I2I Landscaping exercise

Standard membrane feeding assay (SMFA) and PK/PD modelling for endectocides.

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Abbreviations

3D	Three dimensional
AI	Active ingredient
BOHEMIA	Broad One Health Endectocide-Based Malaria Intervention in Africa
CDC	Centre for Disease Control
CI	Confidence Interval
HPPD	4-hydroxyphenylpyruvate dioxygenase
IRS	Indoor residual spraying
IVM	Ivermectin
LLINs	Long-lasting insecticidal nets
MDA	Mass drug administration
MoA	Mode of action
PCR	Polymerase chain reaction
PD	Pharmacodynamics
PK	Pharmacokinetics
SMFA	Standard membrane feeding assay
SOPs	Standard operating procedures
WHO	World Health Organisation

Terminology

Endoparasitic: Lethal to parasitic organisms inside the body, such as worms.

Ectoparasitic: Lethal to parasites outside the body, including mosquitoes, ticks.

Efficacy: The killing effect of the compound over time.

Duration of mosquito killing effect/Time under the curve: The concentration of the compound in the blood over time and how long it remains lethal to mosquitoes.

Pharmacokinetic (PK) profile: The drug-concentration in blood over time. Depends on the recommended safe dosing regimens as described by the manufacturer and food and drug administration (FDA) standards.

Pharmacodynamic (PD) profile: The compound concentration-effect curve on the target species.

Pharmacokinetic/pharmacodynamic (PK/PD) modelling: Techniques that combine the PK and PD profile of a compound to describe the lethal effect of the compound over time on the mosquito.

Hazard ratio: The likelihood of mortality compared to a control group.

Summary

Aim and key questions addressed	- Compounds that can be safely administered to humans or animals to target blood-feeding arthropods. - An impact on individual mosquito longevity can largely impact the mosquito vectorial capacity, thereby interrupting disease transmission. - Endectocides are proposed as a complementary tool alongside currently existing vector control tools for malaria.
Context	- Field administration
Test item	- Endectocidal drugs
Mosquito population	- Wild populations
Number of mosquitoes per replicate	- N/A
Endpoints measured	- Mosquito lethality - PK/PD profile of endectocides
Exposure time	- N/A
Holding time	- N/A
Indicative of personal protection	- No
Suitable chemistries	- Currently no approved endectocide for malaria control, however, majority of research is based on ivermectin.
Appropriate controls	- N/A
Relevant stage of production pipeline	- N/A
Characterisation of output	- Well categorised for uses other than malaria vector control.
Accessibility	- Need for mass drug administration programmes
Cost	- Cost associated with mass drug administration programmes

Level of validation and characterisation of outputs	- Standard membrane feeding assays are widely used, but validation for use with endectocides needs to be completed.
Outstanding questions, gaps and priorities	- No official guidance has been generated from the sufficient evidence, despite numerous funding streams being dedicated to generating an evidence base. - It is urgent to generate the evidence basis which confirms this mechanism of action is possible and epidemiological impact is achievable.
Key references, related SOPs, guidelines and publications	- I2I Landscaping Exercise, Endectocidal drugs overview (I2I., 2023) - I2I Landscaping Exercise, Clinical trials for endectocides (I2I., 2023) - Billingsley, P., Binka, F., Chaccour, C., Foy, B. D., Gold, S., Gonzalez-Silva, M., … Zulliger, R. (2020). A roadmap for the development of ivermectin as a complementary malaria vector control tool. American Journal of Tropical Medicine and Hygiene, 102(Suppl 2), 3–24. https://doi.org/10.4269/ajtmh.19-0620 - World Health Organization. (2021). Preferred product characteristics: Endectocide products for malaria transmission control

Overview

An endectocidal drug is a compound that can be safely administered to humans or animals to target blood-feeding arthropods. This tool is already being exploited in veterinary medicine to treat ectoparasites, such as ticks or fleas, in domestic and non-domestic animals and is also successfully deployed in public health to treat endoparasites, such as worms. Its impact is proposed through mass drug administration (MDA) during high transmission seasons. An impact on individual mosquito longevity can largely impact the mosquito vectorial capacity (the rate at which future infections arise from a currently infective mosquito), thereby interrupting disease transmission. Endectocides are proposed as a complementary tool alongside currently existing vector control tools for malaria.

