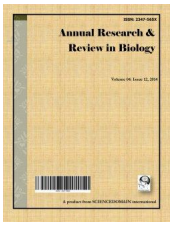

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Insecticide Susceptibility Profile of *Anopheles gambiae* s.l. from Ikot-Ekpene, Akwa Ibom State, Nigeria

K. N. Opara[1*], M. S. Ekanem[1], N. I. Udoidung[1], F. M. Chikezie[1], G. Akro[2], L. P. Usip[1], D. E. Oboho[1] and M. A. Igbe[3]

[1]Department of Animal and Environmental Biology, University of Uyo, Uyo, Nigeria.
[2]Ministry of Health, Akwa Ibom State, Nigeria.
[3]Federal Ministry of Health, National Onchocerciasis Elimination Programme, Abuja, Nigeria.

Authors' contributions

This work was carried out in collaboration between all authors. Authors KNO, MSE, NIU, FMC, GA, MAI, LPU and DEO designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors KNO, MSE, NIU, FMC and GA managed the analysis of the study. Authors KNO and FMC managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Background: Mosquito resistance to routinely used insecticides is threatening malaria vector control strategies in sub-Saharan Africa. This study reports the susceptibility of wild populations of *Anopheles gambiae* s.l. from Ikot-Ekpene, Akwa Ibom State, Southern Nigeria to insecticides.
Methods: WHO standard methods were used to detect knock-down and mortality in wild female *Anopheles* mosquitoes collected from 4 rural communities in Ikot-Ekpene. The WHO diagnostic doses of 0.05% deltamethrin, 0.05% lambdacyhalothrin, 0.75% permethrin and 5% malathion were used. Bioassays were performed on non-blood-fed mosquitoes of ages 2 to 3 days old. Post exposure mortality after 24 hours and knock-down values for KDT_{50} and KDT_{95} were calculated.

**Corresponding author: E-mail: nkopara@yahoo.com;*

Results: According to WHO criteria, insecticide resistance was not recorded at any of the studied sites and for any of the tested insecticide. Knock-down within 1 hour post insecticide exposure ranged from 98.7% to 100%. Mortality after 24 hours post exposure was 100% for all insecticides except lambdacyhalothrin which averaged 99.1%. There was high variability in KDT_{50} and KDT_{95} values across the sites.
Conclusion: Since the local *Anopheles gambiae* populations were susceptible to all the insecticides tested, vector control campaigns employing the use of any of the insecticide would not be compromised; however, there is need for continued monitoring to ensure early detection of resistance.

Keywords: Malaria; vectors; organophosphate; pyrethroid; assay; Ikot Ekpene; Nigeria.

1. INTRODUCTION

Malaria is still regarded as a public health problem in some 97 countries and territories in the tropics and subtropics [1]. Globally, approximately 214 million cases of malaria occur annually and 3.2 billion people are at risk of infection [2]. Approximately 438, 000 deaths were attributed to malaria in 2015, particularly in sub-Saharan Africa, where an estimated 90% of all malaria deaths occur [2]. These deaths are mostly among infants and children [3,4].

The most important vectors of malaria in sub-Saharan Africa are *An. gambiae*, *Sensu stricto*, *An. arabiensis* and *An. funestus* and they occur in sympatry across most of their range [5]. To alleviate the scourge of this disease, global initiatives to control malaria include preventive and curative measures such as vector control, use of bed nets, such as Long lasting insecticidal nets (LLINs), indoor residual spraying (IRS) with insecticides, use of mosquito repellents and prompt treatment with artemisinin-based combination therapy (ACT) [3,6]. There are documented evidence about the efficacy of IRS and LLINs in the reduction of vector populations and of malaria morbidity and mortality [7-9].

Malaria vector control using either LLIN or IRS relies on the continued susceptibility of *Anopheles* mosquito vectors to a limited number of insecticides approved for their control [10]. Sadly, there are reports that malaria vectors are developing resistance to commonly used insecticides [11,12] and this is more widespread in West Africa, where it has been incriminated with the use of insecticide in public health for mosquito control and in agriculture for pesticide control of agricultural pests [13]. Twelve insecticides are considered effective and safe for use in IRS, these insecticides are grouped under 4 classes namely, organochlorines, organophosphates, carbamates and pyrethroids [14].

