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Insecticides as Strategic Weapons for Malaria Vector Control

Mauro Prato[1], Amina Khadjavi[1], Giorgia Mandili[1], Valerio G. Minero[2] and Giuliana Giribaldi[1]
[1]*Dipartimento di Genetica, Biologia e Biochimica, Facolta' di Medicina e Chirurgia, Universita' di Torino*
[2]*Dipartimento di Medicina e Oncologia Sperimentale, Facolta' di Medicina e Chirurgia, Universita' di Torino*
Italy

1. Introduction

Malaria is a parasitic disease confined mostly to the tropical areas, caused by parasites of the genus *Plasmodium* and transmitted by mosquitoes of the genus *Anopheles*. Annually, nearly a million human deaths, mainly of children ≤5 years of age, are registered among 500 million cases of clinical malaria, whereas 2.37 billion people are estimated to be at risk of infection by *P. falciparum*, the most virulent among *Plasmodia* (Guerra et al., 2008). In 2007, the Bill and Melinda Gates Foundation, rapidly endorsed by the World Health Organization (WHO) and the Roll Back Malaria association, claimed for malaria eradication as the primary goal to be prosecuted (Roberts & Enserink, 2007). In order to achieve such an ambitious objective, several strategies are being adopted, involving multidisciplinary areas such as treatment, chemoprevention, vaccine research, health system assessment and of note vector control (Greenwood, 2008; Khadjavi et al., 2010). Indeed insecticides, which have already been essential components of previous malaria control programs, are supposed to play a key role in the new eradication program, where they will be employed either for indoor spraying or treated bednet approaches (Greenwood, 2008; Khadjavi et al., 2010).

The present chapter will review the status of insecticides currently used for malaria vector control, along with present evidence on their benefits and risks in relation to the available alternatives. After a brief description of the *Plasmodium* life cycle, occurring either in mosquito vector (sexual reproduction) or in human host (asexual replication), the insecticides currently allowed by WHO for malaria vector control, including organophosphates (OP) for larval control and organochlorines (OCs), pyrethroids (PYs) and carbamates (Cs) for the control of adult mosquitoes, will be described; formulation, side effects and cost-effectiveness will be discussed. A special attention will be paid to 1,1,1-trichloro-2,2-di(4-chlorophenyl)ethane (DDT), which is presently used by approximately fourteen countries, while several others are preparing to reintroduce it. Nevertheless, the concerns about the continued use of DDT and the recent reports of high levels of human exposure associated with indoor spraying amid accumulating evidence on chronic health effects will be taken in account. Furthermore, the big issue of growing resistances to the

toxic action of current insecticides, which are spreading almost worldwide, will be focused in a dedicated paragraph. Finally, the existing and future alternatives, either chemical or non-chemical, to the insecticides currently in use will be analyzed focusing on repellents and genetic control. Taken altogether, the data shown in the present chapter could be useful to the reader to better know the present and the future tools available for malaria vector control, in the context of the ongoing malaria eradication program.

Informations on available insecticides, formulations, side effects, resistance, cost-effectiveness, and alternatives have been obtained from literature searches, by using the search engines Scopus and Pubmed. Due to the complexity of the subject, only the most relevant studies were selected, and reviews were prioritized. Old literature was accessed electronically, or hard copies were obtained from libraries. Information on human exposure and health effects is based on reviews published over the past five years and supplemented with recent studies on exposure due to indoor spraying and treated bednets.

2. *Plasmodium* life cycle

Plasmodium species all share the same life cycle, which occurs either in human host (asexual cycle) or in mosquito vector (sexual cycle), as represented in Figure 1.

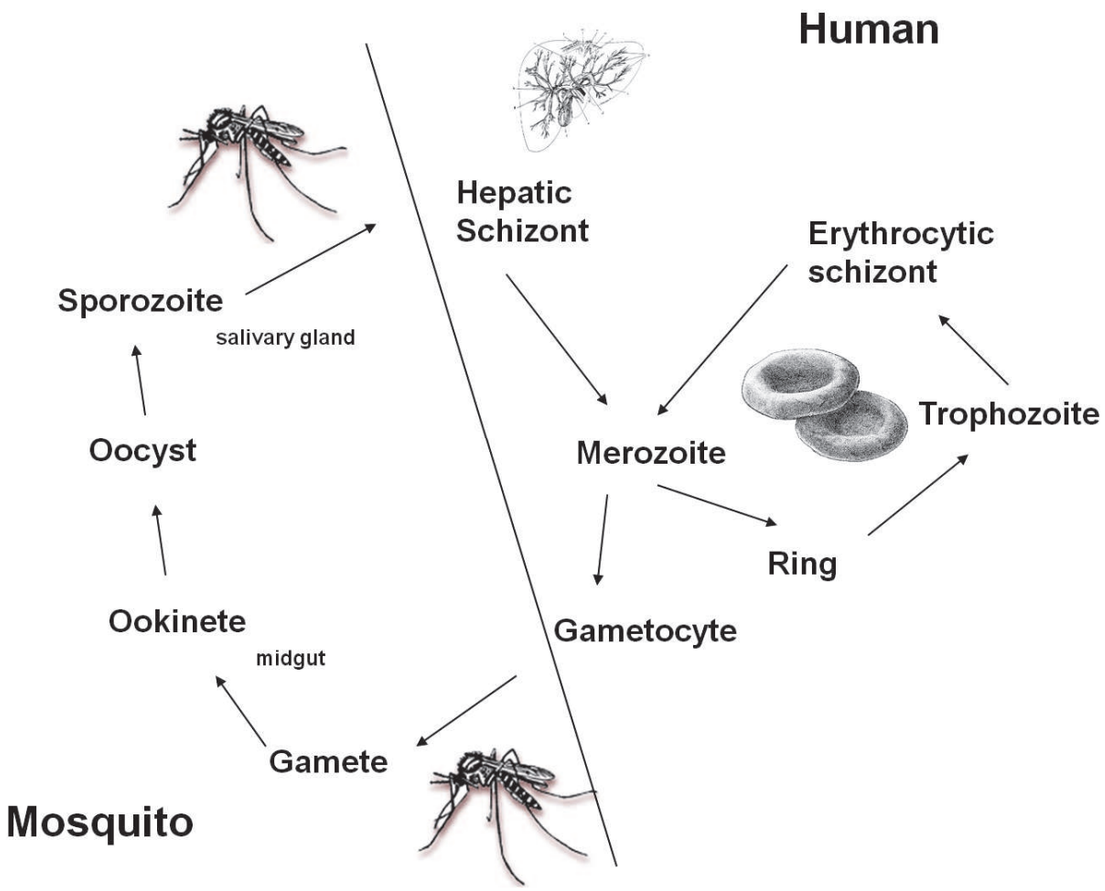

Fig. 1. *Plasmodium* parasite life cycle.

