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Pileggi VN, Braga GC, Bellissimo-Rodrigues F, Souza JP. A rapid review of personal protective measures for preventing Zika virus infection among pregnant women. [Submitted]. Bull World Health Organ. E-pub: 21 July 2016. doi: http://dx.doi.org/10.2471/BLT.16.182592

A rapid review of personal protective measures for preventing Zika virus infection among pregnant women

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(Submitted: 20 July 2016 – Published online: 21 July 2016)

Abstract

Background. Zika virus (ZIKV) infection during pregnancy is associated with fetal microcephaly and other severe malformations, and the World Health Organization declared that this situation is a Public Health Emergency of International Concern in 2016. ZIKV is predominantly transmitted by *Aedes* spp. mosquitos, and although there is no direct evidence regarding personal protective measures targeting ZIKV infection, there is indirect evidence from studies of other arboviruses that share the same vector (e.g., dengue and chikungunya fever). Therefore, this rapid review evaluated personal protective measures that aim to prevent *Aedes* spp. mosquito bites and/or infection with related arboviruses.

Methods. We searched the PubMed and Embase databases and grey literature for the last 10 years (up to March 25, 2016) to identify studies that evaluate the safety, efficacy, and effectiveness of personal protective measures. A total of 17 studies were included.

Findings. Mosquito repellents (e.g., N,N-Diethyl-meta-toluamide/DEET, icaridin/picaridin, and IR3535) effectively prevented mosquito bites at different dosages, although these repellents were not evaluated in the context of preventing arbovirus infections. Citriodora extracts provided low efficacy. Nets that were treated using permethrin and deltamethrin effectively prevented dengue. There was no evidence of any teratogenic effects from the in vitro and animal model studies that evaluated these substances. Only DEET and permethrin were tested in clinical trials that included pregnant women, and both substances were considered safe.

Conclusion. Although definitive data is lacking, insecticide-treated nets and mosquito repellents may be considered safe and potentially effective measures for preventing ZIKV infection during pregnancy.

Key words: Zika virus, dengue, arboviruses, pregnancy, prevention, insect repellents, insecticide-impregnated nets, insecticide-impregnated clothes.

1. Background

Zika virus (ZIKV) is an arbovirus from the *Flaviviridae* family that was originally described in Uganda during 1947.[1, 2] Although ZIKV is predominantly transmitted by *Aedes* spp. mosquitos, inter-human transmission has been documented through the parenteral, perinatal, and sexual routes.[3-6] After the identification of ZIKV, small outbreaks and isolated cases of a self-limited fever have been attributed to ZIKV infection throughout Africa, with specific episodes in Uganda (1969, 1970), Nigeria (1971, 1975), Sierra Leone (1972), Gabon (1975), Central African Republic (1979), Senegal (1988, 1991), and Cote d'Ivoire (1999). During the 1970s, ZIKV reached southern Asia, where it has been described in Malaysia, Pakistan, and Indonesia (1977–1978), as well as in Micronesia (2007) and Cambodia (2010).[7-13]

The clinical signs and symptoms of ZIKV infection include fever, rash, headache, conjunctival hyperemia, myalgia, and joint pain. These signs and symptoms usually last for 3–7 days and then spontaneously resolve. However, serological evidence indicates that up to 80% of infected people do not report any clinical symptoms.[14]

ZIKV was first observed in Brazil during 2014, and was likely passed through Polynesia from Asia. The first reports described the virus in the Brazilian states of Bahia and São Paulo, although the virus rapidly spread to the states of Rio de Janeiro, Alagoas, Rio Grande do Norte, Pará, and Maranhão. This rapid spread was likely related to these states being infested with *Aedes aegypti*.[7, 15-18] Interestingly, despite only causing self-limited benign disease in Africa, ZIKV infection in some parts of Asia (Micronesia) and Brazil is associated with life-threatening conditions, such as Guillain-Barre syndrome. For example, at least 48 cases of Guillain-Barre syndrome during 2015 in the state of Bahia may have been related to ZIKV infection, and another 130 cases were described in the state of Pernambuco.[19, 20] The most severe complications of ZIKV infection are fetal microcephaly and severe malformations after maternal infection during pregnancy, and these complications have only recently been described in Brazil and Polynesia (2014–2015). As >4,000 suspected cases of ZIKV-related microcephaly have been reported to Brazilian health authorities, the World Health Organization (WHO) declared that this situation is a Public Health Emergency of International Concern in February 2016.[20-25]

Although theoretically effective, population-level vector control measures (e.g. eliminating mosquito breeding sites) are difficult to implemented and sustain, and in many instances have failed to control other diseases that are transmitted by the same vector (e.g., dengue and chikungunya fever). Therefore, it is possible that personal protective measures (e.g., mosquito repellents and insecticide-treated nets [ITNs] and clothes) could help prevent ZIKV infection during pregnancy.[18] As there are limited data regarding measures to protect against ZIKV infection, this rapid review aimed to assess the effectiveness, efficacy, and safety of personal protective measures for preventing *Aedes* spp. mosquito bites and/or related arbovirus infections among pregnant women.

2. Methods

During March–April 2016, we performed a "rapid review" of the literature; this review format uses accelerated systematic review methods to adapt to the current outbreak. This review evaluated three domains (efficacy, effectiveness and safety) and used a two-phase search strategy to identify studies that evaluated personal protective measures that targeted *Aedes* spp. bites or related arbovirus infections among pregnant women. For each domain, we initially searched the PubMed, and Embase databases for previous literature reviews and systematic review protocols that were published

during the last 10 years. For domains with one or more relevant literature reviews, the reviews were assessed using the A Measurement Tool to Assess Systematic Reviews (AMSTAR) checklist, and were adapted to strengthen their methods, focus the search on specific preventive measures, search additional databases, and update the previous search results. For domains with a relevant review protocol but no relevant literature reviews, the protocol was adapted and implemented for the present review. For domains with no previous reviews or protocols, a primary literature search was performed. The full search strategy for each domain is described in Supplementary File 1.

The population of interest was pregnant women, or teratogenic animal studies if no human results were available. Teratogenic studies evaluate substances and provide general information regarding the effects of prenatal exposure on the pregnant test animal and the developing fetus. These studies typically use pregnant rats, mice, or rabbits (and their offspring) to evaluate reproductive effects, structural abnormalities, and toxicity when the substance has not been tested in humans.

The interventions of interest were defined as personal protective measures that were designed to protect against mosquito bites and infection with related arboviruses. These interventions included clothes and nets that were treated using permethrin or deltametrin, topical repellents (e.g., DEET/N,N-Diethyl-meta-toluamide, icaridin, IR3535, and citriodora), and spatial repellents (e.g., transfluthrin coils, metofluthrin coils, D-allethrin coils, pyrethrin coils, metofluthrin emanators, and transfluthrin emanators). The outcomes of interest were defined as a reduction in mosquito bite frequency and/or symptomatic or asymptomatic dengue, chikungunya, or Zika disease that was confirmed using serological or virological tests. We also evaluated adverse events (including teratogenicity) in the pregnant women and tested animals; control patients and animals were defined as having received a placebo or no treatment.

All reports that were published in English, French, Italian, Portuguese, and Spanish were considered eligible for inclusion. The inclusion criteria are shown in Supplementary File 1. Two independent reviewers screened each title and abstract, and the full texts of potentially relevant articles were retrieved for further evaluation. A third reviewer was consulted to address unresolved disagreements between the reviewers. As there were few reports regarding Zika disease and personal protective measures at the time of our search, we also considered other arboviruses that are transmitted by *Aedes* spp. mosquitos as proxies for ZIKV (e.g., dengue, chikungunya, and yellow fever). Relevant data from the included studies were extracted using domain-specific Microsoft Excel spreadsheets.