There is currently no approved endectocide for the control of malaria and most of the research to establish an evidence base is on ivermectin, a broad-spectrum drug with an extremely well-established safety profile. Ivermectin has been used to treat onchocerciasis and lymphatic filariasis in country-wide MDA campaigns since the 1980s and has formed the cornerstone of neglected tropical disease elimination (Amazigo, 2008; Ottesen, Hooper, Bradley, & Biswas, 2008).

The initial lethal effect of ivermectin on mosquitoes was first identified in 1985 (Pampiglione, Majori, Petrangeli, & Romi, 1985) and the first controlled human study was conducted in 2010 (Chaccour, Lines, & Whitty, 2010). The application of ivermectin as an endectocide against mosquitoes for malaria control has gained much momentum since the creation of the Ivermectin Roadmappers consortium in 2017 (Billingsley et al., 2020). In 2021, the World Health Organization (WHO) announced its preferred product characteristics of an endectocidal drug, based largely on the current body of evidence on ivermectin (World Health Organization, 2021).

Recent studies have identified and established the pharmacokinetic and pharmacodynamic profile of ivermectin against Anopheles mosquitoes (Chaccour, Hammann, & Rabinovich, 2017;

Smit, Ochomo, Waterhouse, et al., 2019) which have been followed by safety and clinical trials in the field (Foy et al., 2019; Smit et al., 2018).

Define Accepted Methodologies

Are there existing standard operating procedures (SOPs)/Guidelines detailing methodologies?

Currently there are no officially approved or endorsed methodologies, standard operating procedures (SOPs) or guidelines on establishing the efficacy of an endectocidal drug. The gold standard approach is the 'standard membrane feeding assay', which has been adopted to demonstrate the efficacy of drugs/compounds to kill mosquitoes. Small scale and late-stage research have adopted this approach.

Standard membrane feeding assay (SMFA)

There are two types of standard membrane feeding assay for endectocide drugs:

- The in-vitro feeding assay whereby artificial bloodmeals are spiked with known concentrations of the drug and fed to mosquitoes through artificial feeding methods, such as a Hemotek membrane feeding system. Mosquito mortality is then observed, and survival is assessed to determine the lethality of the compound, at known concentrations.
- The in-vivo feeding assay sees human participants administered with the drug/compound and mosquitoes fed on blood draws at defined periods. Alternatively, mosquitoes are directly allowed to feed on the arms of participants, administered with the drug (direct skin feeding).

The former (in-vitro assay) is the most adopted method and has shown no difference in mosquito-killing effect when compared with direct skin-feeding in an ivermectin clinical trial (Smit, Ochomo, Aljayyoussi, et al., 2019).

The assay data is analysed using survival analysis through either of the following analytical methods:

- Probit survival analysis
    - A form of regression analysis which assesses binary outcome data such as survival and generates a "lethal concentration" based on the survival data at specified single timepoints/endpoints.
- Cox regression analysis
    - The outcome of the Cox regression analysis is a hazard ratio, describing the likelihood of mortality associated with the specific concentrations tested in the membrane feeding assay. Pharmacokinetic/pharmacodynamic (PK/PD) modelling can be applied to generate the entire concentration-effect curve of a drug.

The concentration-effect curve of a drug describes the lethality of a drug (likelihood of mortality, compared to a control) over increasing concentrations of the drug. This is often a sigmoidal relationship and can be predicted for the entire concentration-effect curve by a non-linear predictive model.

In addition to defining the mosquito-killing effect of a compound, the efficacy also of an endectocide depends on its pharmacokinetic profile. The PK/PD profile of a drug demonstrates the length of time that the compound is effective at killing mosquitoes, following administration. Whilst SMFA generated hazard ratios generate the concentration-effect curve of a drug, PK/PD modelling must be applied to that curve to simulate the lethality to mosquitoes over time, within a population. The most extensive work detailing the PK/PD simulations are detailed for ivermectin (Smit, Ochomo, Waterhouse, et al., 2019) and the pharmacology considerations are described (Chaccour et al., 2017). Further modelling on veterinary drugs describe the PK/PD modelling for use as an endectocide (Meredith, Furuya-Kanamori, & Yakob, 2019).

Emax, EC50, Hill slope estimation – PK/PD modelling

- Estimation of the PD parameters of a drug uses mosquito survival data to identify the parameters associated with the lethal effect.
- Emax: Maximal lethal effect (Expressed as a hazard ratio)
- Hill slope: Steepness of the concentration-effect curve
- EC50: Concentration of drug in the blood required to kill 50% of mosquitoes.