Parasites are transmitted to humans by the females of the *Anopheles* mosquito species. There are about 460 species of *Anopheles* mosquitoes, but only 68 transmit malaria. *Anopheles gambiae*, found in Africa, is one of the major malaria vectors. It is long-living, prefers feeding on humans, and lives in areas near human habitation (Rogier & Hommel, 2011). The malarial infection begins when the sporozoite stage of the parasite, that resides within the salivary gland of the mosquito, halts in the host liver (Menard, 2005). This happens when an infected female bites a healthy person and takes its blood meal, injecting a small amount of saliva into the skin wound. Male mosquito does not feed on blood, hence only female serves as a vector. The saliva contains anti-haemostatic and anti-inflammatory enzymes that disrupt the clotting process and inhibit the pain reaction. Typically, each infected bite contains 5-200 sporozoites which proceed to infect the human vector. Once in the human bloodstream, the sporozoites only circulate for a matter of minutes before infecting liver cells.

2.1 Liver stage in man

After circulating in the bloodstream, sporozoites migrate to the liver and finally infect a hepatocyte, after crossing several Kupffer cells and hepatocytes (Trieu et al., 2006). The sporozoites rapidly grow in size absorbing nourishment to form a large round schizont. The schizont divides by schizogony, a type of asexual reproduction, in which multiple fissions result in the formation of a number of small, spindle-shaped uninucleate cells called merozoites (Rogier & Hommel, 2011). Schizonts rupture and merozoites are released into the sinusoids or venous passages of the liver. This phase of asexual reproduction is called pre-erythrocytic schizogony. The merozoites are immune to medicines and host natural resistance. After a development stage in liver, during which there are no clinical symptoms of disease, merozoites are released into the blood and enter the erythrocytic portion of their life-cycle. A single schizont can produce thousands of merozoites by asexual reproduction.

2.2 Erythrocytic stage in man

The merozoites feed on erythrocytes, become rounded and modify into a trophozoite. During growth, a vacuole appears in the centre of merozoites and the nucleus is pushed to one side; this modification, that is known as “ring stage”, gives it a ring-like appearance. This food vacuole secretes some digestive enzymes, which break down haemoglobin into proteins and haematin. Proteins are used by the parasite as nourishment source, whereas haematin is converted into a waste product called haemozoin, a lipid-enriched ferriprotoporphyrin IX crystal avidly phagocytosed by host immune cells. As a result of phagocytosis, several monocyte functions are impaired, including oxidative burst, bacterial killing, antigen presentation, coordination of erythropoiesis. Moreover, the production of several pro-inflammatory molecules, including cytokines, chemokines and matrix metalloproteinases, as well as the production of anti-apoptotic molecules, such as heat shock protein-27, is enhanced. The overproduction of these host molecules as a response to a parasite product has been proposed to play a crucial role in clinical progress towards complicated malaria, including cerebral malaria, respiratory distress, and placental malaria (Prato et al., 2005, 2008, 2009, 2010a, 2010b, 2010c; Giribaldi et al., 2010; Khadjavi et al., 2010; Prato et al. 2011a, 2011b; Prato 2012; Giribaldi et al., 2011). During their growth, the trophozoites metamorphose into schizonts (Rogier & Hommel, 2011). Schizont appears after a period of about 36 to 40 hours of growth and represents the full-grown trophozoite. The

nucleus of schizont divides in the next 6 to 8 hours to form 12 to 24 daughter nuclei of new merozoite cells in the erythrocyte. This phase of asexual multiplication is known as erythrocytic schizogony. One erythrocytic cycle is completed in 48 hours. Thereafter, the merozoites burst from the red blood cell, and proceed to infect other erythrocytes. The parasite remains in the bloodstream for roughly 60 seconds before entering into another erythrocyte, restarting the process (Cowman & Crabb, 2006). This infection cycle occurs in a highly synchronous fashion, with roughly all of the parasites throughout the blood in the same stage of development. The toxins are liberated into the blood along with the liberation of merozoites. The toxins are then deposed in the liver, spleen and under the skin, so that the host gets a sallow colour. The accumulated toxins cause malaria fever: the patient suffers from chills, shivering, sweating and high temperature. The fever lasts for six to ten hours and then it comes again after every 48 hours with the liberation of a new generation of merozoites. During the erythrocytic stage, some merozoites increase in size to form two types of gametocytes, the macrogametocytes and microgametocytes. The macrogametocytes (female) are large, round with the food laden cytoplasm and a small eccentric nucleus. The microgametocytes (male) are small, with clear cytoplasm and a large central nucleus. This process is called gametocytogenesis. The specific factors and causes underlying this sexual differentiation are largely unknown. The gametocytes take roughly 8–10 days to reach full maturity and do not develop further until they get sucked by the appropriate species of mosquito. If this does not happen, they degenerate and die, because they require lower temperature for further development.

