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Protocol

Are encapsulated pesticides less harmful to human health than their conventional alternatives? Protocol for a systematic review of *in vitro* and *in vivo* animal model studies

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ABSTRACT

Background: The gradual increase in the global population has led to the rising demand for agricultural products worldwide. This required the introduction of environment- and public health-friendly advanced technologies for plant protection to guard yields from pest destruction in a sustainable way. Encapsulation technology is a promising procedure to increase the effectiveness of pesticide active ingredients while reducing human exposure and environmental impact. Despite the presumed favorable properties of encapsulated pesticide formulations on human health, it is necessary to systematically assess whether they are less harmful to human health than conventional pesticide products.

Objectives: We aim to systematically review the literature to answer the question of whether micro- or nano-encapsulated pesticide formulations exert different degrees of toxicity than their conventional (not-encapsulated) counterparts in *in vivo* animal and *in vitro* (human, animal, and bacterial cell) non-target models. The answer is important to estimate the possible differences in the toxicological hazards of the two different types of pesticide formulations. Because our extracted data will come from different models, we also aim to perform subgroup analyses to investigate how toxicity varies across different models. A pooled toxicity effect estimate will also be performed by *meta*-analysis when appropriate.

Methods: The systematic review will follow the guidelines developed by the National Toxicology Program's Office of Health Assessment and Translation (NTP/OHAT). The protocol adheres to the Preferred Reporting Items for Systematic Reviews and meta-analyses Protocol (PRISMA-P) statement. PubMed (NLM), Scopus (Elsevier), Web of Science Core Collection (Clarivate), Embase (Elsevier), and Agricola (EBSCOhost) electronic databases will be comprehensively searched in September 2022 to identify eligible studies using multiple search terms of "pesticide", "encapsulation" and "toxicity" along with their synonyms and other words that are semantically related. The reference lists of all eligible articles and retrieved reviews will be manually screened to identify additional relevant papers.

Eligibility criteria: We will include peer-reviewed experimental (non-target *in vivo* animal model and *in vitro* human, animal, and bacterial cell cultures) studies published as full-text articles in English language that simultaneously investigate the effect of any micro- or nano-encapsulated pesticide formulation, applied in all ranges of concentrations, duration, and routes of exposure, and its corresponding active ingredient(s) or its conventional non-encapsulated product formulation(s) used in the same ranges of concentrations, duration, and routes of exposure on the same pathophysiological outcome. We will exclude studies that examine pesticidal activity on target organisms, cultures of cells isolated from target organisms exposed *in vivo* or *in vitro*, and those using biological materials isolated from target organisms/cells.

Study appraisal and synthesis: Studies identified by the search will be screened and managed according to the review inclusion and exclusion criteria in the Covidence systematic review tool by two reviewers, who will also

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blindly extract the data and assess the risk of bias of included studies. The OHAT risk of bias tool will be applied to evaluate the quality and risk of bias in the included studies. Study findings will be synthesized narratively by important features of the study populations, design, exposure, and endpoints. If findings make it possible, a *meta*-analysis will be performed on identified toxicity outcomes. To rate the certainty in the body of evidence, we will use the Grading of Recommendations Assessment, Development and Evaluation (GRADE) approach.

1. Introduction

1.1. Background

The global population will likely exceed nine billion people by 2050, requiring an estimated 35 % to 56 % more food (van Dijk et al. 2021). The rising demand for agricultural products is one of the key challenges of sustainable food production (Tilman et al. 2011). One of the most important tools of global food security has been the use of agricultural pest management practices, without which crop losses would be on average 30 % higher than that at present (Savary et al. 2012).

Pesticides are chemicals extensively used around the globe to eliminate undesirable living organisms including bacteria, fungi, weeds, snails, insects, rodents, and worms. They are particularly used in agriculture for protecting crop and increase agricultural output, but are also utilized in public health to control vector-borne diseases and to eradicate domestic pests. Pesticides may be classified according to their target species into different groups, such as insecticides, herbicides, nematicides, fungicides, rodenticides, acaricides, molluscicides, repellents, etc. (Yadav and Devi 2017).

Although pesticide products are considered easy to use, cost-effective and reliable tools of maintaining high agricultural productivity, it has become known that excessive and uncontrolled pesticide application has undesirable effects on the ecosystem, on non-target species, including aquatic organisms, amphibians, honeybees or humans (Damalas and Eleftherohorinos 2011; Meka et al. 2021). The spectrum of adverse health effects resulting from exposure to pesticides is extremely wide, reflecting the diverse range of active pesticidal substances. It is well-recognized that certain pesticides can cause acute and chronic side effects in exposed individuals. The adverse effects include diseases of the respiratory system, immunological disorders, central and peripheral nervous system disorders, alterations in the (neuro)endocrine system, metabolic and reproductive dysfunctions, birth defects, or even a wide range of malignant diseases, especially lung, prostate, and colon cancer as well as multiple myeloma (Mostafalou and Abdollahi 2017; Parks et al. 2022; Varghese et al. 2021). Epigenetic changes and oxidative stress are two of the most recently proposed mechanisms of action (Teodoro et al. 2019). Non-dietary exposure to pesticides and consumer exposure through residues in food and beverages is inevitable. For this reason, coupled with the fact that pesticide use is increasing, pesticide exposure continues to pose a significant global public health concern (Kim et al. 2017).