In Nigeria, the control and elimination of malaria focuses on the use of LLINs, IRS, intermittent preventive treatment (IPT) and environmental management [15]. Consequently, the National Malaria Elimination Programme (NMEP) has scaled up indoor residual spraying to achieve 85% coverage in 20% of eligible structures in Nigeria in 2014 [16]. To supplement LLIN distribution and environmental management in Nigeria, IRS activities were progressively expanded in the seven World Bank supported malaria Booster states of Bauchi, Gombe, Kano, Jigawa, Rivers, Anambra and Akwa Ibom from 2009 – 2014 [16]. For the success of this IRS programme there is need to collect baseline data from different ecological setting on the susceptibility status of *An. gambiae* s.l., the principal vectors of malaria in Nigeria to the approved insecticides. This becomes imperative so as to guard against the development of increasing insecticide resistance. This study presents baseline data on the insecticide susceptibility status of *An. gambiae* s.l. from rural communities in Ikot Ekpene Local Government Area (LGA) of Akwa Ibom State, Nigeria.

2. MATERIALS AND METHODS

2.1 Study Sites

The study was carried out in randomly selected four rural communities, Ikot Osurua (N05.16506, E007.67252), Mbiaso (N05.21508, E007.67106), Ikot Out (N05.21436, E007.71820), Abak Oko (N05.14689, E007.74239) of Ikot-Ekpene Local Government Area of Akwa Ibom State, Nigeria (Fig. 1). These communities have long period of rain (April – October) and a shorter dry season (November to March). The inhabitants of these communities are subsistence farmers.