2.3 Life cycle in mosquito

When a female *Anopheles* sucks the blood of a malaria patient, the gametocytes enter along with blood, reaching the stomach and leading to formation of gametes (Aly et al., 2009). Only the gametocytes survive inside the stomach, while the other stages of the parasite, as well as the erythrocytes, are digested. Two types of gametes are formed: the microgametocytes (male) become active and their nucleus divides to produce 6 to 8 haploid daughter nuclei. The nuclei arrange at the periphery. The cytoplasm gives out same number of flagella-like projections. A daughter nucleus enters in each projection. These projections separate from the cytoplasm. This process of formation of microgametes is called exflagellation. From each microgametocyte, 6 to 8 flagella-like active microgametes are formed. The megagametocyte (female) undergoes some reorganization and forms megagametes. Fertilization of the female gamete by the male gamete occurs rapidly after gametogenesis. The fertilization event produces a zygote that remains inactive for some time and then elongates into a worm-like ookinete or vermicule. The zygote and ookinete are the only diploid stages. The ookinete penetrates the wall of the stomach and comes to lie below its outer epithelial layer. It gets enclosed in a cyst formed partly by the zygote and partly by the stomach of mosquito. The encysted zygote is called oocyst. The oocysts absorb nourishment and grow to about five times in size. They protrude from the surface of the stomach as transparent rounded structures. Over a period of 1–3 weeks, the oocyst grows to a size of tens to hundreds of micrometes. During this time, multiple nuclear divisions occur. As a consequence of oocyst maturation, the oocyst divides to form multiple haploid sporozoites. Each oocyst may contain thousands of sporozoites and groups of sporozoites get arranged around the vacuoles. This phase of asexual multiplication is known as sporogony. In the mosquito, the whole sexual cycle is completed in 10 to 21 days. Finally the

oocyst bursts and sporozoites are liberated into the haemolymph of the mosquito. They spread throughout the haemolymph and eventually reach the salivary glands and enter the duct of the hypopharynx. The mosquito now becomes infective and sporozoites get inoculated or injected into the human blood when the mosquito bites, starting a new life cycle. It is estimated that a single infected mosquito may contain as many as 200,000 sporozoites.

3. Insecticides used for malaria vector control

The most prominent classes of insecticides are organochlorines (OCs), organophosphates (OPs), carbamates (Cs), and pyrethroids (PYs). In general, they act by poisoning the nervous system of insects, which is fundamentally similar to that of mammals. A small amount of pesticide can be fatal for an insect, primarily because of its small size and high rate of metabolism. Such an amount is not fatal for humans, but it may still harm. Since the similarities between the nervous system structures make it nearly impossible to design insecticides affecting only insect pests, insecticides may affect non-pest insects, people, wildlife, and pets. Some insecticides harm water quality or affect organisms in other ways; for example, the insecticide carbaryl (a C insecticide, further discussed below) is listed as a carcinogen by the state of California. The newer insecticides are designed to be more specific and less persistent in the environment (Toxipedia, 2011).

3.1 Organochlorines

Chemical structure of OCs is various, but they all contain chlorine, which places them in a larger class of compounds called chlorinated hydrocarbons. These compounds, including DDT, represent a typical example of the potential risks and benefits of insecticide use. OCs have serious unintended consequences, despite the advantage of being cheap and effective against target species. OCs alter and disrupt the movement of ions such as calcium, chloride, sodium, and potassium into and out of nerve cells, but, depending on their specific structure, they may also affect the nervous system in other ways. OCs are very stable, slow to degrade in the environment, soluble in fats (and are therefore readily taken up by insects), and seemingly harmless to mammals; for this reason, at one time, OCs are thought to be ideal. Unfortunately, persistence and fat solubility are very undesirable: OCs can bioaccumulate in the fat of large animals and humans by passing up the food chain. The global use and transport of OCs result in the contamination of wildlife around the globe, including Arctic and Antarctic regions where these insecticides are not used. A decline in the number of birds that prey on animals exposed to DDT is one of the first signs of the unintended consequences. Unexpectedly, DDT causes a thinning of the bird eggshells and results in the death of newborns. OCs like DDT are now largely banned in industrialized countries but they are still manufactured and used in developing countries where they are exposed by the formers.

3.1.1 1,1,1-trichloro-2,2-di(4-chlorophenyl)ethane

DDT is an OC insoluble in water but soluble in most organic solvents, fats, and oils. DDT is not present naturally, but is produced by the reaction of chloral (CCl_3CHO) with chlorobenzene (C_6H_5Cl) in the presence of sulfuric acid, which acts as a catalyst. DDT is a persistent organic pollutant that is extremely hydrophobic and strongly absorbed by soil, where its half life can range from 22 days to 30 years depending on conditions. Routes of

loss and degradation include runoff, volatilization, photolysis and aerobic and anaerobic biodegradation. When applied to aquatic ecosystems DTT is quickly absorbed by organisms and by soil or it evaporates, leaving little amount of DDT dissolved in the water itself (Agency for Toxic Substances and Disease Registry, 2002)

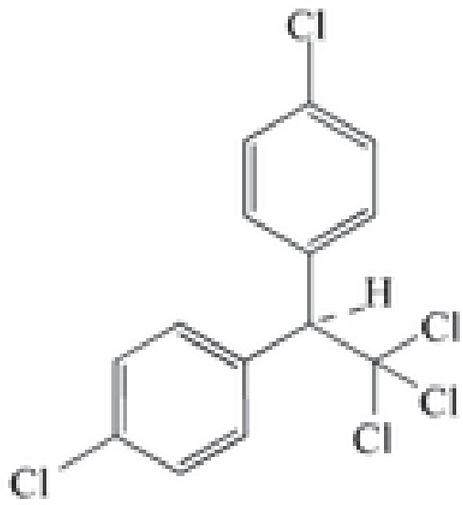

Fig. 2. DDT.

In insects DTT opens sodium ion channels in neurons, causing them to burn spontaneously. This effect leads to spasms and eventual death. For this reason, insects with certain mutations in their sodium channel gene are resistant to DDT and other similar insecticides. DDT resistance is also conferred by up-regulation of genes expressing cytochrome P450 in some insect species (Denholm et al., 2002). In 1955, the WHO commenced a program to eradicate malaria worldwide, relying largely on DDT. The program was initially very successful, eliminating the disease in Taiwan, much of the Caribbean, the Balkans, parts of northern Africa, the northern region of Australia, and a large swath of the South Pacific and dramatically reducing mortality in Sri Lanka and India (Harrison, 1978). However, widespread agricultural use led to resistant insect populations. In many areas, early victories partially or completely reversed, and in some cases rates of transmission even increased (Chapin & Wasserstrom,1981). The program was successful in eliminating malaria only in areas with "high socio-economic status, well-organized healthcare systems, and relatively less intensive or seasonal malaria transmission" (Sadasivaiah et al., 2007). In tropical regions, DDT was less effective due to the continuous life cycle of mosquitoes and poor infrastructure. It was not applied at all in sub-Saharan Africa due to these perceived difficulties.

Through genotoxicity or endocrine disruption DDT may affect human health. DDT may be directly genotoxic, but may also induce enzymes to produce other genotoxic intermediates and DNA adducts [45].