Technological development together with enhanced environmental and public health protection measures in recent decades has set out new directions for pesticide manufacturers to enable the agricultural sector to compete with increasing demand in a sustainable manner (Nicolopoulou-Stamati et al. 2016). There is a continuous need for improving pest management techniques that meets the increasingly rigorous requirements not only for farmers, but also for plant protection regulatory bodies and consumers. One way to fulfill this need has been to develop new pesticide carriers that increase the overall efficiency of pesticide products while reducing their unwanted effects (Singh et al. 2020). Research and development of modern carriers for pesticides are stimulated by the facts that the development and registration of a novel active ingredient are expensive and take a long time to authorize (Whitford et al. 2006). In addition, many pesticide active ingredients are difficult to dissolve in aqueous media, they degrade under UV light even before absorption, or are difficult to get enter plants. Moreover, the European Union has recently tightened its rules on the registration of pesticides by voting in favor of a new licensing strategy inspired by the so-called precautionary principle, which could ultimately ban up to a quarter of pesticide active ingredients on the European market (European Commission, 2022).

The need to develop pesticidal products that are both safe and effective is driving efforts to reformulate pesticides. Controlled release technology is a strategy where the active constituent is chemically attached to or physically incorporated in a polymer matrix by different techniques. The use of this technology allows the active ingredient to be released over a defined period, thereby increasing the duration of time during which the pesticide is effective while reducing undesirable side effects associated with exposure to high concentrations of the active ingredient (Bahadir and Pfister 1990). Packaging existing active ingredients into micro- or nano-sized carriers is one of the most popular methods used in controlled-release formulations (Nair et al. 2010; Nuruzzaman et al. 2016; Singh et al. 2020). These carriers can be in the form of particles, oily substances (emulsions), and degradable plastics (polymers) (Zhao et al. 2020). The fate of the active ingredients depends on the properties of the vehicle produced by micro- or nanotechnology.

The first microencapsulated pesticide formulation (Penncap-M) consisting of polyamide microcapsules loaded with methyl parathion was approved by the Environmental Protection Agency (EPA) for commercial marketing in 1974 (American Chemical Society, 1974), while the application of nano-encapsulated pesticides only became widespread after 2010 (Kah et al. 2013). Since then, both methods have conquered the global pesticide market (Pires-Oliveira et al. 2020). In parallel, there has been a considerable amount of subsequent research demonstrating the increasingly advanced encapsulation technology and suggesting its beneficial properties (Adisa et al. 2019; Nuruzzaman et al. 2016; Xu et al. 2022). Encapsulated pesticides are believed to have a variety of enhanced features that include reduced acute human exposure to the active ingredients due to their slower, more gradual release to the environment, reduced degradation, elimination of organic solvents, and, ultimately, increased efficacy at lower doses (Nuruzzaman et al. 2016).

1.2. Rationale

Although encapsulated pesticides are believed to have advantageous properties in terms of controlled release of the active ingredient and the consequent potentially lower level of acute exposure of non-target species, the reduced and thus prolonged release of the active ingredient may contribute to chronic exposure (Kah et al. 2018), and the carrier may also modify the effect of the active ingredient. The toxicological characterization of encapsulated pesticide formulations requires more attention to the combined effects of ingredients in chemical mixtures, posing a challenge to current pesticide regulatory frameworks that mainly focus on the toxicity of active ingredients (Villaverde et al. 2018). Despite the increasing market penetration of encapsulated pesticides in recent years (Pires-Oliveira et al. 2020), no systematic study has yet been carried out to compare the toxic effects of conventional and encapsulated pesticides.

The proposed systematic review will shed light on the harmfulness of encapsulated pesticides and provide information for environmental and human health risk assessment and, if proves necessary, for decision making on appropriate preventive measures.

1.3. Aim and objectives

1.3.1. Review question

Do micro- or nano-encapsulated pesticide formulations (E) exert different degrees of toxicity (O) than their conventional (not-encapsulated) counterparts (C) in *in vitro* and *in vivo* non-target animal models (P)?

1.3.2. PECO statement

Table 1.

1.3.3. Objectives

The specific objectives of this study are to:

(i) identify full-text research articles published in peer-reviewed scientific journals comparing the toxic effects of micro- or nano-encapsulated pesticides with that of their active ingredients or conventional formulations in experimental models (*in vivo* animal studies, including *ex vivo* analysis of their results, and *in vitro* human, animal, and bacterial cell culture studies) that are not pesticide-targets,

(ii) summarize and compare the extent of these effects to conclude whether encapsulated pesticides present a different level of toxicity to non-target species than their not-encapsulated counterparts.

