

Susceptibility of *Anopheles funestus* strains with contrasting insecticide-resistance phenotypes to HPPD inhibition by nitisinone

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Abstract

The spread of insecticide resistance in malaria vectors highlights the need for complementary strategies targeting biological processes distinct from those of conventional insecticides. In this context, 4-Hydroxyphenylpyruvate dioxygenase (HPPD), a key enzyme in the tyrosine catabolic pathway, has gained interest as a potential target for vector control because its inhibition has been shown to induce post-blood-meal mortality in several blood-feeding arthropods. However, the role of the HPPD in one of the main malaria vector *Anopheles funestus* and its suitability as a vector control target remains uncharacterised. This study evaluated the effect of HPPD inhibition in two *An. funestus* laboratory strains with contrasting insecticide-resistance phenotypes: the susceptible FANG and pyrethroid-resistant FUMAZ strains. Mosquitoes were fed on blood meals supplemented with increasing concentrations of an HPPD inhibitor nitrophenol (NTBC), and post-blood-meal mortality was assessed over 72 hours. Dose-response relationships and LC_{50} values were estimated for each strain. NTBC induced dose-dependent mortality in both *An. funestus* strains, whereas blood-feeding rates remained comparable to those of untreated controls. The resistance phenotype of FUMAZ did not prevent mortality following HPPD inhibition. Importantly, FUMAZ exhibited a lower LC_{50} than FANG (LC_{50} : FUMAZ = $0.729 \mu M$, $CI_{95\%}$ = [0.6832; 0.7770]; FANG = $1.234 \mu M$, $CI_{95\%}$ = [1.193; 1.276]; F test, $F = 212, 1(1; 88)$, $p < 0.0001$), indicating greater susceptibility to NTBC under the experimental conditions. These results demonstrate that NTBC induces dose-dependent mortality in *An. funestus* following blood-meal exposure. Further investigation of HPPD function using genetic approaches could determine whether its disruption can produce a comparable phenotype.

Background

Vector control remains one of the principal components of malaria prevention and has historically relied largely on interventions targeting adult mosquitoes, particularly insecticide-treated nets and indoor residual spraying [1, 2]. Nevertheless, the performance of these strategies is increasingly challenged by the emergence and spread of insecticide resistance among major malaria vectors [3, 4]. *Anopheles funestus* is of particular concern because of its important contribution to malaria transmission in many regions of sub-Saharan Africa [5, 6, 7]. These challenges highlight the need to identify alternative biological pathways that can be exploited for vector control through distinct mechanisms from those targeted by conventional neurotoxic insecticides. One promising approach is to target physiological processes that are essential for mosquito survival, such as blood meal processing. Blood meal digestion is essential in hematophagous arthropods, providing nutrients required for key biological functions, including reproduction, immune responses, and metabolic homeostasis [8, 9]. The digestion of a protein-rich blood meal generates a substantial increase in free amino acids that must be processed efficiently to maintain metabolic homeostasis. Among these amino acids, tyrosine poses a particular challenge resulting from its accumulation and crystallisation within the midgut [9, 10, 11]. Consequently, the regulation of tyrosine degradation following blood feeding is essential for the survival of blood-feeding insects. The tyrosine degradation pathway involves several enzymes, including tyrosine

aminotransferase (TAT) and 4-hydroxyphenylpyruvate dioxygenase (HPPD), which sequentially convert tyrosine into downstream metabolites [10, 12]. The activity of this pathway increases following blood feeding, reflecting the physiological need to process the large quantities of amino acids released during digestion. Disruption of this metabolic process therefore represents a potential vulnerability that differs fundamentally from the neuronal targets of most conventional insecticides. Pharmacological studies on HPPD inhibition have demonstrated the important role of tyrosine degradation in several disease vectors, including *Glossina*, *Aedes*, *Culex* and *Anopheles*, supporting HPPD as a promising biochemical target for mosquito control [13, 14, 15]. However, susceptibility to HPPD inhibition cannot necessarily be extrapolated across mosquito species. Blood-meal processing kinetics are species-dependent. Recent work in *Aedes aegypti*, showed temporal changes in free amino acids after blood feeding, with tyrosine accumulation during egg maturation [16]. In *Anopheles*, blood feeding induces a coordinated digestive response involving many proteins and enzymes, followed by extensive amino acid metabolism. Complete digestion of blood takes approximately 48 hours in *An. gambiae* and 60 hours in *An. funestus* [16, 17]. These differences in the tyrosine catabolism could potentially alter the exposure of the mosquito to the metabolic consequences of HPPD inhibition. As research increasingly explores novel strategies for malaria vector control, it is important to ensure that major vector species beyond *An. gambiae* are also considered. Here, we determined whether the response to HPPD inhibition is maintained in the two *An. funestus* laboratory strains with different insecticide-resistance backgrounds (FANG and FUMOZ) using the β -triketone nitisinone, a Food and Drug Administration (FDA)-approved drug for human use that acts as an inhibitor of HPPD [18]. *Anopheles funestus* FANG is a susceptible strain and *An. funestus* FUMOZ exhibits metabolic insecticide resistance, providing a contrasting genetic and physiological background for assessing the effect of pharmacological HPPD inhibition.

