1   **Repurposing the orphan drug nitisinone to control the transmission**

2   **of African trypanosomiasis**

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33 **Abstract**

34   Tsetse transmit African trypanosomiasis, which is a disease fatal to both

35 humans and animals. A vaccine to protect against this disease does not exist so

36 transmission control relies on eliminating tsetse populations. Although

37 neurotoxic insecticides are the gold standard for insect control, they negatively

38 impact the environment and reduce insect pollinator species. Here we present a

39 promising, environment-friendly alternative that targets insect tyrosine

40 metabolism pathway. A bloodmeal contains high levels of tyrosine, which is toxic

41 to haematophagous insects if it is not degraded. RNAi silencing of either the first

42 two enzymes in the tyrosine degradation pathway (TAT and HPPD) was lethal to

43 tsetse. Furthermore, nitisinone (NTBC), an FDA-approved tyrosine catabolism

44 inhibitor, killed tsetse regardless if the drug was orally or topically applied.

45 However, it did not affect bumblebee survival. A mathematical model shows that

46 NTBC could reduce the transmission of African trypanosomiasis in sub-Saharan

47 Africa, thus accelerating current elimination programmes.

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Introduction

Human African trypanosomiasis (HAT), also known as sleeping sickness, is a parasitic disease caused predominantly by the parasite *Trypanosoma brucei gambiense*. These parasites are transmitted when infected tsetse flies (*Glossina spp.*) blood feed. HAT currently affects 3,500 people/year; most patients live in the Democratic Republic of the Congo and an estimated 70 million people remain at risk of infection in sub-Saharan Africa[1]. Tsetse also spread animal African trypanosomiasis (AAT), which causes high mortality rates in livestock and consequently severely limits animal production[2]. As no vaccine for either HAT or AAT exist, and efficacious treatments are often difficult to obtain, tsetse population control remains essential to limit the spread of trypanosomiasis. In the last decades, tsetse control tools such as aerial spraying of insecticides (pyrethroids), visual and odour baited traps, insecticide-treated livestock, live traps, insecticide-impregnated traps and targets, and sterile male releases have been employed[3–7]. Despite such efforts, AAT and HAT persist and both economic development and public health continue to be jeopardised[8]. Consequently, a novel complementary strategy to control these parasitic diseases is highly desired. Tsetse, like other blood-feeding arthropods, ingest large quantities of blood and often exceed twice their body weight in a single meal[9]. Since more than 80% of blood dry weight consists of proteins, large quantities of amino acids are released in the midgut during bloodmeal digestion[10]. Previously, we showed that blocking tyrosine catabolism after a bloodmeal is lethal in mosquitoes, ticks and kissing bugs due to the accumulation of toxic quantities of tyrosine[11]. However, inhibiting tyrosine catabolism in non blood-feeding insects is harmless, which further provides evidence for the essentiality of this pathway for haematophagy[11]. In the present

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83   work, we evaluated how tsetse physiology was controlled by two enzymes in the

84   tyrosine catabolism pathway: tyrosine aminotransferase (TAT) and 4-

85   hydroxyphenylpyruvate dioxygenase (HPPD). The drug nitisinone (NTBC) is an

86   HPPD inhibitor currently used to treat patients suffering from the genetic disease

87   tyrosinemia type I[12] and is under clinical evaluation for the treatment of

88   alkaptonuria[13]. NTBC was lethal to blood-fed tsetse flies. NTBC treatment, either

89   administered orally as an endectocide or topically, causes the accumulation of

90   tyrosine and 4-hydroxyphenyl lactic acid (HPLA) metabolites, which leads to initial

91   fly paralysis followed by tissue destruction within 18 hours of the bloodmeal. Our

92   results provide evidence that NTBC could be used as an eco-friendly synergistic

93   strategy alongside current tsetse control practices.

94

95   **Results**

96   **Tyrosine detoxification is essential in tsetse**

97   Tyrosine catabolism is a highly conserved pathway (Fig. 1A) in most

98   eukaryote and prokaryote species with only a few exceptions such as the pea

99   aphid, *Acyrthosiphon pisum*[14] and trypanosome parasites[15]. The genes encoding

100   *TAT* and *HPPD* were identified in five *Glossina* species[16], as well as in all

101   hematophagous arthropod species with sequenced genomes (Supplementary

102   Figure 1). Transcript knockdown using RNA interference, of either *TAT* or *HPPD*

103   genes, was lethal to flies once they fed on bloodmeal (Fig. 1B and Supplementary

104   Figure 2). This lethality was further validated by feeding flies with blood spiked

105   with mesotrione, an HPPD inhibitor widely used as a selective herbicide on corn

106   crops under the brand name *Callistro®*, Syngenta (Supplementary Figure 3A). No

107   differences in susceptibility to mesotrione were observed between fly sex or age

108   (data not shown). The mesotrione concentration that killed 50% of the insects 24

109   hours after administration (LC$_{50}$) was 357.7 µM (CI: 222.5-512.4) (Supplementary

110   Figures 3A and 3C). This concentration is ~30X higher than the drug concentration

111   detected in human plasma (4 µg/ml (11.78 µM)) after volunteers received an oral

112   dose of 4 mg/kg body weight[17].

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114   **NTBC ingestion is lethal to tsetse**

115   Hypertyrosinemia Type I (HT-1) is a severe human genetic disease caused

116   by a mutation in the gene encoding for the last enzyme of the tyrosine catabolism

117   pathway, fumarylacetoacetase (FAH). This mutation causes the accumulation of

118   toxic metabolites in blood and tissues. The only drug available to minimise the

119   effect of HT-1 is the orphan drug nitisinone (NTBC / Orfadin®). NTBC is an HPPD

120   inhibitor that prevents a build-up of toxic products derived from

121   fumarylacetoacetate accumulation[18]. NTBC is remarkably safe to use with few

122   reported side effects in <1% of patients[19]. When NTBC was artificially fed to tsetse

123   flies in the bloodmeal, it was ~173 times more potent than mesotrione with a LC$_{50}$=

124   2.07 µM (CI: 0.709 to 4.136) (Fig. 1C and Fig. 1D). This lethal concentration is ~12

125   times lower than the concentration of NTBC persisting in human plasma (8 µg/ml

126   (24.3 µM)) after administering a standard therapeutic oral dose (1 mg/kg). The

127   NTBC half-life in human plasma is 54 hours[17], so assuming linear drug degradation,

128   human blood would remain toxic to tsetse up to ~7 days after a single dose in the

129   therapeutic range. These results suggest that NTBC used as endectocide (here

130   defined as a drug or insecticide that is administered to humans or animals to

131   control parasites) could be used to rapidly decrease tsetse populations as part of a

132   drug-based vector control strategy. Furthermore, it did not matter if the tsetse flies

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133   were infected with *T. brucei*; NTBC lethality remained unaltered in infected flies

134   (Supplementary Figure 4).

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136   **Tsetse fed on NTBC-treated mice die**

137   To evaluate the potential of using NTBC as an endectocide, the *in vivo*

138   efficacy of NTBC was assessed. Colony-reared tsetse (*G. pallidipes*) were allowed to

139   feed on rats that had been orally treated with different NTBC concentrations. We

140   observed that after feeding on rats receiving an oral dose of NTBC equal to 1 mg/kg

141   (the therapeutic dose for humans with Tyrosinemia type I) killed *G. pallidipes* as

142   reflected in our previous data (Fig. 1E). These data provide direct evidence that

143   NTBC could be used as an endectocide for tsetse control.

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145   **NTBC causes insect paralysis and systemic tissue destruction**

146   Treatment of tsetse with either mesotrione- or NTBC-spiked blood

147   presented the same unique physiological changes, which has two distinct phases

148   we classified as early stage and death phenotype. The early-stage phenotype was

149   observed 8-10 hours after NTBC (or mesotrione) ingestion in the bloodmeal; i.e.

