed with earthworms, different species, such as Lumbricus rubellus and others, that are found on natural soil, should be used as model species.
Two papers tested mixture toxicity using E. andrei (de Santo F. B.Guerra N.Vianna M. S.Torres J. P. M.Marchioro C. A.Niemeyer J. C., 2019 and StepiÄ‡ S.Hackenberger B. K.Velki M.Hackenberger D. K.LonÄariÄ‡ Z.2013). The mixtures were the following: Metsulfuron-methyl+mineral oil, endosulfan+metolachor and temephos+metolachor.
The pesticides used were insecticides, herbicides and fungicides, however, not many papers tested fungicides, when comparing with the other tested pesticides. This is a gap that can be explored further.
Insecticides: imidacloprid, thiacloprid, Actara, Titanium, clothianidin, Confidor, Calypso, acetamiprid, Mospilan, thiamethoxam, Epik, chlorpyrifos -comm. Form., endosulfan-comm. Form., Pentachlorophenol, parathion, pentachlorophenol, azinphos-methyl, pirimiphos-methyl, pyrethroid, deltamethrin, fipronil, endosulfan, temephos, dimethoate, clothianidin - commercial formulation.
Fungicides: hexachlorobenzene, fentin, benomyl, carbendazim, carboxin+thiran
Herbicides: Roundup, Original Trop, Zapp, Qi622, Crucial, Metsulfuron-methyl, Metsulfuron-methyl+mineral oil, Glyphosate-comm. Form., paraquat, phenmedipham, Roundup FG, Mon 8750, metolachlor, Diuron, fluazifop-p-butyl.

Fimetaria

Author name		Claudia de Lima e Silva, PhD
Topic description (see Table)		Terrestrial Ecosystem
Search	Used filter 1:	ecosystem
	Used filter 2:	none
	Complete search strategy	fimetaria; soil (genre of the species *Folsomia fimetaria – Collembola; springtail,* which is one of the model species for testing)
Selection	Total number before/after TiAb screening	N=2/1
	Reason for exclusion 1	Not clear data
Description	Type of research	Field studies and experimental (lab study),Insecticide, Fungicide, Herbicide, Acaricide Bactericide, Larvicide, Molluscide, Rodenticide, different endpoints
	Population	fimetaria; soil
	Exposure	Exposure was quantified in days in soil. The quantification of the exposure (endpoints) are on Outcome row.
	Comparator	Availability of information abstract, since this is the piece of information that we are analysing. Therefore if the abstract doesn't provide the following: endpoint analysed, outcome, concentrations of the pesticides and concentrations that caused effect on the studied species, type of substrate, name of the species and not only the taxonomic group, duration of exposure (e.g.), the paper is excluded, since without this information is not possible to be sure that the authors followed standardized (not all the times guidelined) procedures when performing the test. All the tests performed in laboratory using soil invertebrates use control as a comparator. Filed tests are each designed individually, and most of the times, the comparator is related to data present on the scientific literature.
	Outcome	Lethality and reproduction
Recommendation	Future needs	Other tests such as multigeneration tests, decomposition tests, and bioaccumulation tests are needed
	Full systematic review	Yes, based on SOLES database
	Your interest	
References	CSV-format and Endnote-format	Csv-format
Text proposal (100-300 words):		

None of the papers performed a field test with the species. In this search only two papers were found (Schnug et al., 2014 and de Lima E. Silva C. et al., 2021). No multispecies, nor mixture toxicity tests were performed with this species.
Folsomia fimetaria is a sexually reproducing euedaphic species, found in natural soils in temperate regions. It is an important species, since there is the sexual component, that enables to test the genders, and infer the impact it might have on the population density in the field.
The insecticides used were: imidacloprid and thiacloprid.

Foetida

Author name		Claudia de Lima e Silva, PhD
Topic description (see Table)		Terrestrial Ecosystem
Search	Used filter 1:	ecosystem
	Used filter 2:	none
	Complete search strategy	foetida; soil (genre of the species *Eisenia foetida/fetida – Anellida; earthworm,* which is one of the model species for testing)
Selection	Total number before/after TiAb screening	19/5
	Reason for exclusion 1	Different topic, e.g. vermicomposting (N=2)
	Reason for exclusion 2	Not enough information on the abstract concerning the name of the pesticide or concentration, or exposure time
	Reason for exclusion 3	Acute toxicity test – this type of test is not used as a guidelined test. (I will add the reference for the synopsis)
Description	Type of research	Field studies and experimental (lab study),Insecticide, Fungicide, Herbicide, Acaricide Bactericide, Larvicide, Molluscide, Rodenticide, different endpoints
	Population	foetida; soil
	Exposure	Exposure was quantified in days in soil or filter paper. The quantification of the exposure (endpoints) are on Outcome row.
	Comparator	Availability of information abstract, since this is the piece of information that we are analysing. Therefore if the abstract doesn't provide the following: endpoint analysed, outcome, concentrations of the pesticides and concentrations that caused effect on the studied species, type of substrate, name of the species and not only the taxonomic group, duration of exposure (e.g.), the paper is excluded, since without this information is not possible to be sure that the authors followed standardized (not all the times guidelined) procedures when performing the test. All the tests performed in laboratory using soil invertebrates use control as a comparator. Filed tests are each designed individually, and most of the times, the comparator is related to data present on the scientific literature.
	Outcome	damage to lipid membranes; DNA damage; production of protein carbonyls; toxicity; bioaccumulation; cellular toxicity