The present review did not evaluate the risk of bias in the individual studies, and did not evaluate the summary measures and synthesis of the results. However, the emerging nature of the Brazilian Zika outbreak and the need for additional information regarding ZIKV infection makes it reasonable to evaluate all potentially eligible studies without excessive exclusion of potentially relevant studies.

3. Results

Domain #1 – The efficacy of repellents against *Aedes* spp. mosquito bites

We identified one relevant literature review that was published in 2013,[26] and assessed it using the AMSTAR checklist. The review included 26 arm-cage assays, large-room evaluation tests, and field trials. After updating and implementing the original search strategy, we identified two additional

studies.[27, 28] The studies aimed to understand the protection time and percent coverage for specific repellents formulations, and efficacy of at least 90% was used to conclude that they provided acceptable protection against *Aedes* spp. bites. This cut-off was used by most of the assessed studies, although there is no consensus or evidence regarding a correlation between this level of efficacy and the prevention of arbovirus infection.

DEET was considered the standard repellent to protect against mosquito bites. The conventional concentrations of DEET (20–25%) provided an efficacy of approximately 90% for protecting against *Aedes* spp. bites, and this protection lasted 1–8 h (based on the dosage). Icaridin concentrations of 10–20% typically provided 6–7 h of protection against *Aedes* spp. bites. However, one study of 20% icaridin revealed that it provided 4 h of complete protection against mosquito bites, although this protection was reduced to 1.5 h when it was combined with ethanol and vanillin. IR3535 concentrations of 7.5–20% provided approximately 6–8 h of protection against *Aedes* spp. bites. One study evaluated six reports that tested citriodora using the arm-cage assay, although citriodora-based repellents were not effective for protecting against *Aedes* spp. bites.

Domain #2 – The in-field effectiveness of the repellents

Two randomized controlled trials (RCTs) assessed the effectiveness of deltamethrin-treated curtains for preventing dengue (which is also transmitted by *Aedes* spp.), and found that these curtains significantly reduced the prevalence of dengue.[29, 30] Both RCTs compared houses that used insecticide-treated curtains or standard care, and provided moderate-quality evidence (Table 5). In 2006, Kroeger et al. used 50 mg/m^2 of deltamethrin and evaluated the prevalence of IgM against the dengue virus in humans.[30] The authors did not find a significant difference between the control and intervention groups, but did observe that the intervention reduced mosquito populations in neighboring control clusters (the spill-over effect), and that infestation was less common in houses that were closer to the treated houses. In 2013, Pino et al. used 80 mg/m^2 of deltamethrin and evaluated the prevalences of single and multiple dengue infection in humans.[29] The insecticide-treated curtains were associated with a reduction in the numbers of dengue virus infections in one area, intra-domiciliary dengue transmissions, and multiple infections. The authors also found that dengue virus-infected *Aedes aegypti* females were less common in the intervention homes.

Domain #3 – The safety of the personal protective measures

We included 12 reports that included standard toxicity assessment protocols: 5 studies that included pregnant women[31-35] and 7 studies that used animals[36-42] (Tables 6 and 7, respectively).[43] As studies that include pregnant women can be ethically unfeasible, animal studies with teratogenic protocols[43] were also considered for the safety evaluation.

One RCT of DEET concluded that use during the second and third trimesters was safe (use during the first trimester was not evaluated), did not have any teratogenic effects, and only resulted in skin warmth as a side effect.[32] Similarly, three animal studies revealed that DEET was safe during pregnancy, and did not induce any signs of toxicity or congenital abnormality.[39, 40, 42]

No studies tested the safety of icaridin among pregnant women. Two studies of rats and rabbits used the toxicity protocol and did not detect any significant adverse effects in the reproductive, embryonic, or fetal parameters that were evaluated.[37, 38] One WHO report from 2006 evaluated the use of IR3535 and concluded that it was safe for use during pregnancy.[44] No other data

regarding IR3535 were available from the PubMed and EMBASE databases. A study of citriodora extract in mice revealed that moderate consumption of *Lippia citriodora* (as an infusion or tea) appeared to be safe during pregnancy and did not have toxic effects on the embryo's development.[41] Deltamethrin is usually used as an insecticide and pesticide (to protect against scabies and mosquito bites), and one study of rats revealed neurotoxic effects, such as impaired cerebellar development leading to motor skills deficits.[36]

Four reports evaluated permethrin use in pregnant women (Table 8); two reports were related to permethrin-treated nets, one study evaluated a 4% lotion for scabies, and one study evaluated a 1% lotion for head lice. Both studies of the lotions suggested that topical treatment using permethrin was safe during the second and third trimesters.[34, 35] The two reports regarding ITNs evaluated their ability to protect against malaria. One systematic review of 6 studies found that the ITNs were effective at preventing malaria and decreasing mosquito exposure.[45]

The 12 reports regarding the safety of the repellents during pregnancy provided low-to-moderate quality evidence.[32-42, 45] Although we did not find direct evidence of DEET's safety during the first trimester, one study with moderate-quality evidence indicated that DEET was safe during the second and third trimesters.[32] Icaridin and picaridin are similar substances that were tested in animal models,[37, 38] but were not tested in pregnant women. Although we could not evaluate the safety and effectiveness of IR3535, this substance was reviewed by the WHO and may be an alternative treatment for pregnant women.[43] Citriodora did not effectively protect against *Aedes* spp. and was only evaluated in a low-quality study.[41] Nets that were treated using permethrin and deltamethrin were effective for preventing dengue. Among the in vitro and animal model studies, we did not identify any evidence of teratogenic effects that were associated with the substances that we evaluated. Permethrin was tested in an RCT that involved pregnant women, and the findings from that trial indicate that it is safe in that population.[34]

4. Discussion

This rapid review evaluated data regarding the efficacy, effectiveness, and safety during pregnancy of personal protective measures for preventing *Aedes* spp. mosquito bites or related arbovirus infections. Our results indicate that several mosquito repellents (i.e., DEET, Icaridin/picaridin, and IR3535) were effective for preventing *Aedes* spp. bites, although these repellents had not been evaluated in the context of preventing arbovirus infections. Curtains that were treated using permethrin and deltamethrin were also effective in reducing the incidence of dengue. Although we did not find any evidence of teratogenic effects in the in vitro and animal model studies, only DEET and permethrin were tested in RCTs that included pregnant women, and both substances were considered safe. Nevertheless, these repellents and substances are supplied in different concentrations and dosages, and it is important that the user understands how to properly use these substances, in order to maximize their personal protection.

Controlling the population of *Aedes* spp. mosquitos remains the best measure for protecting against the related arbovirus infections (e.g., yellow fever, dengue fever, chikungunya, and ZIKV infection). Thus, mosquito reproduction and ZIKV spread can be blocked by relatively simple measures, such as eliminating stagnant water (i.e., cleaning houses, empting pots, covering water tanks) and proper waste management and sanitation. However, these measures can be difficult to effectively implement, as the affected countries often have complex political, social, and behavioral environments. Furthermore, the worldwide spread of *Aedes aegypti* confirms that it is difficult to control this vector and ZIKV infection. Moreover, pregnancy is a short period and individuals' personal

efforts to prevent ZIKV infection may be effective during this period. Therefore, empowering women by providing them with personal preventative measures may allow them to be less reliant on their neighbors' and countries' complex behavioral and political decisions.