150   the flies remained alive (evidenced by red eyes) but were unable to move (often

151   upside-down; Supplementary Video 1, Supplementary Figure 13). Exposure to

152   bright light or tarsal stimulation often produced only a short burst of leg waving.

153   Small, white, rhomboidal crystals (previously identified as tyrosine crystals[11])

154   were observed on the outside surface of the midgut epithelium against the body

155   wall cuticle. Also, dark brown melanin-like deposits had formed in different

156   tissues, such as the fat body, salivary glands and flight muscle (Fig. 2A and Fig. 2B).

157   Disorganization of tissues in the digestive tract, such as the proventriculus and

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158   midgut was evident (Fig. 2C), and likely contributed to intestinal fragility,

159   compromised digestion and leaking of the midgut content into the haemocoel.

160   The death phenotype was observed between 15-18 hours after treatment

161   and was characterised by a prolonged shift in eye colour from red to golden brown

162   (Fig. 2D). Treated tsetse showed extreme cuticular thinning and complete loss of

163   abdominal elasticity. The abdomen was strikingly distended, dark and filled with

164   blood that had leaked into the haemocoel due to damage of the gut epithelium (Fig.

165   2E). The ingested blood was darkened as though it had been oxidized. Most of the

166   internal tissues and organs (e.g. gut, testes, ovaries, salivary glands, Malpighian

167   tubules and flight muscle) were completely destroyed and could not even be

168   recognized in the abdomen during dissection, suggesting extensive autolysis; only

169   the hindgut, rectum, and trachea remained identifiable. Furthermore, the fat body

170   also disappeared and only lipid droplets could be seen floating in the haemocoel

171   (Supplementary Figure 5). From the outside, the thorax appeared normal, but

172   upon dissection, we observed the flight muscles had detached from the thorax and

173   become melanised, which explains why tsetse quickly lost the ability to fly.

174

175   **NTBC leads to an accumulation of free tyrosine and 4-hydroxyphenyl lactic**

176   **acid**

177   To better understand the metabolic changes in tsetse exposed to NTBC, the

178   haemolymph of NTBC-treated (and control) flies was collected at different time-

179   points. Metabolomics analysis revealed increased levels of tyrosine and 4-

180   hydroxyphenyl lactic acid (HPLA, Fig. 3). The knockdown of *TAT* gene, which is

181   expected to reduce levels of 4-hydroxyphenylpyruvate (HPPA) and, in

182   consequence, produce less HPLA, caused the same lethal phenotype observed

183   upon HPPD inhibition. Together, this suggests tyrosine accumulation is the likely

184   cause of the flies' death. Furthermore, feeding the flies with blood spiked with

185   HPLA or the injection of HPLA into the fly haemocoel did not affect their survival

186   (Supplementary Table 1). In contrast to tyrosine and phenylalanine, the titre of all

187   other detected amino acids was reduced (Fig. 3C and Fig. 3D), which may reflect a

188   lower digestion rate in NTBC-treated flies.

189

190   **NTBC-associated damage does not depend on phenoloxidase activation or**

191   **haem, but protein concentration in the bloodmeal**

192   As extensive tissue melanisation was a result of NTBC exposure, we

193   assessed whether systemic phenoloxidase (PO) activation led to tissues damage.

194   PO produces melanin, but also reactive intermediates and cytotoxic quinones that

195   could harm the flies if produced in excess[20]. To test this, we blocked PO activity by

196   feeding tsetse on blood containing NTBC (0.01 mg/ml) and phenylthiourea (0.1

197   mg/ml), a PO inhibitor[21]. The addition of phenylthiourea to the bloodmeal failed to

198   revert the lethal phenotype suggesting that PO activation is not required for NTBC

199   death (Supplementary Figure 6).

200   Another potential cause of toxicity could be haem. Haem is a product of

201   blood (haemoglobin) digestion and becomes toxic by amplifying reactive oxygen

202   species[22]. To investigate if haem contributed to the lethal phenotype, flies were fed

203   with horse serum (red blood cells were removed to reduce haemoglobin)

204   supplemented with mesotrione. Fly mortality remained high even in the absence

205   of haemoglobin, which suggested haem was not involved in the toxicity of HPPD

206   inhibitors. Furthermore, this experiment demonstrated that the protein content in

207   serum (around 60 mg/ml)[23] is high enough to produce toxic levels of tyrosine

208   (Supplementary Figure 7).

209   In order to investigate the toxicity of HPPD inhibitors, flies were fed with

210   sugar supplemented with a fixed concentration of NTBC (lethal when added to

211   bloodmeal) and different concentrations of bovine serum albumin (BSA) as the

212   sole protein source. As expected, flies that fed on NTBC-spiked sugar (protein-free)

213   did not die, whereas NTBC toxicity showed a clear dependence on the protein

214   content of the meal. The observed LC_{50} was 12.5 mg/ml of protein (CI: 11.09-

215   13.67) (Supplementary Figure 8).

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217   **Topical application of NTBC kills tsetse**

218   To investigate if NTBC-induced mortality was restricted to ingestion, we

219   tested if the drug could be absorbed through the insect cuticle. Topically applying

220   NTBC to the fly thorax caused high mortalities after tsetse took a bloodmeal, with

221   a LD_{50} of 39 picomoles/fly (CI: 9-90) (Fig. 1D and F), 24 hours after bloodmeal

222   ingestion. We compared this NTBC dose against a standard pyrethroid insecticide

223   (deltamethrin) that tsetse are highly susceptible to and the LD_{50} was 0.116

224   picomoles/fly (CI: 0.093-0.145) (Supplementary Figure 9), which means that

225   deltamethrin is 336 times more potent than NTBC.

226   In the field situation where HPPD inhibitors could be absorbed through the

227   insect cuticle, fly contact with the drug could occur either before or after a

228   bloodmeal. Thus, it is important to know how long NTBC activity persists in insects,

229   particularly if tsetse feed after cuticular exposure. High fly mortality was observed

230   even when a tsetse ingested a bloodmeal six days following a single topical

231   application of NTBC (Supplementary Figure 10). To address the opposite situation

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232   where blood-feeding precedes topical exposure to NTBC, we assessed fly mortality

233   at different times post-bloodmeal. Topically applied NTBC reduced tsetse survival

234   up to 48 hours after a bloodmeal (Supplementary Figure 11), which implies that

235   bloodmeal proteins were digested after two days (as predicted for tsetse) and

236   excess tyrosine had been catabolized. In contrast to NTBC, topical application of

237   mesotrione was not lethal, which could be due to reduced penetration through the

238   tsetse cuticle (Supplementary Figure 12).

239

240   **NTBC is not metabolised by insect P450 enzymes**

241   Metabolic resistance due to increased rates of insecticide metabolism by P450s can

242   cause resistance liabilities for new compounds. However, nitisinone appears to

243   only be moderately metabolised by CYP3A4 in humans[24], with little oxidative

244   metabolism by other liver CYP enzymes[25]. We have incubated nitisinone with

245   microsomes extracted from tsetse, *Aedes* and *Anopheles* and failed to detect

246   evidence of metabolism as measured by substrate depletion/turnover

247   (Supplementary Table 2). Furthermore, we find no obvious metabolism

248   by recombinant *An. gambiae* CYP6P3, a P450 with broad substrate specificity

249   similar to CYP3A4, and associated with cross-resistance to pyrethroids and other

250   insecticides[26]. Overall, this suggests that nitisinone may be a weak substrate for

251   P450 metabolism in these insects. This does not preclude the evolution of

252   metabolic resistance, but suggests it may be less likely to emerge rapidly.

