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Protocol of the systematic literature review on the state of biocide resistance in wild vector populations in the EU and neighbouring countries

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Abstract

Globalisation, climate change, and altered land use have facilitated the spread of disease vectors into new areas, making these diseases a significant challenge for European public and veterinary health authorities. In many cases, vector control is the primary strategy to prevent transmission, as vaccines or effective treatments may be lacking. Methods such as biocide application are commonly used to control vector populations, but the widespread use of biocides has led to the development of resistance in vector species. This resistance, which reduces the effectiveness of biocides, complicates efforts to manage vector-borne diseases, increasing the public and veterinary health burden. Resistance to biocides used in public and veterinary health has been reported globally. Understanding the scope of biocide resistance in wild vector populations is essential for adapting and improving control strategies. This systematic literature review protocol aims to review the current status of biocide resistance in wild vector populations within the EU/EEA, excluding outermost regions, and to provide an overview of the mechanisms of resistance observed in these species, supporting more effective vector control interventions.

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Summary

Vector-borne diseases, such as dengue, Zika virus disease, West Nile virus infection, and Lyme borreliosis, are increasingly threatening public and veterinary health in Europe. These diseases are spreading to new areas due to globalisation, climate change, and land use changes. The introduction of new vector species and alterations in the capacity of local species to transmit pathogens are exacerbating the risk. As effective vaccines or treatments are often unavailable, vector control, particularly through biocides, is a key strategy to prevent disease transmission. However, the widespread use of vector control biocides, along with agricultural pesticides, has led to resistance in many vector populations, reducing the effectiveness of these control measures and increasing the burden on health systems. Understanding the extent and mechanisms of biocide resistance in wild populations is crucial for effective vector control. This protocol outlines a systematic literature review aimed at assessing the biocide resistance status of wild vector populations in Europe and identifying the mechanisms underlying resistance in affected species. The review will help public health and veterinary authorities develop more effective, adaptive control strategies. The research questions guiding this review are: (1) What is the status of biocidal resistance in field populations of arthropod vectors in Europe? (2) What are the mechanisms of resistance in these populations?

The eligibility criteria for this systematic literature review focus on publications that assess biocide resistance in specific arthropod vector groups: mosquitoes, sand flies, biting midges, and ticks. Studies are included if they report biocide resistance using specific bioassay methods, including WHO and CDC bioassays for mosquitoes, sand flies, and midges, as well as tests like the larval packet and adult immersion tests for ticks. Studies that assess metabolic resistance (via synergist bioassays) or target-site resistance (via biochemical or molecular assays) are also eligible. Biocide resistance in wild populations is measured through discriminating concentration assays, where mortality rates (MR) are compared against known thresholds to determine susceptibility or resistance. The lethal concentration (LC₅₀/LC₉₀) and resistance ratios (RR) are used to further evaluate resistance levels.

The review will include studies published from January 2022 onward, building on the first ECDC report covering data from 2000 up until January 2022. To gather relevant literature, databases like Web of Science™ and PubMed will be searched, using specific terms related to biocide resistance, geography, and vector species. Grey literature will also be obtained from the One Health VectorNet Entomological Network (OHVEN).

The selection process involves two independent reviewers screening titles and abstracts for eligibility. Excluded studies include those using lab colonies, new biocides evaluations, assays not specified, or focusing solely on molecular mechanisms without field data. Data extraction will be done by one reviewer, with 5% of the extracted data quality-checked by a second reviewer.

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1 Introduction

1.1 Background and terms of reference as provided by the requestor

This contract was awarded by EFSA to: VectorNet

Contractor: VectorNet, AVIA-GIS, Zoersel, Belgium

Contract title: European network for medical and veterinary entomology (VectorNet 3)

Contract number: SPECIFIC CONTRACT No 01 implementing Framework contract No EFSA/2023/OP/0009 (OC/EFSA/BIOHAW/2023/05)

1.2 Interpretation of the terms of reference

Vector-borne diseases represent a significant and growing health threat across Europe, requiring urgent attention. Diseases such as dengue, Zika virus disease, West Nile virus infection, leishmaniasis, Lyme borreliosis, bluetongue, and Schmallenberg disease pose substantial challenges to public health and veterinary services in local communities. Historically, these diseases were predominantly confined to tropical and subtropical regions. However, due to factors such as globalisation, climate change, and changes in land use, areas previously unaffected by these diseases are now at risk. This is due to the introduction, settlement, and spread of new vector species, as well as changes in the vector capacity of local species. As a result, vector-borne diseases now pose a unique challenge to national public health and veterinary authorities, as well as to key European bodies like the European Centre for Disease Prevention and Control (ECDC) and the European Food Safety Authority (EFSA).