Population simulation modelling

- Population simulation techniques can be used to simulate the PK/PD profile of a drug, according to human or animal population dynamics. Software such as IQRtools includes features to enable such simulation modelling (Schmidt, 2020).

Are these sufficiently detailed?

Methodologies are described in varying detail in academic research papers.

- The SMFA is an established method used for purposes other than for an endectocide efficacy testing experiment and has been described elsewhere at length.
- Detailed methods are available to assess the PK profile of drugs and compounds in clinical literature, however the development of pharmacodynamic methods against mosquitoes are more newly established.
- The most detailed and described methods for this exist in the form of published academic papers and require tailoring to compound mode of action.

Do these methods require specialised/non-standardised equipment and/or training?

The *in-vitro* and *in-vivo* standard membrane feeding assays are methods that require specialised training on the use of the following techniques. Several potential blood feeding devices can be used and have been tried and tested in different settings.

- Hemotek artificial feeding system – A novel mosquito feeding system, designed, and developed in 2012 and consisting of a feeder component which heat a metal plate, which is attached to a reservoir where blood is deposited. A cellulose membrane is attached to the reservoirs allowing mosquitoes to pierce and feed.
- Membrane blood feeding system – Several earlier blood feeding systems have been developed in the field, the most used for infection assays of mosquitoes consists of a primitive version of the Hemotek: glass vials with parafilm membrane attached are held together on a wooden frame and each vial is surrounded by a jacket of warm water (thick plastic tube), which is attached to a water bath, circulating water at a specified temperature.
- New versions of this primitive method are being developed, including 3D printed versions of blood feeding systems (Witmer et al., 2018).
- Mass-rearing of mosquitoes: To expose mosquitoes to a compound within a bloodmeal, the ability to mass rear enough mosquitoes is required.
- Bloodmeal preparation: Expertise in calculating concentrations and dilutions of preference is required in generating bloodmeals of known drug concentration. In addition, pipetting small volumes (microliters) is required. Weighing solids (powered drugs) may be required, depending on the stability form of the compound.
- Interpretation of knocked down and dead mosquitoes when scoring mosquito mortality daily.
- Male, female interpretation of mosquitoes and ability to aspirate large numbers, to remove male mosquitoes and evenly distribute mosquitoes between test cages.
- Removal of unfed mosquitoes immediately following bloodmeal ingestion. Critical to the interpretation of mosquito feeding experiments is the removal of unfed mosquitoes, identified by the lack of a full abdomen.

Non-standardised equipment and consumables

- Mass mosquito rearing equipment required to generate mosquitoes for testing.
- Laboratory facilities (Sterile fume hoods, with negative pressure).

- Equipment to generate bloodmeals, including P10, P200 and P1000 pipettes and sterile pipette tips.
- Access to sterile blood (animal or human, preferably human blood).

Facilities

- Cold chain supply for the storage of test compounds, depending on longevity and stability requirements.
- Insectary facilities with a humidity and temperature-controlled environment.

Training

Specialised training in pharmacology and PK/PD modelling. Depending on the level of modelling proposed, a varying level of training would be required to sufficiently meet the needs of the study. An understanding on the pharmacokinetics of the compound and how to convert mosquito mortality data into a full PK/PD profile would require basic training on PK/PD modelling and must be overseen by a trained expert in pharmacology.

Software/tools

PK/PD simulation software and expertise in pharmacology to simulate PK/PD profile of compound, such as IQRtools (Schmidt, 2020).

Are there issues with the methods or their interpretation?

Issues with methods: SMFA

- Labour and time intensive to rear mosquitoes and prepare for blood membrane feeding experiments.
- Requires large numbers of mosquitoes per experiment.
- Requires multiple replicates of survival assays to generate sufficiently powered results.
- Requires daily score counts over a minimum of 12-day period.
- Requires insectary space and mosquito cages for rearing and follow-up of experiment.

- High level of data management required.

Issues with interpretation: SMFA

- Different interpretations between knocked-down or dead mosquitoes.
- Interpretation of fed and non-fed mosquitoes should be clearly defined and uniform between experiments.
- The removal of non-fed mosquitoes is essential during the experiment. Varying interpretations between blood fed or sugar fed mosquitoes. Meticulous detail required to ensure the removal of all non-feds.
- Low sample size of mosquitoes can affect the power to detect any difference in mortality between control and exposed mosquitoes. Caution should be exercised where sample sizes are below 100.
- The significance level assumed during Cox regression analysis can define the outcome samples size, depending on the cut-off point of p-value adopted.