253

254   **NTBC is highly stable under environmental conditions**

255   Considering uses for NTBC in the field, we examined several stressors that

256   could degrade drug activity and reduce its toxicity. An NTBC solution was

257   subjected to ten freeze-thaw cycles, prolonged ambient temperature storage (5

258   weeks) or different exposures to light (Supplementary Table 3). These NTBC test

259   samples (final concentration in blood 3 μM=1 μg/ml) were screened for activity

260   by spiking them into a tsetse bloodmeal as a type of activity bioassay. In all cases,

261   fly mortality remained unchanged, thus indicating NTBC does not quickly degrade

262   and is stable under simple, room temperate storage conditions. In agreement with

263   our results, Barchanska *et al.*[27] recently demonstrated that NTBC show

264   considerable stability under different experimental conditions such as pH of

265   solution, temperature, time of incubation and ultraviolet radiation.

266

267   **NTBC does not affect the insect pollinator, *Bombus terrestris*.**

268   Bees are the world's most important pollinator of food crops[28], and several

269   reports have shown that bee populations are directly and indirectly affected by

270   insecticides[29]. To determine the environmental impact of NTBC on off-target

271   species, we investigated the mortality of phytophagous pollinators when exposed

272   to NTBC. Colony-reared *Bombus terrestris* (the buff-tailed bumblebee) were fed *ad*

273   *libitum* with NTBC-spiked sugar as a sole hydration source; bee mortality was

274   assessed over 10 days. Mortality rates did not differ between NTBC-treated and

275   control bees during this sustained exposure, despite feeding NTBC at doses as high

276   as 50 μg/ml (152 μM) and providing pollen as a protein source (Fig. 1G).

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278   **A mathematical model supports use of NTBC for tsetse control**

279   Based on our results and the pharmacokinetics of NTBC in humans[17], we

280   evaluated the predicted impact of using NTBC for tsetse control by mathematical

281   modelling. Our models suggest that targeting humans in trypanosomiasis endemic

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282   areas with NTBC as an endectocide could control disease transmission with tsetse

283   species that have a preference for feeding on humans (anthropophagic). This

284   strategy would require drug administration with a maximal interval of every 40

285   days to be effective (Fig. 4A). However, tsetse flies can also feed on livestock or

286   wildlife. Therefore, we modelled a scenario of NTBC being simultaneously given to

287   livestock and humans. We found that tsetse population control is achieved more

288   readily if both humans and livestock are treated, and that a frequency up to 90 days

289   between consecutive drug administrations would still achieve control (Fig. 4B).

290   This approach would also capture tsetse species that preferentially feed on

291   livestock.

292

293   **Discussion**

294   When blood-sucking insects digest a bloodmeal, large amounts of tyrosine

295   are produced, which would be potentially toxic if it were not for the first two

296   enzymes in the tyrosine degradation pathway, TAT and HPPD[11]. Here we provide

297   evidence that tyrosine detoxification is essential for tsetse survival post-bloodmeal

298   and have further investigated the possible mechanisms underlying this lethal

299   phenotype. Furthermore, two commercially available HPPD inhibitors were

300   evaluated as novel tsetse control interventions: mesotrione and NTBC. We

301   concluded that NTBC is the best candidate for future field trials due to its low-dose

302   efficacy and environmental sustainability. This drug presents unique features

303   when compared with current insecticides as it specifically targets blood-feeding

304   insects, has low toxicity to mammals and can be used effectively against tsetse via

305   two delivery routes. NTBC kills tsetse upon cuticular application, similar to

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306   pyrethroids, and it can be safely administered to a mammalian host to kill tsetse

307   upon blood-feeding.

308   The endectocide ivermectin has been evaluated *in vivo* as a potential tsetse

309   control tool. *G. palpalis gambiensis* fed on cattle treated with a single ivermectin

310   dose showed decreased survival ranging from 21-84% for the therapeutic dose

311   (0.2 mg/kg), and from 78-94% for a dose 10 times greater (2 mg/kg)[30]. However,

312   it is important to highlight that another tsetse species, *G. palpalis palpalis,*

313   remained unaffected by an ivermectin dose of 2 mg/kg (4% mortality after 25

314   days)[31], and no mortality was observed when flies fed on ivermectin-treated (0.5

315   – 1.0 mg/kg) guinea pigs and goats[32]. Similarly, no effect on fly mortality was

316   observed when *G. tachinoides* fed on pigs treated with ivermectin[33]. These

317   differences may be due to pharmacokinetic variations in the different host species

318   (guinea pig, goat, cattle, and pigs) rather than *Glossina spp*-specific susceptibilities.

319   In our study, 50% of fly mortality was observed 24 hours after tsetse took a single

320   bloodmeal from rats that had received 1 mg/kg of NTBC. The surviving 50% were

321   severely compromised and had lost the ability to fly. Compared to published data

322   on ivermectin-induced fly mortality, dose-matched NTBC is more potent as it kills

323   the flies faster than ivermectin. NTBC is also classified as a safer drug for mammals;

324   the oral LD_{50} for NTBC in rats is >1,000 mg/kg[34] while ivermectin is >100 times

325   more toxic presenting a LD_{50}=10 mg/kg[35].

326   As observed in the different dose-response curves, it is not only the

327   concentration of drug ingested or applied topically that is important, but also the

328   quantity of blood ingested and the digestion rate of bloodmeal proteins. This is due

329   to the particular mode of action of HPPD inhibitors. These drugs are not toxic to

330   hematophagous arthropods during starvation or when insects are fed with low

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331 protein content diets, but their toxicity is caused by the accumulation of tyrosine

332 derived from hydrolysis of dietary proteins. This implies that any factor affecting

333 bloodmeal digestion, such as temperature, may also affect HPPD inhibitor toxicity

334 and consequently how long the blood-fed arthropods take to die.

335   Our data shows neither the extensive melanisation observed during the

336 early phenotype nor the haem in the bloodmeal trigger the lethal phenotype in

337 tsetse. Similarly, in *R. prolixus* HPPD/PO and HPPD/Dopa Decarboxylase (DDC)

338 double-gene silencing, did not rescue lethality observed in *HPPD*-silenced

339 insects[11,36]. These similar results in tsetse reinforce the idea that PO activation and

340 excessive melanin synthesis are not involved in killing hematophagous insects.

341 Together with metabolomics, these results point to tyrosine accumulation (and

342 precipitation in haemocoel and tissues) as the primary cause of insect death.

343 Furthermore, feeding the flies with NTBC-spiked sugar supplemented with only

344 BSA was lethal, and confirmed that tyrosine accumulation, from dietary protein

345 digestion, caused the lethal phenotype. However, it is worth noting that the

346 quantity of protein to be lethal upon HPPD inhibition may depend on the tyrosine

347 content of each protein; bovine BSA tyrosine content is 3.3 % (20/608 residues).

348 Moreover, NTBC proved to be very stable under different environmental

349 conditions. Together, these results expand the possibility of using NTBC mixed

350 with protein in attractive targeted sugar baits (ATSBs) to control different vector-

351 borne diseases.

352   When assessing the feasibility of topically using NTBC to control tsetse flies,

353 a high mortality was still observed 144 hours after NTBC application

354 (Supplementary Figure 10). The longevity of this drug activity is striking and very

355 encouraging for field-based vector control. In lab-reared colony flies, most tsetse

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356 die of dehydration/starvation after such an extensive starvation period and, of

357 those remaining alive, many are too weak to feed when offered a bloodmeal. In

358 wild tsetse, where fly activity and nutritional demands are greatly increased, flies

359 are less likely to survive 144 hours of starvation so the sustained lethality of NBTC

360 is even more encouraging. However, when comparing tsetse lethality to

361 deltamethrin, NTBC is far less potent when topically applied to tsetse. This is not

362 surprising as deltamethrin is a neurotoxin, while NTBC acts by a different, and

363 albeit slower, mechanism linked to digestion and amino acid catabolism. However,

364 the disadvantage to deltamethrin is significant; potency comes at a price.

