

Discovery to Development: Insecticides for Malaria Vector Control

James A. Turner^{*ab}, Colin N. E. Ruscoe^b, and Trevor R. Perrior^b

Abstract: This report provides an outline of a program for the discovery of new public health insecticides for malaria vector control. The status of malaria vector control is first reviewed in terms of the chemical, physical chemical, and biochemical properties of the current WHOPEs-recommended and approved vector control agents. This review provides a basis for a discussion on the critical need for discovery and development of multiple new chemical malaria vector control agents with novel and diverse modes of action. The Innovative Vector Control Consortium (IVCC) New Active Ingredient Target Product Profile (TPP) describes the essential attributes for a successful new malaria vector control agent and then serves as the basis for development of a discovery cascade. The cascade addresses these attributes experimentally at each stage of the discovery process – from design and assembly of an appropriate collection of chemicals for screening, through development of testing protocols to sort candidates, and into the detailed profiling of advanced pre-development candidates against TPP requirements. In addition, this program defines a staged development system to provide intermediate guidance to the insecticide explorer regarding the progress of their discovery program against the ultimate product goal.

Keywords: Insecticide · Malaria · Resistance · Screening compound selection filters · Vector

Introduction

Malaria is a debilitating and life-threatening disease that seriously affects more than 200 million people each year. The disease is caused by any one of five species of *Plasmodium* parasites, which are transmitted to humans through bites from female *Anopheles* mosquitoes. The World Health Organisation (WHO) provides an annual ‘World Malaria Report’^[1] and a ‘Malaria Fact Sheet’^[2] which detail the global impact of malaria upon human health and provides information on the causes, treatments, and control issues associated with the disease (also see the ‘Global Technical Strategy for Malaria 2016–2030’).^[3] The most recent reports include some alarming statistics; for example, approximately 3.2 billion people (half of the world’s population) residing in 97 countries are at risk of developing malaria; in 2015 there were over 438,000 deaths attributed to malaria, with 90% of these occurring in sub-Saharan Africa; the majority of deaths (70%)

are children under the age of 5 with one child dying each minute from malaria in Africa.

Although these statistics make grim reading, enormous progress has been made over the past century in reducing the devastating effects caused by malaria. Malaria eradication efforts in the 1900s eliminated the disease from the United States as well as most of Europe, Latin America and parts of Asia. More recently the WHO reports that, since 2000, “malaria death rates among populations at risk fell by 60% globally among all age groups, and by 65% among children under 5”.^[2] For all its dire consequences, malaria is an entirely preventable disease. Key components for controlling malaria include prevention of transmission through vector control, and prompt treatment with combination drug therapies. Two methods have been shown to be highly effective for control of the malaria vector: indoor residual spraying (IRS) with insecticides and the use of insecticide-treated nets (ITN), in particular long-lasting insecticide-treated nets (LLIN).^[1,4,5] When properly deployed, these methods provide individual protection from mosquitoes and proven community-wide protection from malaria transmission as a result of the suppression of the number of parasite-infected insects.^[1] A recent report estimates that, between 2000–2015, 663 million cases of malaria have been averted through malaria disease control efforts. The vast majority of these avoided cases were as a result of the use of vector control methodologies – an impressive 68% from use of ITN and 10% from IRS.^[5]

The ITN and IRS vector control methodologies are ultimately dependent upon the availability of a varied group of effective insecticide tools. Ideally, there would be a selection of chemicals to choose from, each of which would belong to a different class of chemistry and function through a unique mechanism of action at the biomolecular level within the mosquito. But the current situation for chemical malaria vector control is far from ideal. Currently, thirteen insecticides are recommended for IRS use for malaria vector control by the World Health Organisation Pesticide Evaluation Scheme (WHOPES).^[6] But these thirteen insecticides belong to only four unique chemical classes, organochlorines, organophosphates, carbamates, and pyrethroids (Fig. 1), and function through only two distinct mechanisms of action: inhibition of acetylcholinesterase (organophosphates, carbamates) and disruption of voltage-gated sodium channels (DDT, pyrethroids).^[7] The situation is even more critical for ITN uses: there are only six WHOPEs-recommended insecticides for ITN, all of which are members of a single class of chemistry (pyrethroids) and therefore share a common target site of action.^[8] To emphasise further the issue of available chemical tools, no new WHOPEs-recommended insecticide has been developed for use in public health for malaria vector control in over 30 years.

Unfortunately, the lack of chemical diversity within the approved list of malaria vector control agents results in an ideal situation for development of insecticide resistance in mosquitoes. Continual use of

*Correspondence: J. A. Turner^{ab}

E-mail: turnerja1@q.com

^aPO Box 683728

Park City, UT 84068, USA

^bPublic Health External Scientific Advisory Committee

Innovative Vector Control Consortium

Liverpool School of Tropical Medicine

Pembroke Place

Liverpool L3 5QA, UK

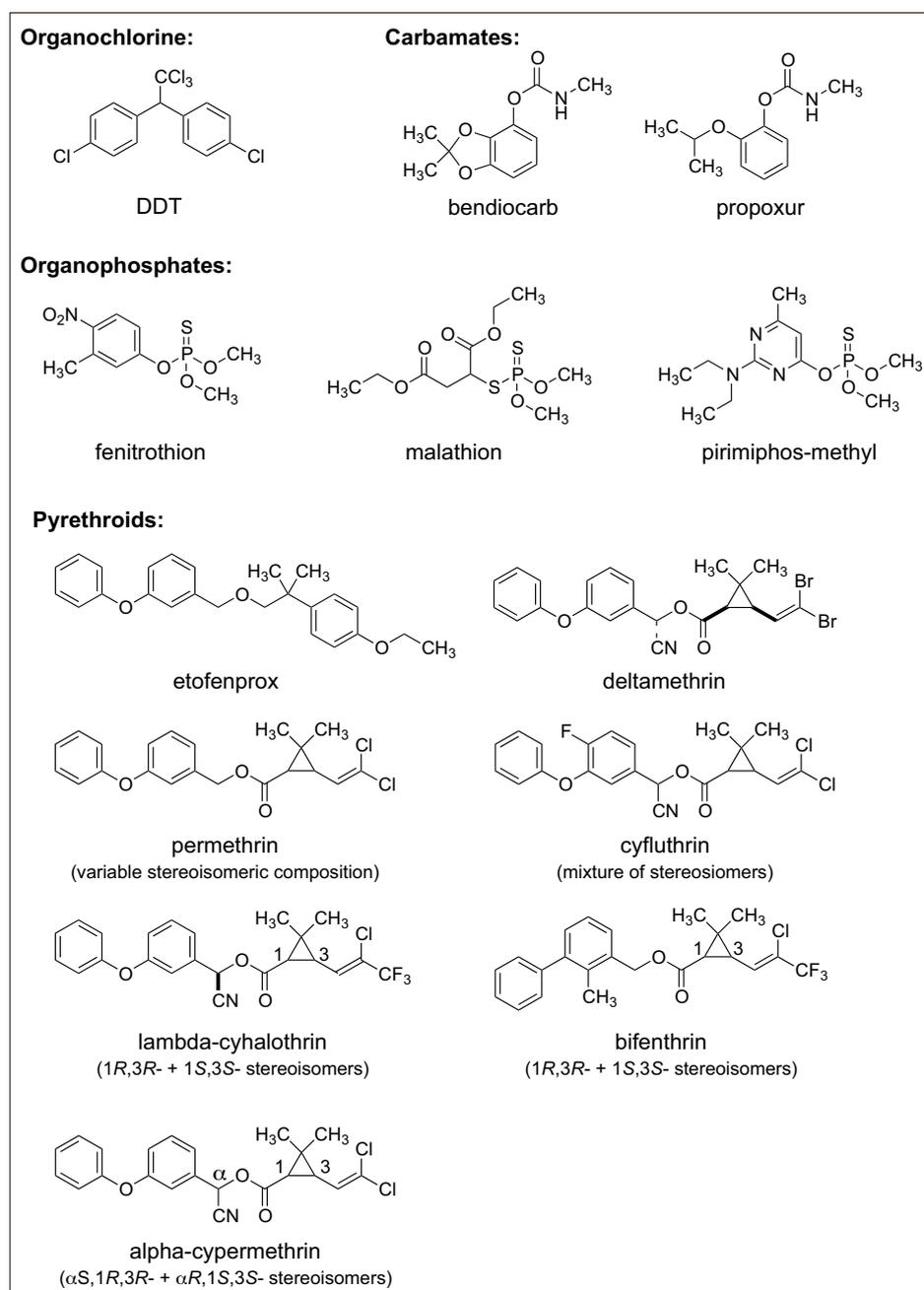


Fig. 1. WHOPEs approved chemicals for malaria vector control.