Recommendation	Future needs	Use of different endpoints such as survival and reproduction
	Full systematic review	Yes, based on SOLES database
	Your interest	I have worked since 2015 with earthworms
References	CSV-format and Endnote-format	Csv-format

Text proposal (100-300 words):

None of the papers performed a field test with the species, and none of them performed a multispecies test. Reasons for that can be the degree of complexity for a field test using earthworms, and the number of factors that can influence the outcome; space. A multigeneration test using earthworm is a long experiment, nonetheless, there is a gap on the scientific literature concerning this type of data. Tests where the species is exposed to the compound, and the next generation is allowed to recover, and bioaccumulation and other endpoints are measured are also not present in the literature.

In all of the tests performed, none of them used multi toxicity approach, which leaves a gap. The multi toxicity approach is important, mainly when using earthworms, because this is the reality they encounter in the agricultural fields.

The pesticides used were insecticides and fungicides, with no tests assessing toxicity of herbicides or others.

Besides the gaps in the scientific literature there is much debate on the difference between the species: Eisenia andrei and Eisenia foetida, with some researchers (in person communication) indicating that only a molecular test can really distinguish between both. In Europe, most of the tests use Eisenia andrei, also known as compost worm.

Insecticides: deltamethrin; DDT; DDD; DDE; imidacloprid and RH-5849.

Fungicides: triadimefon; difenoconazole; propiconazole and hexachlorobenzene.

Folsomia

Author name		Claudia de Lima e Silva, PhD
Topic description (see Table)		Terrestrial Ecosystem
Search	Used filter 1:	ecosystem
	Used filter 2:	none
	Complete search strategy	folsomia; soil (genre of the species *Folsomia candida – Collembola; springtail,* which is one of the model species for testing)
Selection	Total number before/ after TiAb screening	N=47/24 Observation: 3 papers for F. candida were added from previous keyword searches (see below) that tested multiple species
	Reason for exclusion 1	Already in the database due to other searches using different keywords, e.g. hypoaspis and minuta. N=1
	Reason for exclusion 2	Not related to the topic, since it works with other topics: pore water exposure and mineralisation. N=3
	Reason for exclusion 3	Not enough information in the abstract. N=20
Description	Type of research	Field studies and experimental (lab study),Insecticide, Fungicide, Herbicide, Acaricide Bactericide, Larvicide, Molluscide, Rodenticide, different endpoints
	Population	folsomia; soil
	Exposure	Exposure was quantified in days in soil. The quantification of the exposure (endpoints) are on Outcome row.
	Comparator	Availability of information abstract, since this is the piece of information that we are analysing. Therefore if the abstract doesn't provide the following: endpoint analysed, outcome, concentrations of the pesticides and concentrations that caused effect on the studied species, type of substrate, name of the species and not only the taxonomic group, duration of exposure (e.g.), the paper is excluded, since without this information is not possible to be sure that the authors followed standardized (not all the times guidelined) procedures when performing the test. All the tests performed in laboratory using soil invertebrates use control as a comparator. Filed tests are each designed individually, and most of the times, the comparator is related to data present on the scientific literature.
	Outcome	Lethality and reproduction; avoidance; reproduction/survival; toxicity;

		reproduction/survival at different temperatures; gene expression; GST; TER and reproduction
Recommendation	Future needs	Other tests such as multigeneration tests, decomposition tests, and bioaccumulation tests are needed
	Full systematic review	Yes, based on SOLES database
	Your interest	I am a specialist in Collembola
References	CSV-format and Endnote-format	Csv-format

