Determination of the discriminating concentration of chlorfenapyr (pyrrole) and Anopheles gambiae s.l. susceptibility testing in preparation for distribution of Interceptor® G2 insecticide-treated nets

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Abstract

Background

Following agricultural use and large-scale distribution of insecticide treated nets (ITNs), malaria vector resistance to pyrethroids is widespread in sub-Saharan Africa. Interceptor® G2 is a new dual active ingredient (AI) ITN treated with alpha-cypermethrin and chlorfenapyr for the control of pyrethroid-resistant malaria vectors. In anticipation of these new nets being more widely distributed, testing was conducted to develop a chlorfenapyr susceptibility bioassay protocol and gather susceptibility information.

Methods

Bottle bioassay tests were conducted using five concentrations of chlorfenapyr at 12.5, 25, 50, 100 and 200µg AI/bottle in ten countries in sub-Saharan Africa using 13,639 wild collected *An. gambiae* s.l. (56 vector populations per dose) and 4,494 pyrethroid susceptible insectary mosquitoes from 8 colonized strains. In parallel, susceptibility tests were conducted using a provisional discriminating concentration of 100µg AI/bottle in 16 countries using 23,422 wild collected pyrethroid resistant *An. gambiae* s.l. (259 vector populations). Exposure time was 60 minutes, with mortality recorded at 24, 48 and 72 hours after exposure.

Results

Median mortality rates (up to 72h after exposure) of insectary colony mosquitoes was 100% at all five concentrations tested, but the lowest dose to kill all mosquitoes tested was 50µg AI/bottle. The median 72h mortality of wild *An. gambiae* s.l. in 10 countries was 71.5%, 90.5%, 96.5%, 100% and 100% at concentrations of 12.5, 25, 50, 100 and 200µg AI/bottle, respectively. Log-probit analysis of the five concentrations tested determined that the LC_{95} of wild *An. gambiae* s.l. was 67.9µg AI/bottle (95% CI: 48.8-119.5). The discriminating concentration of 203.8µg AI/bottle (95% CI: 146-359) was calculated by multiplying the LC_{95} by three. However, the difference in mortality between 100 and 200µg AI/bottle was minimal and large-scale testing using 100µg AI/bottle with wild *An. gambiae* s.l. in 16 countries showed that this concentration was generally suitable, with a median mortality rate of 100% at 72h.

Conclusions

This study determined that 200µg AI/bottle chlorfenapyr in bottle bioassays is the most suitable discriminating concentration for monitoring susceptibility of wild *An. gambiae* s.l., using mortality recorded up to 72h. Testing in 16 countries in sub-Saharan Africa demonstrated vector susceptibility to chlorfenapyr, including mosquitoes with multiple resistance mechanisms to pyrethroids.

Results

Median mortality rates of pyrethroid susceptible colony strains across eight countries were 100% at 72 hours post-exposure to each of five chlorfenapyr concentrations tested in bottle bioassays, with 50µg AI/bottle being the lowest concentration to kill every mosquito tested (Fig. 2).

Figure 2. Percentage mortality (72h) after exposure to each of five concentrations (12.5, 25, 50, 100, 200µg AI/bottle) of chlorfenapyr in bottle bioassays using pyrethroid susceptible colony mosquito strains in eight countries.

A clear positive response with increasing mean mortality rates at every chlorfenapyr concentration was observed among wild *An. gambiae* s.l. (Fig. 3). The median 72h mortality was 71.5%, 90.5%, 96.5%, 100% and 100% at 12.5, 25, 50, 100 and 200µg AI/bottle respectively. Log-probit analysis determined the LC50 as 7.7µg AI/bottle (95% confidence interval (CI): 5.5–9.8), LC95 as 67.8µg AI/bottle (95% CI: 55.2–89.4), and LC99 as 166.9µg AI/bottle (95% CI: 120.4-266.5). The discriminating

concentration was calculated at either 203.4µg AI/bottle (95% CI: 166–268) using the method of Lees et al or 333.8µg AI/bottle (95% CI: 241–533) using the WHO approach.

Figure 3. Median percentage mortality of wild *An. gambiae* s.l. 72 hours after exposure to chlorfenapyr at concentrations of 12.5, 25, 50, 100 and 200µg AI/bottle in bottle bioassay in 10 countries.

Figure 4. Percentage mortality of wild *An. gambiae* s.l. 60 mins, 24h, 48h and 72h after exposure to the provisional discriminating concentration of chlorfenapyr at 100µg AI/bottle in 16 countries.

At the provisional discriminating concentration of 100µg AI/bottle, large variation in percent mosquitoes knocked-down at 60 minutes was observed (Fig. 4), although the median value was low at 38.0% (IQR (interquartile range): 8.0-66.6%), demonstrating the slow acting nature of pyrrole insecticides compared to pyrethroids [29]. Results confirmed that a holding time of 72h post-exposure is required, with median mortality of 96.7% (IQR: 82.0-100) at 24h compared to 100% (IQR: 100–100) at 72h. While the median mortality was 100% after 72h, there were many outliers when mortality was < 98%, indicating that 100µg AI/bottle may not be a suitable discriminating concentration. Tests conducted with 200µg AI/bottle in 10 countries produced similar trends to the 100µg concentration, reaching a median of 100% (IQR: 100–100) mortality at 72h (Fig. 5).

Figure 5. Percentage mortality of wild *An. gambiae* s.l. 60 mins, 24h, 48h and 72h after exposure to chlorfenapyr at 200µg AI/bottle in 10 countries.

In this study it was not always possible to closely regulate testing and holding temperature however, the temperature was consistently < 25°C only in Madagascar and Zimbabwe. However, it should be noted that in Madagascar mortality was < 98% with 100 or 200µg AI/bottle in seven sites where testing and holding temperatures were regularly below the WHO recommended range of 27 ± 2°C. Molecular species identification indicated that the predominant species tested in the dose-ranging studies were *An. gambiae* in DR Congo and Madagascar, *An. coluzzii* in Mali, mixed *An. gambiae*/ *coluzzii* in Ghana and Nigeria, and *An. arabiensis* in Ethiopia, Kenya, Senegal and Uganda (Table S1). All wild malaria vectors used for chlorfenapyr susceptibility tests were found to be resistant to pyrethroid insecticides, except for a few locations in Madagascar (Table S1).