The present review revealed that DEET and icaridin effectively protected against *Aedes* bites. For example, Lupi et al. reported that 20% DEET provided the best efficacy (10 h of protection) and that citriodora had lower efficacy, compared to other products.[26] Sorge et al. also reviewed repellent use among children, and reported that 20% IR3535 and 20% icaridin provided 4–6 h of protection against *Aedes* bites. Sorge et al. also reported that DEET was the only repellent that was confirmed to be safe for use by pregnant woman, although some countries (e.g., Canada) do not recommend using DEET at higher concentrations (30–50%) during pregnancy.[46] Similar to our findings, they concluded that the three most frequently recommended personal protective measures are ITNs, mosquito repellent, and insecticide-treated clothes.

Effectiveness against arbovirus infection was only evaluated in studies of insecticide-treated curtains, which provided moderate-quality evidence and suggested that deltamethrin-treated curtains effectively prevented dengue and mosquito bites. However, as there are no studies that have specifically evaluated ZIKV, evidence from these dengue studies may be useful for developing ZIKV-specific preventative measures. Moreover, women can minimize their contact with insecticide-treated curtains, which might result in fewer toxicity-related events. For example, a 2005 WHO report concluded that pyrethroids could be safely used in insecticide-treated mosquito nets,[44] and that report also emphasized that these substances firmly attached to the fabric and provided effect protection for a period of several days.

Although we used standard systematic review methods, the present rapid review has several limitations that warrant consideration. First, we only searched a limited number of databases, although we were able to evaluate reports that were published in six languages. Second, the studies that we identified did not specifically evaluate the measures' effectiveness against Zika disease, and efficacy was evaluated based on the ability to protect against *Aedes* spp. bites (the primary vector of ZIKV infection). Third, we did not discover any evidence regarding safety during the first trimester. Nevertheless, given the risks of ZIKV-related microcephaly and Guillain-Barre syndrome, it may be prudent to frequently use mosquito repellants, although this use should be guided by the application instructions for the chosen repellent, in order to avoid issues with toxicity.

5. Conclusion

Although definitive data are lacking, ITNs and mosquito repellents, such as DEET, icaridin and IR3535, may be considered safe and potentially effective measures to protect against ZIKV infection during pregnancy. Future field testing should be performed to confirm these findings.

Table 1: DEET repellent efficacy against Aedes mosquitos, ordered by study design and mosquito species (modified and updated from Lupi E, et al. 2013 (26)

Study ID *(Year)*	Mosquito	Number of Subjects	Repellent substance	Dosage	Mean Complete Protection Time	Percentage Protection		Reference
						%	Time interval	
Arm-in-cage-studies								
Tawatsin A, et al. 2001	*Ae. Aegypti*		DEET 25%	0.1 ml/30cm^2 = 0.0033	8h	76	8h	(47)
			DEET 25% + Vanillin 5%		8h			
Tawatsin A, et al. 2001	*Ae. Aegypti*		DEET 20% in ethanol	0.66mg/cm^2	9.7h	100	6h	(47)
Kim DH, et al. 2002	*Ae. Aegypti*		DEET			100	5 min	(48)
						99	30 min	
						97	1h	
Fradin MS, et al. 2002	*Ae. Aegypti*		DEET 23.8% (Off! Deep woods)		301 min			(49)
			DEET 20% (Sawyer controlled release)		234.4min			
			DEET 6.65% (Off! Skintastic)		112.4min			
			DEET 4.75% (Off! Skintastic for Kids)		88.4 min			
Badolo A, et al. 2004	*Ae. Aegypti*		DEET	ED50 (0.05µg/cm^2) ED90 (20.8µg/cm^2)				(50)
Kim SI, et al. 2004	*Ae. Aegypti*		DEET	0.1mg/cm^2		100	30min	(51)
Tuetun B, et al. 2005	*Ae. Aegypti*		DEET 25%	1ml/30cm^2	3.5h			(52)
			DEET 25% + Vanillin 5%		5.5h			
			DEET 14.5% (Off!)		3.5h			
Amer A, et al. 2006	*Ae. Aegypti*		DEET 20% in complex formulation	0.1ml/30cm^2	360min	45.9	480min	(53)
			DEET 20% with 5% Vanillin in ethanol	0.1ml/30cm^2	270min	75.7	480min	
	Ae. Aegypti		DEET 20% ethanol	0.1ml/30cm^2	240min	86.5	480min	
Carroll SP, et al. 2006	*Ae. Aegypti*		DEET 10% (Off! Skintastic SPF 30 Lotion)	1g/600cm^2	120min			(54)
	Ae. Aegypti		DEET 30% (Cutter outdoorsman lotion)					
Chang KS, et al. 2006	*Ae. Aegypti*		DEET	0.025mg/cm^2		100	10min	(55)
Tawatsin A, et al. 2006	*Ae. Aegypti*		DEET 10%	0.1ml/30cm^2	7.5			(56)
Kamsuk K, et al. 2007	*Ae. Aegypti*		DEET 25% in ethanol solution	0.1ml/30cm^2	3.5h			(57)
			DEET 25% + Vanillin 5%		5.5h			
Webb CE, et al. 2007	*Ae. Aegypti*		DEET 7.2% (Aerogard)	1g/forearm	360min			(58)
Tuetun B, et al. 2008	*Ae. Aegypti*		Ethanolic DEET solution 25%	0.1ml/30cm^2	3.5h			(59)

		DEET 14.25% (Off!)		3.5h			
		DEET 20% (Sketolene Lotion		4h			
		DEET 28.5% (Insect Block 28)		4.5h			
Witting-Bissinger BE, et al. 2008	*Ae. Aegypti*	DEET 7% (Cutter Skinsations)	1ml/600cm^2		98.5	1h	(60)
					88.7	2h	
					88	3h	
					73	4h	
					74.7	5h	
					58.4	6h	
Witting-Bissinger BE, et al. 2008	*Ae. Aegypti*	DEET 15% (Sawyer products)			99.6	1h	(60)
					99.5	2h	
					100	3h	
					96.1	4h	
					95.2	5h	
					89.4	6h	
Frances SP, et al. 2009	*Ae. Aegypti*	DEET 20%	0.43mg/cm^2	195min			(61)
Misni N, et al. 2009	*Ae. Aegypti*	DEET 10%	0.4g/25cm^2		100	4h	(62)
					88.8	6h	
					77.1	8h	
Thomas S, et al. 2009	*Ae. Aegypti*	DEET 5%	1g/forearm	114min			(63)
Garud A, et al. 2011	*Ae. Aegypti*	DEET 20%	1mg/cm^2	6h			(64)
Kwon HY, et al. 2011	*Ae. Aegypti*	DEET 10% in ethanol	0.05mg/cm^2		100	10min	(65)
					86	30min	
					73	1h	
					51	90min	
					49	120min	
					28	150min	
Mittal PK, et al. 2011	*Ae. Aegypti*	DEET 12% cream	10mg/cm^2	4h	97.2	4h	(66)
				>4h	100	4h	
Kim SI, et al. 2012	*Ae. Aegypti*	DEET 5%		127.5min			(67)
		DEET 15%		247.5min	90	0	
Rodriguez SD, et al. 2015	*Ae. Aegypti*	DEET 98.11% (Repel 100 insect repellent)	0.5ml	10h	90	0	(28)
					82	30min	
					85	120min	