365 Deltamethrin is more toxic to mammals and killing is non-selective and it draws a

366 heavy environmental penalty. All insects including pollinators and other non-

367 target species are indiscriminately killed. Furthermore, deltamethrin can cause

368 severe toxicity in aquatic ecosystems[37]. In contrast, NTBC toxicity profile is low

369 (high doses are tolerated in humans and other mammals[34]) and it selectively kills

370 blood-feeding arthropods, making it environment-friendly and more socially and

371 ethically acceptable to employ. Despite this, pyrethroid insecticides remain the

372 most efficacious vector control strategy for many hematophagous arthropod

373 populations, although increasing reports of insecticide resistance compromise

374 their efficacy[7,38–40]. Since NTBC targets a pathway separate to the neurotoxic

375 insecticides, such as pyrethroids and ivermectin, its use could complement current

376 vector control tools and reduce the emergence of insecticide-resistant populations.

377   We conclude that both NTBC and mesotrione induce rapid death in blood-

378 fed tsetse, although the potency and the duration of their effects are very different.

379 For these reasons, we propose using NTBC (but not mesotrione) to develop new

380 and complementary vector control tools that target tsetse and other blood-feeding

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381 arthropod populations. The versatility of NTBC is also noteworthy. This orphan

382 FDA approved drug could be used as a standalone technology to control tsetse and

383 other vector populations as an endectocide, or it could complement (or provide an

384 alternative to) the use of standard insecticides. Thus, NTBC is a safer and more

385 environment-friendly alternative to insecticide-based vector control as it is not

386 toxic to mammals and selectively kills only blood-feeding arthropods.

387

388 **Materials and methods**

389 **Ethics**

390   Male (4–6 weeks old, 180-250g in weight) Wistar rats sourced from Animal

391 Rearing Unit and Containment Unit (ARCU) at International Centre of Insect

392 Physiology and Ecology (*icipe*) were used. The animals were housed in standard

393 plastic mice/rat cages (Thoren Caging Systems, Inc., Hazleton, PA, USA) with wood

394 shavings as bedding material. The rodents were maintained on commercial pellets

395 (Unga® Kenya Ltd, Nairobi, Kenya), and water was provided *ad libitum*. Animal

396 use and all accompanying procedures and protocols were in accordance with *The*

397 *Guide for the Care and Use of Laboratory Animals* (Institute for Laboratory Animal

398 Research. 2011). These procedures and protocols were reviewed and approved by

399 Institutional Animal Care and Use Committee (IACUC) of *icipe* (Ref. No.

400 IcipeACUC2018-003).

401

402 **Tsetse**

403   The *Glossina morsitans morsitans* Westwood colony (originally from

404 Zimbabwe) was housed in an insectary at the Liverpool School of Tropical

405 Medicine. Flies were kept at 26°C and 73 ± 5 % relative humidity and a 12 hour

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406   light:dark cycle. The colony was maintained on defibrinated horse blood (TCS

407   Biosciences Ltd., Buckingham, UK) and fed every 2-3 days using artificial silicone

408   feeding membranes. Male and female experimental flies of different ages were

409   housed and fed separately. The *G. pallidipes* (originally from Nguruman, Kenya)

410   from Insectary Unit at *icipe* were maintained at 26°C and 75 ± 4 % relative

411   humidity with 12 hour light:dark cycle. The flies were fed on defibrinated bovine

412   blood using an *in vitro* silicon membrane system. Only teneral *G. pallidipes* were

413   fed on NTBC-treated rats.

414

415   **Bumblebee rearing**

416   Hives of *Bombus terrestris audax* ordered from Agralan (Wiltshire, UK)

417   were kept at 27°C under constant red light and fed *ad libitum* with pollen and 50%

418   sugar water (Ambrosia syrup, EH Thorne®). Only worker bees were used in this

419   study. Five bees from five different colonies were placed into an acrylic box in five

420   biological replicates, totalling 25 bumblebees per each experimental group. NTBC

421   was diluted in sugar solution and placed into the box 24h after the bees

422   acclimatized to laboratory conditions. Bees were allowed to drink *ad libitum* for 10

423   days*. Each group received PBS or a dose of NTBC at concentrations shown to be

424   lethal to tsetse (0.05 mg/ml, 0.005 mg/ml or 0.0005 mg/ml). Solutions were

425   changed every five days. Worker bees were also provided with pollen (protein

426   source) according to their rate of consumption. Bee mortality was scored daily for

427   10 days.

428

429   **Phylogenetic analysis**

17

430   Dendograms showing the phylogenetic relationships of TAT and HPPD

431   among insects were created according to the maximum likelihood method.

432   Confidence values for each branch was determined through bootstrapping at 100.

433   Analysis was performed on the web-based interface on Phylogeny.fr[41,42], using T-

434   Coffee for alignments under the BLOSUM62 scoring matrix and optimised with

435   Gblocks. Dendograms were constructed with PhyML 3.0[43]. The TAT and HPPD

436   protein sequences used in the phylogenetic analyses were taken from either

437   Vectorbase or NCBI using the most recent gene sets available.

438

439   **Synthesis of double-stranded RNA (dsRNA).**

440   Specific primers for *G. m. morsitans* tyrosine aminotransferase (TAT) and 4-

441   hydroxyphenylpyruvate dioxygenase (HPPD) genes were designed using primer-

442   blast software (NCBI. https://www.ncbi.nlm.nih.gov/tools/primer-blast) (Table 1).

443   These primers contained the T7 polymerase binding sequence, required for dsRNA

444   synthesis at 5' end. The green fluorescence protein (GFP) gene amplified from the

445   peGFP-N1 plasmid (NCBI ID: U55762.1) was used as a control dsRNA to assess *in*

446   *vivo* off-target effects. PCR products were sequenced to confirm identity.

447   MEGAscript High Yield T7 Transcription kit (Ambion) was used according to the

448   manufacturer's instructions to synthesize dsRNA. After synthesis, dsRNAs were

449   precipitated by adding an equal volume of ice-cold isopropanol, and the resulting

450   pellets were washed with ethanol, air dried and then the pellet resuspended in

451   ultrapure water. The dsRNAs concentrations were determined

452   spectrophotometrically at 260 nm on a NanoVue™ Plus Spectrophotometer (GE

453   Healthcare, Buckinghamshire, UK) and visualized in an agarose gel (1.5% w/v) to

454   confer dsRNA size, integrity and purity. A speedvac concentrator (ISS110. Thermo

455   Scientific) was used to dry the dsRNAs and they were adjusted to a final

456   concentration of 5 μg/μl in sterile nuclease-free water (NFW).

457

458   **Table 1.** Sequences of the primers used to amplify target genes for RNAi

459   experiments. T7 promoter sequences that were necessary for transcription are

460   underlined.