2. Methods and analysis

The systematic review will follow the guidelines developed by the National Toxicology Program's Office of Health Assessment and Translation (NTP/OHAT) (NTP-OHAT 2019) that provides a standardized methodology to implement the Grading of Recommendations Assessment, Development and Evaluation (GRADE) approach to environmental health assessments (Morgan et al. 2016). The elements of the NTP/OHAT framework tailored to our research question will guide the workflow of this systematic review as visualized in Fig. 1.

This protocol adheres to the Preferred Reporting Items for Systematic Reviews and meta-analyses Protocol (PRISMA-P) statement (Moher et al. 2015; Shamseer et al. 2015). The completed PRISMA-P checklist as modified for Environment International is available in Supplementary Material 1. This protocol has been registered within the International Prospective Register of Systematic Reviews (PROSPERO) database (registration ID: CRD42022308373).

2.1. Eligibility criteria

The eligibility criteria for the studies to be included in the systematic review were formulated based on the components of the PECO statement (population, exposure, comparators, outcome) as described below (Morgan et al. 2018).

Table 1
PECO (population, exposure, comparator, outcome) statements.

PECO Element	Description
Population	• experimental animal models without restriction on species, sex, age and life stage • microorganisms • animal- or human-derived cell lines • *in vitro* models (e.g., 3D tissue models)
Exposure	• micro- or nano encapsulated pesticide formulation(s)
Comparator	• active ingredient(s) or conventional non-encapsulated pesticide formulation(s) of the analogous micro- or nano-encapsulated pesticide formulation(s)
Outcome	• any pathophysiological outcomes, and signs of acute or chronic toxicity

2.1.1. Population

In vivo models will include any experimental animal models without restriction on species, sex, age and life stage. We expect mainly mammalian models using lab-reared animals but do not restrict our search to such studies. Studies where animals are exposed *in vivo*, but some or all of the effects are assessed on derived cells *ex vivo*, will also be included and considered *in vivo* studies. *In vivo* models that serve as targets of the intended pesticidal activity will be excluded.

For *in vitro* studies, we will include any microorganism or any animal- or human-derived cell lines or *in vitro* models (e.g., 3D tissue models). We will exclude studies that examine pesticidal activity on plant cells, plant models, target organisms, cultures of cells isolated from target organisms exposed *in vivo* or *in vitro*, and those using biological materials isolated from target organisms/cells.

2.1.2. Exposure

The exposure of interest will be exposure to any micro- or nano-encapsulated pesticide formulation, applied in all ranges of concentrations, duration, and routes of exposure as described below.

Microencapsulated pesticide formulation: dispersed, generally-one- to hundred-micrometer diameter particles composed of pesticide active ingredient(s) covered (or encapsulated) by protective but permeable wall materials (polymeric coating) that allow controlled release of the active ingredient(s).

Nano-encapsulated pesticide formulation: dispersed, generally-one- to hundred-nanometer particles composed of pesticide active ingredient(s) covered (or encapsulated) by protective but permeable wall materials (polymeric coating) that allow controlled release of the active ingredient (s).

We will consider micro- or nano-encapsulated pesticide formulations whose active ingredient belongs to any of the following categories: insecticides, herbicides, nematicides, fungicides, rodenticides, acaricides, miticides, molluscicides, repellents, piscicides, avicides, bactericides, lampricides, algicides and defoliants.

2.1.3. Comparator

The comparison group will include any animal or *in vitro* model exposed, under the same conditions, to level(s) of the corresponding active ingredient(s) or of the conventional non-encapsulated product formulation(s) equivalent or comparable to the level(s) of the analogous micro- or nano-encapsulated pesticide formulation(s). We will exclude studies with comparison group exposed under different conditions or to non-equivalent or non-comparable level(s) of the corresponding active ingredient(s) or of the conventional non-encapsulated product formulation(s), or studies using no comparison group. Findings of comparative studies the results of which are based on previously conducted studies (i.e., the comparison is based on experiments carried out non-concurrently) will be separately reported due to possible inter-experimental biases. Studies reporting exposure solely to non-encapsulated pesticide formulation(s) will be excluded.

2.1.4. Outcome

To prevent reporting bias and facilitate the harmonization of outcomes and evidence synthesis, the outcomes will not be restricted to results of specific assays or testing guidelines, if we can align specific pesticide exposure to adverse outcomes. Thus, broad outcome domains will be adopted.

Outcomes for *in vivo* models include any pathophysiological outcomes, such as lethality, growth inhibition, changes in body or organ weight, histological changes, organ toxicity, organ dysfunction, behavioral changes, reproductive function, gene mutation, cancer development, and other signs of acute or chronic toxicity.