Conclusions

This study determined that 200µg AI/bottle chlorfenapyr in bottle bioassays is the most suitable discriminating concentration for monitoring susceptibility of wild *An. gambiae* s.l., using mortality recorded up to 72h. Testing in 16 countries in sub-Saharan Africa demonstrated vector susceptibility to chlorfenapyr, including mosquitoes with multiple resistance mechanisms to pyrethroids.

Keywords

Chlorfenapyr, pyrrole, *Anopheles gambiae*, Interceptor G2, CDC bottle bioassay, discriminating concentration, insecticide treated net, insecticide resistance.

Background

The core vector control interventions recommended by the World Health Organization (WHO) to reduce malaria transmission are universal coverage with insecticide-treated nets (ITN) and/or indoor residual spraying (IRS) of houses [1]. An estimated 1.9 billion ITNs were delivered by manufacturers to countries in sub-Saharan Africa between 2004 and 2019, with 213 million ITNs distributed in 2019 alone [2]. Between 2000 and 2015 it is estimated that vector control averted 663 million clinical cases of malaria in sub-Saharan Africa, with ITNs contributing to 68% of that reduction [3]. Pyrethroid insecticides remain the dominant chemical class used on ITNs due to their low cost (<$2 per net), low human toxicity and efficacy against mosquitoes through rapid knock-down, mortality and repellency [4, 5]. Currently there are 15 standard pyrethroid net products that have WHO prequalification (PQ) listing, consisting of seven that contain alpha-cypermethrin, seven deltamethrin and one

permethrin [6]. Following agricultural use and large-scale distribution of pyrethroid ITNs, resistance to pyrethroids in sub-Saharan Africa is widespread, with many countries reporting high resistance intensity which is likely to lead to vector control failure [7-11].

To manage insecticide resistance and effectively control malaria vectors, it is important for ITNs to use insecticides with different modes of action. New 'dual active ingredient' nets treated with two different active ingredients (AIs) have been developed, though to date these all include a pyrethroid as one of the AIs. Examples include Interceptor G2 (treated with chlorfenapyr and alpha-cypermethrin) and Royal Guard (treated with pyriproxyfen and alpha-cypermethrin) ITNs which received WHO PQ listing in 2018 and 2019, respectively [6]. Experimental hut studies of Interceptor G2 ITNs have shown particular promise, with high efficacy and wash durability against pyrethroid resistant malaria vectors demonstrated in Benin, Burkina Faso and Côte d'Ivoire [12-14]. Chlorfenapyr is a pyrrole compound with a non-neurotoxic mode of action that involves uncoupling of oxidative phosphorylation via disruption of the proton gradient [15, 16]. This uncoupling at the mitochondria ultimately results in disruption of ATP (Adenosine 5'-triphosphate) production, cellular death, and organism mortality [17]. Chlorfenapyr is a pro-insecticide, meaning that after uptake by the insect the parent form of chlorfenapyr (CL303630) is metabolized by cytochrome P450 enzymes into the active metabolite (CL303268) [16]. Chlorfenapyr is used in agriculture as a foliar-applied insecticide to control insect and mite pests of various fruit, vegetable, grain, herb, spice and tea crops but is not yet widely used in sub-Saharan Africa and is fairly new for vector control [18].

As of 2020 there was no published guidance regarding chlorfenapyr susceptibility test procedures or discriminating concentration. Insecticide susceptibility tests of malaria vectors are normally conducted using either pre-treated filter papers that are prepared by a WHO collaborating institution (Universiti Sains, Malaysia) and distributed to field sites for use in tube tests or by using bottle bioassay procedures according to United States Centers for Disease Control and Prevention (CDC) defined discriminating concentrations [19, 20]. This delay in guidance was partly due to the non-neurotoxic nature of chlorfenapyr meaning that standard WHO testing protocols needed adaptation. WHO initially proposed 5% chlorfenapyr filter papers for susceptibility testing, but this was later found to be an unsuitable system [21].

U.S. President's Malaria Initiative (PMI) funding supports regular insecticide resistance monitoring in partnership with national malaria control programs (NMCPs) in sub-Saharan Africa to assist with national vector control decision making. In anticipation of Interceptor G2 nets being distributed in sub-Saharan Africa, chlorfenapyr susceptibility testing using a modified bottle bioassay protocol was conducted to determine a suitable discriminating concentration and to gather baseline susceptibility information.

Methods

Study sites and chlorfenapyr dosages tested

Experiments were conducted using five concentrations of chlorfenapyr at 12.5, 25, 50, 100 and 200µg AI/bottle. Tests were conducted on wild uncharacterized *An. gambiae* s.l. in 10 countries in sub-Saharan Africa, out of which eight countries conducted additional tests with colonized pyrethroid susceptible strains. The countries included, The Democratic Republic of Congo (4 sites), Ethiopia (1 site), Ghana (3 sites), Kenya (2 sites), Madagascar (10 sites), Mali (11 sites), Nigeria (3 sites), Senegal (3 sites), Uganda (2 sites) and Zimbabwe (1 site). Locations are shown in Figure 1. In parallel, susceptibility tests were conducted using a provisional discriminating concentration of 100µg AI/bottle in 16 countries. This concentration was chosen based on preliminary bottle bioassay testing by CDC which established a provisional discriminating concentration of 100µg AI/bottle (Dr WG Brogdon, 2017, personal communication). All bioassays were conducted between 2017 and 2020.