a single insecticide or class of insecticides on a local mosquito population results in selection for resistance *via* a genetic change within the mosquito population which can (a) render the insecticide less effective at interaction with the biomolecular target site (target site resistance) or (b) enhance the mosquito's ability to metabolically detoxify the insecticide (metabolic resistance). Indeed, both types of resistance, as well as combinations of the two, have been widely documented against a number of classes of insecticides, including pyrethroids, organophosphates, carbamates, DDT and dieldrin, within wild mosquito populations.^[4,9] Furthermore, a recent review and meta-analysis on the use of pyrethroids in ITN documents the presence of both target site and metabolic

resistance within the wild *Anopheles* mosquito population and points to an expected relationship between such resistance and the effective level of control of the ITN.^[10]

The Bill and Melinda Gates Foundation have initiated an ambitious effort to eradicate malaria, a goal widely supported within the international malaria community and by WHO.^[11,12,13] One consequence of this initiative was the establishment of the Innovative Vector Control Consortium (IVCC), a public-private product development partnership with a mission to "improve the tools and technologies available for malaria ... vector control".^[14,15] A key component of this mission is the discovery and development of new malaria vector control agents which would represent both novel classes of chemistry and new modes-

of-action for vector control. The availability of such novel materials is deemed critical for the prevention of wide-spread insecticide resistance, particularly in ITN uses.^[9] To help guide and direct this discovery and development effort, the IVCC has prepared a detailed set of specifications, known as the New Active Ingredient Target Product Profile (TPP),^[16,17] for new chemical entities intended for use as vector control agents in either IRS or ITN. The TPP details the necessary features of a new vector control insecticide, including its expected performance characteristics (*e.g.* potency, spectrum, speed of action), durability (residuality on multiple surfaces), human and environmental safety profile, acceptability to the purchaser (cost-effectiveness) and the end user.

In this report we provide an outline of a program for the discovery of new public health insecticides for malaria vector control. The IVCC New Active Ingredient TPP serves as the basis for development of a discovery cascade and describes the essential attributes for a successful new malaria vector control agent. The cascade addresses these attributes experimentally at each stage of the discovery process – from design and assembly of a collection of chemicals for screening, through development of testing protocols to sort and rank candidates, into the detailed profiling of advanced development candidates against TPP requirements. In addition, this program defines a staged development system to provide intermediate guidance to the insecticide explorer regarding the progress of their discovery program towards the ultimate product goal.

Discovery Approaches

Since its inception, the pesticide industry has made successful use of *in vivo* assays as the primary tool for discovery of bioactivity and identification of 'lead' classes of chemistry. The long-time strategy of the industry has been to conduct the primary bioactivity screen directly against the actual target organism, or a closely-related surrogate when the target is unavailable.^[18] To understand the rationale for this reliance on the *in vivo* approach, it is instructive to consider the discovery models used by both the agricultural and pharmaceutical industries.

First, appropriate *in vivo* models of human diseases are rarely available in a simple, inexpensive format that would allow for direct screening and sorting of the tens (or hundreds) of thousands of available chemical inputs. Instead, potential target sites (enzymes, receptors) believed to be associated with a desired therapeutic outcome are identified and significant effort

is then made to establish the therapeutic validity of these targets. An appropriate *in vitro* binding assay is developed for the target, typically in high throughput format, and a screening set is designed and assembled to find and sort potential leads. The most promising leads from this assay are then optimised for binding activity against the target protein and the best (most active and most “drug-like”) candidate(s) ultimately tested in an *in vivo* system. The main limitation of this strategy is that these simple target-based assays have not proven to be reliable predictors of activity on the whole organism, since – at least for a novel target – the importance of the target to the disease (‘target validation’) can be uncertain, and furthermore the primary assays do not take into account additional attributes, beyond intrinsic activity, which are essential for whole organism activity (e.g. oral availability, metabolic potential, ability to cross membranes and distribute within the organism). While this is a very simplified analysis of an enormously more complex process, identification of the factors that can limit therapeutic potential may be uncovered only late in the discovery process and only following extensive and expensive activity optimisation efforts.

The challenge of translating *in vitro* activity to *in vivo* is magnified in pesticide discovery, where many additional complicating factors exist. Among the more significant of these are delivery mechanism challenges, host/pest interactions, environmental influences (UV degradation, exposure to elements, e.g. air oxidation), and surface interactions (soil, plant, insecticide-treated net, wall). But ‘phenotypic’ screening directly against a target organism, for example an aphid on a plant or an insect larva eating an artificial diet, in a laboratory or greenhouse environment, poses its own set of issues. Such a system requires a significant quantity of often precious screening chemicals, is labour intensive, and is difficult to miniaturise or scale to the vast numbers of screening candidates often available or required to develop sufficient numbers of quality leads. Thus most primary pesticide discovery is now conducted *via* low-barrier, high-throughput *in vivo* assays, using target pests or indicator organisms as models for the actual targeted pests.^[19] In the case of insecticide discovery, this primary screen frequently includes measuring the kill of *Aedes aegypti* larvae, which are bathed in an aqueous solution of the chemical. A manageable number of ‘actives’ are thus identified and challenged against the target organisms themselves (adult mosquitoes in this case) in secondary assays. In this particular example, continuous exposure of the insect to the chemical minimises the effects of both metabolism and uptake, while still provid-

ing a measure of intrinsic activity and an indication of potential for redistribution. In contrast to the pharmaceutical industry discovery model, ‘actives’ from these simplified *in vivo* assays are relatively rare, but those identified are sure to be addressing a ‘validated’ (i.e. potentially lethal) target, and have at least some molecular properties consistent with those required for therapeutic control. Finally, it is worth noting that assaying directly against the therapeutic outcome maximises the opportunity for serendipitous discovery.

Binding assays do nonetheless serve important roles in the pesticide discovery process. Such assays are valuable in the lead optimisation process where an understanding of the intrinsic activity of a chemical can play a critical role in structure-activity relationship (SAR) development of a lead series and in the identification of the factors limiting expression of bioactivity against the target organism. In addition, a high-throughput *in vitro* assay can serve as an initial sorting tool for selection of chemicals for inclusion in a primary *in vivo* assay.

Discovery Cascade

Malaria vector control insecticides targeting adult mosquitoes are generally utilised either in the form of insecticide treated nets (ITN) or as indoor residual sprays (IRS). The latter are applied to the walls of buildings, to leave a deposit of insecticide that will kill insects resting on the treated surface. These two techniques are targeted

against indoor-biting mosquitoes, i.e. once they have entered dwellings. While there is the potential, and need, for novel techniques to address outdoor biting mosquitoes, these may well utilise products developed for either ITN or IRS. An alternative strategy for malaria vector control is the use of toxic baits, such as attractive toxic sugar baits (ATSB); these consist of a mosquito attractant combined with an appropriate, orally-active insecticide.^[20] In this case the toxicant can probably be based on one of the many existing classes of stomach-acting insecticides that are commonly used for control of agronomically important insects. This paper concentrates solely on discovery of novel chemistry for ITN and IRS use.