Methods

Mosquitoes rearing

The two laboratory mosquito strains *An. funestus* FANG and FUMOZ were used in this study. FANG was originally collected in Angola and known for its complete susceptibility to insecticides [19]. FUMOZ was collected in Mozambique and is known for its metabolic resistance to insecticides [6, 20]. Both strains are maintained at the GLP-approved facility Liverpool Insect Testing Establishment (LITE). These FANG and FUMOZ colonies' upkeep involve offering blood meals twice before egg-laying using a Hemotek Membrane Feeding System (Hemotek Ltd., Blackburn, UK) and with screened human blood procured from NHS bloodbanks. Eggs were floated in purified water and reared at larval densities of 0.24 larvae/mL. Larvae were fed increasing amounts of ground TetraMinV R Tropical Flakes under the following regime: day 1 (day of hatching) 100mg/larvae; then day 2–7 200mg/larvae; then day 8–10 233mg/larvae, with day 10 being the first day of pupation. Adult mosquitoes are reared at 26°C $\pm$ 2°C, in 70-80% relative humidity and under an 11-hours light/dark cycle with 1-hour dawn/dusk transitions and fed with 10% sucrose solution. The FUMOZ strain resistance phenotype is preserved under selection pressure, with routine selection every third generation using 0.05% deltamethrin-impregnated papers according to the WHO susceptibility bioassay protocol [20].

Nitisinone inhibiting solution preparation and blood feeding setting

Nitisinone (NTBC) was chosen based on its displayed *HPPD* enzyme inhibition properties. HPLC grade $\geq 95\%$ nitisinone powder was dissolved in buffer solution consisting in Sodium hydroxyl (600 μL , 0.5M), Hydrogen Chloride (100 μL , 3M) and Phosphate Buffered Saline (300 μL ; 0.15 M NaCl, 10 mM Na phosphate, pH 7.0) to constitute the stock solution (5mg/mL). Then, serial dilutions were made and tested at concentrations of 10^{-5} , 10^{-4} , and 10^{-3} (mg/mL) to determine the concentration range resulting in high mortality among blood-fed mosquitoes. Following these preliminary tests, different concentrations (10^{-5} mg/ml; $5 \cdot 10^{-4}$ mg/ml; 10^{-3} mg/ml; and $5 \cdot 10^{-3}$ mg/ml) were prepared and tested. A constant volume of each concentration (80 μL) was diluted in 4mL of blood for the blood test (1:50) to minimise the protein dilution effect. At least 60 five-day-old females per concentration and replicate were used for blood feeding. Mosquitoes were allowed to feed artificially for one hour using a Hemotek membrane feeding system. Blood-feeding status was assessed visually after feeding based on abdominal distension and the presence of a visible red blood meal through the abdominal cuticle. The number of blood-fed females was recorded for each concentration to calculate the blood-feeding rate. Mortality was recorded at 24 hours over 72 hours in five different replicates. Control groups received PBS in blood.

Survival experiments

Mosquito mortality was assessed according to the WHO insecticide test procedures [21]: mosquitoes were considered dead if they showed no signs of life, were immobile, unable to fly, or incapable of standing.

Data analysis

The feeding rate was calculated as the percentage of females that successfully blood-fed among the total number of females exposed to the respective NTBC-supplemented blood treatment. Observed mortality was corrected for control mortality using Abbott's formula (Abbott, 1987). The LC_{50} values were estimated by nonlinear regression using sigmoidal dose-response curves. Differences in LC_{50} between FANG and FUMOZ were assessed using an extra sum-of-squares F test comparing a model in which the LC_{50} was shared between strains with a model allowing separate LC_{50} values. Analyses and graphical representations were performed using GraphPad Prism v11.

Results

Nitisinone induces rapid post-blood-meal mortality in *Anopheles funestus*

The use of FANG and FUMOZ strains allowed us to assess NTBC *HPPD* inhibition effect in contrasting metabolic and insecticide-resistance backgrounds. FUMOZ exhibits pyrethroid resistance associated with elevated expression of the duplicated cytochrome P450 genes *CYP6P9a* and *CYP6P9b* [22] in contrast to the susceptible FANG strain. Across the range of NTBC concentrations tested, NTBC-induced

mortality occurred predominantly within 24 h post-blood meal in both FANG and FUMOS strains. On average 94.69 % and 90.91 % of total mortality occurred during the first 24 h in FANG and FUMOS, respectively, with substantially fewer deaths recorded thereafter. (Figure 2).

Nitisinone exposure reduces survival in both susceptible and insecticide-resistant *Anopheles funestus*

Survival analysis revealed a dose-dependent increase mortality following exposure to NTBC-supplemented blood meal in both *An. funestus* strains (Figure 3). Mosquitoes receiving only PBS-supplemented blood showed low and comparable mortality between FANG (0.75%) and FUMOS (0.6%) within the 72 h post-blood meal, whereas survival progressively decreased survival with increasing NTBC concentrations. The greatest separation between treatment groups occurred during the first 24 h.