Text proposal (100-300 words):
None of the papers performed a field test with the species. In this search the species *Folsomia fimetaria* and *Folsomia quadrioculata* were also included, due to the keyword folsomia. However, only *Folsomia fimetaria* is used as an **official** surrogate. One author performed a multi species test with different herbicides (Niemeyer J. C.de Santo F. B.Guerra N.Ricardo Filho A. M.Pech T. M. 2018). Two papers tested mixture toxicity using the following mixtures: Kraft+Score in different temperatures and captan+tiran (Pitombeira de FigueirÃªdo L.Athayde D. B.Daam M. A.van Gestel C. A. M.Guerra G. D. S.Duarte-Neto P. J.EspÃ-ndola E. L. G., 2021// Bandow C.Coors A.Karau N.Rombke J., 2014).
Most of the papers tested insecticides, and other pesticides were Acaricidae, Fungicides and Herbicides.
Insecticides: chlorantraniliprole (CAP); imidacloprid; thiacloprid; Actara; Titanium; clothianidin; Dimethoate; deltamethrin; clorpyrifos; clorpyrifos; Kraft; Confidor; Calypso; Acetamiprid; Mospilan; thiamethoxam; Epik; lindane; chlorpyrifos-methil; fipronil; methoprene; fenoxycarb Teflubenzuron; precocene II; tebufenozide; hexaflumuron; endosulfan-comm. Form.; chlorpyrifos -comm. Form.; clothianidin - commercial formulation
Acaricidae: Spirodiclofen
Fungicides: Score; Kraft+Score; Bravo (500); clorothalonil; captan and captan+tiran
Herbicides: Roundup; Original Trop; Zapp; Qi621; Crucial; Montana; Glyphosate; diuron; Glyphosate-comm. Form.
Fungicide: Score
Observation: a different search using the keywords candida and soil, led to 48 papers, out of which, 46 were already analysed, and the new papers were dealing with a different taxonomic group.
Folsomia candida is a good model organism and is used for Tier 1 ecotoxicology testing of pesticides. However, most of the tests are focusing on survival and reproduction, leaving a gap on the effect on population, such as multigeneration tests. Moreover, there is a gap also concerning the exposure to mixture toxicity using this species.
This species is considered a tramp species, not natural from many types of soils, despite being used as a model organism. In order to bring more realism in testing, other collembolans, such as Sinella curviseta, Heteromurus nitidus, Orchesella cincta could be used as model species.

Hipoaspis

Author name		Claudia de Lima e Silva, PhD
Topic description (see Table)		Terrestrial Ecosystem
Search	Used filter 1:	ecosystem
	Used filter 2:	none
	Complete search strategy	hypoaspis; soil (genre of the species *Hypoaspis aculeifer*, mites, which is one of the model species for testing)
Selection	Total number before /after TiAb screening	7/6
	Reason for exclusion 1	Theoretical paper with no toxicity data (N=1)
Description	Type of research	Field studies and experimental (lab study),Insecticide, Fungicide, Herbicide, Acaricide Bactericide, Larvicide, Molluscide, Rodenticide, different endpoints
	Population	hypoaspis; soil
	Exposure	Exposure was quantified in days in soil, and the amount of food eaten. The quantification of the exposure (endpoints) are on Outcome row.
	Comparator	Availability of information abstract, since this is the piece of information that we are analysing. Therefore, if the abstract doesn't provide the following: endpoint analysed, outcome, concentrations of the pesticides and concentrations that caused effect on the studied species, type of substrate, name of the species and not only the taxonomic group, duration of exposure (e.g.), the paper is excluded, since without this information is not possible to be sure that the authors followed standardized (not all the times guidelined) procedures when performing the test. All the tests performed in laboratory using soil invertebrates use control as a comparator. Filed tests are each designed individually, and most of the times, the comparator is related to data present on the scientific literature.
	Outcome	avoidance/reproduction; Lethality and reproduction at different temperatures; toxicity
Recommendation	Future needs	Test more mixture toxicity with this species, but also, test using food web as an endpoint, in order to see how the impact on these predators can impact the biological pest control in the fields
	Full systematic review	Yes, based on SOLES database
	Your interest	
References	CSV-format and Endnote-format	Csv-format
Text proposal (100-300 words):		

None of the papers performed a field test with the species, or a multispecies, neither a mixture toxicity. A field test using this species would be too much complicated, and it would be advisable to perform mesocosm tests, or multi species test systems in the lab in order to understand better the impact of toxicants on this species.
Few tests were devoted only to assess the toxicity to the species – *Hypoaspis aculeifer*, which leaves a gap on the impact of pesticides on this species.
The following pesticides were used:
Insecticides: deltamethrin; dimethoate; Kraft
Fungicide: Score
Observation: a different search using the keywords aculeifer and soil, led to the same result as hypoaspis and soil.

Enzyme activity of microorganisms

Author name		Philipp Mäder
Topic description (see Table)		Microoganisms – Enzyme activity
Search	Used filter 1:	Ecosystem
	Used filter 2:	n/a
	Complete search strategy	#1 (soil.*, microorganism.*, microb.*, bacter.*, fung.*) AND #2 (enzym.*, activit.*)
Selection	Total number before/after TiAb screening	21/13
	Reason for exclusion 1	Other outcome reported (2)
	Reason for exclusion 2	Not PPP related (1)
	Reason for exclusion 3	Only active ingredient tested or novel PPP (3)
	Reason for exclusion 4	Only selected microorganisms studied (2)
Description	Type of research	Laboratory studies (11), Field studies (2)
	Population	Soil microorganisms
	Exposure	Direct application to soil (11), application to plants (1), seed dressing (1)
	Comparator	Soil without PPP treatment
	Outcome	General inhibition or suppression of enzymatic activity with different impact dependent on PPP concentrations and enzymes.
Recommendation	Future needs	Repeated applications (only performed in 1)
	Full systematic review	No
	Your interest	Not applicable
References	CSV-format and Endnote-format	enzyme activity.txt and enzyme activity.csv