The ITN/IRS New Active Ingredient Target Product Profile^[16] provides definitions for the Essential Attributes (extracted in a simple format in Table 1) needed in a successful new product; all these properties must be met in order for a candidate to advance into development. These Essential Attributes therefore provide direct guidance for the types of properties that must be considered and evaluated during the discovery process and for the design of the screening cascade itself. Furthermore, the TPP provides the context for a staging system (Active, Hit, Lead) with well-defined gates that allow a measure of progress against the TPP at intermediate stages of the discovery effort. The combination of these Essential Attributes and the staging system can then be used to develop a screening cascade for insecticide candidates (Fig. 2). The balance of this dis-

Table 1. Essential attributes of a novel insecticide development candidate for malaria vector control.

| | |
|---------------------------------------|--|
| Activity | topically active (lethal) against adult mosquitoes |
| Speed of Effect | lethality within 72 hours of a 30-minute exposure |
| Potency | at least equivalent to that of permethrin |
| Cross-resistance | active against both target site and metabolically resistant mosquito strains |
| Spectrum | active against multiple mosquito species (minimally <i>Anopheles gambiae</i> and <i>Anopheles arabiensis</i>) |
| ITN Fit | demonstrated ability to formulate for use in bed-nets <ul style="list-style-type: none"> • can be incorporated into or impregnated onto a polymer matrix (ITN) • predicted efficacy on nets, with routine washing, lasting for 5 years (ITN) |
| IRS Fit | demonstrated ability to formulate for use as a surface spray <ul style="list-style-type: none"> • chemically stable (residual activity) for at least 6 months on standard substrates – cement, wood, mud |
| Patent Status | patentable or clear and exclusive freedom to operate |
| Human and Environmental Safety | acceptable toxicological and eco-toxicological profiles (defined in Target Product Profile) |
| Cost of Goods | acceptable cost of goods; with reference to cost/time duration equal to or better than that for permethrin. |

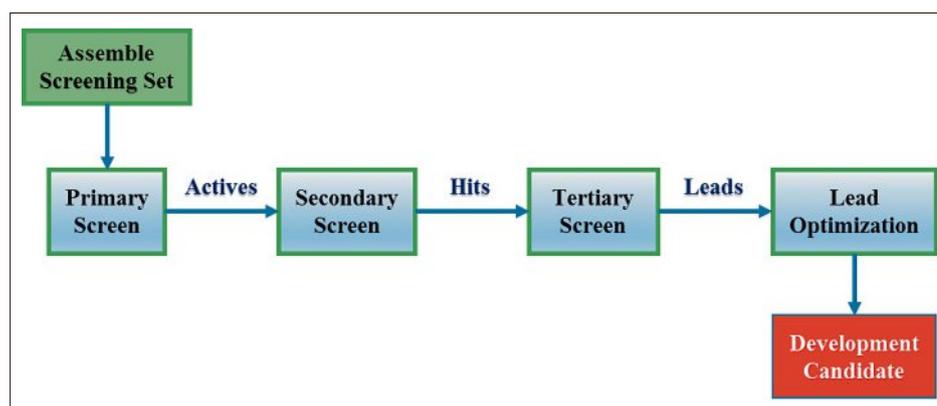


Fig. 2. Screening cascade for a new insecticide.

discussion is directed towards the activities and assays associated with each of these discovery stages for a novel insecticide intended for the control of mosquitoes acting as vectors for human diseases.

Designing the Screening Collection

Physical Properties of Insecticides: Bioavailability Predictors

The importance of the *in vitro* – *in vivo* translation problem discussed above was recognised by Lipinski^[21] in his seminal paper on the physical properties of pharmaceuticals. From an analysis of pharmaceutical databases, Lipinski introduced the concept of ‘drug-like’ molecules and developed what has become known as the ‘Lipinski Rule of 5’ as a guideline for selecting compounds for pharmaceutical screens. The ultimate goal was to enrich the screening set with molecules whose ‘drug-like’ properties would increase the likelihood that lead materials would have both high levels of intrinsic activity *and* be bioavailable. The rules are particularly useful as the properties identified by Lipinski can be easily calculated with acceptable levels of precision – for real or virtual molecules – on the often massive scale needed

to select compounds for screening or combinatorial/parallel synthesis.

Similar molecular property analyses have been conducted on pesticides, with the ultimate goal of providing analogous ‘agrochemical-like’ rule-based guidance for selection of screening inputs. The summary conclusions from two of these analyses are compiled in Table 2. The more comprehensive of these studies was reported by Tice^[22] of Rohm and Haas, who examined the physical property and functional group distribution of a collection of commercial insecticides and herbicides. This analysis is somewhat dated, and as a result does not include some important newer classes of insecticides (*e.g.* diamides and most neonicotinoids), but nonetheless does provide an extensive compilation of selected physical properties of commercial insecticides. A similar property analysis was performed on the entire collection of commercial pesticides by Clarke and Delaney of Syngenta.^[23,24] The latter report is interesting to review since it includes a property analysis not only of commercial pesticides, but also of proprietary sets of screening inputs, *in vivo* actives, and pesticide leads.

In a manner analogous to the Lipinski approach, these physical property analyses were then used to develop sets of

probability-based rules for selection of ‘agrochemical-like’ screening inputs for pesticide discovery (Table 3). An agrochemical probability predictor introduced by Briggs^[25] of Bayer (‘Briggs Rule of 3’ or ‘ground rules of three’) is included alongside the Tice and Clarke-Delaney guidelines, although details of Briggs’ analysis that led to these conclusions have not been published. The selected properties differ within each approach, but they all include molecular weight, a calculated log P value and number of hydrogen-bond donors in the molecule. Some other properties included in these guidelines, such as melting point, water solubility and $\Delta \log P$, undoubtedly play important roles in the inherent bioavailability of a chemical yet are difficult to calculate and thus of limited value as predictive tools.

Interestingly, despite the enormous differences in activity barriers, application methodologies, target organisms and therapeutic outcomes between the various classes of bioactive materials, there is a similarity across the published guidelines for most properties, even across therapeutic areas. Perhaps this is not surprising as a combination of these properties is required to strike an optimal balance between pharmacokinetics/biokinetics and biochemical recognition. In other words, the guidelines do indeed provide a good, if very general, predictor of bioavailability. The striking difference between the Lipinski guidelines and the agrochemical predictors is the recommended number of H-bond donors; the guideline for maximum number of such functionalities is 40–60% lower for agrochemicals than for pharmaceuticals.