For *in vitro* models, any pathophysiological outcomes, such as cell viability, apoptosis, necrosis, DNA damage, chromatid and chromosome aberrations, micronucleus frequency, proliferation inhibition, enzyme activity, membrane potential, reactive oxygen species (ROS) generation,

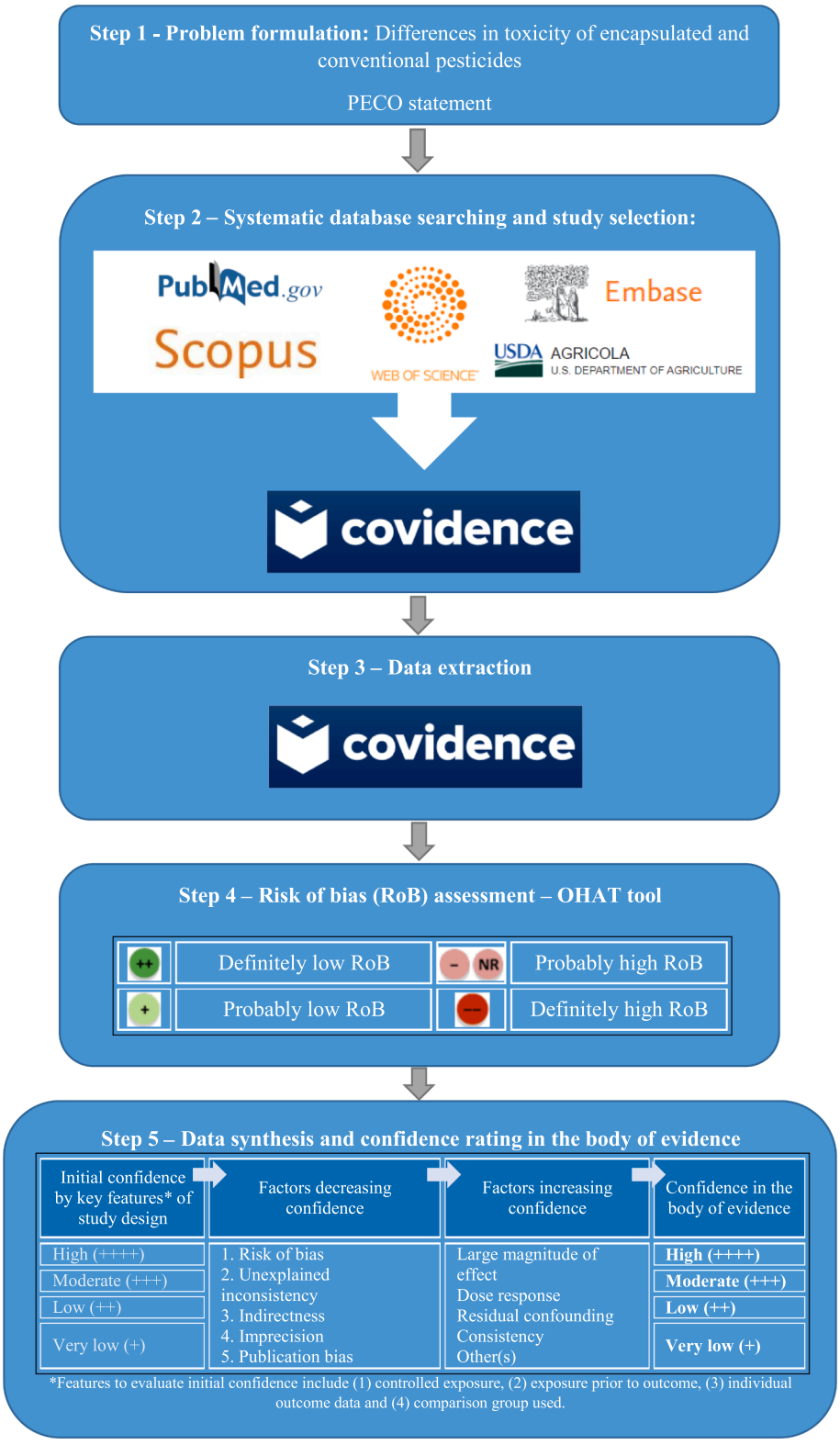


Fig. 1. Planned workflow of the systematic review adapted from the OHAT framework.

and functional changes will be included in the review.

In addition, newly identified outcome measures during the search for both *in vivo* and *in vitro* models will also be considered for inclusion in the review.

2.1.5. *Types of studies*

We will include all experimental studies published as full-text articles in peer-reviewed journals. Articles that do not contain original data, such as review studies, letters to editors, short communications, commentaries, as well as non-experimental studies (e.g., observational epidemiological studies), conference proceedings and presentations, book chapters, and studies published in languages other than English will be excluded from the review; however, the reference lists of reviews will be searched for eligible studies. We will also exclude grey literature,

including academic theses and dissertations, government reports and book chapters. There will be no restriction applied for publication years. Our inclusion criteria could potentially bias the results due to the selection of only English-language and peer-reviewed studies.