Moreover, based on the results of animal studies, DDT is suspected to cause cancer. By epidemiological studies it is worth demonstrated that DDT causes liver, pancreas and breast cancers. Its contribution in the development of leukemia, lymphoma and testicular cancer is still unclear. Other epidemiological studies suggest that DDT does not cause

multiple myeloma or prostate, endometrium, rectum, lung, bladder, and stomach cancers (Rogan & Chen, 2005; Eskenazi, 2009; Spinelli et al., 2007; McGlynn et al., 2008).

3.2 Organophosphates and Carbamates

OP is the general name for esters of phosphoric acid. These compounds were developed in the 1940s as highly toxic biological warfare agents (nerve gases). Modern derivatives, including sarin and VX, were stockpiled by several countries and now present some difficult disposal problems. In their search for insecticides that would target selected species and would be less toxic to mammals many different OPs have been developed. When the OP Parathion was first used as a replacement for DDT, it was believed to be better and more specific. Unfortunately, Parathion short-term (acute) toxicity is greater than DDT, and this characteristic causes a significant number of human deaths. On the other hand, Cs feature the carbamate ester functional group. Although OPs and Cs have very different chemical structures, they share a similar mechanism of action and will be examined here as one class of insecticides. OPs and Cs affect an important neurotransmitter common to both insects and mammals, the acetylcholine, which is essential for communication of nerve cells. Acetylcholine, released by one nerve cell, initiates communication with another nerve cell, but this stimulation should eventually be stopped. The interruption of this communication is made by removing acetylcholine from the area around the nerve cells. Subsequently, acetylcholine is broken down by a specific enzyme, the acetylcholinesterase. OPs and Cs block the enzyme and disrupt the proper functioning of the nerve cells. Hence, these insecticides are called acetylcholinesterase inhibitors. Structural differences between the various OPs and Cs affect the efficiency and degree of acetylcholinesterase blockage. Nerve gases are highly efficient and permanently block acetylcholinesterase, while the commonly used pesticides block acetylcholinesterase only temporarily. The toxicity of these pesticides presents significant health hazards, and researchers continue to work to develop new insecticides that have fewer unintended consequences.

3.3 Pyrethroids

Synthetic PYs, that were first developed in the 1980s, are one of the newer classes of insecticides; they are loosely based upon the naturally pyrethrum found in *Chrysanthemum* flowers and first commercially used in the 1800s. Their use has increased significantly over the last 20 years. The chemical structure of PYs is quite different from that of OCs, OPs and Cs but the primary site of action is also the nervous system. PYs affect the movement of sodium ions (Na+) into and out of nerve cells that become hypersensitive to neurotransmitters. Structural differences between several PYs can change their toxic effects on specific insects and even mammals. PYs are more persistent in the environment compared to natural pyrethrum, which is unstable in light and breaks down very quickly in sunlight.

3.4 Chemical agents in malaria vector control

The historical successful elimination of malaria in various parts of the world has been achieved mainly by vector control (Harrison, 1978). In addition, the Global Malaria Control Strategy emphasized the need for selective and sustainable preventive measures for reducing malaria transmission (WHO, 1993). In order to control vector-borne diseases, control of mosquitoes is the most important aspect. It is accomplished by application of chemical pesticides against adult-stage mosquitoes. Application of insecticides remains the

primary control tool in the majority of vector control programs throughout the world since early nineteenth century (Breman, 2001). In the twentieth century, after the discovery of DDT, a new era of insect control began (Hassall, 1982). DDT was the first synthetic organic insecticide used for effective vector control with reasonable success. DDT was banned by Environmental Protection Agency in 1972, owing to ecological considerations and opening up a debate between groups for or against the ban. However, the ban exempts its use in public health emergencies like outbreaks of malaria. The restriction permits indoor residual sprays (IRS) of DDT in malaria control until an effective, affordable, and safe alternative is available. In September 2006, based on the increasing scientific evidences, finally, WHO gave a clean bill to use of DDT to fight against malaria in Africa and other areas where the vectors are still susceptible to DDT (WHO, 2006a). However, the debate on the use of DDT is still continuing and will continue until a more effective, affordable, and safe alternative tool is made available.

3.4.1 Indoor residual spraying

Indoor residual spraying (IRS) with insecticides continues to be the mainstay for malaria control and represents an application of stable formulations of insecticides to the interior sprayable surfaces (walls and roofs) of houses to kill the mosquitoes. This affects the malaria transmission by reducing the life span of female mosquitoes thereby reducing density of mosquitoes (WHO, 2006b). Insecticide efficacy depends not only on the molecule intrinsic chemical nature and properties but also on certain technical factors, such as susceptibility of the target vector species to different insecticides, quality of indoor spraying (dose dispensation and coverage), and on residual efficacy. Insecticides recommended by WHO for IRS for control of malaria vectors are given in Table 1.

Insecticide compounds and formulations	Chemical type (2)	Dosage (a.i[a]g/m2)	Mode of action	Duration of effective action (months)
DDT WP	OC	1–2	Contact	>6
Malathion WP	OP	2	Contact	2–3
Fenitrothion WP	OP	2	Contact & airborne	3–6
Pirimiphos-methyl WP, EC	OP	1–2	Contact & airborne	2–3
Bendiocarb WP	C	0.1–0.4	Contact & airborne	2–6
Propoxur WP	C	1–2	Contact & airborne	3–6
Alpha-cypermethrin WP, SC	PY	0.02–0.03	Contact	4–6
Bifenthrin	PY	0.025–0.05	Contact	3–6
Cyfluthrin WP	PY	0.02–0.05	Contact	3–6
Deltamethrin WP, WG	PY	0.02–0.025	Contact	3–6
Etofenprox WP	PY	0.1–0.3	Contact	3–6
Lambda-cyhalothrin WP, CS	PY	0.02–0.03	Contact	3–6

Formulations: *CS* capsule suspension; *EC* emulsifiable concentrate; *WP* wettable powder; *OC* Organochlorines; *OP* Organophosphates; *C* Carbamates; *PY* Pyrethroids; [a] *a.i.* active ingredient

Table 1. Insecticides recommended for IRS against malaria vectors.