In FANG, mortality was significantly higher at 1.52 μ M NTBC compared with the control group (Cox proportional-hazards model, HR = 3.93, CI_{95%} [2.68; 5.19], $p = <0.0001$) (Figure 3.a). A similar pattern was observed in FUMOS, although mortality occurred at lower NTBC concentrations. Compared with the mosquitoes receiving only PBS-supplemented blood meal, mortality was significantly increased at 0.61 μ M (HR = 4.43, 95% CI_{95%}: [2.17; 6.71], $p < 0.0001$) (Figure 3.b). Overall, increasing NTBC concentration was associated with a progressive reduction in mosquito survival after blood feeding.

Kaplan–Meier survival curves showing the probability of survival following ingestion of NTBC-supplemented blood meals in *An. funestus* FANG (a) and *An. funestus* FUMOS (b). Each curve corresponds to a specific NTBC concentration, with sample size in bracket.

Nitisinone-induced mortality differs between susceptible and insecticide-resistant *Anopheles funestus* strains

A difference in NTBC susceptibility was observed between the two *Anopheles funestus* strains. The insecticide-resistant FUMOS strain exhibited a lower LC₅₀ than the susceptible FANG strain (LC₅₀: FUMOS = 0.729 μ M, CI_{95%} = [0.6832; 0.7770]; FANG=1.234 μ M, CI_{95%} = [1.193; 1.276]; F test, $F=212.1(1; 88)$, $p<0.0001$) (Figure 4.b). These findings demonstrate a dose-dependent increase in mortality in both strains, with steep dose–response curves indicating a rapid decline in survival over a defined range of NTBC concentrations. Furthermore, NTBC concentration did not significantly affect blood-feeding success, with blood-feeding rates remaining comparable to the control group across all concentrations tested (Mann Whitney test, $p > 0.05$; Figure 4.a).

Discussion

The identification of novel vector-control targets requires validation across the major vector species responsible for malaria transmission. In this study, we have explored the effect of HPPD inhibition in two *An. funestus* laboratory strains with contrasting insecticide-resistance phenotypes: the susceptible FANG and pyrethroid-resistant FUMOS strains. Our results have revealed that NTBC induces dose-dependent mortality in *An. funestus* following blood-meal exposure. Previous studies have demonstrated that

inhibition of HPPD using NTBC causes lethal toxicity following blood feeding in arthropod species, establishing the tyrosine degradation pathway as a promising physiological vulnerability for hematophagous insects (Sterkel, 2016; Sterkel, 2021; Haines, 2025). Since then, *HPPD* gene has gained interest as a potential target for mosquito control. NTBC is a FDA-approved drug for the treatment of tyrosinemia type I in humans and was selected for this study based on its established HPPD-inhibitory activity and previously reported activity against hematophagous insects. Recent study showed that nitisinone susceptibility differs among mosquito species, with *An. gambiae* showing greater susceptibility than *Aedes aegypti* and *Culex quinquefasciatus* at several exposure concentrations [13]. These interspecific differences may reflect variation in physiological processes associated with blood feeding and digestion, although these mechanisms were not directly investigated. This is particularly relevant for *An. funestus*, for which the response to HPPD inhibition had not been previously characterised. Dose-dependent mortality observed in the current study is consistent with the role of HPPD in the detoxification of tyrosine released during the digestion of a protein-rich blood meal [11, 15]. Disruption of the tyrosine pathway is expected to cause toxic accumulation levels of tyrosine and ultimately mosquito mortality [11, 15]. An important feature was the rapid onset of mortality following NTBC-supplemented blood, with the majority of deaths occurring within 24 h post blood meal. This early mortality is consistent with previous observations of rapid nitisinone-induced killing in blood-fed mosquitoes [23, 24]. Blood feeding initiates extensive digestion of blood proteins and a substantial increase in amino-acid flux, creating a physiological context in which disruption of tyrosine catabolism may have rapid consequences. Although this study did not directly measure tyrosine or other metabolites, our findings are consistent with previous evidence that NTBC is effective against *Anopheles* mosquitoes following systemic exposure. A recent study showed that, in *An. gambiae*, ingestion of NTBC through a blood meal displayed mosquitocidal activity, with an estimated EC_{50} of 205.31 ng/mL ($\sim 0.624 \mu\text{M}$) and a maximum killing effect at $0.759 \mu\text{M}$ ($\sim 250 \text{ ng/mL}$) [25]. Similarly, Kučera et al. demonstrated nitisinone-induced mortality following membrane feeding, with LC_{50} values of $0,658 \pm 0,096 \mu\text{M}$ at 24 hours [23]. The present study extends these observations to *An. funestus*, where NTBC delivered through the blood meal similarly induced dose-dependent mortality with an LC_{50} of $1,234 \mu\text{M}$ and $0.729 \mu\text{M}$ in FANG and FUMOZ, respectively.