In many cases, preventing and controlling the transmission of vector-borne pathogens relies on vector control, as effective drugs or vaccines may be unavailable. To mitigate the impact of these diseases and interrupt the transmission cycle, a comprehensive vector control strategy is essential. Various control methods exist, such as the use of larvivorous fish or odour-baited traps, though most interventions typically involve the application of biocides—products containing active substances designed to kill, repel, or neutralise unwanted organisms.

A survey conducted in 2021 assessed vector surveillance and control practices across Europe (European Centre for Disease Prevention and Control, 2021). Of the 31 countries engaged in mosquito control, 21 reported using biocides as part of their efforts. Although less common, biocide-based interventions are also frequently employed to manage other vectors, such as biting midges, sand flies, and ticks.

The widespread use of biocides, as well as agricultural pesticides and crop protection products, has led to the development of resistance in many vector populations. This resistance reduces the effectiveness of biocides, undermining their ability to control vector-borne diseases and thus increasing the public and veterinary health burden. Understanding the extent of biocide resistance in wild populations is crucial for local authorities planning vector control campaigns.

Such knowledge enables them to implement effective mitigation measures and adjust strategies to maintain the efficacy of control efforts.

Resistance to many of the WHO-approved biocides used in public health applications has been reported globally. The primary mechanisms of biocide resistance in vectors, predominantly in adult vectors, are generally categorised into two main types: target-site resistance and metabolic resistance. Additionally, behavioural changes in vector species can undermine the effectiveness of biocides used in control efforts. Unlike physiological resistance, behavioural resistance is more difficult to study and measure. Another underexplored mechanism is cuticular resistance, which has been identified in some mosquito species. This form of resistance occurs when the vector's cuticle thickens or its composition changes, reducing the absorption rate of the biocide and thus diminishing its effectiveness.

To aid public health and veterinary officials, as well as those involved in vector control, this protocol outlines a systematic literature review aimed at assessing the current status of biocide resistance in wild vector populations (rather than laboratory insect colonies) within the EU/EEA (excluding the outermost regions and overseas countries and territories) and to provide an overview of detected biocide resistance mechanisms in these vector populations.

1.3 Research question

Within this systematic literature review we aimed to answer the following research questions:

1. What is the **biocidal resistance status** of field populations of arthropod vectors of public and veterinary health importance in Europe?
2. What are the **underlying mechanisms** of biocide resistance present in populations where resistance is detected?

1.4 Eligibility criteria

1.4.1 Population

Publications are included for species of the following vector groups:

- Mosquitoes
- Sand flies
- Biting midges
- Ticks

All arthropod species belonging to one of the four vector groups are eligible for inclusion. Arthropod species outside of these four groups are excluded.

1.4.2 Study design

The biocide resistance assessments of mosquitoes, sand flies and biting midges are included:

- WHO adult susceptibility bioassay
- CDC bottle bioassay
- WHO larvicide susceptibility bioassay

- WHO insect growth regulators test

Biocide resistance assessment studies by means of synergist bioassay (metabolic resistance) or biochemical/molecular assay (target site resistance) will be also included.

For ticks, the biocide resistance assessments include:

- Larval packet test
- Adult immersion test
- Larval immersion micro assay
- Larval tarsal test

In ticks, assessments of biocide resistance by means of synergist bioassay (metabolic resistance) or biochemical/molecular assay (target site resistance) will be included.

1.4.3 Outcomes

Resistance assessments are usually performed using a discriminating concentration (DC) assay. In these assays, individuals from wild populations are subjected to a predetermined biocide concentration and the mortality rate (MR) is measured. For WHO bioassays, populations are considered as susceptible if $MR > 98\%$, possibly resistant if MR is between 90 and 98% and resistant if $MR < 90\%$, while for CDC bottle bioassays $MR < 80\%$ confirms resistance. Another way to measure the efficacy of a biocide is by calculating the concentration which is lethal for 50 or 90% of the wild population (LC50; LC90). Dividing the lethal concentration of a wild population by that of a susceptible one produces a resistance ratio (RR) which indicates whether a population is resistant or not ($RR < 5$: low resistance; $5 < RR < 10$: moderate resistance; $RR > 10$: high resistance). When discriminating concentrations are not predetermined, a comparison is often made with a known susceptible population. This method is also eligible.