Preparation of solutions

Treatment of 250ml Wheaton® bottles was conducted locally in the country of testing using technical grade chlorfenapyr dissolved in acetone. A vial containing 5g of technical grade (99.9% pure) chlorfenapyr was supplied by BASF (Ludwigshafen, Germany) to each country team and a stock solution was prepared at 1mg/ml by weighing 100mg and dissolving with 100ml

acetone. The stock solution was prepared in an amber glass bottle (or clear glass bottle covered with aluminium foil) to avoid exposure to UV light and sealed with a tightly fitting lid to prevent evaporation before being stored at 4°C in a refrigerator for a maximum of three months. A test solution of 200µg/ml was prepared by performing a five times dilution by mixing 10ml of the stock solution with 40ml of acetone. Diluents were serially prepared with 2-fold dilutions of 100µg/ml, 50µg/ml, 25µg/ml and 12.5µg/ml.

Bioassay procedures

Each 250ml glass bottle and its plastic cap were coated with 1ml of insecticide solution by rolling and inverting the bottles according to CDC procedures [20]. In parallel, a negative control bottle was coated with 1ml of acetone. All bottles were dried overnight in the dark and bioassays were conducted within 24h of treating bottles. In general, a total of 80-100 female mosquitoes, aged two to five days old, were exposed for 60 minutes in four replicates of 20-25 mosquitoes, with an additional single replicate of 25 mosquitoes used for the negative control (bottle treated with 1ml acetone). A total of 13,639 wild collected *An. gambiae* s.l. (56 vector populations per dose) in ten countries were tested using five concentrations of chlorfenapyr. While a total of 4,494 pyrethroid susceptible insectary mosquitoes from eight colonized strains were tested. A total of 23,422 wild collected pyrethroid resistant *An. gambiae* s.l. (259 vector populations) were tested at the discriminating concentration of 100µg AI/bottle in 16 countries. After exposure, mosquitoes were transferred to clean paper cups and provided with 10% sugar solution. Mortality was recorded at the end of the 60 minutes exposure and at 24, 48 and 72 hours after exposure. Tests were conducted during the day time with effort made to keep testing and holding conditions within WHO guidelines of 27°C±2°C and relative humidity of 75%±10% [19]. Temperature and humidity were monitored and recorded, however, in several cases could not be accurately controlled, as tests with wild collected mosquitoes were generally conducted in improvised field insectaries which did not have robust temperature and humidity controls.

Mosquito species tested

Insectary-reared pyrethroid susceptible colonies of *An. gambiaesensu stricto* (s.s.) Kisumu strain were used for testing in six countries (Ghana, Kenya, Madagascar, Nigeria, Uganda, Zambia) while *Anopheles coluzzii* Ngousso strain was used in Mali and *Anopheles arabiensis* Adama strain in Ethiopia. Larval collections of wild *An. gambiae* s.l. were made in areas where pyrethroid resistance had previously been detected from temporary sunlit pools between 2017 and 2020 (timing varied by country) using larval dippers. Larvae were subsequently transported to a field insectary where they were reared in water collected from the field and fed with Tetramin® fish food. Emerging adult mosquitoes were provided with cotton wool pads dipped in 10% sugar solution until they were used in insecticide susceptibility tests. Wild *Anopheles* were identified morphologically as *An. gambiae* s.l. in all 16 countries using the key of Gillies and Coetzee (1987) [22]. Molecular analysis to determine species of these test mosquitoes was not conducted. However, *An. gambiae* s.l. collected from the same locations for other purposes were identified to species by PCR using the protocols of either Scott (1993), Santolamazza (2008), or Wilkins (2006) to determine members of the *An. gambiae* species complex [23-26].

Data analysis

Insecticide susceptibility results were presented as unadjusted percentage mortality at the end of 60 minutes and subsequently 24, 48 and 72 hours after bioassay exposure. If negative control mortality was greater than 20%, the data was discarded, and tests were repeated. Box plots are used to present mortality data showing the median and interquartile range, with whiskers representing one and a half times the interquartile range and small circles outside the whiskers considered outliers. PoloPlus (LeOra Software, Parma MO, USA) was used to conduct probit analysis on the logarithmic scale to calculate the concentration of chlorfenapyr needed to kill a defined proportion of mosquitoes, known as lethal concentration (LC). Mortality data (72 hours after exposure) was included for each concentration used in the analysis (12.5, 25, 50, 100, 200µg AI/bottle) to determine the LC_{50}, LC_{95} and LC_{99} (concentration needed to achieve 50%, 95%, and 99% mortality) for wild *An. gambiae* s.l. The LC_{95} value was then multiplied by three to give a discriminating concentration (LC_{95}×3=DC) as described by Lees et al, 2019 [27]. The WHO approach of multiplying the LC_{99} by two was also used to determine a discriminating

concentration [28]. Probit analysis was not conducted with data generated using insectary strains as there was not a sufficient spread of data to fit the probit curve.

Results

Median mortality rates of pyrethroid susceptible colony strains across eight countries were 100% at 72 hours post-exposure to each of five chlorfenapyr concentrations tested in bottle bioassays, with 50µg AI/bottle being the lowest concentration to kill every mosquito tested (Figure 2).

A clear positive response with increasing mean mortality rates at every chlorfenapyr concentration was observed among wild *An. gambiae* s.l. (Figure 3). The median 72h mortality was 71.5%, 90.5%, 96.5%, 100% and 100% at 12.5, 25, 50, 100 and 200µg AI/bottle respectively. Log-probit analysis determined the LC50 as 7.7µg AI/bottle (95% confidence interval (CI): 5.5-9.8), LC95 as 67.8µg AI/bottle (95% CI: 55.2-89.4), and LC99 as 166.9µg AI/bottle (95% CI: 120.4-266.5). The discriminating concentration was calculated at either 203.4µg AI/bottle (95% CI: 166-268) using the method of Lees et al or 333.8µg AI/bottle (95% CI: 241-533) using the WHO approach.