Most studies were performed in the laboratory on agricultural soils. Dehydrogenase (11) and urease (10) were the most common enzymes analysed followed by acid and alkaline phosphatase (8), arylsulfatase (5) and β-glucosidase (5). Other enzymes were catalase (3), amylase (2), invertase (2) and diacetate hydrolase (2). Four out of 13 studies used multiple different PPP with one study applying them simultaneously. The other studies each focused on a single PPP. Next to single doses (4), multiple (between 2 and 7 different) often scaling concentrations (up to 300-fold) were used (9). Three publications used the recommended dose for the pesticide. The main outcome reported in the majority of publications was the suppression of enzymatic activity (Tomkiel et al., 2019) or negative effects on the enzyme activity by soil contaminants (Mahapatra et al., 2017). Two studies reported that dehydrogenase was more tolerant to pesticide addition (Mahapatra et al., 2017, Baćmaga et al., 2016). Other studies reported variable effects dependent on the enzyme (Baćmaga et al., 2018; Wu et al., 2016) or no clear impact at the tested concentration (Tu, 1992 & 1995). This was also reported when the pesticide was administered according to the recommended dose (Borowik et al., 2017) whereas dosages higher than the recommended rate influenced the enzyme activity (Baćmaga et al., 2016). Dosage correlated well with the impact of the pesticide (Borowik et al., 2017) and similarly multiple simultaneous applications had the strongest inhibitory effect on enzyme activity (Borowik et al., 2017; Baćmaga et al., 2019).

Conclusion

In 13 included studies research on the effects of pesticides on enzyme activities of microorganisms were reported. Soil contaminants such as pesticides alter the enzyme activity, most often in a negative way by suppressing specific enzyme activities, with different magnitudes of the effect dependent on the kind of enzyme. The dosage plays an important role but also application at the field rate seems to induce an effect. Important enzymes such as urease and phosphatases are already involved in almost every study, similarly β-glucosidase needs more studies. In combination with the enzymatic activity, it would be interesting to investigate linked aspect such as litter decomposition. The number of studies on multiple simultaneously applied pesticides is still limited, as is the research on the effect of repeated applications on enzyme activity.

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Functional genes of microorganisms

Author name		Philipp Mäder
Topic description (see Table)		Microoganisms – Functional genes
Search	Used filter 1:	Ecosystem
	Used filter 2:	n/a
	Complete search strategy	#1 (soil.*, microorganism.*) AND #2 (functional gene.* OR gene)
Selection	Total number before/after TiAb screening	30/7
	Reason for exclusion 1	Single strain or model organism (8)
	Reason for exclusion 2	Bioremediation (3)
	Reason for exclusion 3	Other outcome (6)
	Reason for exclusion 4	No functional genes involved (6)
Description	Type of research	Laboratory (6), Laboratory and field (1)
	Population	Soil microorganisms
	Exposure	Direct application (7)
	Comparator	Untreated soil
	Outcome	Functional genes can be used as proxy for degradation potential although changes might only be observed in higher concentrations. Some pesticides have a negative effect on functional genes involved in soil functions.
Recommendation	Future needs	Analysis of functional genes in soil health related studies to improve and understand the behaviour of functional genes in the presence/absence of pesticides
	Full systematic review	No
	Your interest	Not applicable
References	CSV-format and Endnote-format	Functional_genes.csv

Five included studies reported on functional genes that are involved in the degradation pathways of pesticides and two studies had a focus on the effect of pesticides on genes involved in N cycling in soils.

Genes involved in the degradation pathway of atrazine were atzA (Shapir et al., 2000) as well as atz and trz genes (Douglass et al., 2017). Douglass et al. (2017) detected multiple combinations of genes in the atrazine degradation with each containing at least atzBC, but no complete set of atz genes. They concluded that several microorganisms must be involved for atrazine degradation as well as cooperative pathways for metabolites. In a more health related study, Shapir et al. (2000) found that the mineralization of atrazine soil with no previous atrazine history expressed longer lag phases (> 17 d) compared to soils in which atzA gene copies were already present (4-5 d).
The gene thiO was identified as a proxy for glyphosate degradation (Hernández et al., 2021) and tfdA as well as cadA genes were used for MCPA degradation (Ditterich et al., 2013, Wirsching et al., 2020). Soil with no previous history of glyphosate use had an increasing expression of the thiO gene after application, while in soils with a glyphosate use history the gene abundance decreased (Hernández et al., 2021). Nevertheless, the amount of thiO copies was positively correlated to the

amount of glyphosate degraded, leading to the conclusion that this gene can be used as a proxy (Hernández et al., 2021). On the other hand, Ditterich et al. (2013) found that total tfdA gene abundance could not reflect the MCPA degradation potential.
In N cycling involved genes were e.g. amoA, nosZ (Teng et al., 2018) or nifH (Sim et al., 2021). The abundance of amoA genes decreased significantly compared to the control after application of chlorothalonil, with discrepancies for other genes involved in soil denitrification (Teng et al. 2018). Similarly, Sim et al. (2021) found a significant effect of two pesticides on the amoA gene abundance.