1.4.4 Temporal delineation

In March 2023, ECDC published a first report of the state of biocide resistance in wild vector populations in the EU and neighbouring countries covering the period 2000 – January 2022. The current SLR will include new references since 1/1/2022. This SLR will be updated on demand.

1.4.5 Search strategy

The following databases will be searched: Web of Science™ and Medline/PubMed and the search terms will include three categories (Appendix A):

- Biocide resistance terms
- Geography (all included countries and a selection of regions)
- Vector terms (common names, synonyms and genera)

Additionally, grey literature will be obtained by contacting the One Health VectorNet Entomological Network (OHVEN) members directly via a questionnaire (Appendix B) and sending them the data extraction form.

www.efsa.europa.eu/publications

1.4.6 Selection process

Title and abstract will be screened independently by two reviewers.

Criteria for exclusion are:

- Testing of new biocides or synergists
- Usage of lab colonies (i.e. populations not collected from the field)
- Use of assays other than bioassays, synergist assays, molecular assays or biochemical assays
- Study of non-target species
- Study only addressing (molecular) mechanisms of biocide resistance without field assays to measure resistance

Disagreements regarding study eligibility will be reviewed and a consensus made. If needed, a third opinion will be sought. The full texts of the resulting studies will then be screened independently for inclusion/exclusion by the one reviewer.

In addition, publications will be excluded if:

- The full text of a publication with results is not accessible.
- No distinction is made between species when conducting the assays.
- Data is compiled in graphs and there is no numeric data to extract.

1.4.7 Data extraction

Data will be extracted by one reviewer according to the data template in Appendix C. After data extraction, 5% of the data extracted from the papers shall be quality checked by another reviewer. Additionally, to entering the coordinates of the study site, the observation location will also be converted to the polygon levels used by VectorNet.

References

European Centre for Disease Prevention and Control (2021). Organisation of vector surveillance and control in Europe, Technical Report. <https://www.ecdc.europa.eu/en/publications-data/organisation-vector-surveillance-and-control-europe> [doi: 10.2900/853486]

APPENDIX A – Search terms

Resistance

((((Insecticid* OR acaricide* OR biocid* OR pyrethroid* OR organophosphat* OR organochlorin* OR carbamate* OR neonicotinoid* OR DDT OR allethrin OR bifenthrin OR cyfluthrin OR cypermethrin OR cyphenothrin OR deltamethrin OR pyriproxyfen OR PBO OR Bti) AND resistan*) OR ((Insecticid* OR acaricide* OR biocid* OR pyrethroid* OR organophosphat* OR organochlorin* OR carbamate* OR neonicotinoid* OR DDT OR allethrin OR bifenthrin OR cyfluthrin OR cypermethrin OR cyphenothrin OR deltamethrin OR pyriproxyfen OR PBO OR Bti) AND susceptib*)) OR "kdr resistance)