Heading the list of Essential Attributes in the ITN/IRS TPP (Table 1) is ‘contact activity’ against an adult mosquito. Thus the mosquito must pick the chemical up from a surface (wall or bed net) and the chemical must then cross the insect cuticle, distribute within the insect to the target tissue(s) and bind with a protein(s) at the

Table 2. Selected physical properties of commercial insecticides (mean values unless noted)

| | Tice ^[22] | Clarke-Delaney ^[23] all pesticides | Clarke-Delaney ^[23] insecticides ^a | WHOPES recommended insecticides ^[6,8,26] |
|-------------------------------|----------------------|--|---|--|
| # of pesticides | 243 | 1380 | not specified | 13 |
| molecular weight | 324 | 297 | 210–500 | 361 |
| log P | 3.5 ^b | 3.0 ^c | 0.9–6.6 ^c | 4.9 |
| H-bond donors ^d | 0.4 | | | 0.15 |
| H-bond acidity ^e | | 0.3 | 0–0.7 | |
| H-bond acceptors ^f | 4.1 | | | 3.5 |
| H-bond basicity ^g | | 1.4 | 0.7–1.6 | |
| rotatable bonds | 6.1 | | | |

^a10–90th percentile range; ^blog P; ^cElog P – mean of three prediction methods; ^dSum of N-H and O-H; ^eAbraham A; ^fSum of N and O atoms; ^gAbraham B

Table 3. Probability-based ('drug-like') bioavailability predictors

| | Lipinski ^[21] | Tice ^[22] (insecticides) | Briggs – Rules of 3 ^[25] | Clarke- Delaney ^[24] |
|-------------------------------|--------------------------|-------------------------------------|-------------------------------------|---------------------------------|
| molecular weight | <500 | >150–<500 | 300 ± 100 | 200–400 |
| log P or alog P | <5 | ≥0–≤6.5 | 3 ± 2 | ≤4 |
| H-bond donors ^a | <5 | <2 | <3 | ≤2 |
| H-bond acceptors ^b | <10 | ≥1–8 | | |
| rotatable bonds | | ≤12 | | |
| melting point | | | <200 °C | <200 °C |
| Δ log P | | | <3 | ≤2 |
| log water solubility | | | | 2 ± 1 |
| pKa | | | | 7 ± 2 |

^aSum of N-H and O-H; ^bSum of N and O atoms

target site. The discussion above regarding physical properties of chemicals generally addresses the distribution, mobility, and binding of a chemical within a biological system. Yet *specific* physical requirements for cuticular penetration into an insect are not well understood nor well modelled. The situation is further complicated as a number of so-called 'residual' insecticides are not actually active through cuticular penetration but rather through ingestion, a route not appropriate for adult mosquito to control *via* either IRS or ITN. The cuticular structure is quite complex, with a waxy lipophilic exterior transitioning to a polar interior. The waxy layer is the critical barrier to chemical penetration and, as an apparent consequence, polar insecticides such as the neonicotinoids (*e.g.* imidacloprid, log P = 0.57^[26]) are rarely effective as contact treatments. In contrast, the highly lipophilic pyrethroids (permethrin, log P = 6.1^[26]), which readily partition into the cuticular wax, are potent topical insecticides. Beyond this rather generic requirement for

lipophilicity, it is difficult to provide further guidance regarding properties associated with cuticular penetration.

Finally, it is informative for this discussion of bioavailability predictors to compare the physical property analyses and guidelines described above with the actual property values of the WHOPES recommended insecticides for IRS^[6] and ITN^[8] (Table 2, Table 4).^[26] This dataset is limited to only 13 compounds, and dominated by pyrethroids, yet it is clear from this comparison that the physical properties (both mean and range) of the WHOPES insecticides fall squarely within the guidelines of the predictors.

Recommended Guidelines for Screening Candidates for Discovery of IRS / ITN Insecticides

The 'bioavailability predictors' described above have proven useful as primary filtering tools for potential screening sets in pesticide discovery and, thus, we have adapted these to a set of recommenda-

tions for vector-control insecticide discovery (Table 5). It is important to emphasise that these guidelines are intended solely for discovery of *leads*, and not *products*, from sets of chemical screening inputs.

That there should be a preferred molecular weight range is interesting. Insecticides with higher molecular weights than those proposed in the guidelines are known (*e.g.* certain pyrethroids, spinosyns, diamides), but, as said, these guidelines are intended for selection of materials as potential *leads*, not *products*. Oprea at AstraZeneca has introduced the concept of 'lead-like' space and its use as an opportunity predictor in drug discovery.^[27,28] A total of 96 pharmaceutical lead-product couplets were examined to determine the effect on molecular properties during the optimisation process. The analysis showed that, on average, the molecular weight increased by 79 Da during optimisation of a lead to a product (molecular weight increases as large as 200 Da were noted), and, even more dramatically, optimisation resulted in a mean increase in

Table 4. WHOPES recommended insecticides for malaria vector control^[6,8] (MW and log P values from *The Pesticide Manual*^[26])

| Insecticide | Use | Chemical Class | MW | log P | H-bond acceptors | H-bond donors |
|--------------------|-----------|-----------------|-------|-------|------------------|---------------|
| DDT | IRS | organochlorine | 354.5 | 6.2 | 0 | 0 |
| bendiocarb | IRS | carbamate | 223.2 | 1.72 | 5 | 1 |
| bifenthrin | IRS | pyrethroid | 422.9 | >6 | 2 | 0 |
| cyfluthrin | IRS / ITN | pyrethroid | 434.3 | 6 | 3 | 0 |
| alpha-cypermethrin | IRS / ITN | pyrethroid | 416.3 | 6.94 | 3 | 0 |
| deltamethrin | IRS / ITN | pyrethroid | 505.2 | 4.6 | 3 | 0 |
| etofenprox | IRS / ITN | pyrethroid | 376.5 | 6.9 | 3 | 0 |
| fenitrothion | IRS | organophosphate | 277.2 | 3.43 | 5 | 0 |
| lambda-cyhalothrin | IRS / ITN | pyrethroid | 449.9 | 7 | 3 | 0 |
| malathion | IRS | organophosphate | 330.4 | 2.75 | 6 | 0 |
| permethrin | ITN | pyrethroid | 391.3 | 6.1 | 3 | 0 |
| pirimiphos-methyl | IRS | organophosphate | 305.3 | 4.2 | 6 | 0 |
| propoxur | IRS | carbamate | 209.2 | 1.56 | 4 | 1 |

Table 5. Guidelines for selection of screening candidates for insecticide discovery

| Property | Guideline |
|-------------------------------|--|
| molecular weight | 200–400 |
| alog P | 3–6 |
| H-bond donors | ≤1 |
| H-bond acceptors | ≤8 |
| pka | 7 ± 2 |
| undesirable elements | no metals or boron |
| reactive functionalities | eliminate alkylating and arylating agents, aldehydes |
| basic moieties | eliminate 1°, 2° or 3° aliphatic or aromatic amines |
| acidic moieties | eliminate carboxylic acids, electron deficient phenols |
| ionized molecules | eliminate salts |
| metabolizable functionalities | minimize alcohols, phenols, electron-rich aromatics |

log P of 1.25 units (range = +0.5–4). His rationale for such significant differences was that the typical goal of optimisation is to increase potency; this usually means addition of binding recognition elements thus increasing complexity, which in turn increases both molecular weight and lipophilicity. Furthermore, he points out that if one starts with leads that are already towards the upper limit of the ‘drug-like’ properties (Lipinski, Tice, *etc.*) then the optimisation process may result in materials whose log P and molecular weight fall outside of the drug-like range. Finally, from a pragmatic standpoint, molecular weight might be considered to be a very crude indicator of molecular complexity and thus, indirectly, the ultimate cost of the active ingredient, which should be minimised for a vector-control insecticide.

The remaining guidelines reflect the lipophilic requirement to enable insect cuticular penetration, the need to purge the screening set of undesirable functionalities (metals, reactive chemicals), and a desire to enrich the screening pool with metabolically robust materials. Most of these properties can be calculated from the molecular structure, but others, such as chemical reactivity, are more difficult to define and eliminate. In these instances, it may be more practical to purge such materials from the ‘active’ list following the primary assay.