Gene	NCBI/ Vector Base ID	Forward primer	Reverse primer
Green fluorescence protein	U55762.1	<u>TAATACGACTCACTATAG GG</u>ACGTAAACGGCCACAA GTTC	<u>TAATACGACTCACTATAG GG</u>CTTGTACAGCTCGTCC ATGCC
Tyrosine aminotransferase	GMOY012 088	<u>TAATACGACTCACTATAG GGAGA</u>CGAAGTGACTGCC GGTCTACG	<u>TAATACGACTCACTATAG GGAGA</u>TTCACGAGGCACT GTTAGCAC
Hydroxyphenylpy ruvate dioxygenase	GMOY012 145	<u>TAATACGACTCACTATAG GGAGA</u>ATCGCAGCCAATA TCGTGGTG	<u>TAATACGACTCACTATAG GGAGA</u>CTTTAATTTTGGT GCGGCTGTGC

461

462   **Gene silencing**

463   The protocols described by Walshe *et al.*[44] were followed for RNAi

464   knockdown. Briefly, *G. m. morsitans* were injected in the thorax with 10 μg of each

465   target gene dsRNA (2 μl of 5 μg/μl stock). Control insects were injected with 10 μg

466   of GFP dsRNA. Injection needles were handmade using pulled micro-haematocrit

467   glass capillary tubes (2.00 mm outside diameter) (Globe Scientific) mounted inside

468   200 μl yellow micropipette tips and sealed with Araldite epoxy glue. Flies were

469   injected 24 hours after receiving a bloodmeal to increase survival rates. Flies were

470   chilled for 10 minutes on ice to immobilize them and 2 μl of dsRNA was injected

471   into the dorsolateral surface of the thorax. Injected flies were allowed to rest for

472   24 hours before the next bloodmeal. Following injections, flies were fed every 2-3

19

473 days on sterile, defibrinated horse blood. Three days after dsRNA injection, the

474 digestive systems were excised to determine the efficacy of gene-silencing as

475 evaluated by quantitative RT-PCR.

476

477 **RNA isolation and cDNA synthesis**

478 *G. m. morsitans* flies were immobilized on ice and the digestive system was

479 dissected into ice-cold PBS. The total RNA was extracted using TRIzol reagent

480 (Invitrogen), according to the manufacturer's instructions. Following treatment of

481 the extracted mRNA with TURBO DNase (Ambion), first-strand cDNA synthesis

482 was performed using 1 μg total RNA with "Superscript III First-strand Synthesis

483 System for RT-PCR Kit" (Invitrogen) and poly-T primer, according to the

484 manufacturer instructions. The cDNAs were stored at -80°C until use.

485

486 **Quantitative polymerase chain reaction (qPCR)**

487 Specific gene-specific qPCR primers were designed to amplify a different

488 region from that amplified by the RNAi primers to prevent dsRNA amplification.

489 They were designed to span different exons so to easily detect contaminating DNA

490 amplification and their efficiency was experimentally tested (Table 2). *Glossina α-*

491 *tubulin* and *β-tubulin* genes were used as references (housekeeping) genes.

492 Quantitative PCR was performed using a MxPro – Mx3005P thermocycler (Agilent

493 Technologies) with Brilliant III Ultra-Fast SYBR® Green QPCR Master Mix (Agilent

494 Technologies) under the following conditions: 95 °C for 15 minutes, followed by 40

495 cycles of 95 °C for 15 seconds, 60 °C for 30 seconds and 72 °C for 30 seconds and a

496 final extension of 72 °C for 10 minute. Target gene expression levels were assessed

497 as $2e^{-\Delta CT}$ values ($\Delta C_T = C_T$ gene of interest – C_T housekeeping gene) were used to

498   evaluate the expression levels of the genes in the different experimental groups.

499   (dsTAT- or dsHPPD-injected flies and dsGFP injected flies) [45].

500

501   **Table 2.** Sequence of the primers used to amplify target genes by Real-Time PCR.

Gene	Vector Base ID	Forward primer	Reverse primer	% Efficiency
Alpha tubulin	GMOY004645	TGTATGTTGTAT CGTGGTGATGT	GAATTGGATGGTG CGTTTAGTTT	100,6
Beta tubulin	GMOY000148	CCATTCCCACGTC TTCAGTT	GACCATGACGTGG ATCACAG	96,6
Tyrosine amino transferase	GMOY012088	CCTAGCAATCCG TGTGGTAGTG	TAACCGCTATGTG CTGCGAAC	103
Hydroxyphenyl pyruvate dioxygenase	GMOY 012145	CTAAAGAACGTG GAGCAACTGTG	TCCACAAAAGTGT GAGTCGT	106,5

502

503

Oral dosing of mesotrione and nitisinone

505   Mesotrione (2-[4-(Methylsulfonyl)-2-nitrobenzoyl]cyclohexane-1,3-dione;

506   PESTANAL analytical standard; Sigma-Aldrich) and nitisinone (NTBC, 2-[2-nitro-

507   4-(trifluoromethyl)benzoyl]cyclohexane-1,3-dione; PESTANAL, analytical

508   standard; Sigma-Aldrich) were solubilized in sterile PBS (NaCl 0.15 M, Na-

509   phosphate 10 mM, pH 7.0) to different final concentrations (pH was re-adjusted

510   to 7.0 with 1 M NaOH). One volume of the drugs, or PBS as control, was then mixed

511   with nine volumes of sterile defibrinated horse blood and these spiked bloodmeals

512   were fed to male and female flies. Only fully engorged flies were used. Final

21

513   concentration in blood for mesotrione were: 2, 1.5, 1, 0.75, 0.5, 0.25, 0.1, 0.05 and

514   0.01 mg/ml. Final concentration in blood for NTBC were: 0.05, 0.025, 0.01, 0.005,

515   0.0025, 0.00075, 0.0005, 0.00025, and 0.0001 mg/ml).

516

517   **Confocal microscopy**

518   Teneral (<24h old) male tsetse were fed on horse blood (TCS Biosciences)

519   and three days later were offered a second meal containing horse serum with or

520   without 500 ng/mL NTBC. Fifteen hours post-feeding, tsetse were anaesthetized

521   on ice and midguts (with attached proventriculi) were dissected out in ice-cold PBS

522   and immediately fixed in fresh 4% paraformaldehyde for 1 hour at room

523   temperature. Tsetse survival of each group was determined 72 hours post-NTBC

524   administration. Fixed tissues were washed in PBS (and stained with SiR-actin

525   (1:1000 dilution, Cytoskeleton Inc.) for 3 hours at room temperature (RT) and

526   posteriorly incubated in 500 ng/mL DAPI for 10 minutes at RT. Tissues were

527   finally suspended in 1% (w/v) low-melting agarose at ~40°C mixed with Slowfade

528   Diamond oil (Molecular Probes) on a slide. Slides were imaged using a Zeiss LSM-

529   880 confocal laser scanning microscope and tissues were 3D-reconstructed from a

530   series of z-stacks at intervals of 1.3 μm (Zeiss Company).

531

532   **Drug treatment of trypanosome-infected flies**

533   Newly emerged (24–48 h. post-eclosion), teneral male *G. m. morsitans* were

534   fed an infected bloodmeal containing *T. brucei* (strain Antat 1.1 90:13)[46] at 10^6

535   parasites per ml of blood. Newly emerged flies are highly susceptible to

536   trypanosome infections. Nine days after infection, when procyclic trypanosomes

537   should be established in the midgut, the flies were fed with blood spiked with

538   NTBC as indicated above.

539

Oral dosing of NTBC supplemented with phenylthiourea

541   *G. m. morsitans* were fed blood spiked with NTBC (0.01 mg/ml) and

542   phenylthiourea (PESTANAL analytical standard; Sigma-Aldrich. 0.1 mg/ml) as

543   indicated above.

544

Oral dosing of NTBC in serum

546   Horse red blood cells were removed by centrifugation at 1000 x g for 5 min.

547   The serum was collected, supplemented with mesotrione as indicated above and

548   subsequently fed to *G. m. morsitans*.