					86	240min	
		DEET 25% (OFF deep woods insect repellent VIII)	0.5ml		94	0	
					83	30min	
					86	120min	
					71	240min	
		DEET 7% (cutter skinsations insect repellents)	0.5ml		89	0	
					78	30min	
					83	120min	
					70	240min	
Barnard DR, et al. 2004	*Ae. albopictus*	DEET 7% (Skinsations Spray)	1ml/650cm^2	5h			(68)
		DEET 15% (Off! Spray)		7.2h			
Yang P, et al. 2005	*Ae. albopictus*	DEET 4%	2µg/cm^2	>6h			(69)
Tawatsin A, et al. 2006	*Ae. albopictus*	DEET 10%	0.1ml/30cm^2	8h			(56)
	Ae. albopictus	DEET 7% (Cutter Skinsations)	1ml/600cm^2		98.3	1h	(60)
					100	2h	
					95.3	3h	
					94	4h	
					91	5h	
					86.9	6h	
Witting-Bissinger BE, et al. 2008	*Ae. albopictus*	DEET 15% (Sawyer products)			99.3	1h	(60)
					100	2h	
					100	3h	
					96.3	4h	
					96	5h	
					88.1	6h	
						0	
						30min	
						120min	
						240min	
Rodriguez SD, et al. 2015	*Ae. Albopictus*	DEET 98.11% (Repel 100 insect repellent)	0.5ml	10h	90	0	(28)
					93	30min	
					86	120min	

					86	240min	
		DEET 25% (OFF deep woods insect repellent VIII)	0.5ml		93	0	
					87	30min	
					85	120min	
					73	240min	
		DEET 7% (cutter skinsations insect repellents)	0.5ml		80	0	
					82	30min	
					82	120min	
					70	240min	
Yoon JK, et al. 2014	*Ae. Albopictus*	DEET 24%(Insectan Spray, Green Cross, Yongin, South Korea)	1.5ml		100	3h	(27)
					99.5	4h	
					97.9	5h	
					90.3	6h	
<u>Large room evaluation</u>							
Tawatsin A, et al. 2001	*Ae. Aegypti*	DEET 25%	3ml/lower leg (782-826 cm^2)	8h	100	6h	(47)
		DEET 25% + Vanillin 5%			100	6h	
	Ae. albopictus						
<u>Field trial</u>							
Trongtokit Y, et al. 2004	*Ae. Aegypti*	DEET 20%		3h	82.7	5h	(70)
Tawatsin A, et al. 2006	*Ae. Aegypti (1.2%)*	DEET 10%	2ml/750cm^2		100	9h	(71)
Tawatsin A, et al. 2006	*Ae. Aegypti (99.9%)*	DEET 10%	2ml/750cm^2		100	1-5h	(71)
Mittal PK, et al. 2001	*Ae. Aegypti*	DEET 12% cream	10mg/cm^2	6.75h	96.2	11h	(66)
Thavara U, et al. 2001	*Ae. albopictus*	DEET 20% in ethanol	0.76-0.84 mg/cm^2		97.3-100	1h	(72)
					98.9-100	2h	
					97.5-100	3h	
					95.9-100	4h	
					94-100	5h	
					95.7-100	6h	
					100	7h	
					100	8h	

Table 2: Icaridin repellent efficacy against Aedes mosquitos, ordered by study design and mosquito specie

Study ID *(Year),* [ref]	Mosquito	Number of Subjects	Repellent substance	Dosage	Mean Complete Protection Time	Percentage Protection		Reference
						%	Time interval	
Arm-in-cage-studies								
Badolo A, et al. 2004	Ae. *aegypti*		Icaridin	ED50 0.02ug/cm^2 ED90 63.8ug/cm^2				(50)
Amer A, et al. 2006	*Ae. aegypti*		Bayrepel 20%® in complex formulation	0.1ml/30cm^2	240min	29,7%	480min	(53)
			Bayrepel 20%® with 5% Vanilin in ethanol		90min	55%	480min	
			Bayrepel 20%® with ethanol		90min	75%	480min	
Barnard DR, et al. 2004	*Ae. albopictus*		Icaridin 10%	1ml/650cm^2	5.7h			(68)
Large room evaluation Field trial								
Naucke TJ, et al. 2007	*Ae. aegypti*		Picaridin 10%® Lotion	1.5g/600cm^2	458.7 min	95% 90% 85%	6h 7h 8h	(73)
			Picaridin 20% Spray	1g/600cm^2	461min	95% 90% 85%	6h 7h 9h	

Table 3: IR3535 repellent efficacy against Aedes mosquitos, ordered by study design and mosquito specie

Study ID *(Year),* [ref]	Mosquito	Number of Subjects	Repellent substance	Dosage	Mean Complete Protection Time	Percentage Protection %	Percentage Protection Time interval	Reference
Arm-in-cage-studies								
Thavara U, et al. 2001	*Ae. aegypti*		IR3535 20% in ethanol solution	0.67mg/cm^2	9.8h			(72)
Fradin MS, et al. 2002	*Ae. aegypti*		IR3535		22.9min			(49)
Barnard DR, et al. 2004	*Ae. albopictus*		IR3535 7.5% Avon-Skin-So-Soft Bug Guard plus IR3535® cream	1ml/650cm^2	1.8h			(68)
Tuetun B, et al. 2005	*Ae. aegypti*		IR3535 12% Kor Yor IR lotion® IR3535 10% Kor Yor IR gel® IR3535 10% Sketolene Roll on® IR3535 20% Beauti spray®	0.1ml/30 cm^2	2h 1.5h 1h 2h			(52)
Tawatsin A, et al. 2006	*Ae. aegypti* *Ae. albopictus*		IR353510%	0.1ml/30 cm^2	6.7h 7.8h			(56)
Tuetun B, et al. 2008	*Ae. aegypti*		IR3535 10% Sketolene Roll on® IR3535 10% Kor Yor IR gel® IR3535 12% Kor Yor IR 15 ®lotion IR3535 12.5% Johnson's Baby clear Lotion Anti-mosquito® IR3535 20% Beauti spray®	0.1ml/30 cm^2	1h 1.5h 2h 1h 2h			(59)
Field trial								
Thavara U, et al. 2001	*Ae. aegypti*		IR3535 20% in ethanol solution	0.77-0.84mg/cm^2		98.6-100% 98.9-100% 97.5%-100% 100% 94-100% 97.8-100% 100% 100%	1h 2h 3h 4h 5h 6h 7h 8h	(72)

Naucke TJ, et al. 2007	*Ae. aegypti*	IR3535 10%® Spray	1g/600cm^2=1,66	411min	95%	6h	(73)
					90%	6h	
		IR3535 15%® Spray			85%	7h	
			1g/600cm^2	454.8min	95%	6h	
		IR3535 10%® Lotion			90%	6h	
					85%	6h	
		IR3535 15%® Lotion	1.5g/600cm^2=2.5	425.3min	95%	4h	
					90%	5h	
		IR3535 20%® Spray			85%	6h	
			1.5g x /600 x cm^2	455.3min	95%	6h	
					90%	6h	
					85%	6h	
			1g/600cm^2	436.5min	95%	6h	
					90%	7h	
					85%	7h	

Table 4: Citriodora repellent efficacy against Aedes mosquitos, ordered by study design and mosquito specie