At the provisional discriminating concentration of 100µg AI/bottle, large variation in percent mosquitoes knocked-down at 60 minutes was observed (Figure 4), although the median value was low at 38.0% (IQR (interquartile range): 8.0-66.6%), demonstrating the slow acting nature of pyrrole insecticides compared to pyrethroids [29]. Results confirmed that a holding time of 72h post-exposure is required, with median mortality of 96.7% (IQR: 82.0-100) at 24h compared to 100% (IQR: 100-100) at 72h. While the median mortality was 100% after 72h, there were many outliers when mortality was <98%, indicating that 100µg AI/bottle may not be a suitable discriminating concentration. Tests conducted with 200µg AI/bottle in 10 countries produced similar trends to the 100µg concentration, reaching a median of 100% (IQR: 100-100) mortality at 72h (Figure 5).

In this study it was not always possible to closely regulate testing and holding temperature however, the temperature was consistently <25°C only in Madagascar and Zimbabwe. However, it should be noted that in Madagascar mortality was <98% with 100 or 200µg AI/bottle in seven sites where testing and holding temperatures were regularly below the WHO recommended range of 27±2°C. Molecular species identification indicated that the predominant species tested in the dose-ranging studies were *An. gambiae* in DR Congo and Madagascar, *An. coluzzii* in Mali, mixed *An. gambiae*/*coluzzii* in Ghana and Nigeria, and *An. arabiensis* in Ethiopia, Kenya, Senegal and Uganda (Table S1). All wild malaria vectors used for chlorfenapyr susceptibility tests were found to be resistant to pyrethroid insecticides, except for a few locations in Madagascar (Table S1).

Discussion

In this study, susceptibility tests conducted with a pyrethroid susceptible colony and wild *Anopheles* species confirmed that chlorfenapyr is a slower-acting insecticide when compared with neurotoxic pyrethroids [29]. While pyrethroids are characterized by rapid knock-down of susceptible mosquitoes within a few minutes, chlorfenapyr-induced knock-down at 60 minutes post-exposure was generally low (albeit highly variable). Despite the low levels of knock-down, more than 90% of chlorfenapyr-induced mortality occurred within 24h of exposure, but 72h holding period was required to consistently reach >98% mortality at 100 and 200µg AI/bottle. It has previously been shown that 25µg AI/bottle was sufficient to kill 100% of a susceptible colonized strain of *An. arabiensis* but mortality rates were significantly lower with wild *An. arabiensis* with 100µg and 200µg AI/bottle producing mortality >98% [33]. Results presented here are in keeping with this trend, with pyrethroid susceptible colony strains killed at lower concentrations than wild *An. gambiae* s.l. Inbreeding over a period of several decades reduces the overall fitness of reference strains, therefore it is important to conduct testing against wild mosquitoes before widespread use for vector control [34]. Other factors which may contribute to higher toxicity with insectary colonies in bioassays could be related to the mode of action which is intertwined with mosquito metabolism [35]. The circadian rhythm of colonies can be different to wild *Anopheles* populations either due to rearing under reverse photoperiod or due to daytime blood-feeding, which would result in greater metabolic activity during daytime [36, 37]. Therefore, there could be greater

impact of the insecticide on insectary colonized mosquitoes with daytime exposures because they are more active during the day than wild mosquitoes that are more active during the night under natural conditions.

Others have proposed a discriminating concentration of 50µg AI/bottle based on bottle bioassays performed with susceptible *An. gambiae* Kisumu strain and wild collected *An. arabiensis*, *An. gambiae* s.s. and *An. funestus* from western Kenya [38]. In our study, 50µg AI/bottle was sufficient against susceptible colony mosquitoes but mean mortality of wild *An. gambiae* s.l. across ten countries was only 93% (95% CI: 86.3-99.5) after 72h. Large-scale susceptibility testing using the interim discriminating concentration of 100µg AI/bottle against thousands of *An. gambiae* s.l. in 16 countries showed that this concentration was generally suitable, with a mean of 98.7% mortality (95% CI: 98.1-99.3). Other studies in Faranah Prefecture of Guinea with 100µg AI/bottle produced 100% mortality with wild *An. gambiae* s.s., while in the Agréby-Tiassa Region of south-east Côte d'Ivoire, the same concentration only produced 95.5% mortality [39, 40]. Using the formula of Lees et al. (2019) we determined that 200µg AI/bottle was a suitable discriminating concentration and we believe this concentration will produce fewer cases of false resistance reporting than with 100µg AI/bottle. It is recognized that susceptibility testing with chlorfenapyr will produce more variable results than with neurotoxic insecticides due to the mode of action being linked with the metabolism of the insect, and this variability was most evident at lower concentrations. WHO recommend a temperature of 27±2°C be closely adhered to when conducting chlorfenapyr bioassays [35]. In this study (and probably in general) it was not always possible to closely regulate testing and holding temperature and this may have been a factor in the generally lower mortality in Madagascar. This highlights the need to repeat bioassays to confirm resistance over several time points, particularly when resistance is being reported for the first time. To minimize the occurrence of false resistance reporting, we propose that tests should always be conducted in parallel with a well characterized colony strain to try and detect issues with under-dosing or low testing temperature. We also recommend that before reporting chlorfenapyr resistance for a site, experiments should be repeated at the same location at least three times in different months and consistently result in less than 90% mortality. Ideally any findings of potential resistance would be supported by molecular studies (for example transcriptome sequencing to identify upregulated and downregulated genes) to identify mechanisms of resistance.