549

Feeding fructose supplemented with BSA and NTBC

551   Teneral, male *G. m. morsitans* were sorted into 10 cages and starved for 48

552   hours. Bovine serum albumin (BSA) fraction V (stock concentration at 200 mg/ml

553   (w/v) in PBS) was serially diluted into sterile 0.1% (w/v) fructose in PBS to create

554   final concentrations of 34, 25, 20, 18, 15, 12, 10, and 5 mg/ml BSA. Control flies

555   were fed with either diluent alone (0.1% fructose) or the diluent spiked with

556   NTBC. All experimental groups were exposed to a NTBC dose of 0.001 mg/ml

557   (lethal when delivered with blood). Flies were offered a meal at 72 hours post-

558   emergence and mortality was tracked daily for one week.

559

Administering HPLA to tsetse by feeding or injection

23

561   Newly emerged, male *G. m. morsitans* were sorted into 10 cages containing

562   25 flies/cage. At 48 hours post-emergence, each cage was fed on defibrinated

563   horse blood spiked with distilled water (control) or a serial dilution of DL-p-

564   hydroxyphenyllactic acid (HPLA: Aldrich-Sigma cat no. H3253). All dilutions were

565   made with the same ratio: 25 µl of additive to 1.975 ml of blood. The

566   concentrations of HPLA tested were 0.05, 0.025, 0.01, 0.005, 0.0025, 0.0075,

567   0.0005, 0.00025, and 0.0001 mg/ml. All dilutions were kept on ice until feeding to

568   avoid chemical degradation. Fresh HPLA stock concentration was 2 mg/ml in

569   sterile water. After 3 days, flies were offered a normal bloodmeal to test if HPLA

570   impaired a second feed. To administer HPLA by injection, one-week old male flies

571   that had taken three previous bloodmeals (to ensure good health and adequate

572   rehydration) were separated into 10 cages. Each group received 2 µl of the

573   selected HPLA concentration via thoracic injection. Stock HPLA was 2 mg/ml and

574   the concentrations injected were 1.0, 0.5, 0.1, 0.05, 0.01, 0.005, 0.001, 0.0005, and

575   0.0001 mg/ml with sterile water injected as a control. Flies were allowed to

576   recover for 24 hours and then offered a normal bloodmeal. Fly mortality was

577   monitored throughout in both experiments for a week after HPLA administration

578   and no group exceeded 10% mortality.

579

580   ***In vivo* oral nitisinone administration to rats**

581   Rats received an oral dose of NTBC by gavage solubilized in sterile PBS. The

582   doses administered were 0.1, 0.2, 0.5, 1.0, and 2.0 mg/kg, with controls receiving

583   an equal volume of PBS. After one and half hours, the rats were anaesthetized with

584   an intraperitoneal injection of 80 µl of 3% ketamine and Xilazin 0.33% diluted in

24

585   sterile PBS. Neither ketamine nor Xilazin influence tsetse mortality. Groups of male

586   *G. pallidipes* were fed on either PBS or NTBC-treated rats.

587

588   **Topical application of mesotrione and NTBC**

589   Mesotrione and NTBC were solubilized in 100% acetone at different

590   concentrations (Mesotrione: 10, 5, 2.5 ,1, 0.5, and 0.1 mg/ml. NTBC: 1, 0.5, 0.25,

591   0.125, 0.0625, 0.03175, 0.0156, 0.0078, and 0.0039 mg/ml). One microliter was

592   topically applied to the ventral surface abdomen of cold-anaesthetized *G. m.*

593   *morsitans*, either immediately before ingesting a blood meal, at different times

594   before or after a bloodmeal. The control flies received 1 µl of 100% acetone. Only

595   fully engorged flies were used.

596

597   **Metabolomic analysis**

598   Male *G. m. morsitans* were dissected at different times (0, 5 or 10 hours)

599   after ingesting horse blood spiked with NTBC 0.01 mg/ml or PBS. The flies were

600   chilled for 10 min at 4°C and kept on ice. The legs of 20 flies were severed and

601   collected into 1.5 ml plastic tubes containing 300 µl of 0.9% NaCl and

602   13.34 µM phenylthiourea (internal standard) in ultrapure water. The samples

603   were vortexed for 1 min, centrifuged for 10 min at 14000 rpm and the supernatant

604   was collected. The supernatants were then filtered (Millipore 0.22 µm syringe

605   filter) to remove cells, such as haemocytes, present in the haemolymph. Filtered

606   samples (150 µl total volume) were transferred to a 1.5 ml tube containing 50 µl of

607   chloroform, 75 µl of methanol and 75 µl of water containing B-methylamino-L-

608   Alanine 53.4 µM as an internal standard for normalization. After centrifugation,

609   the polar and apolar phases were collected and dried in a speed vac concentrator

25

610   (ISS100. Thermo Scientific). These samples were stored at -80 until analysis. Five

611   samples in total were collected from two independent experiments for each group

612   and time point. See Supplementary Information for details about LC-MS and LC-

613   MS/MS analyses.

614

615   **Metabolomic pathway analysis and heat map**

616   The mean relative abundances of all identifiable metabolites for each time-point

617   for both treated and untreated flies were divided by the mean abundance of the

618   metabolite at the corresponding time-point from untreated flies. Results were

619   expressed in fold change values for each individual metabolite, with the untreated

620   group serving as the baseline. Thus, metabolites with abundance fold-changes

621   greater than one were said to be up regulated in flies treated with NTBC as

622   compared to control, whilst metabolites with abundance fold change less than one

623   were said to be down regulated.

624   The abundance fold-change values were then plotted onto a heatmap using

625   gplot's heatmap.2 program (version 3.0.1.1, http://cran.r-project.org). The

626   heatmap colour scale was limited from 0 to 4, with additional non-cosmetic

627   options as follows: distfun = function(x) dist(x, "manhattan"), hclustfun =

628   function(x) hclust(x, "centroid"), Colv = FALSE.

629   Subsequent to this, metabolic pathways were assembled using Pathway

630   Collages from BioCyc[47,48], focused on pathways relevant to tyrosine degradation,

631   carbon metabolism, and amino acid biosynthesis focused on. Fold-changes from all

632   identified metabolites were overlaid onto this 'combined' metabolic pathway, and

633   statistically significant metabolites (as determined by the two-way ANOVA test as

634   described previously) were highlighted. Metabolites that were identified from LC-

26

635   MS/MS but were not identifiable within the 'combined' metabolic pathway, were

636   not mapped. Pathways that did not have any LC-MS/MS-identified metabolites

637   mapped to it were removed from the 'combined' pathway.

638

639   ***In vitro* comparative metabolism of nitisinone against microsomal**

640   **preparation from blood-feeding insects**

641   **Microsome preparation**

642   Mosquito (*Anopheles gambiae (*Kisumu) and *Aedes aegypti* (New Orleans)

643   microsomes were prepared from young female flies (48 to 72 hours old) as

644   described by Kasai *et al.*[49] and Inceoglu *et al.*[50] with some modifications. Briefly,

645   preparation of the adult mosquito microsomes (~200 individuals per colony) were

646   done by removing the heads to avoid enzyme inhibition by xanthommatin eye

647   pigments[51]. Mosquitoes were snap-frozen in liquid nitrogen with a sieve ~2 mm

648   mesh size. Small steel spheres were gently mixed with the mosquitoes to

649   fractionate their bodies and to separate the heads, legs, and wings parts from the

650   joint abdominal-thoracic components. The thorax and the abdomen were then

651   washed with 3 ml of pre-chilled 0.1 M KBP pH 7.4 homogenization buffer (HB)

652   containing 1x protease inhibitor (Roche Complete ULTR). The complex was

653   homogenized in 20 ml of HB using 40 ml glass Dounce homogenizer with a loose B

654   pestle (Wheaton Science, Millville, NJ) for 20 strokes. The separation of the

655   homogenate into cytosolic and microsomal extracts was achieved by

656   centrifugation steps at 4°C. The initial centrifugation was performed at 10,000 x g

657   for 15 min to remove insoluble debris. A second centrifugation at 200,000 x g for

658   1 h was performed to pellet the microsomes. The microsomal pellet was re-

659   suspended in 2ml of ice-cold suspension buffer (HB containing 20% glycerol).