Study ID *(Year),* [ref]	Mosquito	Number of Subjects	Repellent substance	Dosage	Mean Complete Protection Time	Percentage Protection		Reference
						%	Time interval	
<u>Arm-in-cage-studies</u>								
Barnard DR, et al. 2004	*Ae. Aegypti*		65% PMD (Repel Lotion)	1ml/650cm^2	7.8h			(68)
Amer A, et al. 2006	*Ae. Aegypti*		20% EO in complex formulation	0.1ml/30cm^2	150min	59.4	480min	(53)
Carroll SP, et al. 2006	*Ae. Aegypti*		PMD 10% (Off! Botanicals)	1g/600cm^2	124min			(54)
			PMD 20% (Repel OLE Lotion)		307min			
Maguranyi SK, et al. 2009	*Ae. Aegypti*		E. citriodora oil	1ml/forearm	<20min			(74)
Phasomkusolsil S, et al. 2010	*Ae. Aegypti*		EO#1	100µl	0min			(75)
			EO#2		30min			
Kim SI, et al. 2012	*Ae. Aegypti*		Lemon eucalyptus essential oil 5%		22.5min			(67)
			Lemon eucalyptus essential oil 15%		52.5min			
			EO: Vanillin 5%:5%		60 min			
			EO: Vanillin 15%:5%		67.5min			

Table 5: Effectiveness of interventions for reducing transmission of human arboviruses

Study ID *Country (Year), ref*	**Study design**	**Intervention**	**GRADE (Quality of evidence)**	**Before Intervention**		**After Intervention**		
				Intervention	Control	Intervention	Control	OR (95% CI)
Prevalence of IgM against DENV in humans								
Kroeger A, et al.2006[30]	Cluster RCT	ITC (Deltamethrim 50mg/m2)	Moderate[+]	64/398 (16%)*	62/300 (21%)*	27/330 (8%)	56/310 (18%)	0.40 (0.25-0.66)
Prevalence of DENV infection in humans								
Lorono-Pino MA, et al.2013[29]	Cluster RCT	ITC (Deltamethrim 80mg/m2)	Moderate[++]	197/800 (24.6%)	199/793 (25.1%)	61/722 (8.4%)	106/727 (14.6%)	0.54 (0.39-0.75)
Prevalence of Single DENV infection in humans								
Lorono-Pino MA, et al.2013[29]	Cluster RCT	ITC (Deltamethrim 80mg/m2)	Moderate[++]			38/78 (48,7%)	40/78 (51,3%)	
Prevalence of Multiple DENV infection in humans								
Lorono-Pino MA, et al.2013[29]	Cluster RCT	ITC (Deltamethrim 80mg/m2)	Moderate[++]			10/34 (29,4%)	24/34 (70,6%)*	*p=0.016

ITC: Insecticide treated curtain

[+]: Downgraded to moderate quality due to the fact that serological assessments were carried out only in a subsample of one of the study sites (Trujillo, Venezuela)

[++]: Downgraded to moderate quality due to process of randomization and no masking

*: Individuals, statistically non significant difference

Table 6 : Summary of the evidence in humans

STUDY	Type of Study	Level of Evidence	Intervention	Participants	Number/local	Prevention to:	Outcomes	Results	Safety/Efficacy (Commentary)	FDA Categorie
Gamble C. et al. 2007[45]	Sist.Review (6RCT – 5RCT analyse) 2-Individual 3-Cluster	1a	ITN x No nets (No treatment) [ITN – with permethrin or Cyfluthrin]	All Gravidity	6418(Africa) + 223 (Thailand)	Malaria (*Anopheles gambiae*)	Anaemia Birth Weight LowBirthWeight Fetal loss Pre-term delivery Placenta malaria	ITN Groups: birth weight +55g (mean) ↓23% Low birth weight ↓33%Miscarriages /Stillbirths in first pregnancy ↓23% Placental parasitemia	Safety - ? decrease bad outcomes of Malaria disease. Plausability: ITN is effective to prevent Malaria, decreasing mosquito expose. Permethrin used also in clothes against *Aedes aegypti.*	No classif. Approved
McGready R, et al. 2001[32]	RCT double blind	1b	DEET + Tanaka x Tanaka [Tanaka is a local cosmetic paste used as a carrier for the repellent]	Pregnants (3-7month) daily use in 2nd and 3rd trimesters Infants 1st year (liveborn singletons 81% - 597 of 741)	897 Thailand	Malaria (*Anopheles gambiae*)	Toxicologic Report in pregnant women (Skin, gastrointestinal and neurologic side effects Birth Outcomes Congenital Abnormalities	DEET group : Pregnant: Skin Warnth (RR=1.39, IC95%1.27-1.52) Scabies (RR=0.69, IC95% 0.50-0.96) Similar born outcomes (livebirths, abortions, stillbirths, birth weight) Infants: No difference – neurology performance and growth. No difference of congenital abnormalities (6 cases per group)	Safety – recommended in 2nd and 3rd trimesters of pregnancy. No evidence in 1st trimester. Side effect detected was Skin warmth. No teratogenic effects	No classif. Approved

Menéndez C, et al. PLoS One. 2008[33]	RCT Double-blind	1b	ITN+Placebo x ITN+ IPTp (Sulphadoxine-pyrimethamine)	Pregnants Infants 1st 2 months	1030 Mozambique (Africa)	Malaria (*Anopheles gambiae*)	LowBirthWeight Anaemia Birth Weight Fetal loss (Stillbirths/Abortions) Pre-term delivery Placenta malaria Hospital admission and outpatient visit	IPTp was not associated with reduction of anaemia, low birth weight or placental infection malaria in pregnancy No difference the groups	Safety - ? The study aimed to evaluate IPTp performance, but evidenced the ITN effect. Plausability: ITN is effective to prevent Malaria, decreasing mosquito expose. Permethrin used also in clothes against *Aedes aegypti.*	No classif. Approved
Mytton OT, et al. BJOG 2007[34]	Retrospective Cohort	2b	Benzyl benzoate lotion 25% x Control Permethrin lotion 4% x Control	Pregnants (all gestational ages – 10,9% in 1st trimesters)	444 BBL 196Permethrin (Thailand)	Scabies	Birth Outcomes Congenital Abnormalities	Similar born outcomes (livebirths, abortions, stillbirths, birth weight) No difference of congenital abnormalities	Safety – the study suggest that topical treatment with BBL and permethrin are safe in second and third trimesters of pregnancy. Permethrin used also in clothes against *Aedes aegypti.*	B Approved
Kennedy D, et al.. 2005[35]	Case control	3	Permetrin 1% crème rinse	226 pregnant woman (113 in each group)	Pregnant Woman	Head lice	Live birth Spontaneous Abortion Major malformations Birth Weight	There were no differences between the groups. Despite the small sample size, it		

				does appear that permethrin products are relatively safe to apply for lice infestation during pregnancy. It also appears that simply giving women reassuring evidence- based information will not necessarily make them feel comfortable enough to use a particular product during pregnancy.

Table 7 : Summary of the evidence in animals

STUDY	Type of Study	Level of Evidence	Intervention	Participants	Number / local	Prevention to:	Outcomes	Results	Safety/Efficacy (Commentary)	FDA Categorie
Kumar K, et al. 2013[36]	Case-control	3B	0.75 mg/kg body wt/day deltamethrin	12	PregnantAlbino wistar rats	Insecticide	In DLT exposed rats, a significant overexpression of reelin was observed in the cells of the external granule cell layer (EGL) and internal granule cell layer along with an ectopic expression of reelin in the EGL as well as in the migrating granule cells just below the EGL, revealing an arrest of granule cell migration in this zone. Disorientation and hypertrophy of the Bergmann glial fibres further hampered the journey of the granule cells to their final destination. Possibly reelin overexpression also caused misalignment of the Purkinje cells and inhibited the neurite growth leading to a significant decrease in the spine density, main dendritic length and width of the dendritic arbour. Thus, it is proposed that the DLT exerts its neurotoxic effects possibly via the intra-cellular accumulation and low release of reelin leading to an impaired granule cell and Purkinje cell migration inhibition of neurite outgrowth and reduced spine density. Such impaired cerebellar development leads to motor coordination deficits.			
Astroff AB, et. al. 1999[37]	RCT	1b	Icaridin KBR3023 – 90 d skin exposure 20days in gravid rat	young adult male Sprague Dawley rats.	27 (13 x 14 control)	toxicity	Skin effects Reproductive effect	No reproductive toxicity		No Classif. Approved