Insecticide selection pressure from agriculture is generally regarded as an important early driver of insecticide resistance in malaria vectors [41, 42]. Chlorfenapyr resistance has been reported in several species of crop pest due to agricultural use in Asia, Europe, North America and Australia, [43-45]. While statistics from sub-Saharan Africa are scant, there appears to be little usage of pyrrole insecticides for agricultural pest control, with supply by BASF limited to Kenya for control of rose pests in greenhouses (Dr S Stutz, BASF 2020, personal communication). However, it is likely that generic formulations will become more widely available, for example chlorfenapyr residues have already been detected on cabbages in Botswana [46]. Limited agricultural use of chlorfenapyr in sub-Saharan Africa would help to preserve susceptibility of the vector to this important insecticide by limiting selection pressure to public health use. The only report of potential chlorfenapyr resistance to date is from Côte d'Ivoire by Kouassi et al. which showed that 200µg AI/bottle killed less than 98% of *An. gambiae* s.l. in five of 15 sites, with possible resistance recorded in Bouake, Gagnoa, Nassina, Sakassou and San Pedro (Kouassi et al. 2020) [47]. This could be a sign of chlorfenapyr resistance in some parts of Côte d'Ivoire, but further phenotypic and genotypic data should be collected to confirm this finding. Fortunately, results from our study in 16 countries in sub-Saharan Africa have shown no signs of chlorfenapyr resistance.

Cluster-randomized control trials of Interceptor G2 ITNs in Tanzania and Benin are nearing completion, pilot distribution evaluations are underway in Burkina Faso, Rwanda, Mali, Mozambique and Nigeria, and a MedAccess partnership has reduced the price of 35 million Interceptor G2 nets by 40% [31, 32] which is expected to greatly increase the availability of these products. With millions of Interceptor G2 nets being distributed, it is essential for a discriminating concentration to be determined and susceptibility testing to be regularly conducted to ensure there is no cross-resistance through existing mechanisms and to monitor any developing resistance. In a time where new insecticides are desperately needed, it is vitally important for timely susceptibility protocols for new active ingredients. Results from this study have been included as part of the WHO expert committee to determine a suitable discriminating concentration and a WHO recommendation is expected in 2021.

Conclusion

Studies showed that 200µg AI/bottle chlorfenapyr in bottle bioassays is a suitable discriminating concentration for monitoring susceptibility of wild *An. gambiae* s.l., with mortality recorded up to 72h. Testing in 16 countries in sub-Saharan Africa demonstrated malaria vector susceptibility to chlorfenapyr at all sites, including mosquitoes with multiple resistance mechanisms to pyrethroids.

Abbreviations

AI = Active ingredient

ATP = Adenosine 5'-triphosphate

CDC = United States Centers for Disease Control and Prevention

CI = Confidence interval

IQR = Interquartile range

IRS = Indoor residual spraying

ITN = Insecticide-treated nets

NMCP = National Malaria Control Programme

PMI = US President's Malaria Initiative

PQ = Prequalification listing

WHO = World Health Organization

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Availability of data and materials

All data analysed during this study are included in this published article and its supplementary information files.

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

RMO was involved in the design of the study, provided technical support, interpreted data, conducted analysis and wrote the manuscript.

AS, YY, JC and DD conceived the study design, provided technical support, reviewed data and edited the manuscript.

FW, FRA, SC, SLM, OF, MO, MB, EA, J-DR, HM, AS, LG, BA, KO, IB, RD, VG, GY, KV and EF were involved in collection of data and reviewed the manuscript.

JC and JSA provided programmatic support and edited the manuscript.

All authors read and approved the final manuscript.