660   Protein concentration was measured in triplicate by the Bradford method [52] with

661   Bio-Rad reagents using bovine serum albumin as a protein standard.

662

663   **Tsetse microsomes**

664   Virgin female *G. m. morsitans* (n=174) were maintained on three

665   bloodmeals over the week, and then starved for six days to reduce bloodmeal

666   contaminants during tissue homogenization. The intact thorax and abdomen were

667   removed by a pair of forceps, snap frozen in liquid nitrogen and homogenized as

668   described with mosquito microsomes. Because of the low P450 content in

669   insecticide-susceptible flies and mosquitoes, it was not possible to determine

670   P450 content by the traditional CO-reduced spectral assay[53], thus protein

671   concentrations were adjusted to 4 mg/ml using 0.1 M Potassium Phosphate buffer

672   (pH 7.4) to normalize for protein content. Recombinant *Anopheles gambaie*

673   CYP6P3 enzyme was used as a positive control for P450s microsomal activities.

674   All microsome preparations, in comparison to CYP6P3, were tested for O-

675   dealkylation activities against the P450's generic substrate "Diethoxyfluorescein"

676   before the set-up of the NTCB metabolism assay that identifies P450s enzyme

677   activities in microsomal extracts. Briefly, for activity assays, 200μl of reaction

678   media were set up in triplicate containing 0.5 mM NADPH, 5 μM

679   diethoxyfluorescein, 4 mg/ml microsomes or with mixture of 0.1 μM CYP6P3, 0.8

680   μM cytochrome *b5* and incubated at 30°C for 15 min. The enzyme activity was

681   recorded versus negative control (no NADPH) as 147.93±32, 408.64±45.96,

682   51.5±2.63 and 372±9.8 relative fluorescence units (RFU) for tsetse, *Ae. aegypti, An.*

683   *gambiae*, and CYP6P3 respectively. This showed the suitability of microsomes,

684   CYP6P3 and NADPH for running the NTBC comparative metabolism. See

685   Supplementary Information for details about insecticide metabolism experiments.

686

687   **NTBC stability under environmental stressors.**

688   NTBC was diluted in sucrose 10% to a final concentration of 0.01 mg/ml

689   and stored at either 4°C or 25°C in dark or transparent 1.5 ml polypropylene tubes.

690   One volume of NTBC was then mixed with nine volumes of sterile defibrinated

691   horse blood (final NTBC concentration: 0.001 mg/ml) and these spiked

692   bloodmeals were used to weekly screen against tsetse throughout a five-week

693   period.

694   Additionally, NTBC was diluted in sucrose 10% (w/v) to a final

695   concentration of 0.01 mg/ml and subjected to ten freeze-thaw cycles. Following

696   temperature stress, one NTBC volume was mixed with nine volumes of sterile

697   defibrinated horse blood (final concentration: 0.001 mg/ml) and these spiked

698   bloodmeals were fed to tsetse. Mortality was monitored over 24 hours.

699

700   **Mathematical modelling**

701   A discrete time (1-day time step) compartmental model was constructed to

702   describe the key processes underlying the transmission of *Trypanosoma brucei*

703   *gambiense*. This parasite has a broad reservoir of host animals, so these were

704   categorised according to whether they were livestock or wildlife. The transmission

705   between three host types in total (humans, livestock and wildlife) and the tsetse

706   fly vector was simulated (see Supplementary Information). The impact of vector

707   biting behaviour was examined by apportioning the split of bites on humans,

708   livestock and wildlife randomly across the full possible range (500 iterations) and,

29

709   through simulation, determining the frequency of NTBC application required to

710   drive the basic reproduction number (R0) below unity.

711

712   **Statistical analysis**

713   Tsetse survival was scored daily post-treatment. Statistical analysis and

714   graphics were performed using Prism 6.0 software (GraphPad Software, San Diego,

715   CA). The data from multiple experiments were combined into a single graph. The

716   log-rank (Kaplan-Meier) test was used to evaluate significant differences in

717   survival between the experimental and control groups. Dose-response curves

718   were done using a plot of non-linear fit for log of inhibitor vs. normalized response

719   (variable slope). LD_{50} and LC_{50} were calculate using Probit analysis (POLO Plus

720   version 2.0). The exact *n* (number of insects) in each experimental replicate used

721   to calculate the statistic in different experiments are detailed in table S1.

722

723   **Acknowledgements**

735

736

Author contributions

MS: conceived the project, designed and performed experiments and wrote the paper. **LRH**: designed and performed experiments and wrote the paper. **AC-S**: performed the experiments with *T. brucei*-infected flies and microscopy analysis. **VOD and DM**: performed *in vivo* experiments. **RJVA and SMB**: performed the experiments with bees. **CR**: performed the phylogenetic analysis and related experiments. **MS, MSS, JM, SQ and SW**: performed metabolomics and analysed the data. **NGE:** performed the experiments related to NTBC stability. **HI and MIP**: performed P450 assays. **LY**: performed the mathematical modelling. **PLO**: conceived the project and provided financial support. **AAS**: conceived the project, designed the experiments, wrote the paper and provided financial support. All authors were involved in proofreading and manuscript correction.

Competing interests. The authors declare no competing financial interests.

Figure legends

Fig. 1. Inhibiting tyrosine catabolism is lethal for tsetse, but not for bees.
a Tyrosine catabolism pathway. PAH: Phenylalanine hydroxylase; HgD: Homogentisate 1,2-dioxygenase; MAAI: Maleylacetoacetate isomerase; FAH: Fumarylacetoacetase. **b** Survival of *G. m. morsitans* injected with dsRNA for TAT and HPPD. Two independent experiments were performed, each with n=10-13, 12-16 and 12-14 for dsGFP (control-CTRL), dsTAT and dsHPPD, respectively. Five insects of each group were dissected on day 3 post-bloodmeal (PBM) to assess gene knockdown efficiency (Supplementary figure 2). **c** Survival of *G. m. morsitans* fed with horse blood supplemented with NTBC or PBS (Control. 9:1; v/v). Three to six independent experiments were performed for different doses, each with n =10-25 insects. **d** Dose-response curves for *G. m. morsitans* survival 24 hours PBM (Post-bloodmeal): NTBC feeding and topical application assays (Media ± SEM. All doses applied are shown in Supplementary figure 3B). **e** Male *G. pallidipes* survival after feeding on PBS- or NTBC-treated rats. Doses are presented as mg of NTBC per kg of rat body weight. Three independent experiment were performed for different doses, each with n=20-40. **f** Survival of NTBC topically treated *G. m. morsitans* after a bloodmeal. Three independent experiments were performed for different doses, each with n=10-20 insects.

31

769 **g** Bees survival maintained with 50% of sugar solution supplemented with NTBC (or PBS)
770 and fed with pollen. Two independent experiments were performed, each with n=25
771 insects**.**

772

773 **Fig. 2 Tsetse phenotypes observed upon NTBC treatment.**
774 **a** Fat Body. **b** Flight muscle. **c** Bright field (left) and fluorescence 3D-reconstructed
775 (middle) overviews of the anterior midgut (AM), proventriculus (PV), crop (C) and
776 oesophagus (E) from tsetse fed either without (Control) or with NTBC. Fluorescence
777 channels merge SiR-actin staining (yellow) and DAPI (magenta). Scale bars: 200 μm
778 (100X). Detailed sections (right) of transversal muscular fibres from the outer muscular
779 tissues in the anterior midgut region. Scale bars=20 μm (630X). **d** and **e** Death phenotype.
780 Flies are already dead and the abdomens are back and filled with undigested blood.