Conclusion

Seven studies were included for research on the role of functional genes for the effects of pesticides. This field of research is still relatively new and not much used, more studies need to be conducted to validate the use of functional genes as a proxy for microbial pesticide degradation. The available studies showed good correlations and potential for some of the functional genes and therefore more studies are needed to confirm these preliminary findings. Further knowledge about the behaviour of the functional genes is necessary for example to monitor how repeated applications change the gene expression or how it changes when a pesticide is reintroduced after a period of non-use.

Microcosm studies

Author name		Paula Tourinho
Topic description (see Table)		Microcosm
Search	Used filter 1:	Ecosystem
	Used filter 2:	
	Number of studies before/after TiAb screening	(microcosm.* OR mesocosm.* OR terrestrial model ecosystem OR soil core.*) AND (soil OR terrestr.*)
Selection	Number of studies before/after TiAb screening	276/58
	Reason for exclusion 1	Other outcome reported (180)
	Reason for exclusion 2	Aquatic studies (29)
	Reason for exclusion 3	Pest management (5)
	Reason for exclusion 4	Non-primary study (2)
	Reason for exclusion 5	Not microcosm study (2)
Description	Type of research	Micro and mesocosms studies in the lab and field.
	Population	Microorganisms, soil invertebrates, terrestrial plants.
	Exposure	Soil or litter spiked or sprayed with PPP in the lab, sprayed PPP in the field, seed dressing treatment.
	Comparator	Control treatments (non-spiked soils), uncontaminated field soils.
	Outcome	Main PPP effects were observed in microorganism community and microbial activity. The number and selection of species also affect the results (e.g., the presence of invertebrates affecting plant biomass or organic matter decomposition).
Recommendation	Future needs	More studies on mixture toxicity of PPP, and use of multi-species tests
	Full systematic review	Yes
	Your interest	No systematic review on this topic was found in the literature
References	CSV-format and Endnote-format	microcosms.csv

Included studies

The majority of micro- and mesocosms studies (27) assessed the effects of PPP on microorganisms alone. The effects on microorganisms in exposures containing other organisms were less frequent. Three studies assessed the effects of microorganisms and plants, and other 3 studies in combination with soil invertebrates. Only 2 studies assessed the effects on microorganisms, plants, and invertebrates together. The joint exposure of invertebrates and plants to PPP were observed in 15 studies, and 2 studies evaluated the effects on 2 or more invertebrate species. The number of single species studies of invertebrates (earthworm) and plants were 5 and 4, respectively. The endpoints analyzed in microorganisms were microbial activity, as carbon and nitrogen mineralization, quantification of soil enzymes, and decomposition activity. Microbial community composition and abundance was also a frequently reported endpoint. In soil invertebrates, survival, biomass, and reproduction were the most assessed endpoints. In

terrestrial plants, the uptake of PPP is vastly observed, while growth and germination were less assessed. The fungicide carbendazim was the most studied PPP with 8 studies, all belonging to a group of papers from the same project. The insecticide imidacloprid and the herbicide glyphosate followed appearing in 7 and 6 studies, respectively. The herbicide atrazine and conzole fungicides were found in 5 microcosm studies each. Only 8 out of 58 studies have assessed the joint effects of PPP. The majority of the studies were conducted under lab-controlled conditions. These were especially experiments assessing the effects on microorganisms in dark condition. Eight studies with carbendazim were tested in a field validation experiment. Only 5 studies were conducted in semi-field and field conditions.

Observed effects of PPP in microcosms

The effects of PPP to organisms at low concentrations were observed in a number of micro and mesocosm studies. The LC50 and EC50 (egg production) for imidacloprid in ladybird beetle *Coccinella septempunctata* was 4 and 26 g/ha, respectively (Yu et al., 2014). These values are lower than recommended application doses, and therefore, the authors conclude that this PPP poses a risk to *C. septempunctata*. Also, imidacloprid showed high toxicity towards earthworms with LC50 ranging from 5-25 mg/kg, while no effects on microbial decomposition was observed up to 1400 mg/kg (Kreutzweise et al., 2008).

The effects of carbendazim were observed in several species. Species abundance composition was affected with EC50 of 0.1-0.5, 0.9, and 2 kg/ha for enchytraeids, nematodes, and earthworms, respectively (Moser et al., 2004a, 2004b; Moser et al., 2007; Rombke et al., 2004). Earthworm biomass was the most sensitive endpoint in two studies, with EC50 of 1.3-1.9 mg/kg (Burrows et al., 2014; Rombke et al., 2004). The EC50 values for the effect of carbendazim on bait-lamina consumption ranged between 2.0 and 56 kg/ha. (Forster et al., 2004).