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Figures

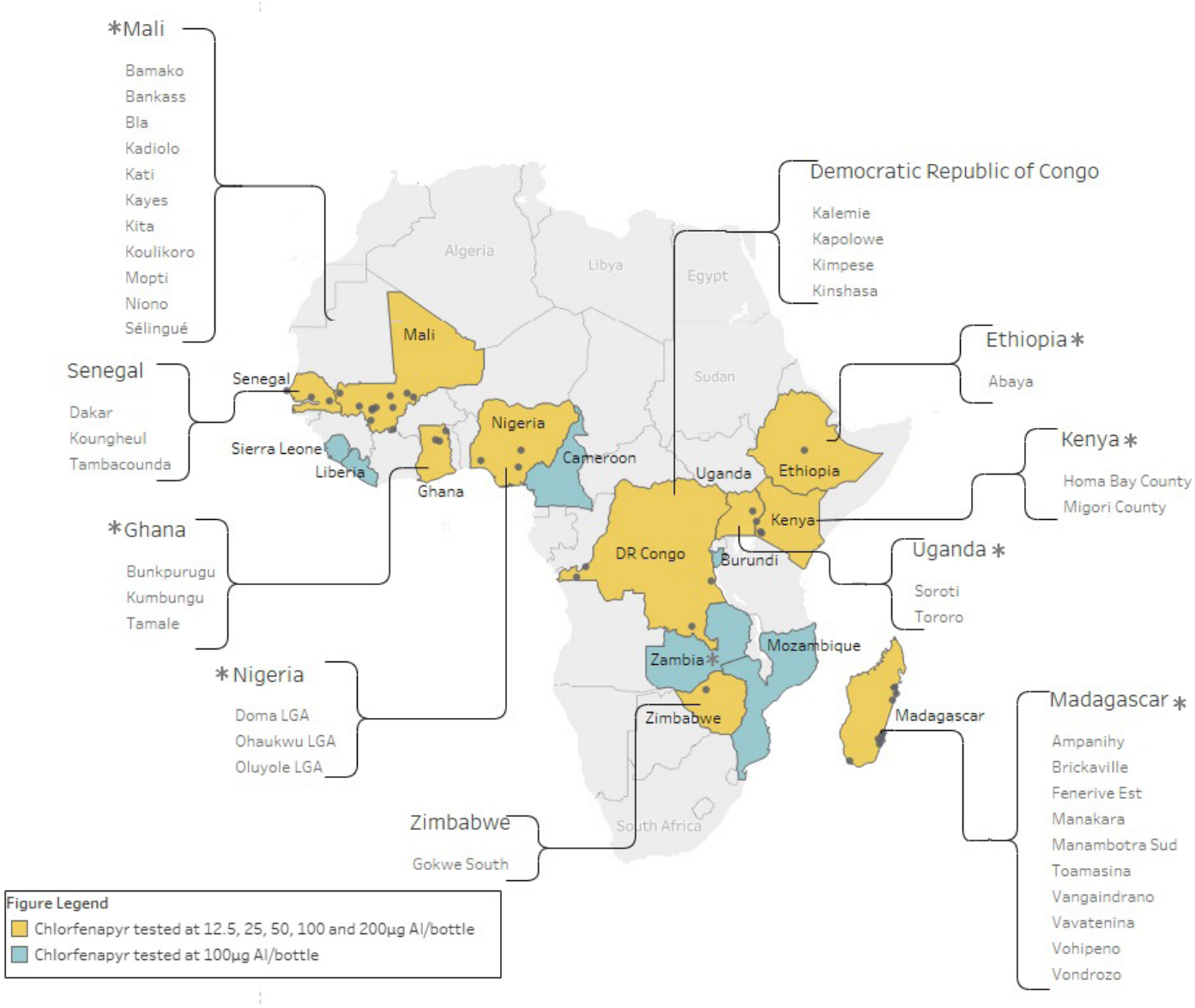


Figure 1

Locations of insecticide susceptibility testing sites where mortality of wild An. gambiae s.l. was measured in bioassays following exposure to a full (yellow) or limited (blue) range of concentrations of chlorfenapyr. *denotes countries where a susceptible insectary strain was also tested with the full range of concentrations.

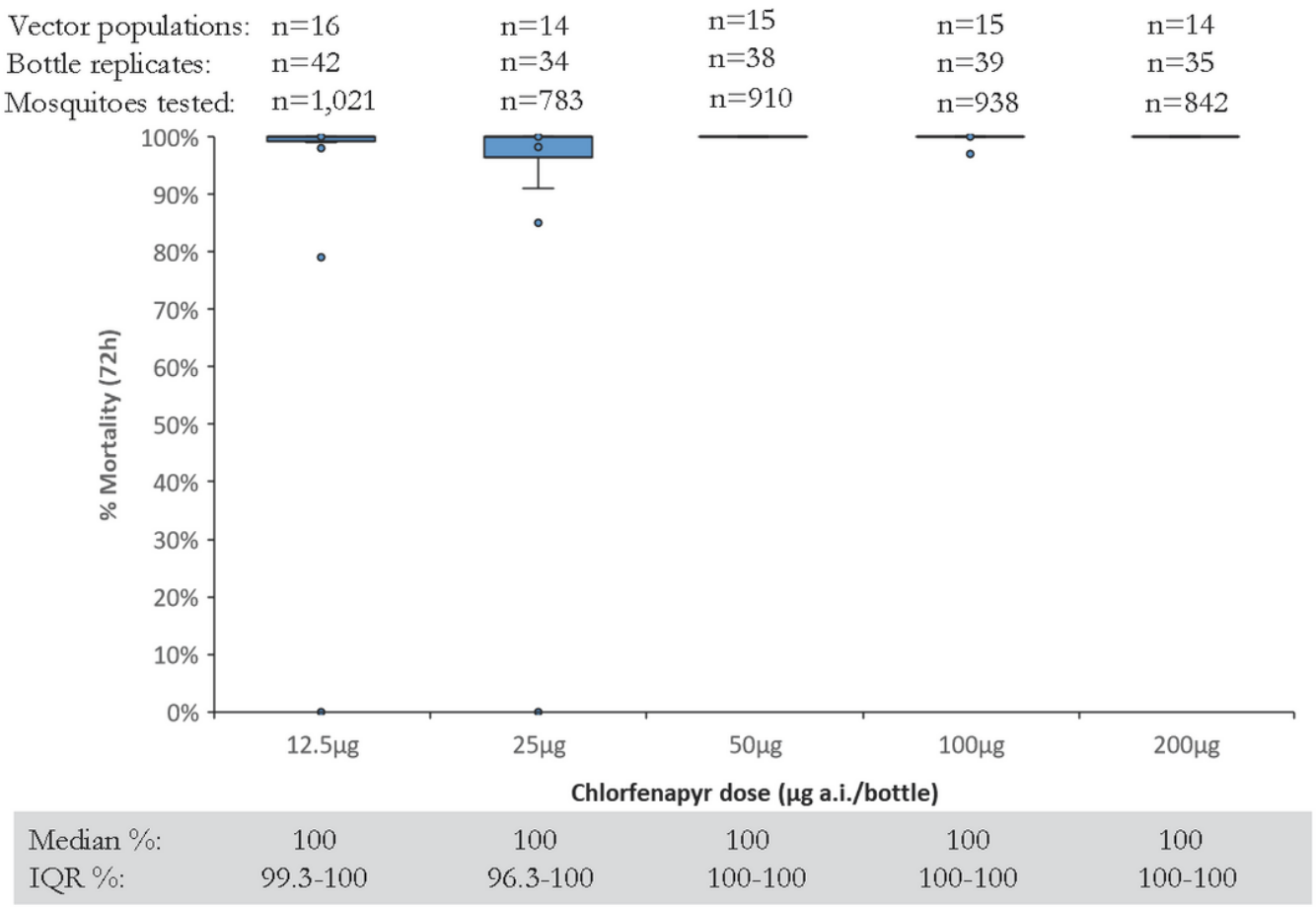
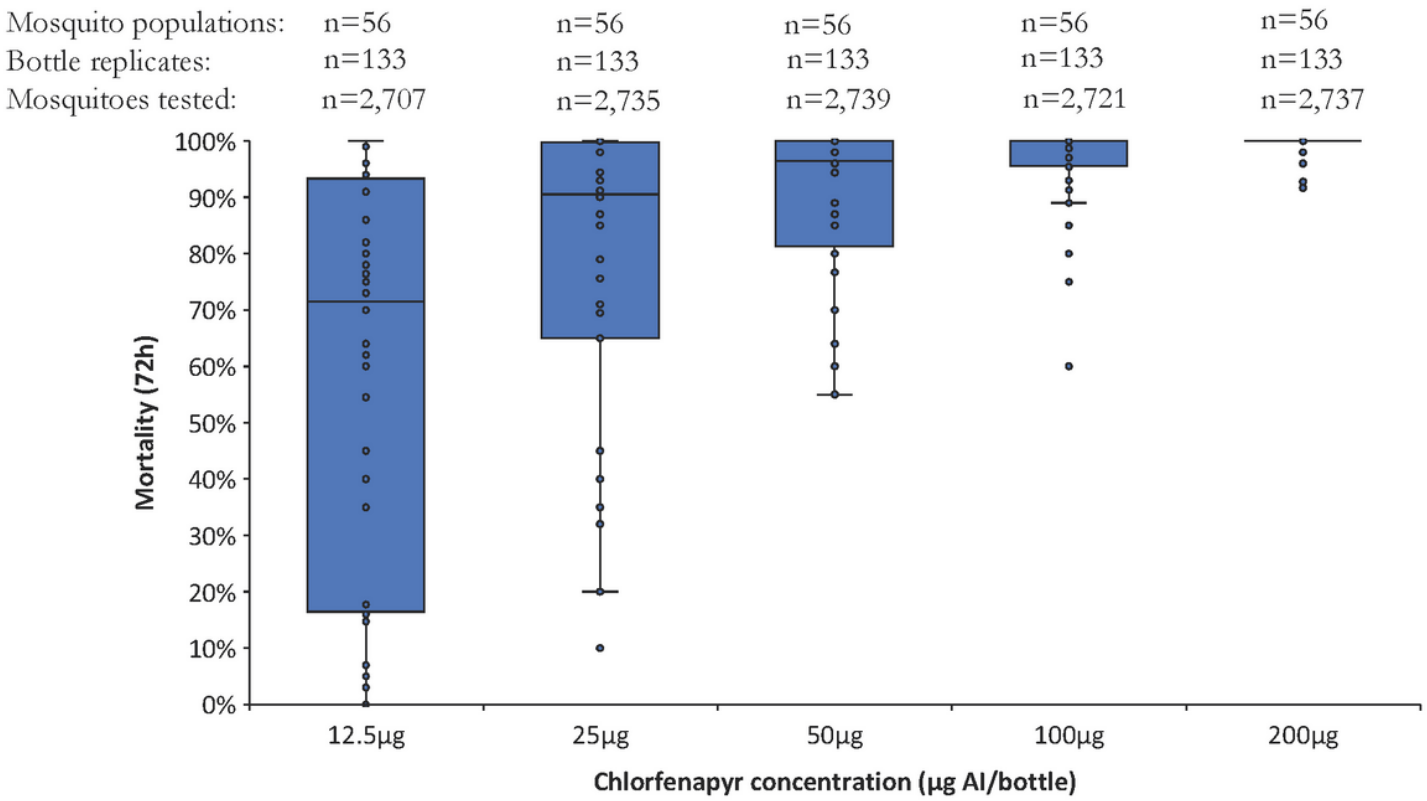