Issues with methods: PK/PD modelling

- Different methodologies can be adopted to simulate the PK/PD profile of a compound, depending on the training and software available.
- The availability of pharmacokinetic parameters differs between drugs. The availability of pharmacological or clinical data ranges between therapeutic compounds. This can affect the certainty of parameters during PK/PD simulations.

Issues with interpretation: PK/PD modelling

- A lack of standardisation in generating PK/PD profiles means that interpretation can differ according to the pharmacology training of the individual. Model assumptions underpinning PK/PD modelling can differ between researchers and institutions.

What AIs or combinations of AIs have the tests been used for?

Several compound classes have been tested for their efficacy against *Anopheles* mosquitoes, which span a range of mode of actions, target sites and hosts. A systematic literature review of compounds assessed for mosquitocidal efficacy against *Anopheles* mosquitoes, included articles published up until November 2020 (Khaligh et al., 2021). Details of each study that demonstrated an impact on Anopheles species are specified in the review. The compounds identified included the macrocyclic lactones: Doramectin, eprinomectin, ivermectin and moxidectin. Additionally, results from the veterinary drugs of the isoxazoline class identified a killing effect of both Afoxolaner, Fluralaner (Miglianico et al., 2018). More recently, HPPD inhibitors, Nitisinone, tembotrione and mesotrione have been tested for a killing effect against *Anopheles* species (Haines et al., 2023; Sterkel et al., 2016; Vergaray Ramirez, Sterkel, Martins, BP Lima, & L Oliveira, 2022).

The large majority of PK/PD modelling for endectocidal drugs have been performed on ivermectin (Slater et al., 2014; Smit, Ochomo, Waterhouse, et al., 2019).
PK/PD modelling for a potential endectocidal drug has been applied to four classes of veterinary drug to simulate their potential as ectoparasitic drugs for malaria transmission control. The PK profiles of the Avermectins, isoxazolines, milbemycins, spinosyns were applied to mathematical simulation modelling (Meredith et al., 2019).

Are they validated, for which AIs/entomological effects, and to what extent?

- The standard membrane feeding assay, as previously referenced is a validated method used to assess the toxicity of compounds to mosquitoes in a bloodmeal via blood membrane feeding equipment.

- The validation processes of the assay include a positive and negative control. The positive control should contain a defined dose of a known lethal compound and the negative control should be absent of the compound to ensure that the blood used for the experiment is not causing elevated mortality of mosquitoes.
- One published validation of the membrane feeding assay for ivermectin is a comparison between the mosquito killing effect of ivermectin in an artificial membrane feeding system and between arm feeding of human participants, administered with ivermectin (Smit, Ochomo, Aljayyoussi, et al., 2019).
- Survival analysis and Probit analyses is a widely accepted and validated data analytical method to assess lethal effects on organisms, such as mosquitoes.

PK/PD modelling methodologies have been established and validated for pharmacological modelling throughout the development of drugs, over many decades. The adaption of PK/PD modelling for an endectocidal compound for mosquito control has evolved within and continues to be enhanced.

Points of validation for PK/PD modelling for an endectocide include:

- Published PK parameters, defined from years of clinical trial data.
- Comparing predicted simulation data against real-world clinical data, from patient trials or measured human participant sampling.
- Important future validations of the PK/PD modelling for endectocidal compounds will emerge from clinical trial data and be used to validate the models and predictions, which have until previously been simulations of both entomological and epidemiological impact.

What inputs need to be characterised? e.g., samples, mosquitoes, equipment

SMFA experiments

- Number of replicates
- Testing sample size should be characterised but can depend on sample size calculations and preferred power detection.
- Time for survival data to be counted (e.g., 12 days)
- Compound to test (Active ingredient)
- Required solution and methodology used to dissolve compound of interest.
- Blood samples to use in bloodmeal (e.g., horse, human)
- Selection of concentration range to test, to capture the entire concentration-effect curve of the compound.
- Dilutions required, according to selected concentrations.
- Control bloodmeal contents - containing ethanol, or PBS (depending on the solutions required to dissolve compound/active ingredient in solution, in order to match pH of bloodmeals administered in experimental bloodmeals).

PK parameters:

- Absorption and clearance parameters (Defined by clinical literature and information available on the drug/compound)
- Drug half-life, as defined by clinical research literature.

PD parameters:

- Hazard ratios generated for selected concentrations during survival assays on mosquito feeding experiments.