The lower LC_{50} observed in FUMOZ raises the possibility that differences in metabolic capacity may contribute to differential susceptibility to *HPPD* inhibition. FUMOZ exhibits predominantly P450-mediated metabolic resistance to pyrethroids, with elevated expression of the duplicated *CYP6P9a/b* genes [22, 26]. However, it is not known whether these or other resistance-associated enzymes contribute to NTBC metabolism or detoxification, and increased P450 activity would not necessarily be expected to alter NTBC susceptibility in a predictable direction. In contrast, previous studies showed comparable nitisinone susceptibility between the insecticide-susceptible Kisumu and the insecticide-resistant Tiassalé strains in *An. gambiae* [25]. Another study reported substantial mortality following tarsal and topical exposure, with comparable susceptibility observed among the susceptible Kisumu and resistant Tiassalé and VK7 strains, although the latter differed in the method of NTBC delivery [13]. These strains differ in the mechanisms underlying insecticide resistance, with resistance in VK7 and Tiassalé involving

distinct combinations of target-sites and metabolic mechanisms [27, 28]. Although the quantitative susceptibility estimates should not be interpreted as directly comparable across species because of differences in experimental design, exposure conditions, mosquito strains or pharmacological endpoints, the observed variation in NTBC susceptibility raises the question of whether species or strain-specific biological factors contribute to this response, particularly observed in *An. funestus*. A genome-wide analyses have shown substantial population structure within *An. funestus*, with FUMAZ clustering with southern African populations while FANG occupies a distinct position, indicating that the two laboratory colonies differ in their broader genomic backgrounds [29]. Such genomic backgrounds may influence physiological processes relevant to NTBC activity. Together, this study provides evidence that NTBC has a potent effect across *An. funestus* strains with different genetic and insecticide-resistance backgrounds.

A key limitation of this study is the use of laboratory-adapted strains rather than field-derived populations. The challenges associated with maintaining and performing reliable blood feeding in *An. funestus* under laboratory conditions prevented us from extending the experiments to field populations. Thus, whether NTBC produces a comparable lethal effect in genetically diverse wild *An. funestus* populations remains to be determined. In addition, residual variation in blood composition between blood lots could contribute to the observed difference in both FANG and FUMAZ.

Conclusion

This study supports that *HPPD* represents a promising biological target for the development of novel malaria vector-control strategies in *An. funestus*. Chemical inhibition of *HPPD* using NTBC induced strong dose-dependent mortality following blood feeding in both the insecticide-susceptible FANG and insecticide-resistant FUMAZ strains, demonstrating the importance of the tyrosine degradation pathway for post-blood-meal survival in this species. Although differences in NTBC susceptibility were observed between the two laboratory colony strains tested, with FUMAZ exhibiting higher susceptibility, further studies may be needed to elucidate the biological mechanism. Together with previous findings in *Anopheles* mosquitoes, these results support the conservation of *HPPD*-dependent blood-meal physiology across major malaria vectors and provide a rationale for investigating *HPPD* beyond its potential as a chemical control target. Importantly, these findings support further genetic investigation of *HPPD* to determine its suitability as a target for genetic control in *An. funestus*.

Abbreviations

HPPD: 4-Hydroxyphenylpyruvate dioxygenase

NTBC: Nitisinone (2-[2-nitro-4-(trifluoromethyl) benzoyl]-1,3-cyclohexanedione)

FANG: *Anopheles funestus* Angola strain

FUMAZ: *Anopheles funestus* Mozambique strain

LC50: Lethal concentration causing 50% mortality

PBS: Phosphate-buffered saline

HPLC: High-performance liquid chromatography

Declarations

Author Contributions: Conceptualization, S.O.G.Y., Z.S.D; methodology, S.O.G.Y., Z.S.D., TN; validation, T.N.; formal analysis, S.O.G.Y., investigation, S.O.G.Y., Z.S.D., O.N.Z., H.H., G.S., A.A.; data curation, S.O.G.Y.; writing-original draft preparation, S.O.G.Y.; writing-review and editing, All authors; visualization, S.O.G.Y.; supervision, N.T., A.D. and M.N; project administration, A.L, H.M.; funding acquisition, A.D. and T.N. All authors have read and agreed to the published version of the manuscript.

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References

1. Bhatt S, Weiss DJ, Cameron E, Bisanzio D, Mappin B, Dalrymple U, et al. The effect of malaria control on *Plasmodium falciparum* in Africa between 2000 and 2015. *Nature*. 2015;526 7572:207–11; doi: 10.1038/nature15535.
2. Hemingway J, Shretta R, Wells TN, Bell D, Djimdé AA, Achee N, et al. Tools and Strategies for Malaria Control and Elimination: What Do We Need to Achieve a Grand Convergence in Malaria? *PLoS Biol*. 2016;14 3:e1002380; doi: 10.1371/journal.pbio.1002380.
3. Suh PF, Elanga-Ndille E, Tchouakui M, Sandeu MM, Tagne D, Wondji C, et al. Impact of insecticide resistance on malaria vector competence: a literature review. *Malar J*. 2023;22 1:19; doi: 10.1186/s12936-023-04444-2.
4. Wangrawa DW, Odero JO, Baldini F, Okumu F, Badolo A. Distribution and insecticide resistance profile of the major malaria vector *Anopheles funestus* group across the African continent. *Med Vet Entomol*. 2024;38 2:119–37; doi: 10.1111/mve.12706.
5. Morgan JC, Irving H, Okedi LM, Steven A, Wondji CS. Pyrethroid resistance in an *Anopheles funestus* population from Uganda. *PloS one*. 2010;5 7:e11872.