Figure 2

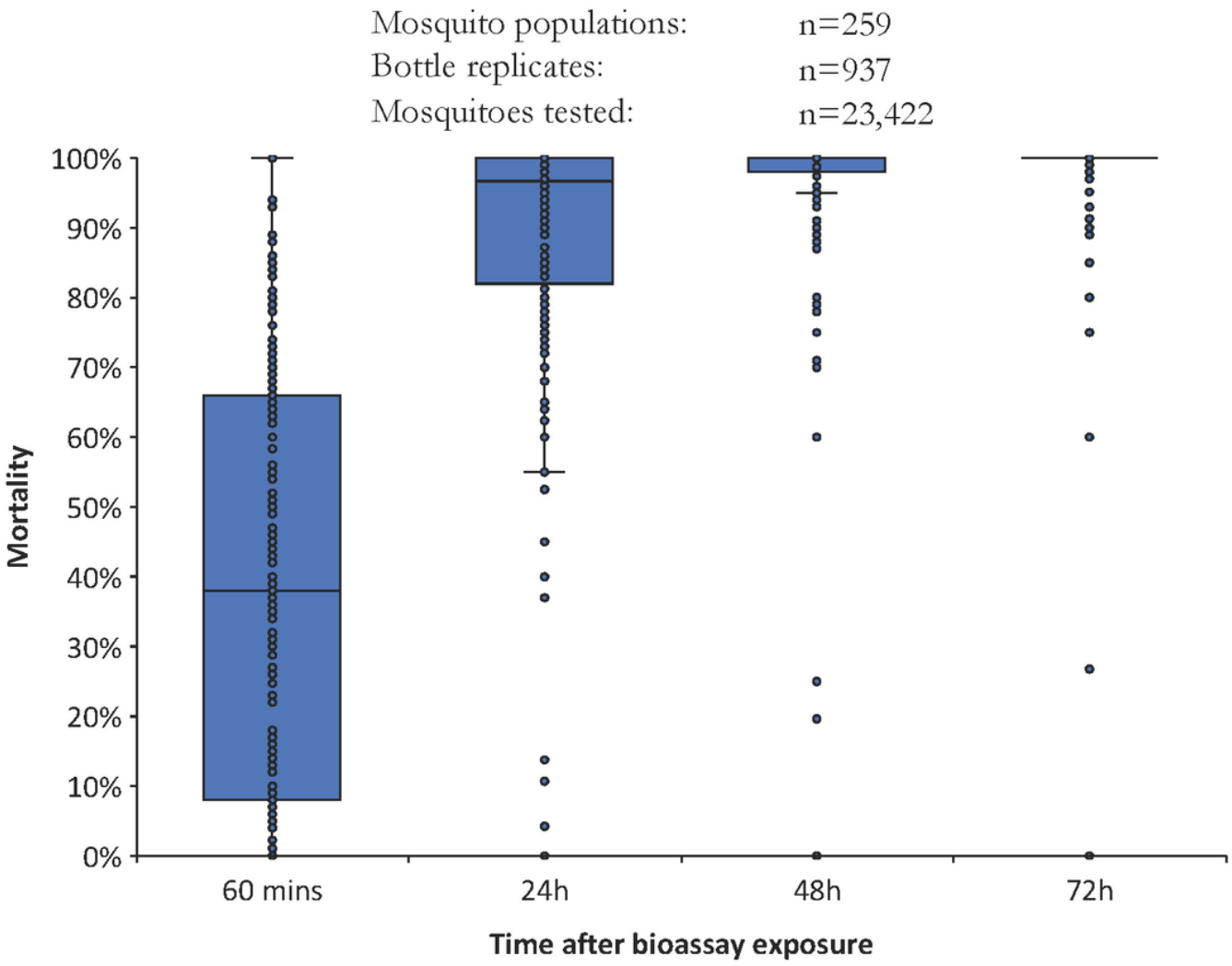
Percentage mortality (72h) after exposure to each of five concentrations (12.5, 25, 50, 100, 200µg AI/bottle) of chlorfenapyr in bottle bioassays using pyrethroid susceptible colony mosquito strains in eight countries.