Geography

Europe* OR "Mediterranean Basin" OR "Mediterranean area" OR Balkan* OR Scandinavia* OR "Iberian peninsula" OR Aland OR Albania* OR Andorra* OR Atlantic OR Austria* OR Belgi* OR "Black sea" OR Bosnia* OR Herzegovina OR Bulgaria* OR Croatia* OR Cypr* OR "Czech Republic" OR Cze* OR Denmark OR Greenland OR German* OR Spain OR Estonia* OR Finland OR "Faroe islands" OR France OR Corsica* OR Greece OR Gibraltar OR Hungary OR Iceland* OR Ireland OR Italy OR Sicil* OR Sardinia* OR Kosov* OR Latvia* OR Liechtenstein OR Lithuania* OR Luxembourg OR Macedonia* OR FYROM OR Malta OR Monac* OR Monegasqu* OR Monteneg* OR Netherlands OR Norway OR Poland OR Portug* OR Slovenia* OR Romania* OR "San Marino" OR Serbia* OR Slovakia* OR Switzerland OR Sweden OR "United Kingdom" OR "British Isles" OR "Great Britain" OR Wales OR England OR Scotland OR Turk* OR "Vatican city" OR Svalbard OR Israel* OR Palestin* OR Jordan* OR Leban* OR Syria* OR Morocc* OR Algeria* OR Tunisia* OR Libya* OR Egypt* OR "Western Sahara*" OR Armenia* OR Azerba* OR Belarus OR Bielorusia* OR Georgia* OR Moldov* OR Russia* OR Yugoslavia* OR Ukrain* OR Ukrayin* OR USSR OR SSSR OR "Soviet Union" OR British OR Irish OR Scottish OR Welsh OR "Channel Islands" OR Jersey OR Guernsey OR Sark OR French OR Greek OR Italian OR Spanish OR Swiss OR Swedish OR Transcaucasia* OR Caucasus OR Danish OR Finnish OR Norwegian OR Baltic OR Czech* OR Hungarian OR Polish OR Mediterranean OR Sahara OR OR Majorca* OR Mallorca* OR Minorca* OR Ibiza OR "North Africa*" OR Kazakh* OR Uzbek* OR Karakalpakstan* OR Crimea* OR Nakhchivan* OR Alsac* OR Abkhazia* OR Adjara* OR Ossetia OR Athos OR Aosta* Friuli* OR Giulia* OR Trentin* OR Adige OR Südtirol* OR "South Tyrol*" OR Găgăuzia* OR Transnistria* OR Madeira* OR Catalan OR Catalonia* OR Adygea* OR Bashkortostan* OR Chechnya* OR Chuvashia* OR Dagestan* OR Ingushetia* OR Karbardin* OR Kalmykia* OR Karachay OR Komi* OR Karelia OR "Mari El"* OR Mordovia* OR Tatarstan* OR Udmurtia* OR Nenets OR Vojvodin* OR Metohija* OR Srpska* OR Andalusia* OR Aragon* OR Asturias* OR Basque OR Cantabria* OR Castile* OR Mancha* OR León OR Extremadura* OR Galicia* OR Rioja* OR Madrid OR Murcia* OR Navarr* OR Valencia* OR Ceuta* OR Melilla* OR "Isle of Man"

Vectors

Ticks OR Ornithodoros OR Ixodes OR Rhipicephalus OR Dermacentor OR mosquito* OR Aedes OR Anopheles OR Culex OR Stegomyia OR Ochlerotatus OR Culicidae OR Culiseta OR Coquillettidia OR sandfly OR sandflies OR "sand flies" OR "sand fly" OR Phlebotominae OR Phlebotomus OR Culicoides OR "biting midges"

APPENDIX B – Questionnaire to be sent to VEN

In the context of the VectorNet project, we are reviewing the biocide resistance status of wild populations of mosquitoes, ticks, sand flies and biting midges in the EU and its neighbouring countries (VEN countries). In March 2023, ECDC published a first report of the state of biocide resistance in wild vector populations in the EU and neighbouring countries covering the period 2000 – January 2022 (European Centre for Disease Prevention and Control. Literature review on the state of biocide resistance in wild vector populations in the EU and neighbouring countries. Stockholm: ECDC; 2023). This questionnaire is an iteration of the previous one sent out in 2022. In case information has been shared in 2022 please indicate this in the questionnaire. In general, we are searching for data on biocide resistance obtained by performing WHO bioassays or CDC bottle tests, synergist assays, molecular assays, and biochemical assays.

Through this questionnaire we would like to know whether the biocide resistance status of the four vector groups is assessed in your country and if data is available and can be shared with VectorNet.

This questionnaire will be sent to the OH VectorNet Entomology Network (OHVEN) members of all EU/EEA countries, the EU candidate countries and potential candidates, and the European Neighbourhood Policy partner countries.

The completion of the questionnaire will take around 15 minutes.

Full Name:
Country you are based in:

1. Did you complete the last biocide review questionnaire in 2022?

- 1.1. No
- 1.2. Yes and no new information (data)
- 1.3. Yes, but new information (data)

2. Is biocide resistance of wild populations of the following vector groups (mosquitoes, ticks, sand flies, biting midges) assessed in your country? The assessment can be done as part of a governmental assignment or as part of e.g. research project at national, regional or local level.

- 1.1. Government-assigned (national, regional and/or local): yes, no, not sure
- 1.2. Institutionally based (e.g. in the context of a research project): yes, no, not sure.