6. Wondji CS, Morgan J, Coetzee M, Hunt RH, Steen K, Black WC, et al. Mapping a quantitative trait locus (QTL) conferring pyrethroid resistance in the African malaria vector *Anopheles funestus*. *BMC genomics*. 2007;8:1–14.
7. Djamouko-Djonkam L, Nkahe DL, Kopya E, Talipouo A, Ngadjeu CS, Doumbe-Belisse P, et al. Implication of *Anopheles funestus* in malaria transmission in the city of Yaoundé, Cameroon. *Parasite*. 2020;27:10; doi: 10.1051/parasite/2020005.
8. Chaves LF, Harrington LC, Keogh CL, Nguyen AM, Kitron UD. Blood feeding patterns of mosquitoes: random or structured? *Front Zool*. 2010;7:3; doi: 10.1186/1742-9994-7-3.
9. Fuchs S, Behrends V, Bundy JG, Crisanti A, Nolan T. Phenylalanine metabolism regulates reproduction and parasite melanization in the malaria mosquito. *PLoS One*. 2014;9 1:e84865; doi: 10.1371/journal.pone.0084865. <https://www.ncbi.nlm.nih.gov/pubmed/24409310>.
10. Sterkel M, Oliveira PL. Developmental roles of tyrosine metabolism enzymes in the blood-sucking insect *Rhodnius prolixus*. *Proc Biol Sci*. 2017;284 1854; doi: 10.1098/rspb.2016.2607.
11. Vergaray Ramirez MA, Sterkel M, Martins AJ, Bp Lima J, P LO. On the use of inhibitors of 4-hydroxyphenylpyruvate dioxygenase as a vector-selective insecticide in the control of mosquitoes. *Pest Manag Sci*. 2022;78 2:692–702; doi: 10.1002/ps.6679.
12. Moran GR. 4-Hydroxyphenylpyruvate dioxygenase. *Arch Biochem Biophys*. 2005;433 1:117 – 28; doi: 10.1016/j.abb.2004.08.015.
13. Stavrou-Dowd ZT, Parsons G, Rose C, Brown F, Lees RS, Acosta-Serrano Á, et al. The β -triketone, nitisinone, kills insecticide-resistant mosquitoes through cuticular uptake. *Parasit Vectors*. 2025;18 1:316; doi: 10.1186/s13071-025-06939-0.
14. Sterkel M, Perdomo HD, Guizzo MG, Barletta AB, Nunes RD, Dias FA, et al. Tyrosine Detoxification Is an Essential Trait in the Life History of Blood-Feeding Arthropods. *Curr Biol*. 2016;26 16:2188–93; doi: 10.1016/j.cub.2016.06.025. <https://www.ncbi.nlm.nih.gov/pubmed/27476595>.
15. Sterkel M, Haines LR, Casas-Sánchez A, Owino Adung'a V, Vionette-Amaral RJ, Quek S, et al. Repurposing the orphan drug nitisinone to control the transmission of African trypanosomiasis. *PLoS Biol*. 2021;19 1:e3000796; doi: 10.1371/journal.pbio.3000796.
16. Kato Y, Oi A, Dohmae N, Obata F, Sakuma C. Temporal changes in amino acid profiles coordinate mosquito physiology and egg maturation in *Aedes aegypti*. *BMC Biol*. 2025;23 1:346; doi: 10.1186/s12915-025-02460-z.
17. Hocking K. S, G. MD. Notes on the Bionomics of *Anopheles gambiae* and *A. funestus* in East Africa.. *Bulletin of Entomological Research*. 1948;39(3):453–65; doi: 10.1017/S0007485300022550.
18. Lock E, Ranganath LR, Timmis O. The role of nitisinone in tyrosine pathway disorders. *Curr Rheumatol Rep*. 2014;16 11:457; doi: 10.1007/s11926-014-0457-0.
19. Hunt RH, Brooke BD, Pillay C, Koekemoer LL, Coetzee M. Laboratory selection for and characteristics of pyrethroid resistance in the malaria vector *Anopheles funestus*. *Med Vet Entomol*. 2005;19 3:271–5; doi: 10.1111/j.1365-2915.2005.00574.x.