The exclusion of basic amines from the screening pool is also worthy of further discussion. Of the 243 commercial insecticides examined by Tice^[22] only 2.8% were found to contain an aromatic amine and none contained a 1°, 2°, 3° aliphatic amine or a 3° aromatic amine. This is in marked contrast to the Lipinski^[21] set where >60% of the 6454 pharmaceuticals contained a 1°, 2°, or 3° aliphatic or aromatic amine. The reason for this striking difference in functional group distribution is not en-

tirely clear but the undesirable nature of basic amines within insecticides probably reflects both a bioavailability requirement, which dictates that materials must not be ionised at physiological and environmental pH, and the high potential for amines to be metabolically oxidised. This is an important attribute to consider when purchasing/ assembling insecticide screening libraries, as commercial offerings have been largely designed to meet the needs of the pharmaceutical industry. Thus one can expect such libraries will contain a disproportionate number of amine-containing compounds that are unacceptable for insecticide discovery purposes.

Finally, while the recommended guidelines may initially appear to be rather restrictive and limiting, we would point out that there are >35 million commercial chemicals available^[29] for screening and a near incalculable number of additional materials which could be prepared from typical organic elements (C, H, N, O, P, S, halogens) *via* either conventional or combinatorial organic synthesis. Rather, we view the guidelines as liberating, as they allow the insecticide prospector to focus on the region of chemical space that offers the greatest opportunity to uncover novel bioactivity. Probably some leads will be missed by using these property filters to design screening sets; but with finite resources, leads always will be missed regardless of the screening approach.

Other Potential Screening Candidate Filters

In addition to physical property filters, additional strategies can be used to enhance the quality of a potential screening set for a vector-control insecticide. Foremost among these is prior evidence of activity in an analogous *in vivo* assay and, specifically, activity against insect species (*e.g.* dipteran). In addition, as discussed above, hits from a biochemically-relevant high-throughput *in vitro* assay would be good candidates for the primary *in vivo* assay.

Primary Assay (Identification of Actives, Fig. 3)

The primary assay is intended to give the test chemical the best chance of showing activity (*i.e.* maximal contact, minimal metabolic potential) against a single insect species. The exact nature of the assay will depend largely on the number of compounds available for testing. The two most typical options are a mosquito larval assay or a simplified contact adult mosquito assay (see Secondary Assay below).

Mosquito larval assay features:

- *Aedes aegypti* larvae contained in a microtiter plate and bathed in an aqueous solution of the screening candidate
- Chemical added as a concentrated solution in an organic solvent (acetone or DMSO) and diluted with water to the desired concentration (5–30 ppm)
- Single dose of the chemical, dose chosen to ensure an acceptably small number of false negatives
- Appropriate doses of relevant standards (positive controls – minimally permethrin and bendiocarb) and negative controls must be included
- Graded as pass/fail, based on number of dead or affected insects in each replicate as compared to controls and standards
- Sufficient number of insects per treatment to ensure statistically reliable results (minimum 5, preferably 10)
- It is important to confirm positive results by repeat tests with the compounds concerned, usually with 2–3 replicates

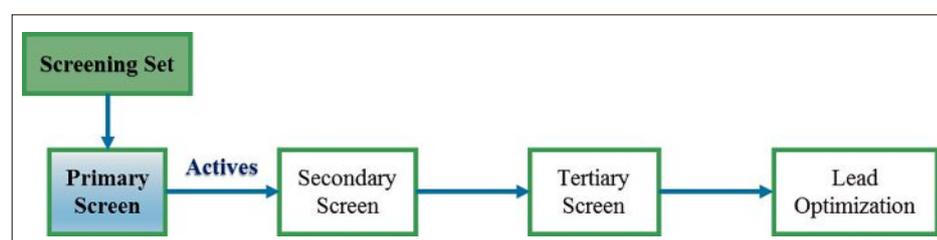


Fig. 3. Primary screen of medium-large sets of chemicals to uncover *in vivo* insect ‘actives’.

The mosquito larval assay is a useful tool to sift through large screening sets, but a significant number of false positives can be anticipated. There are multiple potential mechanisms for lethality in such a low barrier larval assay, many of which are not relevant to adult mosquitoes and others are inconsistent with the requisite topical activity. For these reasons it is *essential* to regard the larval assay as a simple prognosticative tool and avoid the temptation to optimise activity against larvae.

Secondary Assay (Hit Identification, Fig. 4)

The primary purpose of the secondary assay is to uncover relevant lethal activity against a susceptible strain of female adult mosquitoes. The initial assay may be run by topically dosing each individual insect with a solution of the chemical. However, at this stage, an assessment must be made of the lethality resulting from direct pick-up (tarsal contact) of the chemical by the mosquito from an inert surface (glass, plastic, glazed tile). Activity in topical tests does not necessarily correlate with that in direct 'pick-up' contact tests; the latter is more relevant to the use pattern in practice (*i.e.* tarsal contact with ITN or IRS surfaces).

Contact mosquito assay features:

- Susceptible *Aedes* strain acceptable, but *Anopheles* species preferred
- Assay conducted in a confined space (bottle, petri dish, tube, WHO cone *etc.*), to maximise contact of the insects with the treated surface
- A portion or preferably all of the surface is coated with a solution of the chemical and dried to a film
- Female adult mosquitoes added to container and remain in contact with the chemical for a defined period of time – 30 minutes preferred
- The insects are then monitored for the duration of the assay (minimum 24, preferably 72 h)
- Sufficient number of replicates to ensure statistically significant results (minimum 3)

- At conclusion of assay the number of dead/affected/live insects counted to determine % lethality for each replicate
- Appropriate doses of relevant standards (positive controls – minimally permethrin and bendiocarb) and negative controls must be included
- Dose responses must be generated at this phase
- End-point for each chemical reported as an LD₉₀ and referenced to that of the standards and the controls

The approximate speed of action for each confirmed adult active should be determined by varying contact time with the chemical and/or by evaluation at various time points post contact (30 sec.–30 min). Non-lethal affects and symptomatology should be noted – these could provide important information regarding knockdown or repellent potential, possible clues to the mechanism of action (MOA) of the chemistry, or may suggest novel control mechanisms.

Note that activity *via* ingestion or injection of the mosquito is not relevant to the intended uses (ITN, IRS). However, such information may be of value to a particular research program as a means to understand the biokinetic properties of a particular chemical or class of chemicals.

Tertiary Assays (Lead Identification, Fig. 5)

Spectrum of Mosquito Control, and Activity Against Resistant Strains

A primary goal at this stage is to determine the activity of the chemical class against a broader set of mosquito species/strains. The selection may include representative susceptible *Aedes* and *Culex* species but must include *Anopheles gambiae* and *Anopheles arabiensis*. The materials must also be tested against a panel of available and well-characterised insecticide resistant mosquito strains chosen to represent the range of critical field resistance mechanisms (kdr, rdl, metabolism...). Assays against the resistant strains may need to be accomplished through

collaboration with an external partner, *e.g.* the LITE (Liverpool Insect Testing Establishment) at the Liverpool School of Tropical Medicine^[30] or the MR4 program (Malaria Research and Reference Reagent Resource Center).^[31]

Assay Format

- Contact from surface as described above
- Multiple susceptible mosquito species (must include *Anopheles gambiae* and *Anopheles arabiensis*)
- Multiple resistant mosquito strains (*e.g.* Kisumu RDL, Akron, Fumoz, Cayman, Tiassale); this selection will be based on the latest judgements regarding importance of these strains to field resistance mechanisms, and advice on this should be obtained from the IVCC
- Appropriate doses of relevant standards (positive controls – minimally permethrin and bendiocarb) and negative controls must be included
- Sufficient number of replicates to ensure statistically significant results (minimum 3)
- Dose responses must be generated
- End-point for each chemical reported as a breakpoint (LD₉₀) and referenced to that of the standards and the controls

A thorough analysis of the attributes of the chemistry Hit class should be initiated at this stage to fully characterise potential for advancement to Lead status.