PK/PD simulation modelling:

- Population characteristics (Weight, height, number).
- Dosing regimen (Determines starting concentration of PK simulations).
- Dosing regimen schedule (Determines frequency of dosing).
- Uncertainty levels around parameters (e.g., standard practice, 25% within a human population, but can be available from clinical data).

Are endpoints clearly defined and appropriate? Who were they defined by?

The methods described have been obtained from academic research, defined early on by researchers. The validation methods for mosquito killing effect and for PK/PD modelling are established methods with defined endpoints. The endpoints for clinical trials for an endectocide are established but are yet to be validated.

- Mosquito survival following bloodmeal ingestion of a compound is appropriate for determining the mosquito killing effect of a compound.
- Cox regression generated hazard ratios are appropriate for representing mosquito killing effect and are widely accepted in PK/PD modelling techniques.
  Probit analysis on survival data – Also widely adopted for representing the toxicity of a compound.
- Other secondary outcomes can include oviposition, fecundity, knock-down, as defined in entomological studies. Determining these outcomes for a compound ingested in a bloodmeal will also be appropriate and important to define for test compounds.

PK/PD modelling:

- Hill slope of concentration effect curve. An outcome widely accepted in pharmacology when describing a concentration-effect curve of a drug.
- EC50, EC90, EC95 (Concentration required to kill 50, 90 and 95% of exposed mosquitoes) are defined in pharmacology and important in describing the effect of a compound on mosquitoes.
- The length of time a compound and dosing regimen can maintain a mosquito killing effect above hazard ratio of 4 has been suggested as an appropriate epidemiological cut-off point in a malaria transmission modelling study (Burrows et al., 2018).

Are there supporting SOPs? e.g., cleaning SOPs, mosquito rearing SOPs required

- SMFA – Standardized SOPs are available in the literature (Adapted for specific studies).
- Mosquito rearing SOPs previously described elsewhere are also available.
- Mosquito survival analysis is an established methodology with documented instructions available.
- In addition to standard pharmacological techniques, SOPs exist for the simulation of pharmacological profiles of drugs and methodologies are also described in published research.
- No SOPs exist to support methods for pharmacological modelling specifically for endectocidal drugs for mosquito control.

Define Current Use Practices

Does everybody use the same SOP?

- No official defined methodology is currently available.
- All experimental research on potential endectocides is performed through a standard membrane feeding assay, varying in small components of design, such as the apparatus used to administer bloodmeals or the survival follow-up time.
- Published PK/PD modelling studies on endectocidal drugs differ, depending on the proposed dosing strategy of the compound/drug. (For example, livestock administration or route of administration)
- Fundamental PK/PD modelling techniques and simulation methods are adopted throughout PK/PD modelling studies. Adopted techniques may differ, according to technique used and research objectives.

Are there differences of interpretation of the method?

Since no defined methodology is available and no approved compound exists, varying interpretations can exist. Different disease modelling methods demonstrate the varying interpretation of mosquito longevity on transmission outcome. Although there is little difference in the fundamentals of PK/PD modelling, the type of compound will impact the methodologies

adopted. Varying clinical trial designs demonstrate varying interpretations of how to define the efficacy of an endectocidal compound, although many trial designs can be determined by field site or availability of resources and funds.

Are the results obtained largely consistent between studies?

Very little studies have been conducted on potential endectocides to date, however the defined killing effect outcome (LC50) is largely uniform between studies for ivermectin.

Is further development, refinement or validation of the method required? Based on priority, significance, and relevance of method.

Areas of further development required.

- Official defined protocols for the assessment of endectocidal compounds can streamline and optimise future research.
- Generating a screening process for potential compounds can assist the generation of evidence for novel compounds by guiding research objectives.
- Developing effective methods to test compounds on wild mosquito populations, which is currently extremely difficult to achieve (i.e., the development of a forced feeding mechanism).
- Having defined cut-off points, such as sample size and required mortality observations at specified concentrations can also assist future research objectives.

Identify Potential Sources of Variation

What are the sources of variability in the method, and are their means to minimise or characterise these?

Bloodmeal administration technique

Several potential methods exist for administering a drug-spiked bloodmeal to mosquitoes. This is often determined by the funding and resources available but can impact on the administration of results but affecting the potential sterility of the bloodmeal or the potential feeding rate obtained.

Mosquito survival follow-up times

Mosquito survival follow-up times vary, ranging from 1-28 days, most commonly, 12-14 days as per the extrinsic incubation period of malaria parasites in the mosquito gut. The most detailed descriptions of these methods are described by Smit and colleagues (Smit et al., 2018). The primary outcome is the 14-day cumulative mortality of mosquitoes fed 7 days after ivermectin treatment.