20. Williams J, Flood L, Praulins G, Ingham VA, Morgan J, Lees RS, et al. Characterisation of *Anopheles* strains used for laboratory screening of new vector control products. *Parasit Vectors*. 2019;12 1:522; doi: 10.1186/s13071-019-3774-3.
21. WHO. Test procedures for insecticide resistance monitoring in malaria vector mosquitoes. 2018. (<http://www.who.int/malaria>).
22. Wondji CS, Hearn J, Irving H, Wondji MJ, Weedall G. RNAseq-based gene expression profiling of the *Anopheles funestus* pyrethroid-resistant strain FUM0Z highlights the predominant role of the duplicated CYP6P9a/b cytochrome P450s. *G3 (Bethesda)*. 2022;12 1; doi: 10.1093/g3journal/jkab352.
23. Kučera M, Hartmann D, Valdés JJ, Palusová A, Sheshachalam A, Vlot M, et al. Killing of *Anopheles stephensi* mosquitoes by selective triketone inhibitors of 4-hydroxyphenylpyruvate dioxygenase depends on a high protein meal. *Insect Biochem Mol Biol*. 2025;183:104361; doi: 10.1016/j.ibmb.2025.104361.
24. McComic SE, Duke SO, Burgess ERT, Swale DR. Defining the toxicological profile of 4-hydroxyphenylpyruvate dioxygenase-directed herbicides to *Aedes aegypti* and *Amblyomma americanum*. *Pestic Biochem Physiol*. 2023;194:105532; doi: 10.1016/j.pestbp.2023.105532.
25. Haines LR, Trett A, Rose C, García N, Sterkel M, McGuinness D, et al. *Anopheles* mosquito survival and pharmacokinetic modeling show the mosquitocidal activity of nitisinone. *Sci Transl Med*. 2025;17 791:eadr4827; doi: 10.1126/scitranslmed.adr4827.
26. Weedall GD, Mugenzi LMJ, Menze BD, Tchouakui M, Ibrahim SS, Amvongo-Adjia N, et al. A cytochrome P450 allele confers pyrethroid resistance on a major African malaria vector, reducing insecticide-treated bednet efficacy. *Sci Transl Med*. 2019;11 484; doi: 10.1126/scitranslmed.aat7386.
27. Williams J, Ingham VA, Morris M, Toé KH, Hien AS, Morgan JC, et al. Sympatric Populations of the *Anopheles gambiae* Complex in Southwest Burkina Faso Evolve Multiple Diverse Resistance Mechanisms in Response to Intense Selection Pressure with Pyrethroids. *Insects*. 2022;13 3; doi: 10.3390/insects13030247.
28. Edi CV, Djogbénou L, Jenkins AM, Regna K, Muskavitch MA, Poupardin R, et al. CYP6 P450 enzymes and ACE-1 duplication produce extreme and multiple insecticide resistance in the malaria mosquito *Anopheles gambiae*. *PLoS Genet*. 2014;10 3:e1004236; doi: 10.1371/journal.pgen.1004236.
29. Weedall GD, Riveron JM, Hearn J, Irving H, Kamdem C, Fouet C, et al. An Africa-wide genomic evolution of insecticide resistance in the malaria vector *Anopheles funestus* involves selective sweeps, copy number variations, gene conversion and transposons. *PLoS Genet*. 2020;16 6:e1008822; doi: 10.1371/journal.pgen.1008822.