The fungicide pyrimethanil also affected enchytraeid abundance, with an EC50 of 3.48 mg/kg (Bandow et al., 2016).

Glyphosate applied at 10% of field application (to simulate spray drift) changed plant community and decreased the biomass of non-target plants in an outdoor mesocosms (Watrud et al., 2011)

Effects on microbial activity and community were less observed, and in fact, the recovery by microorganisms was observed in many studies. The most common effect was a shift in species composition.

Value of microcosm approach

Microcosms studies aim at providing a more realistic exposure than single-species lab experiments. For example, a higher mineralization of atrazine occurs in a microcosms containing terrestrial plants compared to atrazine mineralization in a microcosm without plants (Bicalho and Langenbach, 2012). Dalton et al. (2010) observed higher sensitive in plant growth to the exposure to glyphosate and atrazine in microcosms than in lab experiments. The comparison of microcosm studies with field experiments, however, has given divergent outcomes, with studies finding a good relation between microcosm and field results (Sousa et al., 2004; Burrow et al., 2004), while other studies have found different results (Storck et al., 2018).

Overall, there is a lack on microcosm studies integrating a high number of species of different trophic levels. The majority of studies have focused on assessing the effects of PPPs to microorganisms, which might be affected only at high concentrations, be stimulated, or recover with exposure time. Carbendazim and imidacloprid were linked to toxic effects in soil invertebrates at relative low exposure concentrations in 8 studies.

Conclusion

In 58 included studies a microcosm or mesocosm approach was used. It provides a scaled experimental setting to bridge controlled lab studies and field testing. This type of studies provides more realistic exposure conditions compared to lab studies. In approximately half of the studies microorganisms were assayed in isolation, whereas other studies used combined settings with other organisms. Carbendazim and imidacloprid were most studied and resulted in the lowest effect concentrations expressed as effect concentration corresponding to the concentration with an observed effect in 50% of the population (EC50). In a similar way for a lethal outcome LC50 is used. For imidacloprid the lowest reported LC50 was 4 g/ha and for carbendazim this value was 0.1 g/h (higher values were reported in other studies). Other PPPs studied showed effects on microorganisms in a micro or mesocosm study were the currently used herbicides glyphosate, atrazine, and the fungicides conzole and pyrimethanil. More studies are needed to explain difference in results of effect of PPP between microcosm and field experiments. There is also a need to study PPP mixtures in this type of experiment set-up.

Plants

Author name		Paula Tourinho
Topic description (see Table)		Plants
Search	Used filter 1:	Ecosystem
	Used filter 2:	-
	Complete search strategy	(terrestrial plant OR non-target terrestrial plant OR lactuca OR allium OR brassica OR avena OR zea OR lolium) AND (phytotox.* OR biomass OR growth OR uptake)
Selection	Number of studies before/after TiAb screening	475/112
	Reason for exclusion 1	Other outcome reported (172)
	Reason for exclusion 2	Pest management studies (109)
	Reason for exclusion 3	Effects on other organisms (55)
	Reason for exclusion 4	Not PPP related (25)
	Reason for exclusion 5	No access (2)
Description	Type of research	Lab (103) and field (11) studies
	Population	Non-target terrestrial plants
	Exposure	Soil, water, and seed treatment
	Comparator	Control treatments
	Outcome	Effects of PPP dependent on endpoint, concentration, exposure route, and species
Recommendation	Future needs	Lack of studies following the standard protocols and/or recommended by EFSA
	Full systematic review	No
	Your interest	Not applicable
References	CSV-format and Endnote-format	plants.csv

A total of 114 studies were selected. These studies were examined in terms of exposure, PPP, plant species, and endpoints.

Exposure

A total of 42 experiments were conducted using aqueous exposure of PPP to plants. These included hydroponic studies, in vitro studies, exposure to elutriates/wastewater, and root immersion tests. Soil and other terrestrial via of exposure (e.g., sand quartz, composts, perlite) were used in 37 experiments. In these experiments, the PPP as aqueous solution were added to the soil matrix or sprayed to simulate a pre-emergence application. Foliar exposure was found in 16 experiments, as spray as a post-emergence treatment, but in 3 studies using as drop application. Treated seeds were used in 13 experiments. Three spray drift studies were found with glyphosate (2) and mecocrop (1).

PPP

A total of 73 compounds were found. The most studied PPP class was herbicides, followed by insecticides and fungicides. Atrazine (20) was the most studied PPP with studies ranging from 1991 to 2021. Even though atrazine is not approved in EU since 2000's, the high number of studies is a reflection of its continued use worldwide. Glyphosate (13) and imidacloprid (10) were the

second and third most studied PPP, respectively. Only few studies assessed the effects of PPP mixtures on plants.