2.2. Search strategy

PubMed (NML), Scopus (Elsevier), Web of Science Core Collection (Clarivate), Embase (Elsevier) and Agricola (EBSCOhost) electronic databases will be comprehensively searched to identify eligible studies published in peer-reviewed scientific journals. The search will be carried out with the assistance of the director of The National Medical Library, United Arab Emirates University, and will be reported in accordance with the PRISMA-S extension (Rethlefsen et al. 2021) and guided by the PRESS Peer Review of Electronic Search Strategies (McGowan et al. 2016). Keywords with synonyms have been selected based on the predefined PECO statement and systematically developed with the help of PubMed and the search term hierarchy in PubMed's Medical Subject Headings (MeSH). The search was further tested in the multidisciplinary database Scopus. The search terms will be combined using Boolean operators (AND, OR) to connect search components of three search domains of "Pesticides", "Encapsulation" and "Toxicity" along with their synonyms and other words that are semantically related (Fig. 2). All search terms will be searched in a combination of the search fields: "title", "abstract" and MeSH/Thesaurus" (when applicable) for the best possible search outcome. In PubMed database, all search terms will be searched in the fields: "title" and "abstract" and in the MeSH (when available, except when included in other MeSH hierarchy). In Scopus database, all search terms will be searched in the fields: "title", "abstract" and "keywords". In Agricola database, all search terms will be searched in the "title", "abstract" and in the "subject" fields. In Embase database, all search terms will be searched in the fields: "title" and "abstract" and in the Emtree (when available). In Web of Science database, all search terms will be searched in the field: "TOPIC" (including title, abstract and keywords), limited to specific Web of Science Research Areas, which are listed in Supplementary Material 2.

In addition, the reference lists of all eligible articles and retrieved reviews will be manually screened to identify additional relevant papers. If the number of retrieved eligible articles will be less than 100, then we will also perform backward snowballing search till new eligible articles are not found in the last 10 searches.

The pilot searches in PubMed and Scopus were conducted from December 2021 - March 2023. The search string developed during pilot searches will be consistently applied in all selected information sources in March 2023 and re-run before the final analysis. The latest (30th March 2023) reproducible search strings with results and search technical notes are provided in Supplementary Material 2. Complete search logs with search details results and search technical notes (search date and any modifications) for all databases will be presented in the review.

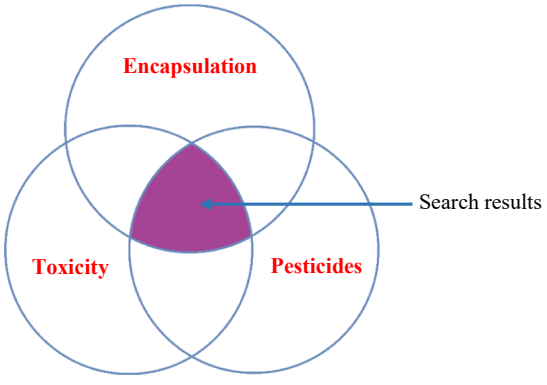


Fig. 2. Graphical representation of the conceptual search domains used to develop the search terms in electronic databases.

2.3. Study records

2.3.1. Selection process

The citations of the search results will be imported into the systematic review software Covidence (Veritas Health Innovation), where the entire screening process will be recorded. After automatic duplication removal in Covidence, the retrieved unique studies will be screened in two stages by two independent reviewers (KRM and MS), based on the predetermined inclusion and exclusion criteria. In the first stage, the title and abstract of the publications will be screened by the reviewers (KRM and MS) independently. Backward snowballing will be manually screened to the reference list of all eligible reviewed articles to identify additional relevant papers that fit the inclusion and exclusion criteria. If too many articles are found, then identifying relevant and highly cited articles may be an alternative. In the second stage, the full texts of publications selected during the first stage will be considered for inclusion also by two independent reviewers (KRM and MS). Discrepancies between the judgments of the two reviewers regarding the eligibility of studies will be resolved by a third reviewer with relevant expertise (KN or BÁ). The screening and conflict-resolving modules in Covidence are blinded. The screening and selection process will be documented in a PRISMA flow diagram as shown in Fig. 3, adopted from The PRISMA 2020 statement (Page et al. 2021). To ensure transparency of the screening process, justification for the exclusion (e.g., wrong study design, wrong population, wrong exposure, wrong comparator, wrong outcome (e.g., intended pesticidal effect) will be reported.