3.4.2 Space spraying

Space spraying/fogging, which is produced by rapidly heating the liquid chemical to form very fine droplets that resemble smoke or fog, is the process of application of a pesticide . It is primarily reserved for application during emergency situations for halting epidemics or rapidly reducing adult mosquito populations resulting in decrease of transmission (CDC, 2009). It is effective as a contact poison with no residual effect. Space spraying must coincide with the peak activity of adult mosquitoes, because resting mosquitoes are often found in areas that are out of reach to the applied insecticides (e.g., under leaves, in small crevices). The best moment to kill adult mosquitoes by fogging is at dusk, when they are most active in forming swarms. The most commonly used products are natural pyrethrum extract, synthetic PYs, and Malathion. WHO recommended insecticides for space sprays are listed in Table 2.

Insecticide	Chemical type	Dosage of a.i[a] (g/ha)	
		Cold aerosol	Thermal fog
Boiresmethrin	PY	5	10
Cyfluthrin	PY	1–2	1–2
Cypermethrin	PY	1–3	–
Cyphenothrin	PY	2–5	5–10
Deltamethrin	PY	0.5–1.0	–
D-phenothrin	PY	5–20	–
Etofenprox	PY	10–20	10–20
Fentirothion	OP	250–300	250–300
Malathion	OP	112–600	500–600
Permethrin	PY	5	10
Pirimphos-methyl	OP	230–330	180–200
Resmethrin	PY	2–4	4
d,d-trans-cyphenothrin	PY	1–2	2.5–5

[a] *a. i.* active ingredient

Table 2. Insecticides suitable for application as cold aerosol ULV sprays or thermal fogs for mosquito control.

3.4.3 Insecticide-treated nets

Mosquito nets effectively prevent malaria transmission by forming a physical barrier between insects and man. Insecticide-treated nets (ITNs), impregnated with PYs, were introduced in the place of untreated nets, that are not a perfect barrier, not only in order to decrease the man–mosquito contact by deterrence or excito-irritability but also to kill the mosquito with its residual insecticidal activity. They are more effective than untreated nets with >70% protection and are proved to be a cost-effective prevention method against malaria (D'Alessandro et al., 1995). WHO-recommended insecticide products for the treatment of mosquito nets for malaria vector control are given in Table 3.

1. Conventional Treatment		
Insecticide	**Formulation**	**Dosage (mg/m^{2} net)**
Alpha-cypermethrin	Suspension concentrate 10%	20–40
Cyfluthrin	Emulsion, oil in water 5%	50
Deltamethrin	Suspension concentrate 1%; Water dispersible tablet 25% and WT 25% + binder [3]	15–25
Etofenprox	Emulsion, oil in water 10%	200
Lambda-cyhalothrin	Capsule suspension 2.5%	10–15
Permethrin	Emulsifiable concentrate 10%	200–500
2. Long-lasting treatment		
Product name	**Product type**	**Status of WHO recommendation**
ICON® MAXX	Lambda-cyhalothrin 10% CS + binder Target dose of 50 mg/m2	Interim

Table 3. WHO-recommended insecticide products for the treatment of mosquito nets for malaria vector control.

3.4.4 Long-lasting insecticidal materials

The rapid loss of efficacy of ITNs due to washing and to the associated low-retreatment rates of the nets limits the operational effectiveness of an ITN program (Lines, 1996). Long-lasting insecticidal nets (LLINs) reduce human–mosquito contact, which results in lower sporozoite and parasite rates. The biological activity generally lasts as long as the net itself (3–4 years for polyester nets and 4–5 years for polyethylene nets) (WHO, 2005). A list of WHO-recommended long-lasting insecticidal mosquito nets for use in public health is given in Table 4. Only five brands of LLINs are currently recommended by the WHO Pesticide Evaluation Scheme, and Olyset® net is the only one which currently granted full recommendation (N'Guessan et al., 2001; Teklehaimanot et al., 2007), while Perma-Net-2.0®, Duranet-®, Net Protect-®, and Interceptor-®, including long-lasting insecticide treatment kits K-OTab1-2-3® and ICON-MAXX® (Sinden, 2007), are approved as an interim recommendation.

Also treatments of screens, curtains, canvas tents, plastic sheet, tarpaulin, etc., with insecticides may provide a cheap and practical solution for malaria vector control. Effectiveness of treated screen and curtains can be comparable to that of mosquito nets. Different types of long-lasting insecticide impregnated materials are under field trials in different countries. The residual insecticides in insecticide-treated wall lining (ITWL) are durable and maintain control of insects significantly longer than IRS and may provide an effective alternative or additional vector control tool to ITNs and IRS (Munga et al., 2009).

4. Insecticide resistance

A major concern on the use of currently available insecticides for malaria control is represented by increasing insecticide resistance (Enayati & Hemingway, 2010). For example, DDT was first introduced for mosquito control in 1946; however, already in 1947 the first cases of DDT resistance occurred, and up to now DDT resistance at various levels

Product name	Product type	Status of WHO recommendation
DawaPlus® 2.0	Deltamethrin coated on polyester	Interim
Duranet ®	Alpha-cypermethrin incorporated into polyethylene	Interim
Interceptor ®	Alpha-cypermethrin coated on polyester	Interim
Netprotect®	Deltamethrin incororated into polyethylene	Interim
Olyset®	Permethrin incorporated into polyethylene	Full
PermaNet ®2.0	Deltamethrin coated on polyester	Full
PermaNet® 2.5	Deltamethrin coated on polyester with strengthened border	Interim
PermaNet® 3.0	Combination of deltamethrin coated on polyester with strengthened border (side panels) and deltamethrin and PBO incorporated into polyethylene (roof)	Interim

Table 4. WHO-recommended long-lasting insecticidal mosquito nets for use in public health.

has been reported for > 50 species of *Anopheles* mosquitoes, including many vectors of malaria (Hemingway & Ranson, 2000). Unfortunately, the introduction of new other insecticides for malaria control, including OPs, Cs, and PYs, improved malaria control strategy only partially, since resistance has tended to follow the switches in insecticides (Hemingway & Ranson, 2000).