Species

Maize (*Zea mays*) was the plant species most studied (42), followed by onion (*Allium cepa*) (14, majority were genotoxicity studies), and lettuce (*Lactuca sativa*) (8) experiments. The genus brassica also had a good representation, especially *Brassica rapa* (9), *Brassica juncea* (9), *Brassica campestris* (6), and *Brassica napus* (5).

Endpoints

Plant growth, analysed as root and shoot weight/height, was found in 54 studies, while germination was assessed in 17 experiments. The uptake of PPP was assessed in 34 studies, with translocation (17), detoxification (2) and metabolite concentration (2) observed in combination with PPP uptake. Studies on oxidative stress (LPO, other) and genotoxicity studies (gene expression, comet assay) were assessed in 17 and 15 experiments, respectively. The effects of PPP in pigments, especially chlorophyll, was found in 17 experiments. Other endpoints were gas exchange, shikimate level, mitotic index, cell viability. Crop yield was assessed in 3 out of 7 field studies.

Conclusion

Exposure concentrations in the range of recommended field dose were applied in many studies of soil exposure and/or foliar spray application. However, toxicity is normally not observed at these doses. Four studies observed toxic effects at recommended field dose: atrazine and difeconazole decreased growth in *Z. mays* and *A. cepa*, respectively, and metazachlor decreased the growth of *B. napus* in two other studies. Only in two field studies toxicity was observed when applied at recommended field doses of glyphosate (Reddy et al., 2010) and pendimethalin (Bandyopadhyay and Choudhury, 2009).

It is also important to highlight that most of the soil studies did not follow the standard protocols (OECD, ISO, USEPA), and only one study (Duffner et al., 2020) used the number of species and tests recommended by EFSA. Moreover, there is a lack of studies using multiple species, as the majority used only one plant species. There is also a lack of mixture toxicity studies. Only 8 out of 112 studies assessed the effects of PPP mixtures. A weakness in this research topic is that most papers do not mention the recommended field doses of the active substances, and therefore it is difficult to know if their concentrations are within the range of field doses.

Overall, there is evidence that PPP exposure affect non-target terrestrial plants, especially herbicides (atrazine, metazachlor, glyphosate, and pendimethalin). However, the effects must be assessed case by case, considering the PPP, plant species, and the exposure method used.

Proisotoma minuta

Author name		Claudia de Lima e Silva, PhD
Topic description (see Table)		Terrestrial Ecosystem
Search	Used filter 1:	ecosystem
	Used filter 2:	none
	Complete search strategy	minuta (genre of the species *Proisotoma minuta – Collembola; springtail,* which is one of the model species for testing); soil
Selection	Total number before/after TiAb screening	4/2
	Reason for exclusion 1	The authors used sea salt solution to dilute the herbicides, which is not really adequate, and not the purpose of our study, since there will be no experiments using sea water.
	Reason for exclusion 2	Not enough evidence related to the presence of pesticides in the field (no concentrations were presented) and the abundance of *P. minuta* in the cotton fields.
	Reason for exclusion 3	-
Description	Type of research	Field studies and experimental (lab study),Insecticide, Fungicide, Herbicide, Acaricide Bactericide, Larvicide, Molluscide, Rodenticide, different endpoints
	Population	minuta; soil
	Exposure	Exposure was quantified in days in soil. The quantification of the exposure (endpoints) are on Outcome row.
	Comparator	Availability of information abstract, since this is the piece of information that we are analysing. Therefore if the abstract doesn't provide the following: endpoint analysed, outcome, concentrations of the pesticides and concentrations that caused effect on the studied species, type of substrate, name of the species and not only the taxonomic group, duration of exposure (e.g.), the paper is excluded, since without this information is not possible to be sure that the authors followed standardized (not all the times guidelined) procedures when performing the test. All the tests performed in laboratory using soil invertebrates use control as a comparator. Filed tests are each designed individually, and most of the times, the comparator is related to data present on the scientific literature.
	Outcome	Toxicity that can mean impact on reproduction and survival

Recommendation	Future needs	Use more this species for testing since it is found in natural and agricultural soils as opposed to *F. candida*
	Full systematic review	Yes, based on SOLES database
	Your interest	I am a specialist in Collembola
References	CSV-format and Endnote-format	Csv-format