Figures

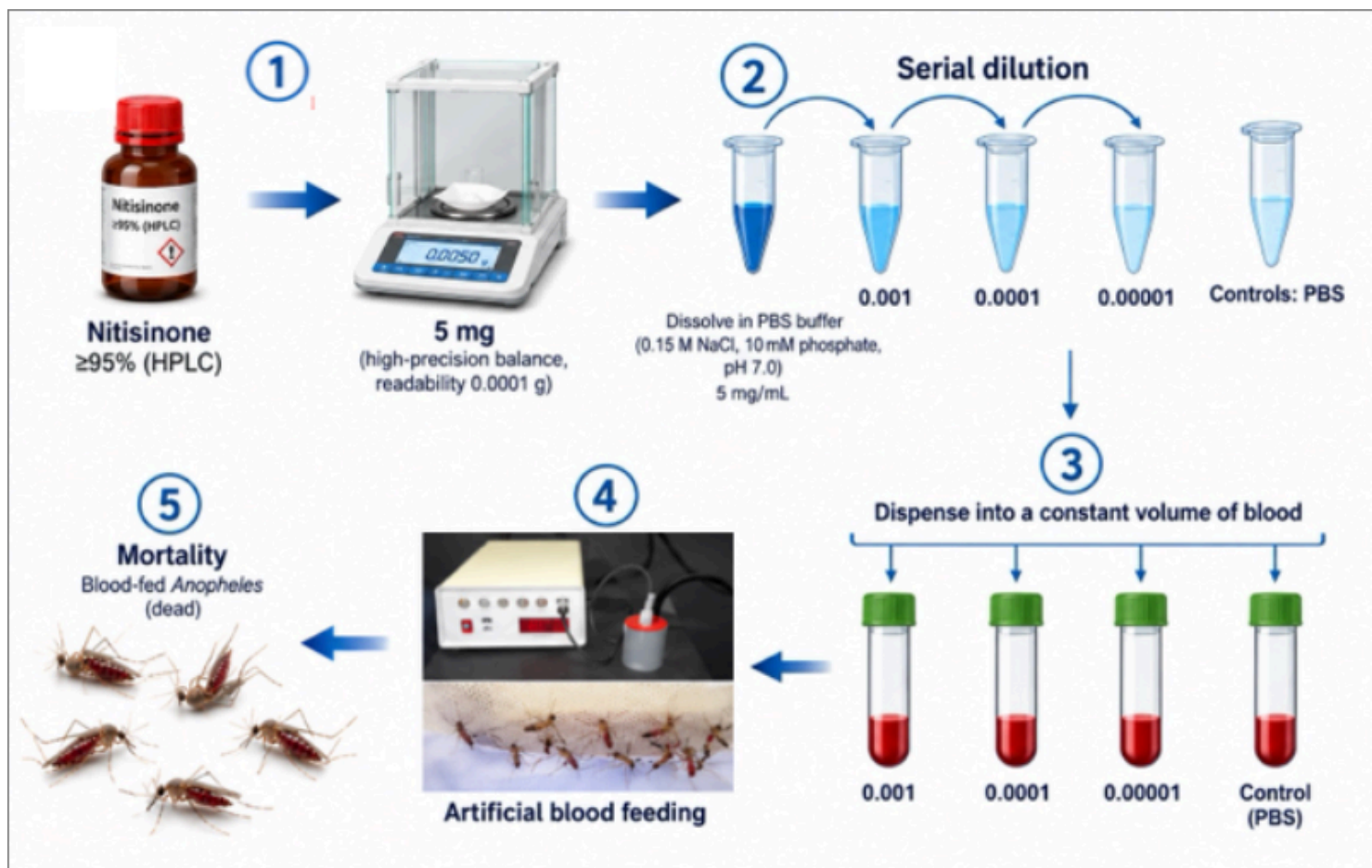


Figure 1

Schematic overview of the experimental workflow: Nitisinone (≥95% HPLC purity) was dissolved in PBS buffer (0.15 M NaCl, 10 mM phosphate, pH 7.0) to prepare a 5 mg/mL stock solution, followed by serial dilution. Each nitisinone dilution was supplemented with a constant volume of blood, with PBS used as the control. The NTBC-supplemented blood meals were offered to *Anopheles* mosquitoes using an artificial membrane-feeding system. (5) Mortality was subsequently assessed among blood-fed mosquitoes every 24 hours over a 72-hour period.

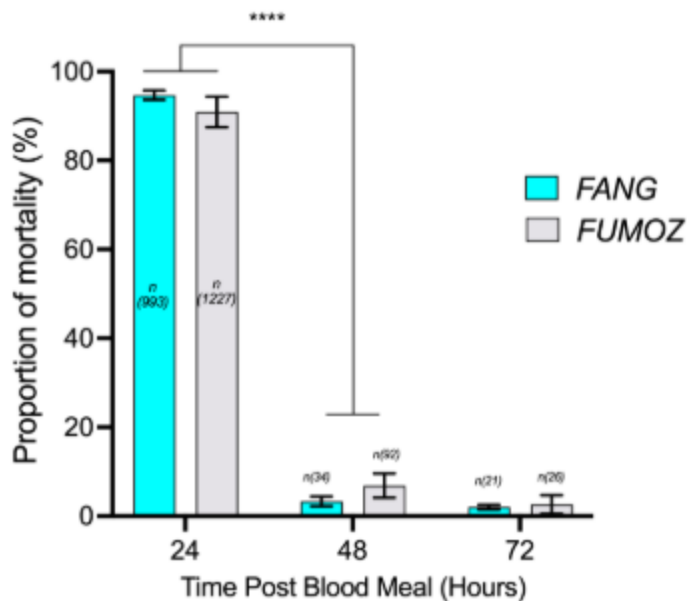


Figure 2

Proportion of total mortality following NTBC-supplemented blood meal. Proportion of mortality time post blood meal. The sample size is indicated in bracket. Most mortality occurred within 24 hours following a blood meal ($p < 0.0001$) in both FANG and FUM0Z strains.