Median %:	71.5	90.5	96.5	100	100
IQR %:	16.4-93.3	65.0-99.8	81.3-100	95.6-100	100-100

Figure 3

Median percentage mortality of wild An. gambiae s.l. 72 hours after exposure to chlorfenapyr at concentrations of 12.5, 25, 50, 100 and 200µg AI/bottle in bottle bioassay in 10 countries.



Median %:	38.0	96.7	100	100
IQR %:	8.0-66.6	82.0-100	98.0-100	100-100

Figure 4

Percentage mortality of wild An. gambiae s.l. 60 mins, 24h, 48h and 72h after exposure to the provisional discriminating concentration of chlorfenapyr at 100µg AI/bottle in 16 countries.

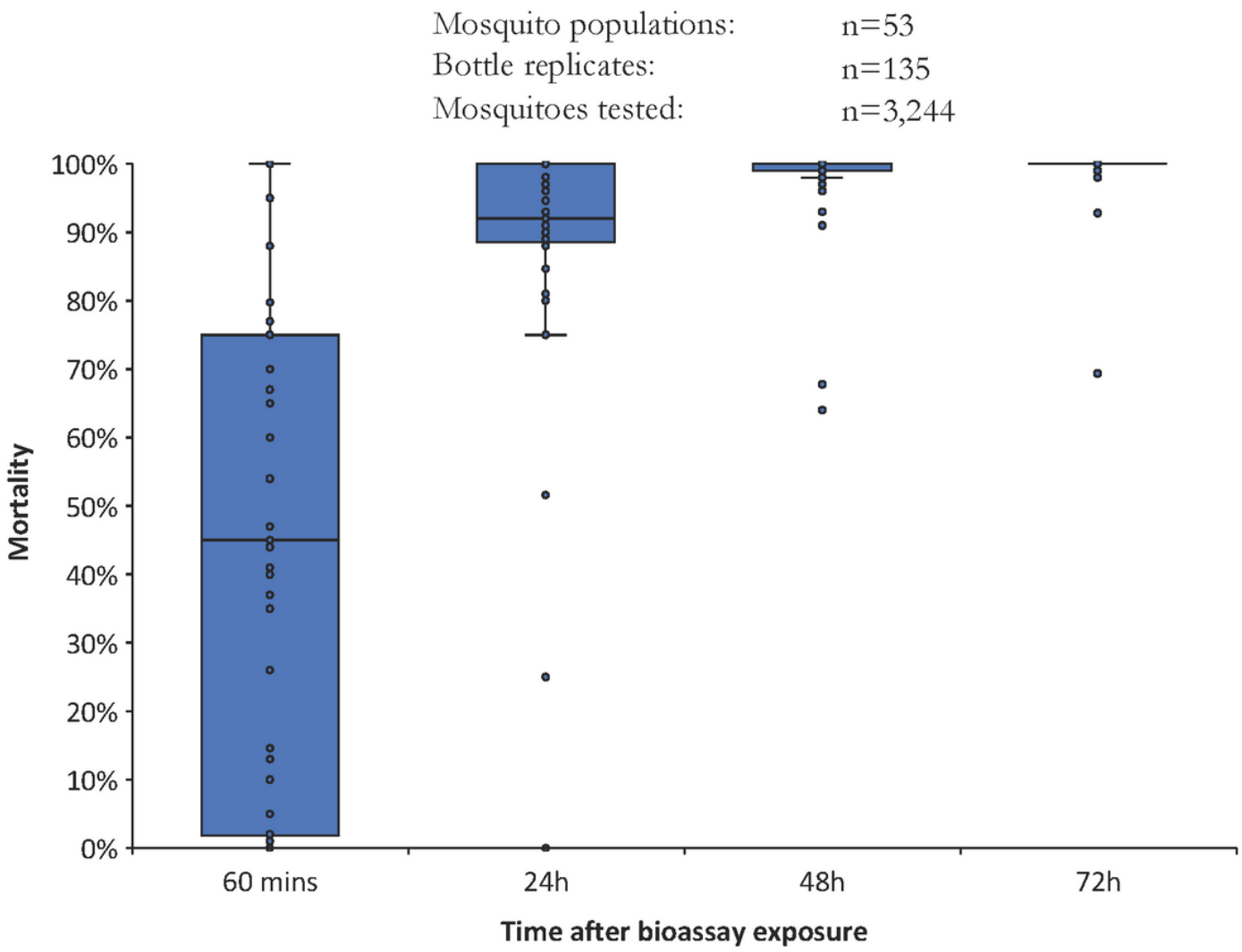


Figure 5

Percentage mortality of wild An. gambiae s.l. 60 mins, 24h, 48h and 72h after exposure to chlorfenapyr at 200µg AI/bottle in 10 countries.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- Supplementaryfiledatabase100200dosesAn.gambiae.xlsx
- Supplementaryfiledatabase12.5200insectary.xlsx
- Supplementaryfiledatabase12.5200wildAn.gambiae.xlsx
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