Astroff AB, et al. 2000[38]	Observational	3	Icaridin KBR3023 20days in gravid	Rats and rabbits gravid	150 rats (120 female) and 126 rabbits (96female)	toxicity	The fetal and litter incidence of external, visceral, models and skeletal malformations and the variations for both the rodent and rabbit studies	There were no significant necropsy findings or any effects reproductive, embryonic, or fetal parameters evaluated. The only significant and a 12-hr photoperiod. gestation clinical signs were dermal effects	Based on the results of this study KBR 3023, 1-(1-methyl-propoxycarbonyl)-2-(2-hydroxyethyl)piperidine, does not demonstrate developmental toxicity, at dermally applied dosage levels limited by the method of application, in either the rat or rabbit.	No Classif. Approved
Abu-Qare AW, et al. 2003[39]	Review (not systematic)	3(?)	DEET Permethrin	Animals (rats, rabbits, pigs)	Did not say how many studies was included	toxicity	Biochemical Markers and pharmacholocal interactions Did not specify outcomes	There were no significant between the markers.	Conclusion: More research is needed examining developmental effects and the susceptibility of children and pregnant women to such combined exposure.	No Classif. Approved
Snodgrass HL, et al. 1982[40]	Observational	3	N,N-Diethyl-m-toluamide (m-Det) DEET	Pregnant rabbits	3 pregnant rabbits 7 days skin exposure	toxicity	potential for transplacental transfer and bioaccumulation of absorbed m-Det the fetus	No evidence of bioaccumulation of the chemical in maternal tissue or individual fetuses.	Conclusiom: It is suggested that m-Det should not present a dermatoxic hazard to man and that topical absorption should be less than 10% of the applied dose.	No Classif. Approved

Wright DM, et al. 1992(42)	RCT		DEET	rats	25 pregnant rats 12 control	Toxicity	Side effects Reproductive outcomes (congenital malformation and body weight)	No difference between the groups. There was no evidence of reproductive or developmental toxicity in any of these assays, but there were signs of neurotoxicity in treated adult male and female rats, which may relate to reports of neurotoxicity in humans heavily exposed to mDET-containing insect repellents.		No Classif. Approved
Shirvan Z, et al. 2016(41)	Cohort	2b	Lippia citriodora	mice	20	toxicity	mortality, morbidity and general appearance, Maternal body weights, congenital abnormalites	There were no significant differences in mean maternal weight gain during pregnancy between groups. Also, no significant differences were observed in mean number of implantation, live and resorbed fetuses between control and treated groups. The prevalence of all types of deformity was low and similar to control group (%1.11).	The results of this study show that moderate consumption of L. citriodora as an infusion or tea appears to be safe to be used during pregnancy and does not have toxic effects on development of mouse embryo.	No Classif. Approved

6. Funding

This review was partially funded by World Health Organization and received in-kind support from the University of Sao Paulo (Brazil).

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7. Supplementary

Search #1 – The Efficacy of repellents against *Aedes* spp mosquitos bites

DEET (N,N-diethyl-m-toluamide), IR3535 (ethyl-butyla-cetyl-amino-propionat, EBAAP), Icaridin (Picadirin or KBR 3023), permethrin or deltametrhin and Citriodora (para-methane-3,8-diol) are repellents or insecticides considered as potentially efficacious against mosquito bites. Previous literature reviews, studies designed as arm-cage, modified arm-cage, large evaluation room test, walk-in exposure room test, in door test and field trial were included. A relevant literature review performed in 2013 was identified (26)and assessed using the AMSTAR checklist (76). We expanded the search strategy used in this review including other search databases to update it until March 2016. Animal studies, studies assessing the efficacy of plants extracts, laboratory in vitro bioassays, as well as oviposition, larvicidal and adulticidal bioassays were not included. We searched the literature databases using the following terms: (a)'mosquito repellents', (b) 'mosquito repellent', (c) 'repellent efficacy'. (Supplementary Box 1 and Figure 1).

Search #2 – The Effectiveness of repellents against *Aedes* spp mosquitos bites

This search strategy was adapted from a Cochrane Collaboration Review Protocol ("Mosquito repellents for malaria prevention - Protocol)"(77). We searched in PubMed and Embase databases. Search strategy was modified from the identified protocol using the words 'dengue', 'chikungunya', 'zika' replacing 'malaria'. It was also used the terms for repellent and personal protection. Searches were conducted in March 2016 using filter for Controlled Clinical Trial, Randommized Controlled Trial and Meta-Analysis. Evidence was graded according to the GRADE working group protocol. (Supplementary Box 2 and Figure 2).

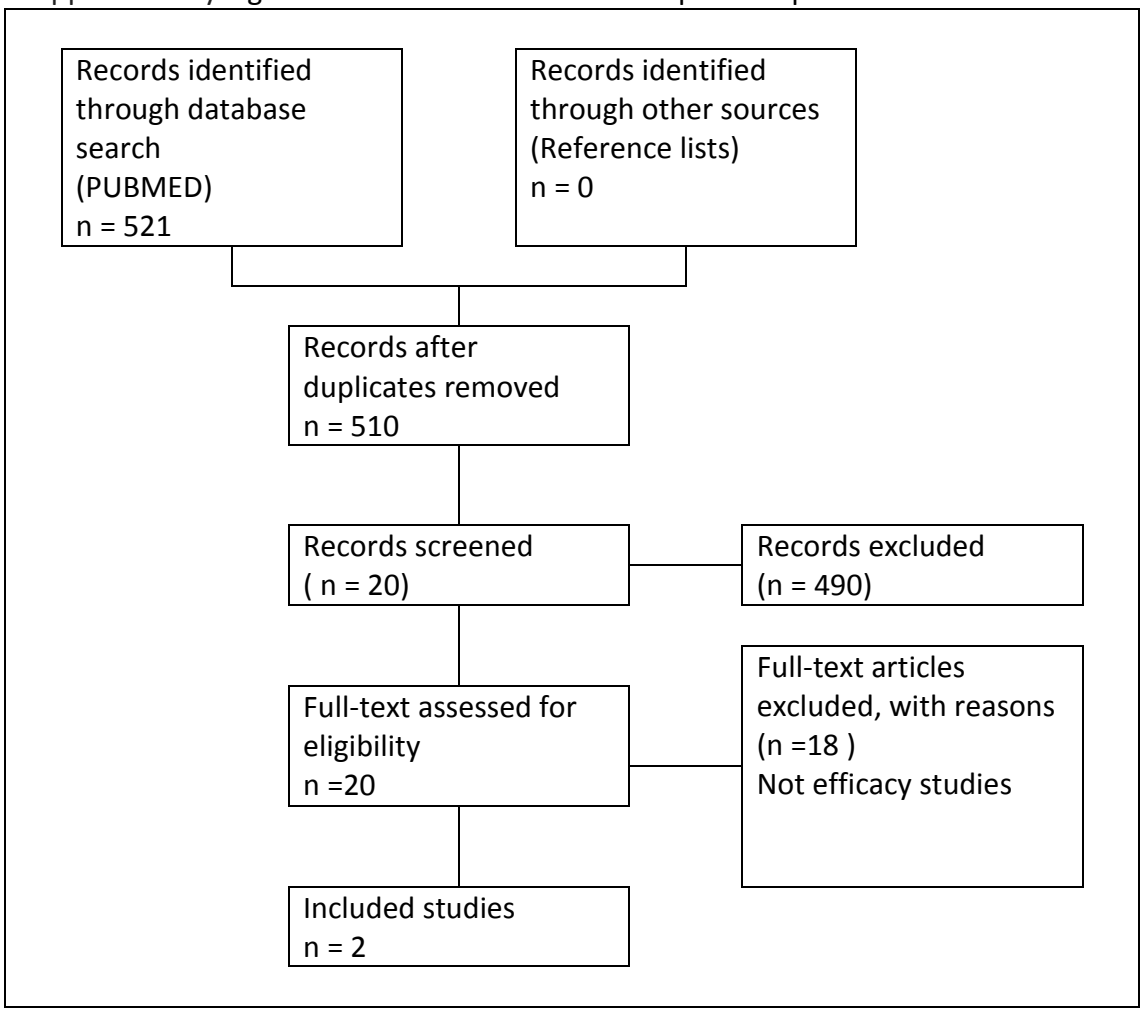
Search #3 – The safety of individual preventive measures against *Aedes* ssp mosquitos bites