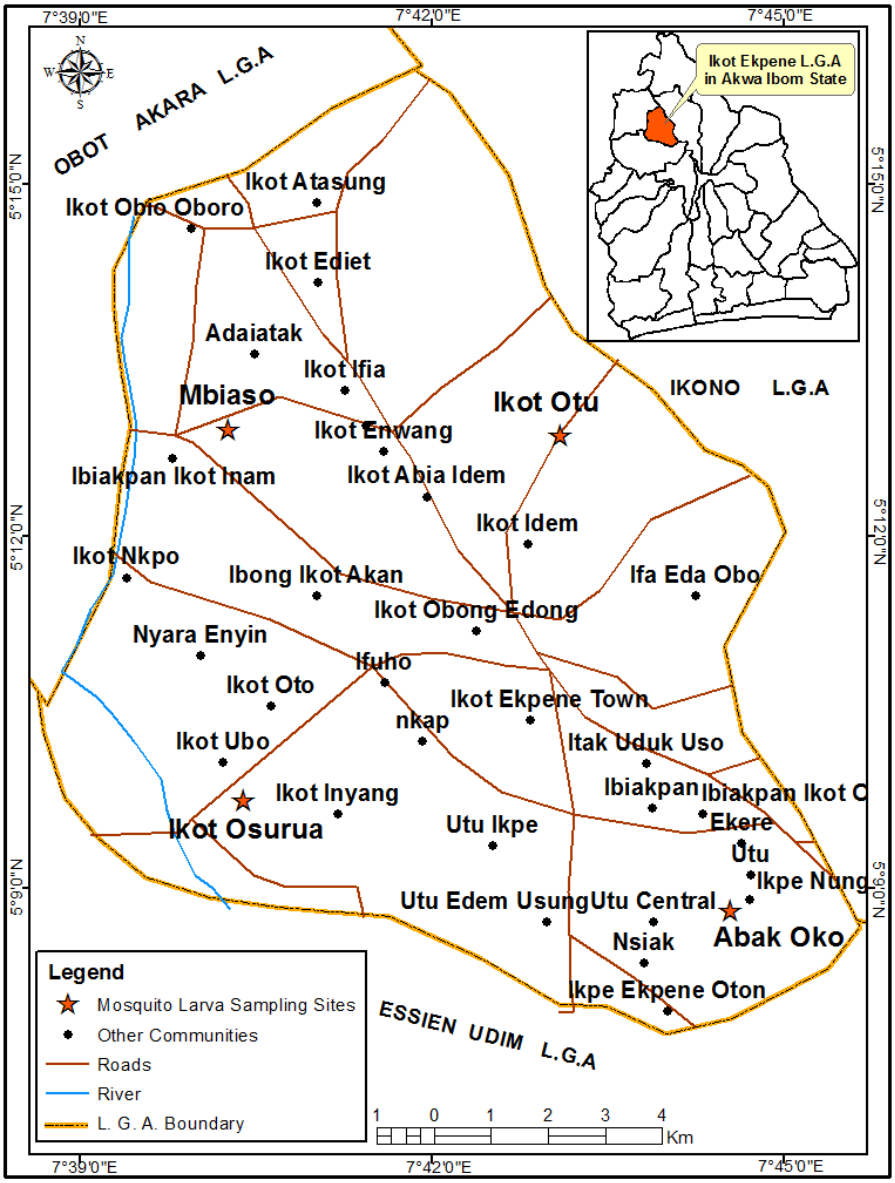

Fig. 1. Map of Ikot Ekpene L.G.A showing mosquito larval sampling sites

2.2 Mosquito Collections

With permission obtained from the community leaders, various sampling sites were identified such as rain puddles, barrels, ponds and stagnant waters, gutters, among others. Immature stages of mosquitoes were collected from the breeding sites (usually at least 4 dips) in the late rains of 2013. The identity of each larval specimen was confirmed as *An. gambiae* s.l. using the keys based on morphological characteristics [17]. Larvae from the different sites were kept alive in separate bottles and transported to the insectary for rearing to adulthood following the methods of [18]. Adults were kept in screen cages with 10% glucose solution provided continuously. Again cages were held at 27-28°C and 75-80% relative humidity.

2.3 WHO Insecticide Susceptibility Assay

Insecticide susceptibility assay was conducted according to [19] protocol, using insecticide susceptibility test kits and impregnated papers (provided by the National Malaria Elimination

Programme (NMEP) Ministry of Health, Abuja). The assay was performed on adult non-blood-fed mosquitoes of 2–3 day old. Briefly-described, batches of 20-25 mosquitoes were exposed to test papers impregnated with permithrin (0.75%), deltamethrin (0.05%) lambdacyhalothrin (0.05%), malathion (5%). Controls included batches of mosquitoes from each site exposed to untreated papers. Control mortalities between 5-20% requires correction using Abbot's formula in line with the test protocols of the WHO [19]. Control mortalities below 5% were ignored and not corrected but any mortality beyond 20% requires that the entire experiment be discarded. All tests were carried out in four replicates. The knock-down effect of each insecticide was recorded every 10 minutes over the 1 hour exposure period. Mosquitoes where then transferred to a recovery tube and provided with 10% glucose solution. Final mortality was recorded 24 hours post-exposure [10].

2.4 Data Analysis

Results from the insecticide susceptibility bioassay were evaluated according to [19] recommendations. By these criteria, resistance is indicated by mortality rate <80% after 24 hours of exposure to insecticide. Mortality rates between 80-97% suggest the possibility of resistance that requires further clarification while mortality greater than 98% are indicative of susceptibility. The KDT_{50} (i.e. time in minutes, required to achieve the knock-down of 50% of the mosquitoes) and KDT_{95} (i.e. time in minutes required to achieve the knock-down of 95% of the mosquitoes) were calculated using standard Probit analysis [20]. Data were analysed using SPSS version 20.

3. RESULTS

A total of 1355 *An. gambiae* s.l. were used in 16 susceptibility test covering 4 communities. After 24 hours the mortality rates of *An. gambiae* s.l. exposed to discriminating doses of the insecticide is presented in Tables 1-5. Control mortalities were below 5% and as such, there was no need for correcting mortalities. Using WHO criteria, no insecticide induced resistance was detected at any site and for any of the insecticides tested. Knock-down within one hour post-insecticide exposure ranged from 98.9% to 100%, while mortality at 24 hours post exposure ranged from 98.7% to 100% (Table 1). Susceptibility test of *An. gambiae* s.l. from the four communities showed that they were highly susceptible to deltamethrin, permithrin and malathion with a mortality rate of 100% (Tables 2-4). Mortality rate in *Anopheles* to lambdacyhalothrin ranged from 98.7% to 100%, indicating full susceptibility.