2.3.2. Data collection process

Two reviewers will independently extract data in Covidence. Data extraction sheets have been developed in Excel 2019. These Excel sheets have been used for pilot testing (see 2.5.) and will be populated in Covidence. Discrepancies will be resolved to utilize the conflict resolution module of Covidence and through discussions with a third reviewer (KN or BÁ) until data extractors reach convergence and agreement. Separate data extraction sheets have been developed for *in vivo* animal and *in vitro* studies, the templates of which are provided in Supplementary Material 3. We will contact the study authors twice in two weeks to retrieve any missing data. If the authors were not responsive or provide unclear data, then we will exclude the study.

2.3.3. Data items

The following items will be extracted from the studies:

- Study design (*in vivo* animal or *in vitro* study);
- Study identification information (title, name of the first author, year of publication, DOI);
- Encapsulated pesticide formulation(s) tested (name, type, ingredients, CAS number, concentration/purity, other characteristics)
- Conventional pesticide(s) or active ingredient(s) tested (name, type of pesticide, ingredients, CAS number, concentration/purity, other characteristics)
- Exposure conditions (for *in vivo* studies: route of exposure/administration, duration of treatment and frequency of dosing, administered doses or concentrations, analytically confirmed doses if reported; for *in vitro* studies: method, duration, and frequency of treatment; administered concentrations, analytically confirmed concentrations if reported; metabolic activation).
- Method to measure outcome (assay type, number of repetitions (i.e., we will consider the number of independent experiments as replicates), statistical analysis performed);
- Primary outcome (quantitative measures of effect or toxicological dose descriptors, such as LD50, LC50, NOEL, LOEL, NOEC, LOEC,

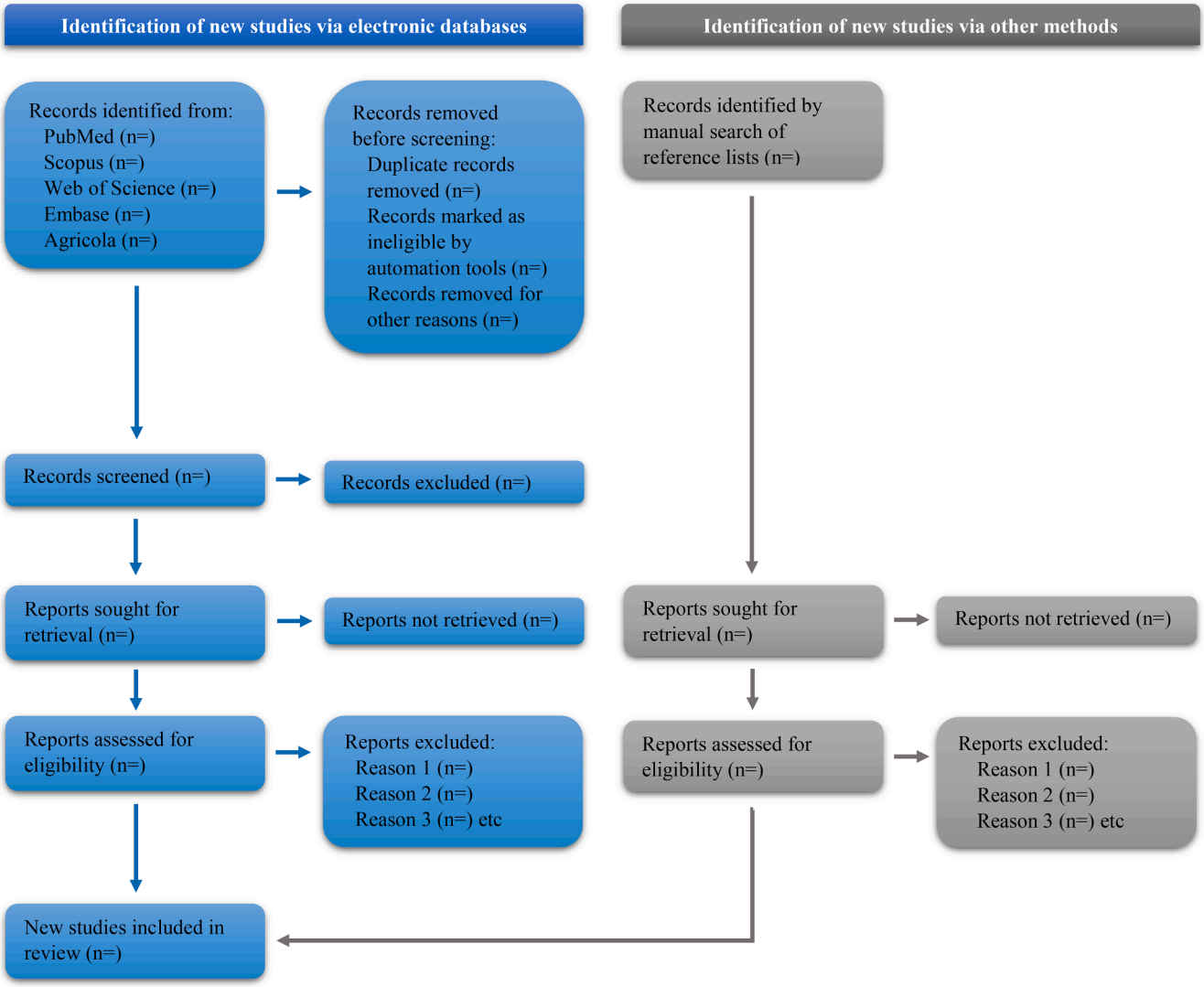


Fig. 3. Example of the PRISMA flowchart to be used in this study.

and other relevant information, e.g. toxicokinetic and toxicodynamic data).