Chemical Properties

- Chemical integrity; purity of sample; isomers and isomeric ratios; isomer interconversions
- Assessment of potential intellectual property position; ability to obtain substance and/or use patents for members of the class
- Physical property measurements (log P, aqueous and organic solubility)
- Chemical stability (hydrolytic, photolytic) evaluated using analytical tools (HPLC, LC/MS) instead of bioassay

Chemistry Breadth

- Evaluated through procurement (purchase or synthesis) and bioassay of additional analogues of Hit class

The intent is to provide a clear indication that development of SAR is feasible (*i.e.* there are multiple examples of potency within the series) and to provide initial guidance for possible downstream lead optimisation. This should not be construed as an effort to optimise the series at this stage.

Synthetic Accessibility:

- Do viable routes exist to generate requisite quantities of multiple, diverse analogues in an optimisation program?
- Any expensive reagents or starting ma-

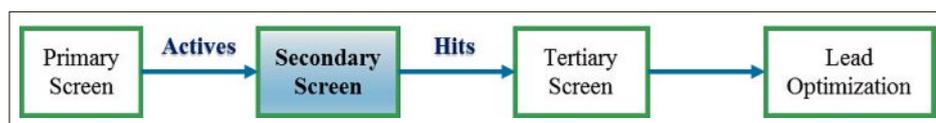


Fig. 4. Characterisation of 'active' chemistries for advancement to 'Hit' status.

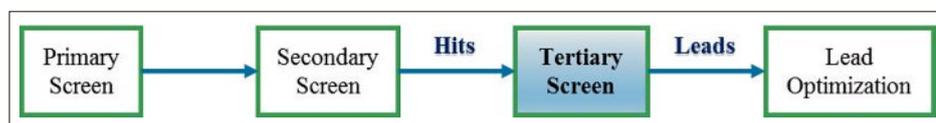


Fig. 5. Characterisation of 'Hit' classes of chemistry for advancement to 'Lead' status or termination.

terials; complex and difficult to separate reaction mixtures/isomers?

Mammalian Toxicology

- Probe with a single dose (≥ 50 mg/kg) acute oral assay in either mice or rats (typically three animals are sufficient for an initial test)
- Assessment of mutagenicity potential of Hit class (Ames test)

Broad-Spectrum Insect Control

- Agricultural pests – representative chewing (Lepidoptera) and sucking (aphids, whiteflies, hoppers) insects
- Public health pests – houseflies and cockroaches
- Animal health pests – flies, ticks

The scope of the analysis should be sufficient to give an indication of the commercial *potential* for insect control beyond mosquitoes; testing should be done in a way (presentation, rates) that allows comparison of activity with relevant commercial standards for these pests. A broader spectrum, which raises commercial attractiveness and the potential for development cost-sharing, is valuable to the IVCC in order to stimulate the interest of possible development partners.

Biochemistry

- Directed toward determination of MOA of Hit class
- Corroborate and support bioassay results with resistant mosquito strains
- Include assays to identify compounds with unacceptable/potentially problematic MOA's:
 - non-specific or reactive chemistry
 - non-selective AChE inhibitors
 - uncouplers of oxidative phosphorylation

Probe Formulations

- Simple formulations, *e.g.* EC (emulsifiable concentrate) or WP (wetttable powder), prepared and evaluated *via* bioassay

Molecular properties analyses may provide important clues regarding effective formulation types or point to potential bioavailability issues (*e.g.* solubility, crystallinity, polarity) within the class.

Development Candidate Selection Phase (Lead Optimisation, Fig. 6)

Many of the activities and assays associated with this stage of the discovery effort will be specific to a particular class of chemistry. The discussion below is intended to provide general guidance of the types of efforts and information needed in this phase. At this stage, and preferably earlier, it is essential that the research project

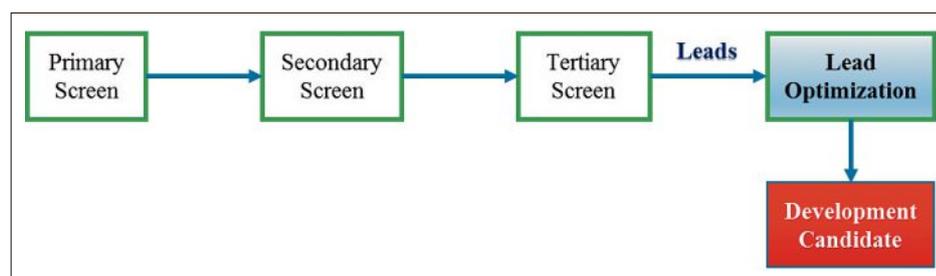


Fig. 6. Lead Optimisation, and selection of Development Candidate(s).

leaders consult with members of IVCC for guidance regarding the assays that are required, sources of expertise, and potential collaborative strategy and resources.

The goal of this stage will be optimisation of the lead chemistry against the TPP attributes. Chemical synthesis and SAR development will be a primary activity and would ideally be supported with appropriate binding assays, biokinetic studies, and computational chemistry efforts. Obviously the discussion regarding preferred physical property values and structural features for screening compound selection is still of importance during the optimisation process. The optimisation will undoubtedly include many trade-offs and in many cases potency will *not* be the sole or most important driver for selection or advancement of a development candidate. It is thus critical to define and focus on the limiting attributes of the lead class and design synthetic analogues and appropriate assays (biological or chemical), and for toxicity to non-target organisms (including man) accordingly. For example, in some series the ability to design and prepare analogues that block insect metabolism may result in higher levels of potency and/or a greater degree of control of metabolically-resistant mosquitoes. In contrast, in other areas of chemistry, the addition of a metabolically-labile functionality might result in selective mammalian detoxification and an improved safety profile.

The synthetic program should also be designed towards ensuring that freedom to operate (FTO) exists for the development candidate. Patent applications (if relevant) should be filed early in this stage and enabling materials prepared and tested to support the claims of the application. At the end of this phase there should be clear evidence that FTO will be attained. The discovery program will undoubtedly necessitate synthesis of quantities of materials for formulation development, toxicological studies, and specialised and intensive biological testing, including field trials. The chemical synthesis program should begin to suggest potential manufacturing routes for ultimate commercial preparation. These should be subjected to 'cost of goods' analyses which, coupled with a

projected use rate, will determine a cost/treatment. Software is available to assist in such analyses; potential development collaborators will need to have expertise in these studies.

Biological assays, beyond the routine contact assays needed to support the SAR development, should be directed towards demonstration of potential efficacy under a 'real world' scenario. As before, all assays must generate dose-responses and include appropriate product standards (WHOPES and commercial). Such studies will require the development of well-defined, commercially-viable formulations for IRS and/or ITN. For ITN uses, the ability to incorporate a lead chemical into and onto a polymeric matrix should also be assessed. Demonstration of residual activity will be required for IRS and/or ITN applications using methods described in WHO guidelines^[32] *e.g.* 'Cone' or, preferably, 'Cylinder' tests and optionally, 'Tunnel' tests for bioassay. Residual effect, suitable for use in ITN, is assessed through simulated bed-net washings using both bioassay of nets, and chemical analyses of washings. IRS residual stability is determined by treatments of a number of representative surface types (mud, concrete, plywood, thatch, bamboo, *etc.*)^[32] with the formulated chemical and assessed again through both biological and chemical endpoints. WHOPES provides well-defined testing protocols for evaluation of insecticide persistence on nets and on substrates typical of those found in the walls and roofs of village residences.^[32–34]

Toxicological studies should continue to focus on probe, single-dose acute oral rodent assays to help guide the optimisation program and to characterise promising candidates. Initial planning should commence (logistics, resource commitments) to conduct the early-stage toxicological studies (acute oral, acute dermal, mutagenicity, carcinogenicity, reproductive toxicology, 90-day chronic) needed to support a registration package for a development candidate. Probe eco-toxicological studies should be initiated against aquatic organisms (fish, algae, *Daphnia*).