In the past, the use of DDT in agriculture was considered a major cause of its resistance in malaria vectors, as many vectors breed in agricultural environments (Mouchet, 1988). At present, DDT resistance is thought to be triggered further by the use of synthetic PYs (Diabate et al., 2002). Indeed, DDT and PYs share a common target, thus facilitating the development of a cross-resistance mechanism (Martinez-Torres et al., 1998). In addition, evidence of increased frequency of resistance genes due to IRS or ITN programs is quite alarming (Karunaratne & Hemingway 2001; Stump et al., 2004): PYs, the only class approved for use on ITNs (Zaim M et al 2000), are being increasingly deployed in IRS programmes in Africa and there has been a dramatic increase in reports of PY resistance in malaria vectors over the past decade (Santolamazza et al., 2008); moreover, PYs are also widely used in the control of agricultural pests worldwide (Ranson et al., 2011).

Typically, two major mechanisms are assumed to be responsible for insecticide resistance: a) changes in the insecticide target site (mutations in the sodium channel, acetylcholinesterase and GABA receptor genes) that reduce its binding; b) increased rates of insecticide metabolism (alterations in the levels or activities of detoxification proteins) and reduced insecticide ability to reach the target site (Hemingway et al., 2004; Ranson et al., 2011).

These mechanisms, alone or in combination, lead to resistance, sometimes at an extremely high level, to all of the available classes of insecticides (Hemingway et al., 2004).

4.1 Target site resistance

As previously discussed, OPs, Cs, OCs, and PYs all target the nervous system (Enayati & Hemingway, 2010). Single base point mutations are the most common cause of target-site resistance, changing the properties of these target sites, and reducing their susceptibility to insecticide binding (Hemingway & Ranson, 2000; Enayati & Hemingway, 2010).

4.1.1 Voltage-gated sodium channel

PYs and OCs target the voltage-gated sodium channel in insect neurons (Davies, T.G. et al. 2007). Insecticide binding delays closure of the sodium channel prolonging action potential and causing repetitive neuron firing, paralysis and eventual death of the insect (Ranson, 2011). Mutations in the sodium channel conferred by DDT and PY resistance are known as knockdown resistance (kdr), so-called because insects with these alleles can withstand prolonged exposure to insecticides without being 'knocked-down' (Hemingway et al., 2004; Hemingway & Ranson, 2000; Ranson, 2011). The kdr is due to changes in the affinity between the insecticide and its binding site on the sodium channel, as a consequence of single or multiple substitutions in the sodium channel gene (Martinez-Torres et al., 1998). 1014 residual aminoacid replacement, which consists in substitution of the leucine residue with an alternative phenylalanine or serine, does not appear to interact directly with the insecticide but is predicted to alter channel activation kinetics (O'Reilly A.O. et al. 2006,Enayati A. and Hemingway J. 2010; Ranson H. et al 2011). However, even though the association between kdr and resistance to PYs and DDT is clear, it is not well understood whether this allele resistance alone is sufficient to lead to control failure (Ranson et al., 2011).

4.1.2 Acetylcholinesterase

The molecular target of OPs and Cs is acetylcholinesterase (AChE) (Enayati & Hemingway, 2010). AChE has a key role in the nervous system, terminating nerve impulses by catalyzing the hydrolysis of the neurotransmitter acetylcholine on the post-synaptic nerve membrane (Hemingway & Ranson, 2000; Hemingway et al., 2004). The insecticides inhibit enzyme activity by covalently phosphorylating or carbamylating the serine residue within the active site (Corbett, 1984). Mutations in AChE gene in OP- and C-resistant insects result in a decreased sensitivity to inhibition of the enzyme by these insecticides (Hemingway & Ranson, 2000).

4.1.3 GABA receptor

The target site of cyclodiene insecticides, such as dieldrin, and of fipronil, a phenyl pyrazole insecticide, is the type A receptor for the neurotransmitter γ-aminobutyric acid (GABA). The GABA receptor is a widespread inhibitory neurotransmission channel in the central nervous system and neuromuscular junctions of insects (Hemingway & Ranson, 2000). GABA receptor binding elicits rapid gating of an integral chloride selective ion channel. Mutations at a single codon in the Rdl (resistance to dieldrin) gene (encoding one receptor subunit), from an alanine residue to a serine or more rarely to a glycine, have been documented in all dieldrin- resistant insect species to date (ffrench-Constant et al., 1998). This mutation appears to confer both insensitivity to the insecticide and a decreased rate of desensitization (Hemingway et al., 2004).

4.2 Metabolic resistance

Metabolic resistance occurs when elevated activity of one or more enzymes results in a sufficient sequester or detoxification of the insecticide before it reaches the target site (Ranson et al., 2011). Increased expression of the genes encoding the major xenobiotic metabolizing enzymes is the most common cause of insecticide resistance in mosquitoes (Hemingway & Ranson, 2000).

Three major enzyme groups are responsible for metabolically based resistance to OCs, OPs, Cs, and PYs: a) glutathione S-transferase (GST), like DDT-dehydrochlorinase, which was

first recognized as a GST in the house fly, *Musca domestica*; b) esterases, often involved in OP, C, and to a lesser extent, PY resistance; and c) monooxygenases, involved in PY metabolism, OP activation and/or detoxication and, to a lesser extent, C resistance (Hemingway & Ranson, 2000).

4.2.1 Glutathione S-transferases

Several studies have shown that insecticide-resistant insects have elevated levels of GST activity, which has been implicated in resistance to at least four classes of insecticides. GSTs are dimeric multifunctional enzymes that play a role in detoxification of a large range of xenobiotics through catalysis of the nucleophilic attack of reduced glutathione on the electrophilic centers of lipophilic compounds. For mosquitoes multiple forms of these enzymes have been reported (Hemingway & Ranson, 2000). Higher enzyme activity is usually due to an increased amount of one or more GST enzymes, either as a result of gene amplification or more commonly through increases in transcriptional rate, rather than qualitative changes in individual enzymes (Ranson & Hemingway, 2004).

The DDT dehydrochlorinase reaction proceeds via a base abstraction of hydrogen, catalyzed by the thiolate anion generated in the active site of the GST, leading to the elimination of chlorine from DDT and generating DDE (Prapanthadara et al., 1995). These GSTs also act as a secondary detoxification route for OPs, resulting in cross-resistance to insecticides such as fenitrothion.