3.1 Knock-down Effect

The knock-down effect of the 4 insecticides determined over a one hour period indicated knock-down was more rapid for pyrethroids (permithrin, deltamethrin, and lambdacyhalothrin) insecticides than the organophosphate (malathion), as represented in Fig. 1.

Table 1. Percentage mortality and knockdown times after 1 hour and 24 hours exposure

Site	Insecticide	No. exposed	% knock-down at 60 min	% mortality after 24 hours
Mbiaso	Deltamathrin	83	100	100
	Permithrin	80	100	100
	Lambdocyhalothrin	74	98.7	98.7
	Malathion	80	100	100
IkotOtu	Deltamathrin	88	100	100
	Permithrin	80	100	100
	Lambdocyhalothrin	92	98.9	98.9
	Malathion	80	100	100
IkotOsurua	Deltamathrin	89	100	100
	Permithrin	80	100	100
	Lambdocyhalothrin	87	98.9	100
	Malathion	80	100	100
AbakOko	Deltamathrin	81	100	100
	Permithrin	80	100	100
	Lambdocyhalothrin	100	100	100
	Malathion	80	100	100
Total	**16**	**1,335**		

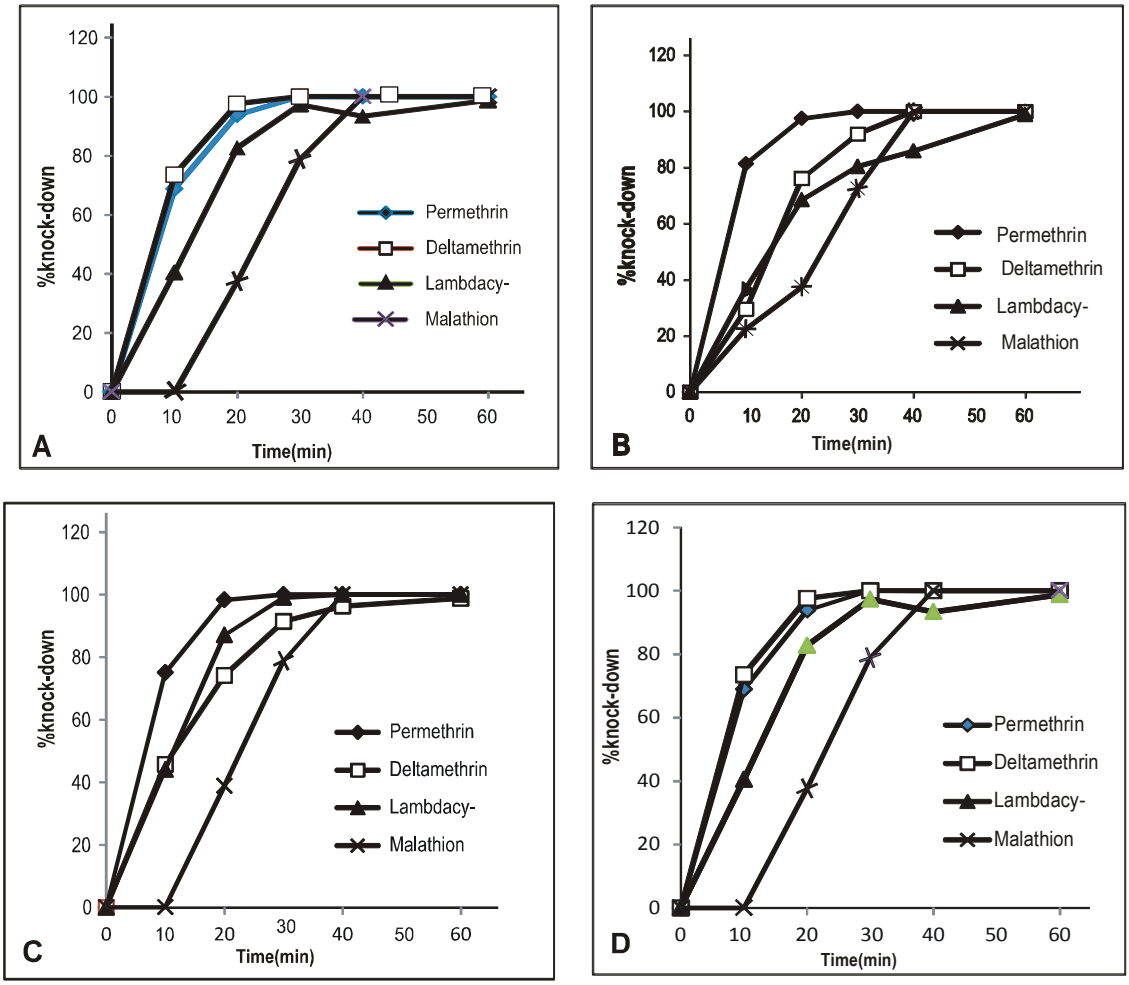

Fig. 2. Rate of knockdown of *Anopheles gambiae* s.l. from Ikot Osurua (A), Ikot Otu (B), Abak Oko (C) and Mbiaso (D) exposed to pyrethroid and organophosphate insecticides

Table 2. Percentage mortality and time of knockdown of *Anopheles gambiae* s.l. exposed to 0.05% of deltamethrin for a period of 60 minutes

Site	No. exposed	No. of replicates	No. dead	% mortality	KDT_{50} (in min)	95% CI	KDT_{95} (in min)	95% CI
Mbiaso	83	4	83	100	7.38	5.26-8.75	16.48	14.26-21.63
IkotOtu	88	4	88	100	13.49	12.08-14.79	31.26	27.67-36.81
IkotOsurua	89	4	89	100	15.17	9.27-20.14	36.73	26.30-92.47
AbakOko	81	4	81	100	11.25	9.16-13.03	38.55	32.58-48.98

Table 3. Percentage mortality and time of knockdown of *Anopheles gambiae* s.l. exposed to 0.75% of permithrin for a period of 60 minutes