The search strategy consisted of using the word 'pregnancy' combined with all repellents found in *Search #2* (DEET, Icaridin, IR3535, Citriodora, Permethrin and Deltamethrin) (Supplementary Box 3 and Figure 3). Since studies with pregnant women were rare, observational studies, trials, review and studies using animals (to evaluate teratogenicity) were included. Studies evaluating the safety of repellents on human skin, nets or clothes treated with insecticide were also included. We excluded studies using plants extracts as substance and insecticides. As several potential repellents (e.g. plant extracts) are compared with DEET only, we have excluded studies that did not use a placebo group.

Supplementary Box 1: Search Strategy for Update the Lupi et al review (from 2013 to 2016)

PUBMED		
#	Terms	Findings
1	repellent efficacy	91
2	mosquito repellent	219
3	mosquitos repellents	211

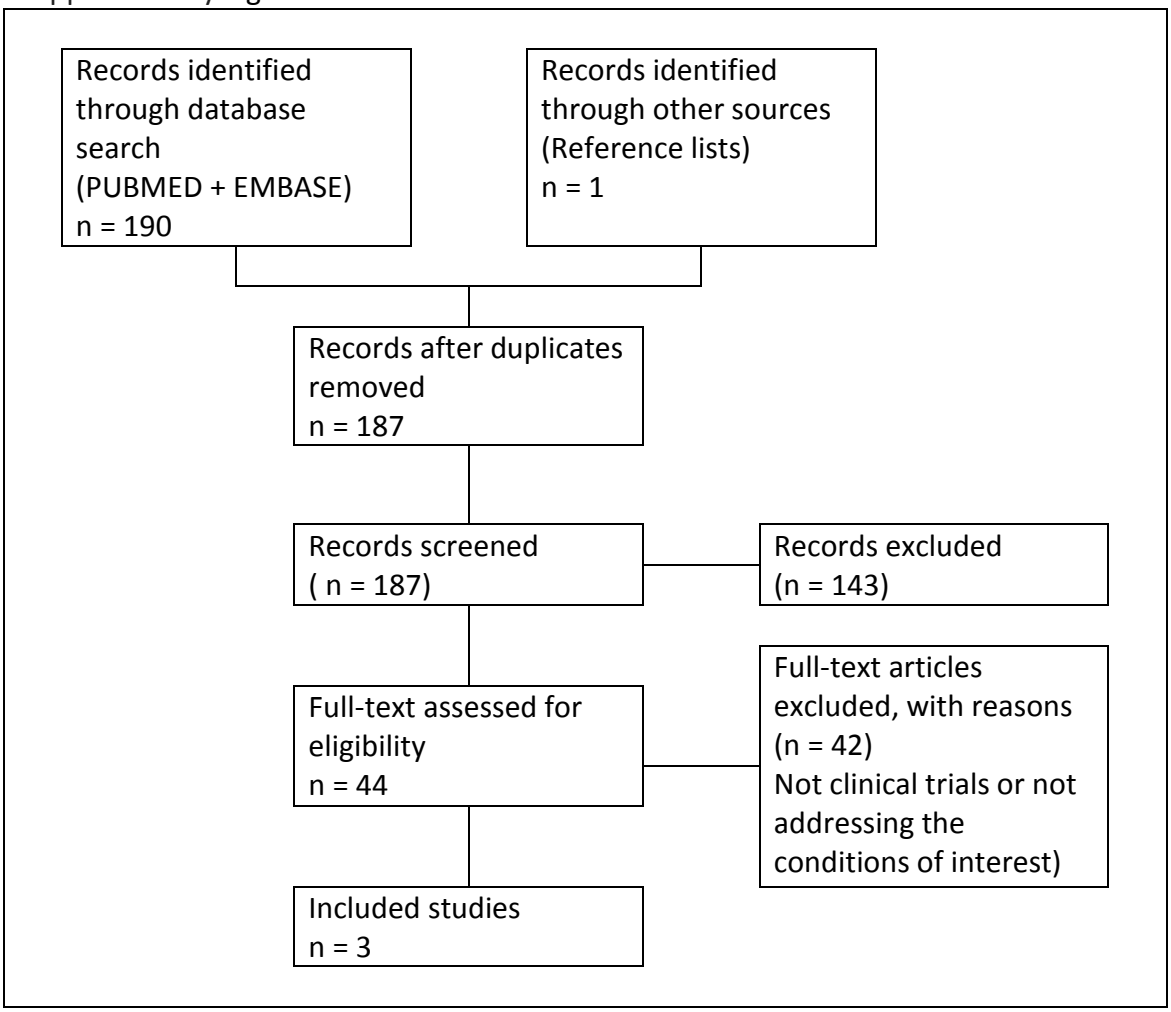
Supplementary Figure 1: The flowchart of review update Lupi et al. 2013



Supplementary Box 2: Search Strategy for Effectiveness review

PUBMED		
#	Terms	Findings
1	Dengue OR Zika OR Chikungunya ti, ab (clinical trial)	163
2	“Insect Vectors”[Mesh] OR vector* ti, ab OR mosquito* ti, ab	1864
3	#1 or #2	1991
4	“Mosquito control” [MESH]	162
5	“Aedes” [MESH]	49
6	#3 or #4 or #5	2035
7	Repellen* ti, ab	83
8	“Insecticide treated clothing” OR ITC	55
9	Spray OR sprays OR lotion* OR gel OR gels OR roll-on* OR wipe* ti, ab	7978
10	Coil* ti,ab	1138
11	“passive emanator*” ti, ab	0
12	“vaporizer mat*” ti, ab	0
13	“electric emanator*” ti, ab	0
14	“personal protection*” ti, ab	25
15	7 or 8 or 9 or 10 or 11 or 12 or 13 or 14	9241
16	6 AND 15	103
EMBASE		
#	Terms	Findings
1	Dengue OR Zika OR Chikungunya ti, ab (clinical trial)	236
2	“Insect Vectors”[Mesh] OR vector* ti, ab OR mosquito* ti, ab	1716
3	#1 or #2	1885
4	“Mosquito control” [MESH]	9
5	“Aedes” [MESH]	59
6	#3 or #4 or #5	1889
7	Repellen* ti, ab	56
8	“Insecticide treated clothing” OR ITC	184
9	Spray OR sprays OR lotion* OR gel OR gels OR roll-on* OR wipe* ti, ab	6629
10	Coil* ti,ab	821
11	“passive emanator*” ti, ab	0
12	“vaporizer mat*” ti, ab	0
13	“electric emanator*” ti, ab	0
14	“personal protection*” ti, ab	21
15	7 or 8 or 9 or 10 or 11 or 12 or 13 or 14	7682
16	6 AND 15	87