Discussion

In this report we have described a screening cascade that can be used for sorting and evaluation of sets of chemicals in order to select a viable candidate for further development for use as a malaria vector control agent. Identification of such a candidate represents only the first step in the rigorous and complex development process leading to successful registration, commercialisation and public acceptance of an insecticide for use in vector control. Many of these development activities are typical of any new pesticide active ingredient and will include such details as the selection and characterisation of final formulation(s), full toxicological and eco-toxicological testing, intellectual property protection, identification of a cost-acceptable process route along with logistics for manufacture of the active ingredient, a final risk assessment, assembly of a data package for submission to regulatory authorities, and preparation of a market access plan (Fig. 7).

An essential step in the development process for a malaria vector control agent is the evaluation of the formulated active ingredient under field conditions. For this purpose, WHOPEs describes a detailed three-phased program (Fig. 8) for evaluation of IRS or ITN targeted insecticides using defined and standardised testing procedures.^[32,33] In the initial stage of this WHOPEs protocol, Phase I, the biological activity of the development candidate is fully characterised *under laboratory conditions*. The candidate is profiled against mosquitoes that are representative of the region where the product is likely to be employed and the efficacy and residuality of the formulated active ingredient, and wash resistance in the case of ITN, determined

on a variety of substrates. These activities are similar to those described under the 'Development Candidate Selection Phase' section above.

The second and third stages (Phase II and III)^[32,33] of the WHOPEs scheme address the issues of translation of laboratory results into real world situations; these trials must be conducted in a region where the malaria vector is endemic. In each phase, the insecticide is either incorporated into, or coated onto, bed nets or sprayed onto the walls of human-occupied residences; the residences are accessible to free-flying native mosquito populations. Phase II field trials are small-scale in nature and are conducted in individual, experimental huts under very carefully controlled conditions; the intent is to validate the laboratory-determined efficacy and residuality of the treatment and to establish the appropriate dose rate. Efficacy, at this stage, is evaluated using the cone bioassay, employing susceptible, laboratory-reared mosquitoes of the local vector species. Phase III field trials are much larger in scope, are directed at determining the effect of the treatment at the whole community level rather than individual residences, and the experimental variables of the trial are, of necessity, much less well-controlled than those of the Phase II trials. The purpose, as in Phase II, is primarily to confirm efficacy and residuality of the product but this phase will also assess the acceptance and ease of use of the net or formulation by the occupants of the community and the applicators as well as document any perceived side effects to the community. The design of the Phase II and Phase III studies is critical, in order to provide statistically significant proof of the efficacy and duration of activity of the insecticide.

WHO will only recommend insecti-

cides for use in IRS or ITN if they have been evaluated through the WHOPEs Phase system described above. Note that the WHOPEs guidelines are intended to evaluate the efficacy of the products against "a fully susceptible *Anopheles* vector species" and, thus, do not directly address the efficacy of ITN products against *resistant* mosquitoes. For this purpose, the WHO Vector Control Advisory Group (VCAG) has published separate guidelines for evaluation of new ITN products against pyrethroid resistant mosquitoes.^[35]

It should be noted that a new process for evaluation and adoption of novel vector control products by WHO is currently under development. Under this procedure, responsibility for such evaluation will be transferred from WHOPEs to a vector control products WHO Prequalification Team.^[36] The Prequalification process, which has not yet been fully defined, will be implemented in 2017.^[37]

It should be clear that the discovery to development process for a new vector control insecticide is multifaceted, time consuming and expensive. The complexity of the overall process demands the collective expertise of individuals from a number of disciplines including chemistry (synthesis, computational, process), biology (entomology, field biology, genetics), biochemistry, toxicology, patent law, environmental science, and formulation science, to name a few. Only the largest industrial organisations will have both the level of experience and the breadth of expertise necessary to successfully navigate the complete path from identification of the conceptual target product profile through delivery of an effective vector control agent to the consumer. It is therefore imperative that the early-stage discovery scientist make a clear and honest assessment of both the

| Lead Discovery | Lead Optimization | Pre-Development | Development and registration | WHOPEs recommendation and local registration |
|---|--|--|---|---|
| <ul style="list-style-type: none"> ▪ Screening chemical inputs against <i>Aedes</i> larvae ▪ Hits screened for activity against adult mosquitoes ▪ Mode of action evaluated ▪ Cross resistance tested | <ul style="list-style-type: none"> ▪ Intensive analogue synthesis ▪ Testing focussed against key attributes <ul style="list-style-type: none"> ○ laboratory activity ○ safety and environmental impact ○ production and patentability ▪ Spectrum of control and cross-resistance fully characterized ▪ Chemical properties evaluated ▪ MOA shown to be acceptable ▪ Probe formulations prepared and tested ▪ Acute mammalian oral toxicology evaluated in probe studies ▪ Mutagenicity potential assessed (Ames) | <ul style="list-style-type: none"> ▪ Define <i>the</i> compound with highest probability of meeting financial and technical criteria for new product including <ul style="list-style-type: none"> ○ ability to produce ○ product performance ○ licence to sell ▪ In-market field trials ▪ Chronic toxicology evaluated ▪ Environmental fate and impact assessed ▪ Formulation(s) selected ▪ Process development defined ▪ Market positioning assessed | <ul style="list-style-type: none"> All studies involve the chosen molecule, its preferred formulations and the defined use patterns <ul style="list-style-type: none"> ▪ In-country, in-house and official trials ▪ Mixtures and sectors ▪ Formulation and pack optimisation ▪ Full chronic toxicology evaluated ▪ Toxicology, eco-toxicology, and regulatory packages developed and submitted ▪ Manufacturing route and logistics for production and launch established ▪ Market access plan established. | <ul style="list-style-type: none"> Submission for WHOPEs recommendation preferred formulation(s) <ul style="list-style-type: none"> • WHOPEs Phase I lab trials • WHOPEs Phase II hut trials • WHOPEs Phase III community trials Final vector control product testing Individual country registration, once WHOPEs approval attained |

Fig. 7. Discovery to Registration Scheme.

| Phase I Laboratory studies | Phase II Small-scale field trials | Phase III Large-scale field trials |
|--|--|---|
| <ul style="list-style-type: none"> ▪ Intrinsic activity ▪ Diagnostic concentration ▪ Behavioral properties (irritant, excito-repellent) ▪ Cross-resistance ▪ Efficacy and residual activity on relevant substrates <ul style="list-style-type: none"> ▫ mosquito nets ▫ other substrates | <ul style="list-style-type: none"> ▪ Intrinsic activity ▪ Efficacy and persistence under different ecological settings <ul style="list-style-type: none"> ▫ blood-feeding ▫ mortality ▪ Dosage of application ▪ Impact on vector behavior <ul style="list-style-type: none"> ▫ deterrence and exophilicity ▪ Handling and application issues ▪ Perceived side-effects ▪ Safety | <ul style="list-style-type: none"> ▪ Efficacy ▪ Residual activity ▪ Operational acceptance ▪ Community acceptance ▪ Safety |

Fig. 8. Phases of the WHOPES evaluation protocol for new insecticides for malaria vector control.

strengths and the limitations of their organisations' research and development capabilities as it relates to the overall goal of delivery of a novel vector control insecticide. This will then allow the discovery scientist to identify missing capabilities and seek appropriate collaborator(s) who have access to the level and types of expertise needed to ensure timely progress along the discovery to development pathway. The IVCC can assist the discovery scientist in conducting the expertise gap analysis as well as in the identification of appropriate discovery and development partners.