Varying numbers of replicates

Mosquito survival experiments are critical in establishing the effect of a compound on survival, however, due to the large random natural variation in survival, replicate experiments are required. Currently there is no official guidance on the number of replicates required.

Variations in insectary conditions

Differences in rearing techniques or conditions can lead to variation in mosquito fitness which can impact on bioefficacy studies.

Laboratory users, counting techniques

Differences in human error and counting techniques.

Many of these differences can be expected when performing research in different settings and environments. Although it is likely that the differences described would be minimized in adjustments made during data analysis, streamlining the methods available can aid those who may wish to test a compound for this purpose.

Differences in methodology of estimating PK/PD parameters

Variations in assumptions adopted during PK/PD modelling, including certainty level assumed or confidence intervals generated.

Assumptions include:

- The ratio of capillary to venous blood concentrations used when applying *in-vivo* clinical pharmacokinetic parameters.
- The level of certainty assumed and included in the modelling impacts the confidence intervals of outcome estimates.
- Population characteristics assumed, e.g., average height and weight, impact resulting drug blood concentration achieved.

Do current method/s need to be adapted for new active ingredients/MoA/types of tools?

- Experimental preparation must be adapted uniquely for each compound or active ingredient being tested, according to its properties in solution, i.e., whether the solution requires alkaline or acid pH to dissolve in solution.
- PK/PD modelling and transmission simulation require adaption to the PK or PD parameters of the compound.

Are new methods required? Identify areas where current method/s are not suitable or sufficient.

- Automation of the SMFA experiment to perform automated mosquito counting, dead and alive rather than individual counting, removing the demand on time and labor.
- An automated screening process of identifying compounds of a similar structure that may have a lethal effect on mosquitoes, i.e., searching for mosquitocidal chemical components.

- A forced feeding chamber or method of blood feeding and exposing wild mosquitoes to compounds to determine effect on local populations, prior to undertaking large-scale clinical trials.

Gaps in biological or other understanding that hinder method development or validation

- The impact of the mosquito killing effect from an endectocidal drug on epidemiological outcome needs to be further defined (The impact on mosquito longevity on transmission outcomes).

Prioritisation – is there an issue that needs to be addressed, what specifics, how urgent is the need?

The first evidence that ivermectin was lethal to mosquitoes was generated four decades ago and yet still no official guidance has been generated from the sufficient evidence, despite numerous funding streams being dedicated to generating an evidence base. The momentum such as that generated by the "ivermectin roadmappers" has no doubt accelerated and streamlined the research evidence basis for ivermectin, however, the timing is not sufficient when considering the current WHO trajectory for malaria case and death estimates. Multiple sources agree that urgent action is necessary and novel methods are an essential component of vector control. Regardless of the outcome of the current clinical trials, the evidence generated thus far should be used as a template for other drugs to be explored in different avenues.

Utilising the drug repurposing trajectory

The role of repurposed drugs in public health is increasing. A potential screening programme could address this, whereby mass-scale drug screening could identify appropriate candidates. The way to establish a potential endectocide would be to focus on a drug that already has an

established safety profile since this aspect would prove the most difficult to address in future research.

Lack of testing ability of wild populations

Very little evidence exists for the killing effect of a drug ingested within bloodmeals on wild mosquito populations. The difficulty in artificially blood-feeding wild mosquito populations makes it almost impossible to generate this evidence. Evidence of insecticide resistance data demonstrates the critical difference between the behaviour of laboratory and wild strains of mosquito and without knowing the impact of a compound on wild populations, it is very difficult to predict the potential impact that a compound may have in the field. Research into techniques to assist the artificial feeding of wild populations to optimize experiments would assist in future research.

Official standardization of protocols

Protocols for assessing novel compounds requires standardisation and publication by the relevant assessment bodies. Preferred attributes should be defined by assessment bodies, by which researchers and academics can assess compound efficacy. There is an urgent need for a streamlined approach for the assessment of endectocidal compounds, despite the complexities of identifying and assessing the efficacy of such a compound.

Discerning the potential role of biotechnology in an endectocide

Much of the mosquito killing effect data from ivermectin suggests its toxicity is time limited. The use of slow-release capsules has been trialed, but this research should be accelerated and trialed for other compounds that have demonstrated early potential. In addition, the cost effectiveness of this should also be considered.

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