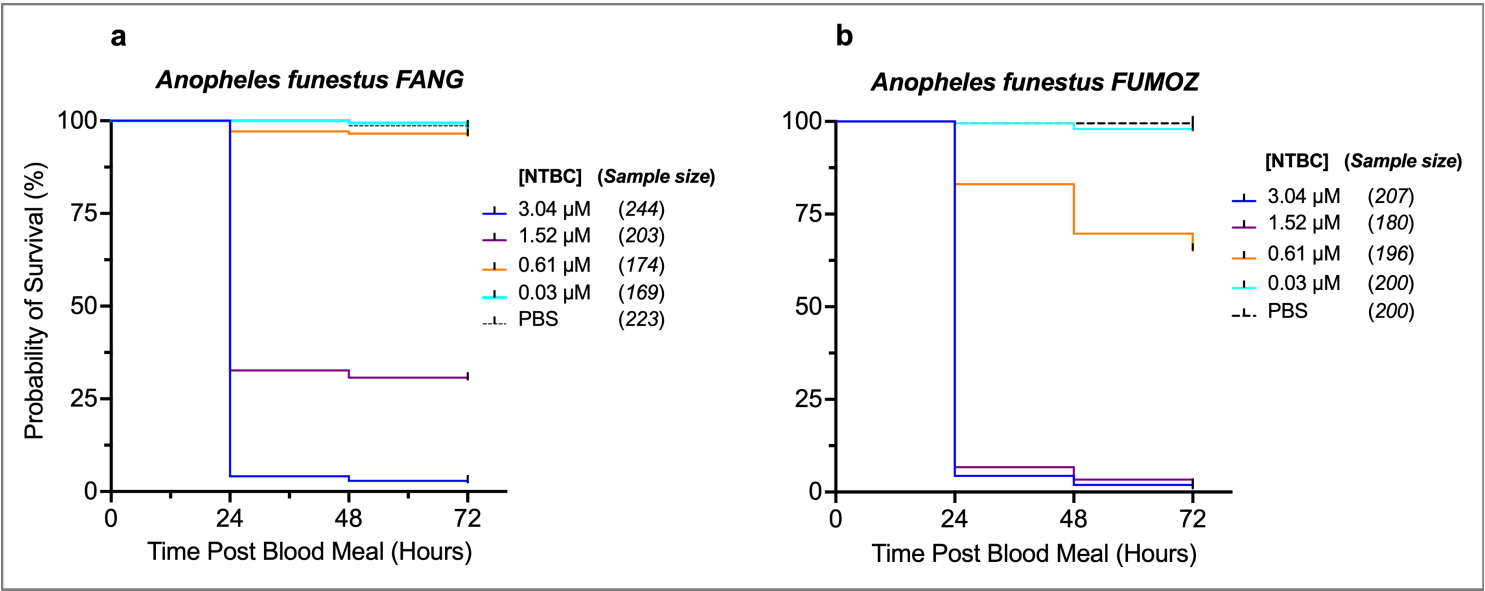


Figure 3

Survival curves for *An. funestus* FANG and *An. funestus* FUM0Z following NTBC-supplemented blood feeding

Kaplan–Meier survival curves showing the probability of survival following ingestion of NTBC-supplemented blood meals in *An. funestus* FANG (a) and *An. funestus* FUM0Z (b). Each curve corresponds to a specific NTBC concentration, with sample size in bracket.

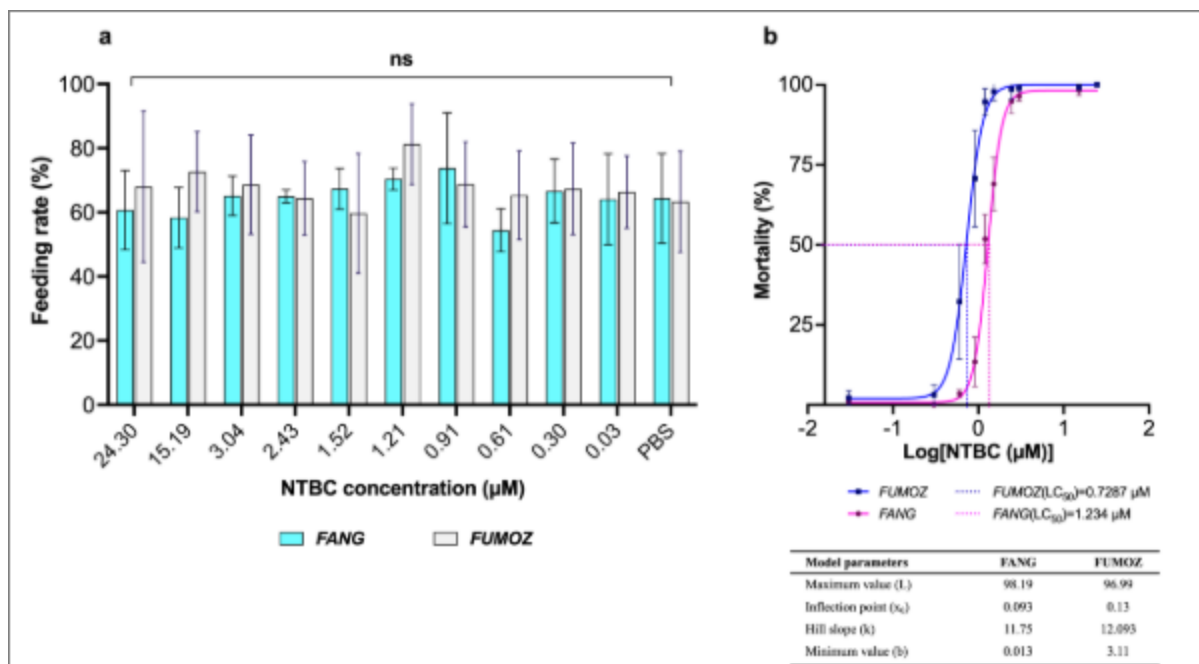


Figure 4

Feeding rate and Dose-mortality curve. **a)** Blood-feeding rate ($\pm$ standard error of the mean) of *Anopheles funestus* FANG and FUM0Z strains at each NTBC concentration tested. A minimum of 60 females per concentration per replicate was used for blood feeding. **b)** Dose-response curves generated for *An. funestus* FANG (pink) and FUM0Z (blue) following ingestion of NTBC-supplemented blood. Data point represents the mean mortality observed, with standard errors indicated. Curves were fitted using a four-parameter logistic (4PL) nonlinear regression model. The fitted curves illustrate a dose-dependent increase in mortality. Dashed lines denote the calculated LC₅₀ values for FANG (pink) and FUM0Z (blue). The table shows the model parameters in both strains. Total sample sizes were $194 \pm 5,6$ and $190 \pm 7,4$ blood-fed females for FANG and FUM0Z, respectively (5 replicates per strain).