In conclusion, the current lack of a sufficient number of unique classes of insecticides for use in IRS and ITN, *i.e.* materials which function by a diversity of biochemical mechanisms, represents a critical threat to the malaria eradication goal. The Innovative Vector Control Consortium (IVCC), funded by donors including the UK and US governments and the Bill and Melinda Gates Foundation, is working with a broad range of partners around the world to develop a new generation of insecticide treated bed nets, indoor residual sprays and tools to manage outdoor disease transmission for use against malaria in developing countries. The IVCC New Active Ingredient TPP describes the essential attributes required for such insecticides and the TPP, in turn, serves as the basis for a discovery cascade that can be used to select a candidate for further development for use as a malaria vector control agent. Discovery scientists are strongly encouraged to maintain a focus on the TPP attributes and use these as a continuous guide in the decision making and compound selection processes.

At the time of going to press, three IVCC-funded novel public health insecticide projects with modes of action that are new to public health are in the pre-development stage, with launch expected between

2020 and 2024. The new active ingredients for vector control will need to be managed by using mixtures of chemistries and/or well-designed rotation plans to optimise their field performance and to delay the evolution of resistant mosquito strains.

Acknowledgement

We would like to thank David Malone, Dr. Nick Hamon and Prof. John Pickett for their review of the manuscript and thoughtful suggestions.

Received: May 15, 2016

- [1] WHO. World Malaria Report: **2015**, http://apps.who.int/iris/bitstream/10665/200018/1/9789241565158_eng.pdf?ua=1
- [2] WHO, 'Malaria - Fact Sheet', **2016**, <http://www.who.int/mediacentre/factsheets/fs094/en/>
- [3] WHO, 'Global Technical Strategy for Malaria 2016-2030', http://apps.who.int/iris/bitstream/10665/176712/1/9789241564991_eng.pdf?ua=1
- [4] A. Enayati, J. Hemingway, *Annu. Rev. Entomol.* **2010**, *55*, 569.
- [5] S. Bhatt, D. J. Weiss, E. Cameron, D. Bisanzio, B. Mappin, U. Dalrymple, K. E. Battle, C. L. Moyes, A. Henry, P. A. Eckhoff, E. A. Wenger, O. Briët, M. A. Penny, T. A. Smith, A. Bennett, J. Yukich, T. P. Eisele, J. T. Griffin, C. A. Fergus, M. Lynch, F. Lindgren, J. M. Cohen, C. L. J. Murray, D. L. Smith, S. I. Hay, R. E. Cibulskis, P. W. Gething, *Nature* **2015**, *576*, 207.
- [6] WHO, 'WHO recommended insecticides for indoor residual spraying against malaria vectors', **2015**, http://www.who.int/whopes/Insecticides_IRS_2_March_2015.pdf?ua=1
- [7] IRAC, 'IRAC MoA Classification Scheme, Version 8.1', **2016**, <http://www.irc-online.org/documents/moa-classification/?ext=pdf>
- [8] WHO, 'WHO recommended insecticide products for treatment of mosquito nets for malaria vector control', **2016**, http://www.who.int/whopes/Insecticides_ITN_Malaria_Feb2016.pdf?ua=1
- [9] WHO, 'Global plan for insecticide resistance management in malaria vectors', **2012**, http://apps.who.int/iris/bitstream/10665/44846/1/9789241564472_eng.pdf?ua=1
- [10] C. Strode, S. Donegan, P. Garner, A. Ali Enayati, J. Hemingway, *PLOS Med.* **2014**, *11*, e1001619.
- [11] R. Feachem, O. Sabot, *Lancet* **2008**, *371*, 1633.
- [12] <http://www.gatesfoundation.org/What-We-Do/Global-Health/Malaria>
- [13] B. Gates, R. Chambers, 'From Aspiration to Action: What will it take to end malaria?', <http://endmalaria2040.org/>
- [14] J. Hemingway, B. J. Beaty, M. Rowland, T. W. Scott, B. L. Sharp, *Trends Parasitol.* **2006**, *22*, 308.
- [15] <http://www.ivcc.com/>
- [16] <http://www.ivcc.com/creating-solutions/our-work/achievements/active-ingredient-portfolio>
- [17] J. Vontas, S. Moore, I. Kleinschmidt, H. Ranson, S. Lindsay, C. Lengeler, N. Hamon, T. McLean, J. Hemingway, *Trends Parasitol.* **2014**, DOI: 10.1016/j.pt.2014.02.005.
- [18] For an excellent review of the history and current status of insecticide discovery see: T. C. Sparks, *Pestic. Biochem. Phys.* **2013**, *107*, 8.
- [19] M. Drewes, K. Tiejn, T. C. Sparks, 'High throughput screening in agricultural research', in: Modern 'Methods in Crop Protection Research', Eds. P. Jeschke, W. Kramer, U. Schirmer, M. Witschel, Wiley VCH, Weinheim, GR, **2012**, 3–20.
- [20] Z. P. Stewart, R. M. Oxborough, P. K. Tungu, M. J. Kirby, M. W. Rowland, S. R. Irish, *PloS One* **2013**, DOI: 10.1371/journal.pone.0084168.
- [21] C. A. Lipinski, F. Lombardo, B. W. Dominy, P. J. Feeney, *Adv. Drug Deliv. Rev.* **1997**, *23*, 3.
- [22] C. M. Tice, *Pest Manag. Sci.* **2001**, *57*, 3.
- [23] E. D. Clarke, J. S. Delaney, *Chimia* **2003**, *57*, 731.
- [24] E. D. Clarke, J. S. Delaney, 10th IUPAC Congress on the Chemistry of Crop Protection, Basle, August, **2002**.
- [25] G. Briggs, 10th IUPAC Congress on the Chemistry of Crop Protection, Basle, August, **2002**.
- [26] Physical property values taken from 'The Pesticide Manual: 17th Edition', Ed. J. Turner, British Crop Production Council, Hampshire, UK, **2015**.
- [27] S. J. Teague, A. M. Davis, P. D. Leeson, T. Oprea, *Angew. Chem. Int. Ed.* **1999**, *38*, 3743.
- [28] T. I. Oprea, A. M. Davis, S. J. Teague, P. D. Leeson, *J. Chem. Inf. Comput. Sci.* **2001**, *41*, 1308.
- [29] The ZINC database is a list of commercially available compounds: <https://docking.org/>
- [30] <http://www.lite-testing-facility.com/>
- [31] <https://www.beiresources.org/ProgramInformation.aspx>
- [32] WHO, 'Guidelines for testing mosquito adulticides for indoor residual spraying and treatment of mosquito nets', **2006**, WHO/CDS/NTD/WHOPES/GCDPP/2006.3 http://whqlibdoc.who.int/hq/2006/WHO_CDS_NTD_WHOPE_GCDPP_2006.3_eng.pdf
- [33] WHO, 'Guidelines for laboratory and field-testing of long-lasting insecticidal nets', **2013**, WHO/HTM/NTD/WHOPES/2013.1 http://apps.who.int/iris/bitstream/10665/80270/1/9789241505277_eng.pdf?ua=1
- [34] <http://www.who.int/whopes/en/>
- [35] WHO, 'Guidelines for testing new LLINs to substantiate claims of efficacy in areas of high insecticide resistance' in 'Report on the Third Meeting of the WHO Vector Control Advisory Group', **2014**, ISBN 978 92 4 150867 4. <http://apps.who.int/prequal/vcp.htm>
- [36] <http://apps.who.int/prequal/vcp.htm>
- [37] http://apps.who.int/prequal/vector_control/about_vcp.htm