2. Who in your country conducts the assessment of biocide resistance? Please list the contact details of all these researchers below, so we can contact them as well if deemed feasible. (Name + e-mail + other contact details (optional))

- 2.1. Mosquitoes
- 2.2. Ticks
- 2.3. Sand flies
- 2.4. Biting midges

3. If you have access to data from biocide resistance assessments, could you share the data with VectorNet? If so, we will contact you once more through e-mail with the specific information we are looking for.

- Yes, I am willing to share the data with VectorNet
- No, I cannot share the data with VectorNet.

4. Here you can provide any additional remark (not the data) related to the assessment of biocide resistance of the four vector groups that you want to share.

APPENDIX C – Database structure

Sheet 1. Includes data from bio-assays, resistance, synergist, molecular and biochemical assays

Code name	Explanation
First Author	Name of the researcher or institution
Year	Publication year
Country	Country of research
Vector_species	Investigated vector species
Location_Address	Research location
Latitude_dec	Latitude (in decimal degrees)
Longitude_dec	Longitude (in decimal degrees)
Year_assessment	Year of insecticide resistance assessment
Bioassay	Bioassay used in the research? (Y/N)
Method	Method of bioassay applied
Biocide	Biocide used for the assessments
Discriminative dose	Did the researchers use the discriminate dose (DD) for the assessment? (Y/N)
Dose_perc	Biocide dose used for the assessment (%)
Exposure_time	Time vector species got exposed to the biocide
Bioassay_sample_size	Number of individuals the biocide was tested on
Bioassay_mortality_perc	Mortality number of the field population
Correction_method	Correction for percentage of mortality (e.g. Abbott's formula)
Mort_control_group_perc	Mortality percentage of the control group
Quality_check	Were the data in line with the pre-set criteria of the test?
Resistance_intensity	Did the researchers test the intensity of the biocide resistance? (Y/N)
5_DD_mortality_perc	Sample size of the 5x DD assay
5_DD_samplesize	Mortality percentage when using five times the discriminating dose
10_DD_mortality_perc	Sample size of the 10x DD assay
10_DD_samplesize	Mortality percentage when using 10x the DD
Synergist_bioassay	Was the effect of a synergist on the resistance phenotype assessed? (Y/N)
Synergist	Synergist used
Sample_size	Number of vector individuals tested for the synergist bioassay
Syn_mortality_perc	Mortality number when performing the synergist bioassay
Molecular_assay	Did the researchers use the molecular assay to assess resistance alleles? (Y/N)
Allele	Which allele was tested for resistance?
Mutation	The group of mutations the mutated allele belongs to
Sample_size_MA	Number of individuals tested for the molecular assay
Allele_frequency_perc	Mutated allele percentage
Biochemical_assay	Did the researchers use the biochemical assay to assess resistance alleles? (Y/N)
Enzyme	Which enzyme was tested for resistance?
Enzyme_activity_field_population	Enzyme activity of the field population
Enzyme_activity_susceptible_population	Enzyme activity of the susceptible population
P_value	Did the enzyme activity of the wild population differ significantly from that of the susceptible population?
Comment	Additional remarks

Sheet 2. Data on bioassays in which LC and RR values are calculated to mark resistance

Code name	Explanation
First author	Name of the first author of the scientific article
Year	Publication year
Country	Country of research
Vector_species	Investigated vector species
Biocide	Biocide used for the assessments
Location_Address	Research location
Latitude_dec	Latitude (in decimal degrees)
Longitude_dec	Longitude (in decimal degrees)
Year_assessment	Year of IR assessment
Method	Method of bioassay applied
LC ₅₀ sus (mg/l)	Biocide concentration which causes 50% mortality of the susceptible population (LC=Lethal Concentration)
SSLC ₅₀ field	Sample size used to calculate the LC ₅₀ of the field population
LC ₅₀ field (mg/l)	Biocide concentration which causes 50% mortality of the field population
SSLC ₅₀ sus	Sample size used to calculate the LC ₅₀ of the field population
RR ₅₀ field_sus	Resistance ratio: LC ₅₀ field / LC ₅₀ sus
LC ₉₀ sus (mg/l)	Biocide concentration which causes 90% mortality of the susceptible population
SSLC ₉₀ sus	Sample size used to calculate the LC ₉₀ of the field population
LC ₉₀ field (mg/l)	Biocide concentration which causes 90% mortality of the field population
SSLC ₉₀ field	Sample size used to calculate the LC ₉₀ of the field population
RR ₉₀ field/sus	Resistance ratio: LC ₉₀ field / LC ₉₀ sus
Comment	Additional remarks