Detoxification of OPs occurs via an O-dealkylation or O-dearylation reaction. In O-dealkylation, glutathione is conjugated with the alkyl portion of the insecticide (Oppenoorth et al., 1979), whereas the reaction of glutathione with the leaving group (Chiang & Sun, 1993) is an O-dearylation reaction. GSTs can also catalyse the secondary metabolism of OP insecticides (Hemingway et al., 2004).

GSTs have no direct role in the metabolism of PY insecticides but they play a very important role in conferring resistance to this insecticide class by reducing oxidative damage and detoxifying the lipid peroxidation products induced by PYs (Vontas et al., 2001). GSTs may also protect against PY toxicity in insects by sequestering the insecticide (Kostaropoulos et al., 2001).

4.2.2 Esterases

Over-production of non-specific carboxylesterases as response to OP and C insecticide selection pressure has been documented in numerous arthropod species including mosquitoes (Hemingway & Karunaratne, 1998). In OP-susceptible insects, the active oxon analogues of the insecticides act as esterase inhibitors, because they are poor substrates with a high affinity for the enzymes. Esterases from resistant insects are more reactive with insecticides than their counterparts from susceptible insects and so they sequester the oxon analogues protecting the acetylcholinesterase target site (Karunaratne et al., 1995). The predominant cause of this excessive enzyme synthesis is amplification of the genes (Mouches et al., 1986; Vaughan & Hemingway, 1995; Vaughan et al., 1995), although up-regulated transcription without an underlying gene amplification event has been reported (Rooker et al., 1996). In some resistant mosquito species, elevated carboxylesterase activity involves rapid hydrolysis of the insecticide, rather than increased sequestration (Hemingway et al., 2004). This mechanism is almost always found in association with Malathion resistance, and gives a much narrower cross-resistance

spectrum (in some cases Malathion-specific) than the amplified esterase-based mechanism. Although the genetic alterations generating these qualitative changes have not yet been identified in mosquito populations, several data obtained from other arthropods suggest that only one or two amino acid mutations may be responsible (Hemingway et al., 2004).

4.2.3 Monooxygenases

Monooxygenases are involved in the metabolism of PYs and in the activation and/or detoxification of OP insecticides (Hemingway & Ranson, 2000). The monooxygenases are a complex family of enzymes found in most organisms, including insects, involved in the metabolism of xenobiotics. The P450 monooxygenases are generally the rate-limiting enzyme step in the chain. Cytochrome P450-dependent monooxygenases are an important and diverse family of hydrophobic, haem-containing enzymes involved in the metabolism of numerous endogenous and exogenous compounds and of virtually all insecticides. It lead to activation of the molecule in the case of OP insecticides, or more generally to detoxification. P450 enzymes bind molecular oxygen and receive electrons from NADPH to introduce an oxygen molecule into the substrate (Hemingway & Ranson, 2000). There are many reports demonstrating elevated P450 monooxygenase activities in insecticide-resistant mosquitoes, frequently in conjunction with altered activities of other enzymes (Hemingway et al., 2004).

4.3 Cuticular resistance

Some mosquitoes have also evolved thicker or altered cuticles, reducing penetration of the insecticide (Stone & Brown, 1969; Apperson & Georghiou, 1975). Obviously this is not the main resistance mechanism used by pests, since the major route of insecticide delivery is by ingestion. However, in malaria control, insecticides are typically delivered on bed nets or on wall surfaces, and uptake of insecticides is primarily through the appendages. Hence an increase in the thickness of the tarsal cuticle, or a reduction in its permeability to lipophilic insecticides, could have a major impact on the bioavailability of insecticide *in vivo* (Ranson et al., 2011).

4.4 Behavioural resistance

Mosquitoes are able to change their behaviour as a result of intensive indoor use of insecticides, but there are currently insufficient data to assess whether these behavioural avoidance traits are symptomatic of genetic or adaptive responses (Bogh et al., 1998).

Several insecticides such as DDT and permethrin influence behavioural changes in the insect by reducing the rate of mosquito entry into houses, by increasing the rate of early exit from houses and by inducing a shift in biting times (Lines et al., 1987; Mbogo et al., 1996; Mathenge et al., 2001). Mosquitoes may also express a change in host preference because their favoured hosts under the ITN can not be reached (Takken, 2002).

In vector control-free areas, mosquitoes are mostly collected in bedrooms. The excito-repellent effect of PYs forces mosquitoes to leave rooms to outdoors, thus explaining the reduction of indoor biting (Takken, 2002). There is a clear need for robust controlled studies to quantify the extent of this behavioural change, and to assess whether scale-up of ITNs and/or IRS could increase importance of outdoor transmission of malaria and new tools against outdoors malaria vectors might be required (Ranson et al., 2011).

5. Future perspectives and possible alternatives to insecticides

Regular monitoring for insecticide resistance is essential in order to react promptly to prevent vector control compromission. Once resistance reaches very high levels, strategies to restore susceptibility are unlikely to be effective (Ranson et al., 2011).
Effective monitoring and decision support systems can be used to detect insecticide resistance at an early stage, which should lead to the implementation of changes in insecticide policy (Sharp et al., 2007). However, the practice of using an insecticide until resistance becomes a limiting factor is rapidly eroding the number of suitable insecticides for vector control (Hemingway & Ranson, 2000) and the choice of unrelated insecticides remains limited (Nauen, 2007).
Rotations, mosaics, and mixtures have all been proposed as resistance management tools (Hemingway & Ranson, 2000): they could delay the development and/or spread of resistance (Curtis C.F. et al., 1998), but cannot prevent it (Penilla et al., 2006).
Efforts are being made to expand the number of available insecticide classes. One initiative is the Innovative Vector Control Consortium (IVCC), a Product Development Partnership, established in 2005 to stimulate the search for alternative active ingredients or improved formulations of insecticides for vector control, and several promising leads are now being evaluated in laboratory and field trials (Ranson et al., 2011; Enayati & Hemingway, 2010). With this goal, also the discovery of new potential targets can be important. For example the sequencing of the *Anopheles gambiae* genome has also been exploited by several groups to identify the range and function of olfactory receptors in the mosquito, with the aim of developing new attractants and repellents (Enayati & Hemingway, 2010).