Site	No. exposed	No. of replicates	No. dead	% mortality	KDT_{50} (in min)	95% CI	KDT_{95} (in min)	95% CI
Mbiaso	80	4	80	100	7.76	5.78-9.20	19.28	16.56-24.60
IkotOtu	80	4	80	100	5.99	3.21-7.76	15.70	13.30-21.13
IkotOsurua	80	4	80	100	8.71	7.13-9.89	18.11	15.74-22.96
AbakOko	80	4	80	100	6.98	4.69-9.51	17.18	14.72-22.49

Table 4. Percentage mortality and time of knockdown of *Anopheles gambiae* exposed to 0.05% of lambdacyhalothrin for a period of 60 minutes

Site	No. exposed	No. of replicates	No. dead	% mortality	KDT_{50} (in min)	95% CI	KDT_{95} (in min)	95% CI
Mbiaso	75	4	74	98.7	11.38	9.59-12.97	31.19	26.85-38.28
IkotOtu	92	4	91	98.9	14.03	11.86-15.96	54.70	45.60-70.79
IkotOsurua	87	4	86	98.9	14.22	8.22-18.92	55.85	38.19-141.58
AbakOko	100	4	100	100	10.94	9.71-12.02	23.99	21.33-28.31

Table 5. Percentage mortality and time of knockdown of *Anopheles gambiae* exposed to 5% of malathion for a period of 60 minutes

Site	No. exposed	No. of replicates	No. dead	% mortality	KDT_{50} (in min)	95% CI	KDT_{95} (in min)	95% CI
Mbiaso	80	4	80	100	22.49	21.05-23.82	35.89	33.02-40.37
IkotOtu	80	4	80	100	24.65	23.33-25.93	37.04	34.32-41.22
IkotOsurua	80	4	80	100	23.39	17.61-28.07	38.78	31.30-76.32
AbakOko	80	4	80	100	22.35	20.90-22.88	35.90	33.09-40.41

For each insecticide, variability in KDT_{50} and KDT_{95} values was observed across study sites. All the mosquitoes showed over 90% knock-down within the one hour exposure period. Exposure times (minutes) which resulted in 50% and 95% knock-down estimated for each insecticide are as follows: deltamethrin KDT_{50} (7.38 to 15.17) and KDT_{95} (16.48 to 38.55); permithrin KDT_{50} (5.99 to 8.71) and KDT_{95} (15.70 to 19.25); lamdacyhalothrin KDT_{50} (10.94 to 14.22) and KDT_{95} (23.99 to 58.85); malathion KDT_{50} (22.35 to 24.65) and KDT_{95} (35.89 to 38.78).