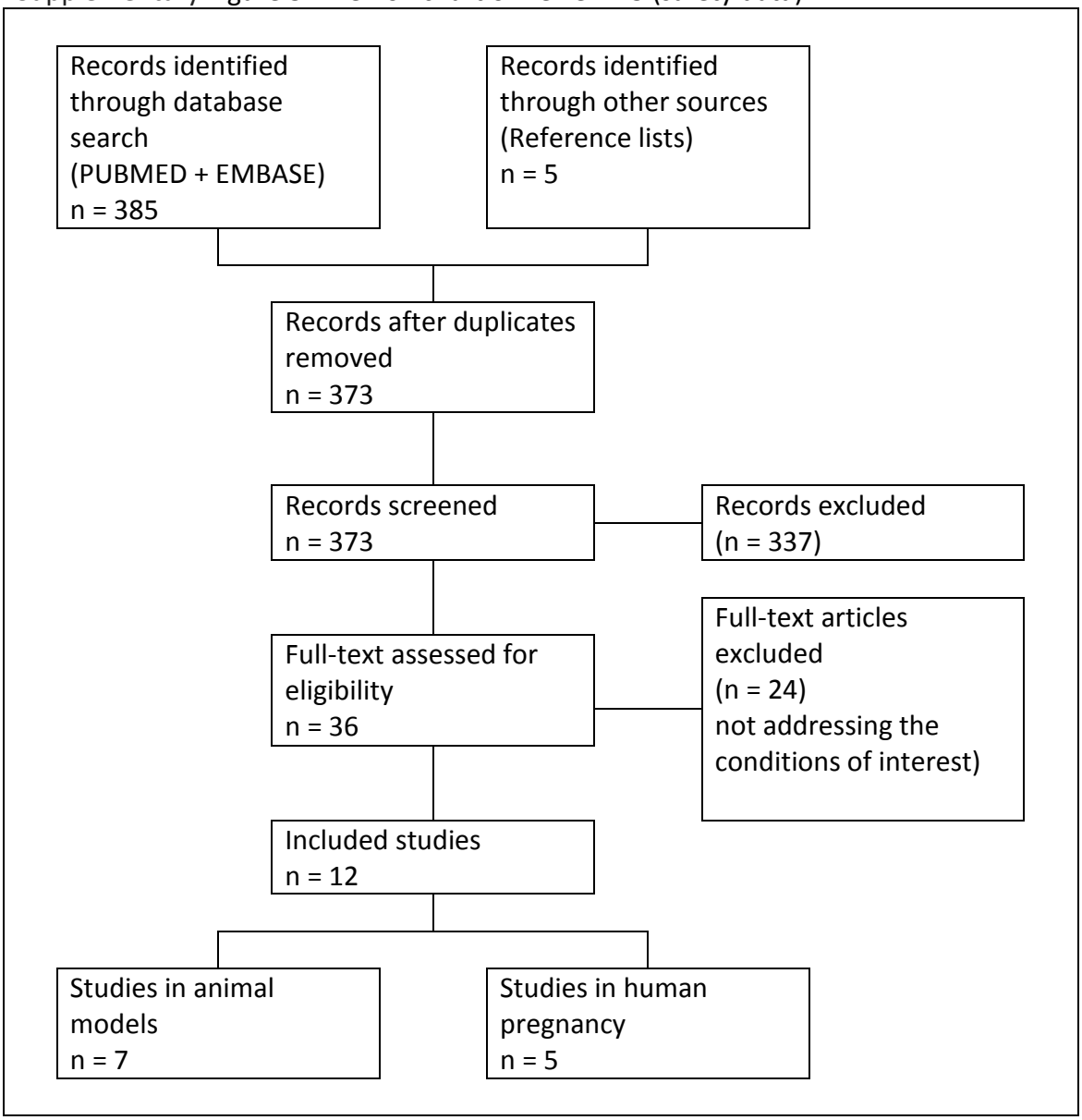
Supplementary Figure 2: The flowchart of Effectiveness Review



Supplementary Box 3: Search Strategy for Review #3 (Safety data)

PUBMED		
#	Terms	Findings
1	DEET AND pregnancy	23
2	Icaridin AND pregnancy	2
3	IR3535 AND pregnancy	0
4	Citriodora AND pregnancy	0
5	Permethrin AND pregnancy	59
6	Deltametrin AND pregnancy	41
7	#1 OR #2 OR #3 OR #4 OR #5 OR #6	125
EMBASE		
#	Terms	Findings
1	DEET AND pregnancy	46
2	Icaridin AND pregnancy	8
3	IR3535AND pregnancy	48
4	Citriodora AND pregnancy	3
5	Permethrin AND pregnancy	118
6	Deltametrin AND pregnancy	37
7	#1 OR #2 OR #3 OR #4 OR #5 OR #6	260

Supplementary Figure 3: The flowchart of Review #3 (safety data)



Summary of findings

Substance	Evidence of Effectiveness	Evidence of Safety	Narrative Summary of Safety Evidence	Comments
DEET	Low (Derived from efficacy studies)	Moderate quality (Studies in human pregnancy and animals). Downgraded due to small number of events and not assessing the use during first trimester pregnancy)	In humans there is one RCT about safety. They recommended in 2nd and 3rd trimesters of pregnancy. There is no evidence in 1st trimester. There are 3 studies using pregnant animals (rats and rabbits) that suggest no tocixity and congenital malformations with the use.	In humans: Side effect detected was Skin warmth. No teratogenic effects
Icaridin	Low (Derived from efficacy studies)	Low quality (No studies in human pregnancy)	There are 2 studies using pregnant animals (rats and rabbits) that showed no toxicity and side effects during the use of the substance. One good quality study (RCT).	Since there is no evidence in pregnant women, we suggest that this repellent should not be the first option for this population.
IR3535	Low (Derived from efficacy studies)	Not retrieved / assessed (Confidential reports from Merck Company) Published reports not found. Absence of studies reporting on use during Pregnancy.	No studies found using this repellent.	Since there is no evidence in pregnant women, we suggest that this repellent should not be the first option for this population.
Citriodora	Low (Derived from efficacy studies)	Low quality (No studies in human pregnancy)	There is one study using mice (cohort) that shows no toxicity and congenital abnormalities. The results of this study show that moderate consumption of L. citriodora as an infusion or tea appears to be safe to be used during pregnancy and does not have toxic effects on development of mouse embryo.	Since there is no evidence in pregnant women, we suggest that this repellent should not be the first option for this population.
Deltamethrin	Moderate (Derived from Cluster randomized trials assessing curtains treated with Deltamethrin)	Low quality (No studies in human pregnancy)	One study using pregnant rats showed neurotoxic effects.	Since there is no evidence in pregnant women, we suggest that this substance should not be used.

Permethrin	Very low (Derived from cluster randomized trials assessing the effectiveness of cloths, curtains and nets treated with permethrin for malaria prevention)	Moderate quality	There are 4 studies in pregnant women. One review and one RCT evaluating insecticides treated nets. They evaluated the efficacy to prevent malaria (anopheles). Two observational studies (one cohort and one case-control) evaluated topical use. They show no side effects in the use for scabies and head lice (2nd and 3rd trimester).	The use is approved by FDA only in the concentration of 1-5% topic. It can also be used in mosquitos nets and clothes (low concentration). Mosquitos nets and clothes could be used by pregnant women.