781

782 **Fig. 3 Metabolomic analysis of tsetse haemolymph upon NTBC treatment at different**
783 **times post-bloodmeal (0, 5 and 10 hours)**.
784 Five samples (in each group and for each time point) collected from two independent
785 experiments were processed for and analysed by mass spectrometry **a** Schematic
786 representation of the tyrosine catabolic pathway highlights the main metabolites
787 accumulated and the decreased production of final products. **b** Peak area normalized to
788 internal standards of differentially regulated metabolites in the tyrosine catabolism
789 pathway**. c** A heat map graphically presents the metabolites identified**. d** Schematic
790 representation of the metabolic pathways indicate metabolites that were differentially
791 regulated in NTBC-treated flies**.**

792

793 **Fig. 4 Simulation-based evaluation of the projected impact of NTBC for tsetse**
794 **control.**
795 The required frequency of applying NTBC to a human population **a** and to human and
796 livestock populations **b** is calculated to control *T. brucei* transmission. The axes denote the
797 proportional split of bloodmeals of the local tsetse vectors between humans, livestock and
798 wildlife. Each point (n=500) represents a random bloodmeal composition of the tsetse fly
799 population and its colour corresponds with the frequency (in days) of application required
800 to reduce the average basic reproduction number to below 1, as determined from a
801 compartmental transmission model.

802

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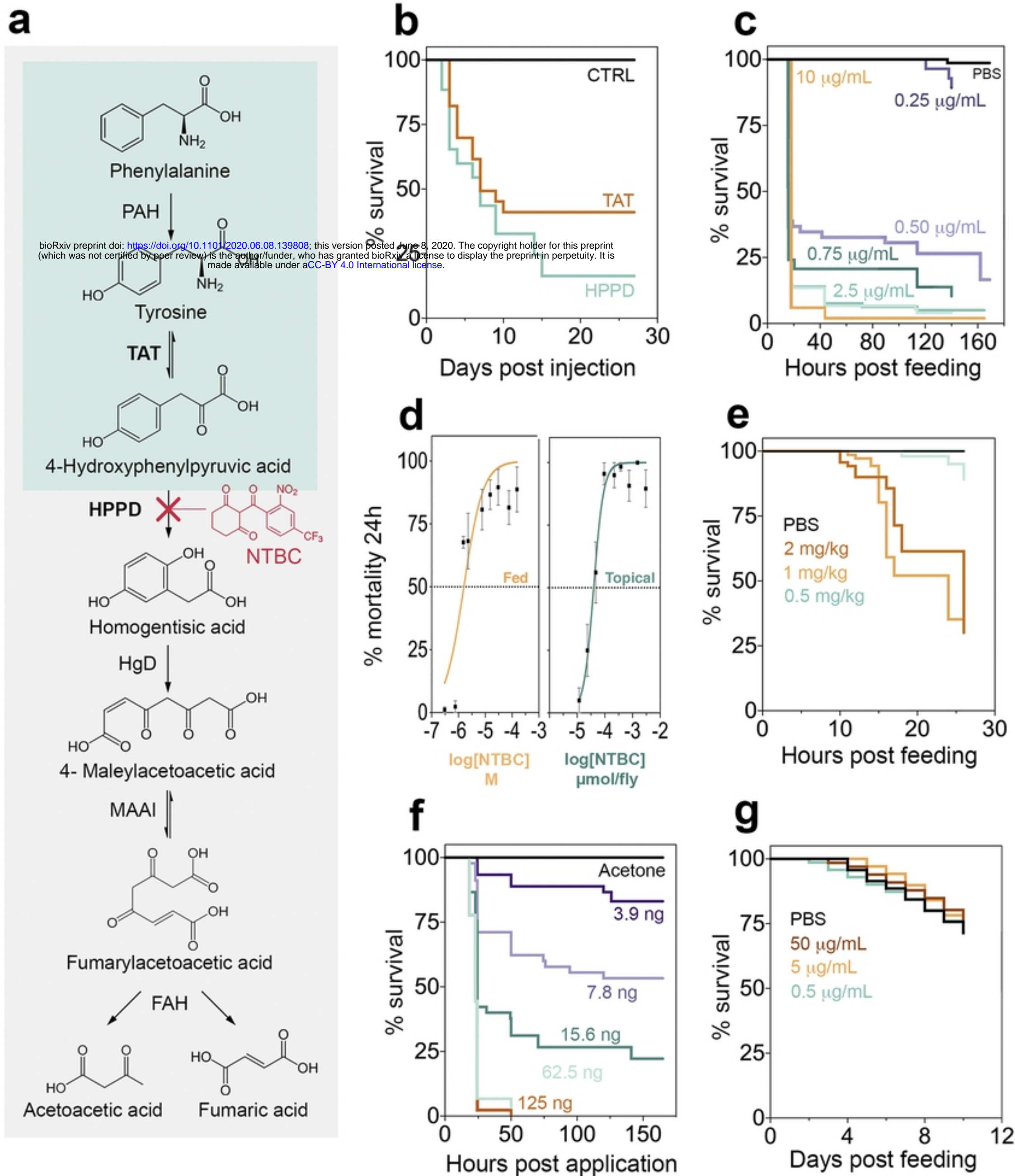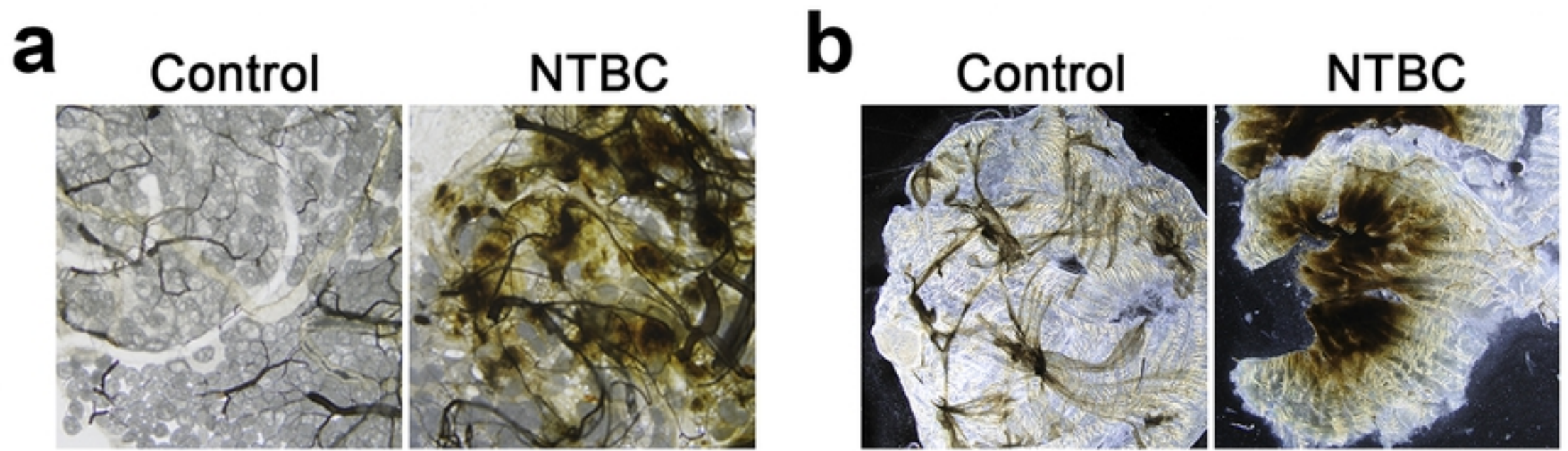
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Figure 1

Figure 2

Figure 3

Figure 4