4. DISCUSSION

The results of the present study have revealed good susceptibility of *Anopheles gambiae* s.l. in the study sites to all the insecticides tested. The implication of this finding is that any vector control programme employing any of these compounds either in the treatment of bednets or other materials or for indoor residual spraying would achieve an appreciable success rate [21]. This observation is consistent with the findings of [10,16,22,23] who reported susceptibility of *Anopheles* mosquitoes to permithrin, deltamethrin, lamdacyhalothrin and malathion insecticides. In contrast, reports by [24-27], indicated resistant of *Anopheles* species to the pyrethroid insecticides. Single, multiple and cross-resistance of *Anopheles* species to insecticides is now considered widespread throughout sub-Saharan Africa, particularly for pyrethroid insecticides [26,28]. Given its widespread distribution and high prevalence, resistance is considered a serious challenge to the effectiveness of current vector control efforts [20,29]. In the present study no evidence of resistance to any of the insecticides tested was found. Previous researchers have reported that exposure of malaria vectors to crop protection and public health insecticides could result in development of insecticide resistance [26,30,31]. Since the inhabitants of these study communities are subsistence farmers, it is possible that the degree of use of crop pesticides and public health insecticides in the area are limited to warrant selection pressure on local *Anopheles* populations among other factors responsible for resistance to pyrethroid insecticides.

The values of KDT_{50} and KDT_{95} in the present study are in consonance with other studies on *An. gambiae* s.l. population that are categorized as susceptible elsewhere [10,16,21,22,32]. The KDT_{50} and KDT_{95} for lambdacyhalothrin and malathion in mosquitoes from Ikot Otu and Ikot Osurua are relatively higher than those of other insecticides tested in the present study, suggesting that knock-down resistance (kdr) mechanisms could be operating in this mosquito populations. The underlying resistance in most cases has been the Kdr mutation [33-36]. The Kdr mechanisms result from mutation in the voltage-gated sodium channel, the target-site for DDT and pyrethroids and is one of the two most important forms of biochemical resistance mechanism, the other being metabolic resistance which occurs when levels of insecticides detoxifying enzymes are elevated or their activity modified, thereby preventing the insecticide from

reaching its site of action [27,29,37]. This study could not confirm the presence of any resistance gene in the *Anopheles* mosquito populations studied since we did not carry out a molecular resistance study. Only phenotypic resistance was studied through susceptibility test [23].

Studies in other parts of Nigeria have shown that the local *Anopheles* populations are resistant to pyrethroids [12,16,25,38-40]. These findings raise concerns on the success of IRS programme implementations in Nigeria. This is premised on the fact that knowledge about insecticide resistance in sub-Saharan Africa countries has emerged from susceptibility test performed as part of specific research studies. Lukwa and colleagues [23] enumerated some limitations of such studies to include: (a) susceptibility tests in most cases are not performed against all insecticide classes, limiting the assessment of the status of cross and multiple resistance; (b) geographic coverage is highly fragmented affecting the ability to infer the national or sub-national status of insecticides resistance; (c) test are not performed on a regular basis (tests were performed mostly once), even though resistance is known to vary seasonally; (d) limited standardization in how mosquitoes are obtained to perform tests.

Even though no resistance to pyrethroids was detected in this study and in view of the facts that resistance has been reported in other parts of the country coupled with the aforementioned limitations of susceptibility tests, Nigeria, in line with the global plan for insecticide resistance management (GPIRM) guideline [29], should strive to integrate resistance management into all control programmes. In addition alternative non-pyrethroid insecticides should be considered.

5. CONCLUSION

To our knowledge, the findings of this study is the first status report on the susceptibility of *An. gambiae* to some selected insecticides in Akwa Ibom State, Nigeria. The results show that *An. gambiae* populations from 4 rural communities of Ikot Ekpene (LGA) of Akwa Ibom State Nigeria were susceptible to all the insecticides. Consequently vector control intervention employing the use of these insecticides in the studied areas would not be compromised by resistance. Furthermore the data in this study will provide a baseline information on which monitoring of development of resistance to insecticide will be anchored.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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