5.1 Chemical alternatives: repellents

In order to push away mosquitoes, which usually are attracted by the moisture, warmth, carbon dioxide or estrogens from human skin, a large spectrum of repellents have been developed and are currently used; these substances, manufactured in several forms, including aerosols, creams, lotions, suntan oils, grease sticks and cloth-impregnating laundry emulsions, are usually applied on the skin or clothes, and produce a vapor layer characterized by bad smell or taste to insects (Brown & Hebert, 1997). The ideal repellent should satisfy several criteria: a) have long-lasting effectiveness; b) do not irritate human skin; c) have a bad odor only to mosquitoes but not to people; d) have no effects on clothes; e) be inert to plastics commonly used, such as glasses or bracelets; f) be chemically stable; and g) be economical (Brown & Hebert, 1997).
The list of main insect repellents, some of which are also used as insecticides, includes N,N-diethyl-3-methylbenzamide (DEET), permethrin, picaridin, indalone, and botanicals.
DEET has been considered the most broad-spectrum and efficacious repellent for sixty years, and is currently used on the skin or clothes. Its mechanism of action is to provide a vapor barrier with a bad odor capable to push down mosquitoes. Among side effects, central nervous system, cardiovascular, cutaneous symptoms have been reported, but generally they were related to overuse o incorrect use of the product (Osimitz & Grothaus, 1995).
Permethrin is a synthetic PY with also repellent properties. Its mechanism of action requires direct contact with the insect; thus it is not recommended for skin application. It is commonly used in agriculture, and can be used on clothing, shoes, bed nets and camping

gear. High doses might induce neurotoxic effects, eye and skin irritation, reproductive anomalies, and immune system alterations (Cox, 1998).

Picaridin (2-(2-hydroxyethil)-1-piperidinecarboxylic acid 1-methylpropyl ester) has been used for almost a decade in Australia, and therefore extended to Europe and America. Like DEET, it produces a repellent vapor barrier. Interestingly, no side effects have been reported, and in the future it might be useful in areas endemic for malaria; unfortunately, at present it is not recommended for children younger than 2 years, the most susceptible target of *Plasmodium* in tropical areas (Solberg et al., 1995).

Indalone (butyl 3,4-dihydro-2,2-dimethyl-4-oxo-2H-pyran-6-carboxylate) is a contact or gustatory repellent, slightly volatile, and contact with the treated surface is required to push away the insects (Brown & Hebert, 1997)

Botanicals contain one of several essential plant oils including oil of lemon eucalyptus, soybean oil, geraniol or oil of citronella. Natural products might be safer for human use than synthetic compounds (Katz et al., 2008). Among natural insect repellents, the most commonly used is oil of citronella, an essential oil extracted from the long narrow leaves of a perennial grass from tropical Asia. However, despite its repellent properties, citronella seems not to be useful for malaria vector control; indeed, it is commercially available only as Natrapel (10% citronella), which unfortunately is not effective against mosquitoes, and as Green Ban (a mix of citronella, peppermint, cajaput grass, myrrh and sassafras), which is the most expensive insect repellent on the market (Brown & Hebert, 1997). Nevertheless, natural plants clearly represent a large, promising and almost yet unexplored area for research of new repellent molecules useful also to malaria community.

5.2 Non-chemical alternatives: genetic control

The development of non-chemical strategies alternative to insecticides and repellents is presently on study. Genetic control appears a promising tool, comprising all methods by which a mechanism for pest or vector control is introduced into a wild population through mating. These include the sterile insect release method or the sterile insect technique (SIT), through which males are sterilized by irradiation or other means and released to mate with wild females, leading them to lay sterile eggs. Additionally, the introduction of genetic factors into wild populations aimed to make pests harmless to humans might be relevant (Pates & Curtis, 2005). Finally novel approaches against vector borne diseases include transgenesis and paratransgenesis to reduce vector competence (Coutinho-Abreu et al., 2010).

For vector transgenesis, the goal is to transform vectors with a gene (or genes) whose protein(s) impair pathogen development. Several mosquito species vectors of different parasites and viruses have been transformed. Some of the transformed mosquitoes were shown capable of blocking pathogen development via tissue-specific expression of molecules impairing the pathogen attachment to the midgut (Ito et al., 2002), or activating some biochemical pathways detrimental to pathogen survival (Franz et al., 2006). Paratransgenesis aims to reduce vector competence by genetically manipulating symbionts. Transformed symbionts are spread maternally or via coprophagy across an insect population (Durvasula et al., 1997). Unfortunately, although these approaches are potentially promising, they remain a complex approach with a limited use (Coutinho-Abreu et al., 2010).

6. Conclusion

The goal to globally eradicate malaria worldwide, established in 2007 by the Bill and Melinda Gates Foundation and rapidly endorsed by the World Health Organization (WHO) and the Roll Back Malaria association, is certainly ambitious. The combination of parallel vector control approaches, either based on current knowledge of benefits and risks of available insecticides or on future research on new promising tools, including chemical agents like repellents or non-chemical strategies such as genetic control, might be helpful in order to reach such an objective. Therefore, it represents an intriguing but hopefully affordable challenge for all the malaria research community.

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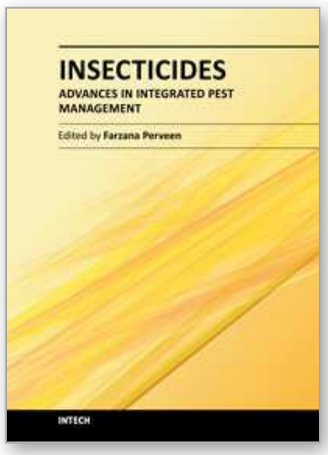

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This book contains 30 Chapters divided into 5 Sections. Section A covers integrated pest management, alternative insect control strategies, ecological impact of insecticides as well as pesticides and drugs of forensic interest. Section B is dedicated to chemical control and health risks, applications for insecticides, metabolism of pesticides by human cytochrome p450, etc. Section C provides biochemical analyses of action of chlorfluazuron, pest control effects on seed yield, chemical ecology, quality control, development of ideal insecticide, insecticide resistance, etc. Section D reviews current analytical methods, electroanalysis of insecticides, insecticide activity and secondary metabolites. Section E provides data contributing to better understanding of biological control through Bacillus sphaericus and B. thuringiensis, entomopathogenic nematodes insecticides, vector-borne disease, etc. The subject matter in this book should attract the reader's concern to support rational decisions regarding the use of pesticides.

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