- Secondary outcome (the level of statistical significance of the difference in effects induced by the micro- or nano-encapsulated pesticide formulations versus their conventional counterparts; dispersion and uncertainty measures of the difference [SD, SE, 95 % CI]).

2.4. Risk of bias assessment

Methodological quality of both *in vivo* and *in vitro* studies will be assessed using the 7 domains proposed by the NTP/OHAT risk of bias (RoB) tool (NTP-OHAT 2019; Rooney 2015). The domains assess randomization, allocation concealment, experimental condition, blinding, incomplete data, exposure characterization, outcome assessment, reporting and other biases related to the methodological structure. Predefined criteria to determine the rating for each RoB are customized to each study type. These criteria outline the study design, conduct, and reporting. A grade (definitely low, probably low, probably high, or definitely high) for risk of bias will be assigned for each domain through answering 11 RoB questions. The overall RoB for a study is the rating given in any of the 7 domains which indicates the highest RoB. So, for example, if a study has either 'Probably low' or 'Probably high' in at least one RoB domain, the overall RoB judgment will be Probably low or Probably high, respectively.

Two reviewers (KRM and MS) will independently assess the RoB in the included studies after a training and pilot testing of the RoB tool. Discrepancies between the judgments of the two reviewers will be resolved by a third reviewer (KN or BÁ).

2.5. Pilot test

Processes of study selection, data collection, and RoB assessment have been piloted using a random sample of studies (1239 out of 12,498 unique records) identified through an extended pilot search including all electronic databases. Results of the pilot database search have been uploaded and de-duplicated in Covidence for further manual screening of the pilot sample. The majority of studies (1223 out of 1239) in the pilot sample have been excluded by two reviewers (KRM and MS) during title/abstract screening in Covidence, leaving 16 studies eligible for full-text screening after conflicts have been resolved by a third reviewer (KN). As a result of full-text screening, 11 studies have been excluded due to selected exclusion criteria. Data have been independently extracted by two reviewers (KRM and MS) from 5 studies that met the inclusion criteria defined in the review protocol. Completed data extraction sheets have been reviewed by the other two reviewers (KN and BA), and discrepancies have been resolved through discussions among the reviewers (KRM, MS, KN and BA) until data extractors have

reached convergence and agreement. The synchronization of the extracted data has been performed by a third reviewer (KN). Afterwards, two reviewers have independently assessed the RoB of the eligible studies using the 11 domains proposed by the NTP/OHAT RoB tool. A grade (definitely low, probably low, probably high, or definitely high) for risk of bias has been assigned for each domain in each of the five eligible studies. Discrepancies between the judgments of the two reviewers have been resolved by a third reviewer (KN), who also summarized the results of the RoB assessment. Results of the pilot study selection, data extraction, RoB assessment and study characteristics are provided in Supplementary Material 4, 5, 6 and 7, respectively. The overall risk of bias rating of the studies identified through the pilot test is shown in Fig. 4.

2.6. Data synthesis

Study findings will be first described and synthesized narratively according to the Synthesis Without meta-Analysis (SWiM) reporting guideline (Campbell et al. 2020). The data from each included study will be grouped, and results synthesized by study design (*in vitro* and *in vivo*), stratified by RoB level, and the result of the comparison (i.e., whether the encapsulated or the conventional pesticide was found more toxic). Data will be described narratively in the text and presented in two tables (one for *in vitro* and one for *in vivo* findings) reporting key characteristics of the included studies (e.g., population, sample size, exposure characteristics, methods, outcomes, and RoB level) and the conclusion of the toxicological comparison.

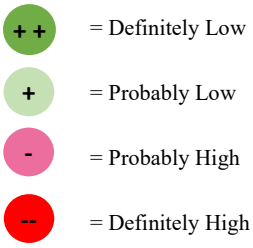
In addition to narrative synthesis, we plan to perform *meta*-analyses (i.e., quantitative syntheses) where there are at least two unique studies of the same study design that are deemed to be sufficiently similar in study subjects, exposures and outcomes and have sufficient quantitative data for analysis. Statistical heterogeneity will be analyzed by I^2 statistic. If statistical heterogeneity is observed (I^2 >=50 % or P less than 0.1), the random effects model will be used. If tests of heterogeneity are not significant, the Mantel-Haenszel method will be used for the fixed effect model. Data will be analyzed by weighted mean differences (95 % CI) or standardized mean differences (95 % CI) for continuous measures, and Mantel-Haenszel methods will be used to calculate pooled relative risks (95 % CI) for dichotomous outcomes. Skewed data and non-quantitative data will be presented descriptively. The *meta*-analysis will be conducted in Stata 16.0 (StataCorp 2019).