Text proposal (100-300 words):
Out of 4 papers, only 2 of them could be used. Within the 2 papers used, other species were also tested: *E. andrei, F. candida, S. curviseta* and *E. crypticus*. The following authors (Bandeira F. O.Alves P. R. L.Hennig T. B.Brancalione J.Nogueira D. J.Matias W. G., 2021) were also used for P. minuta, however they were selected for S. curviseta primarily, but since they also tested P. minuta they were included in this report too. In their paper, they have tested the toxicity og the insecticide clothianidin to the reproduction of P. minuta, but also to other species of soil invertebrates (Eisenia andrei, Enchytraeus crypticus, Folsomia candida).
The following pesticides were used:
Insecticides: clothianidin (as commercial formulation)
Herbicides: Metsulfuron-methyl and as a mixture Metsulfuron-methyl+mineral oil.
Only one paper tested mixture toxicity.
Observation: a different search using the keywords proisotoma and soil, led to the same result as minuta and soil.
There is a lot of potential for P. minuta testing, since this species is found on agricultural soils in temperate zones. This is one of the species suggested to be used, in the ring test for F. fimetaria (P.H. Krogh. 2008. Toxicity testing with the collembolans Folsomia fimetaria and Folsomia candida and the results of a ringtest. Report), and is more likely to be included in the revision of ISO guideline 11.267. Impact of contaminants on this species might approach from the impact expected in the field, therefore the importance of using more this species, instead of F. candida, for soil ecotoxicity testing.

Typhodromus pyri

Author name		Claudia de Lima e Silva, PhD
Topic description (see Table)		Terrestrial Ecosystem
Search	Used filter 1:	ecosystem
	Used filter 2:	none
	Complete search strategy	Typhodromus; pyri (beneficial arthropod; which is one of the model species for testing)
Selection	Total number before/after TiAb screening	11/7
	Reason for exclusion 1	Not related to PPP
	Reason for exclusion 2	Lack of data on the PPP
	Reason for exclusion 3	Not relevant to the topic
Description	Type of research	Field studies and experimental (lab study),Insecticide, Fungicide, Herbicide, Acaricide Bactericide, Larvicide, Molluscide, Rodenticide, different endpoints
	Population	*Typhodromus pyri*
	Exposure	Exposure was quantified in hours or days in the following substrates: Soil, glass plates, leaves, plants. The quantification of the exposure (endpoints) are on Outcome row.
	Comparator	Availability of information abstract, since this is the piece of information that we are analysing. Therefore if the abstract doesn't provide the following: endpoint analysed, outcome, concentrations of the pesticides and concentrations that caused effect on the studied species, type of substrate, name of the species and not only the taxonomic group, duration of exposure (e.g.), the paper is excluded, since without this information is not possible to be sure that the authors followed standardized (not all the times guidelined) procedures when performing the test. All the tests performed in laboratory using soil invertebrates use control as a comparator. Filed tests are each designed individually, and most of the times, the comparator is related to data present on the scientific literature.
	Outcome	Reproduction, oviposition, avoidance, female mortality, general toxicity, abundance, lethal and sub-lethal effects, population reduction
Recommendation	Future needs	Tests with more pesticides, and more tests
	Full systematic review	Yes, based on SOLES database
	Your interest	Not applicable
References	CSV-format and Endnote-format	Csv-format

Text proposal (100-300 words):

Out of 11 papers, only 7 of them could be used. Within the seven papers used, other species were also tested: *Typhlodromus exhilaratus, T. philatus, P. ulmi, Tetranychus urticae* and *Amblyseius andersoni*.

The following pesticides were used:

Insecticides: tebufenozide, clorpyriphus-ethyl, dichlofluanid 500 g kg(-1) kWP, (Euparen) 50WP), quinoxyfen 250 g L(-1) SCC (Legend), cymoxanil 60, mancozeb 200, folpet + 275 g kg(-1) WP [Remiltine F Pepite) (RFP)], whey powder 25% boscalid + 13% pyraclostrobin (Pristine), myclobutanil (Rally), micronized sulfur (92% WP), 75% ethylene bisdithiocarbamate (mancozeb; Manzate); paraffinic oil (JMS Stylet); chlorpyrifos; thiamethoxam; indoxacarb; flufenoxuron; tebufenozide; chlorpyrifos; thiamethoxam; flufenoxuron;

Fungicides: mancozeb; meptyldinocap; myclobutanil; dimehomorph+mancozed; copper compounds folpet and mancozeb

Acaricidae: esferalate.

Four of the papers used mixed toxicity, with different combinations.

Three papers exposed the species in field conditions.

The endpoints used for assessing toxicity of pesticides to *T. pyris* were related to: i) oviposition (reduction for esferalate); ii) avoidance (also positive for esferalate); iii) toxicity, without especification of the endpoint measured (positive for all the other pesticides tested – list above); iv) reduction of population (mancozeb, meptyldinocap, myclobutanil).

The endpoints measured were not really specified, with not many papers focusing on female toxicity, for example, nor in population dynamics, which are important endpoints when analysing the impact on the agricultural landscape, because it (female and population dynamics) has direct impacts on the foodweb, facilitating or not the appearance of pests, that otherwise would be biologically controlled.

There is a gap in the scientific literature concerning the impact of pesticides in T. pyri, not only in general lines, e.g., toxicity to specific endpoints such as reproduction, but also in an large scale: the impact it may have on the biodiversity in the agricultural landscape.