If studies are poorly comparable and are not eligible for *meta*-analysis, the results will only be presented as a narrative synthesis and in tabular format as described above. Differences in sample sizes, which otherwise would be considered in *meta*-analysis, will be addressed in qualitative synthesis, as well.

2.7. Subgroup and sensitivity analyses

Subgroup analysis and *meta*-regression will be performed if possible (i.e., more than one study will be available) and if there is obvious heterogeneity between the included studies. This will be based on different *in vivo* animal and *in vitro* models, exposures, and outcomes. To assess whether the pooled results are affected by a single study, we will carry out a sensitivity analysis by omitting the effect estimate of each study-one by one and by RoB level, and we will recalculate the combined estimates on the remaining studies.

Study ID	D1	D2	D3	D4	D5	D6	D7	Overall
Vignardi et al. 2020	+	+ +	+	+	+ +	+	+ +	+
Demir et al. 2022	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +
Rashidipour et al. 2019	+ +	+ +	+	+ +	+ +	+ +	+ +	+
Zhang et al. 2019	+ +	+ +	+	+	+ +	+ +	+ +	+
Oliveira et al. 2019	-	+ +	+	+	+ +	+ +	+ +	-



D1= Selection bias
D2= Confounding bias
D3= Performance bias
D4= Attrition / Exclusion bias
D5= Detection bias
D6= Selective reporting bias
D7= Other bias

Fig. 4. Risk of bias results for the 5 studies included in the pilot using the OHAT rating tool.

2.8. Confidence rating in the body of evidence

Certainty of the evidence will be assessed following the GRADE (Grading of Recommendations Assessment, Development, and Evaluation) standards (Morgan et al. 2016) as adapted in the NTP/OHAT framework, evaluating individual risk for bias, inconsistency, indirectness, imprecision, and publication bias. As a result, the certainty of the estimated effect will be rated as high certainty of evidence (the true effect lies close to that of the effect estimate), moderate certainty of evidence (the true effect is likely to be close to the effect estimate, but there is a possibility that it is substantially different), low certainty of evidence (the true effect may be substantially different from the effect estimate), and very low certainty of evidence (the true effect is likely to be substantially different from the effect estimate) (Balshem et al. 2011).

Publication bias will be also examined visually by developing and inspecting the funnel plot for asymmetry if there are at least 10 studies included in the *meta*-analysis. A funnel plot usually presents effect sizes plotted against their standard errors or precisions. The funnel plot is expected to be skewed if there is publication bias. We will also perform Egger's linear regression test that regresses the standardized effect sizes on their precisions. The regression intercept should be zero if there is no publication bias. The certainty assessment will be conducted following a group consensus process.

2.9. Discussion

Concerns about the sustainability of agricultural production call for the development of technologies and practices that are not harmful to environmental goods and human health, are efficient for farmers and improve food productivity. As the pesticides are likely to remain the tools of modern agriculture, it is important that strategies are developed to reduce the toxic effects of pesticides on non-target organisms, including humans. Agrochemical companies are continuously expanding their product portfolios by investing in research to develop new chemical compounds with innovative mechanisms of action, including an increasing number of micro- or nanoencapsulated pesticides, which, as a result of recent studies and our pilot testing, appear to be more favorable in terms of their hazard to human health. However, the toxicological hazards of the use of these pesticides have not been comprehensively assessed so far. The proposed systematic review is intended to be the first to explore the toxicological hazards between encapsulated pesticides and their conventional alternatives by identifying and evaluating studies comparing the toxic effects of these formulations.

Identifying the hazard is an early stage in the risk assessment process. The results from this study can provide a basis for regulatory agencies and human health risk assessors to determine the hazard of concern for encapsulated pesticides once the actual level of human exposure and the significance of the toxicity mechanisms are known. The development of this protocol will ensure a proper assessment of the available evidence, providing an up-to-date scientific judgement on the possible harmfulness of encapsulated pesticide formulations.

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CRediT authorship contribution statement

Khadija Ramadhan Makame: Conceptualization, Methodology, Writing – original draft. **Moustafa Sherif:** Conceptualization, Methodology, Writing – original draft. **Linda Östlundh:** Methodology, Writing – review & editing. **János Sándor:** Methodology, Writing – review & editing. **Balázs Ádám:** Conceptualization, Methodology, Writing – original draft. **Károly Nagy:** Conceptualization, Methodology, Writing – original draft.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.envint.2023.107924.

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