Link Function for Binomial Model in Estimating Knockdown Time (KT95 and KT50) of Mosquito Repellents

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ABSTRACT: Estimation of knockdown time (KT) is useful in determining bio-effectiveness of mosquito repellents. Knockdown or not knockdown is a binary variable thus, analysis is done by fitting generalized linear models, based on binomial distribution. Use of appropriate link function in fitting a generalized linear model is crucial especially when estimating quantities such as KT50 and KT95. This study was done to determine the most appropriate link function in fitting generalized linear models to estimate KT50 and KT95. Knockdown activity of metofluthrin 0.005% (w/w) and d-trans-allethrin 0.12% (w/w) was tested under two different physiological conditions (blood fed and sucrose fed) using wild-caught female Culex tritaeniorhynchus mosquitoes from an agro-farming area of the north-western province of Sri Lanka. Coefficient of variation of the observed KT50 and KT95 was less than 5.5%. Both KT50 and KT95 values were estimated by fitting altogether 120 binomial distribution-based generalized linear models with three different link functions namely, logit, probit, and complementary log–log. The G2 statistic was used to test the goodness of fit of the models. However, in order to evaluate the accuracy of all estimated KT50 and KT95 values obtained using the above three link functions, they were compared against corresponding observed values using ANOVA followed by Dunnett mean separation procedure. The probit and logit link functions were found to be appropriate in the estimation of KT50. As the logit link function is commonly used in modeling binary responses, out of the two, logit link function is recommended. Complementary log–log link function was found to be the most appropriate in estimation of KT95. Thus, one link function cannot be recommended in estimating both KT parameters.

Keywords: Bio-effectiveness, generalized linear models, tolerance distribution

INTRODUCTION

The efficacy of an insecticide against particular insect is determined under laboratory and field conditions using various parameters. Out of them estimation of 50% cumulative knockdown (KT50) and 50% lethal dose (LD50) are widely applied parameters. Knockdown is the rapid paralysis of insects causing them to fall down and remain in a state as to be incapable of co-ordinate movements and apparently dead (SLS, 2001). Although KT50 is the popular concept in the comparison of knockdown patterns among different mosquito species, KT95 indicates the accepted maximum tolerance limit of the target insect species against particular concentration of an active ingredient. The generalized liner model based on

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binomial model with probit procedure is recommended for the calculation of KT values for
the testing of the efficacy of mosquito coils (WHO, 2013). Sri Lanka Standards (SLS)
453:2001 section E.5.1 instructs to analyze the obtained knock-down data using PROBIT
procedure, either implemented in a computer programme or PROBIT graph paper by plotting
proportion of knockdown versus knockdown time in minutes. Bliss (1934) reported using
probit link function but logit and complementary log-log link functions have also been used
in fitting of binomial models. Although it is well established that quantities such as LD$_{50}$ is
estimated by fitting binomial models based on probit link function, no adequate literature is
available on appropriate link function for binomial models when establishing KT in general.
Estimating quantities KT$_{50}$ and KT$_{95}$ is crucial because bio-effectiveness of mosquito
repellent product are adjusted specifically based on KT$_{95}$. The objective of this study was to
recommend the best link function for binomial models when estimating KT$_{50}$ and KT$_{95}$ using
three types of link functions namely probit, logit and complementary log-log.

MATERIALS AND METHODS

Data Collection
Two types of commercially available (bio-efficacy approved) mosquito coils containing
metofluthrin 0.005%w/w and d-trans- allethrin 0.12%w/w as active ingredient were used for
the study. A rural area with large paddy fields in Kuliapitiya of Kurunegala district was
selected for the collection of mosquitoes. Cattle baited net trap was used as the sole method
of sampling the test mosquitoes. Mosquitoes belonged to Culex tritaeniorhynchus (a known
vector of Japanese encephalitis in Sri Lanka) found within the cattle traps was used for the
study. From these mosquitoes samples of 20 blood fed and 20 sucrose fed mosquitoes were
exposed to a coil (without active ingredient) to ensure the suitability of them for the efficacy
testing. KT$_{50}$ and KT$_{95}$ was estimated following standard procedure (SLS, 2001) against two
active ingredients metofluthrin 0.005%w/w and d-trans- allethrin 0.12%w/w. Thus KT was
measured under four conditions viz: (i) metofluthrin– blood fed (ii) metofluthrin-sucrose fed
(iii) d-trans-allethrin–blood fed (iv) d–trans-allethrin–sucrose fed under each condition 10
packs were tested. Accordingly three were 40 KT sets.

Model fitting
The general form of the models fitted was $g(x) = \theta_0 + \theta_1 x$ where $g(x)$ denotes the link
function. Link functions considered in the study were logit, probit, and complementary log–
log and they are respectively of the form $\ln\left(\frac{p}{1-p}\right)$, $\Phi^{-1}(p)$, $\ln\{-\ln(1-p)\}$ where $p$ is proportion knockdown and $\Phi^{-1}$ indicates inverse cumulative
standard normal distribution. The variances of the cumulative distribution functions are not
same. In fact the means and the variance of the three distributions, probit, logit and
complementary log–log are respectively $(0, 1)$, $(0, \pi^2/3)$ and $(-\nu, \pi^2/6)$ where $\nu$ is the
Euler constant (Bilder, 2010; Gourdon & Sebah, 2004).

Models were fitted for the data using above three link functions and thus altogether 120
models were fitted. The goodness of fit of the fitted models was evaluated using $G^2$ statistics
(McCullagh & Nelder, 1989). Mean KT$_{50}$ and KT$_{95}$ values estimated from the fitted models.
These estimates were compared with the observed mean KT$_{50}$ and KT$_{95}$ values using one
way ANOVA followed by Dunnett mean separation technique using observed mean as the
control.
RESULTS AND DISCUSSION

The summary of the goodness of fit ($G^2$) with 19°th degree of freedom (df) for 120 fitted models are represented in Table 1. According to the Table 1, all fitted models were adequate (P>0.05) and thus models with any of the three link functions is able to capturing the variability of the response variable. Thus a model with any of those link functions can be considered in estimating important quantities.

Table 1. $G^2$ for different models fitted for KT under different active ingredients and feed

| AI     | Pack no | Feed | Probit | Logit | Clog-log | AI     | Pack no | Feed | Probit | Logit | Clog-log |
|--------|---------|------|--------|-------|----------|--------|---------|------|--------|-------|----------|
| 1      | BF      | BF   | 5.2303 | 2.1639 | 3.7693   | 2      | BF      | 2.3184 | 3.4197 | 5.7782 |
|        |         | (0.9992) | (1.0000) | (0.9999) |         |         | (1.0000) | (1.0000) | (0.9984) |         |
| 2      | BF      | BF   | 2.1639 | 3.3891 | 4.3410   | 2      | BF      | 1.9535 | 2.6837 | 1.9450 |
|        |         | (1.0000) | (1.0000) | (0.9998) |         |         | (1.0000) | (1.0000) | (1.0000) |         |
| 3      | BF      | BF   | 11.9712| 13.7972| 9.3732   | 3      | BF      | 8.2593 | 10.8180| 10.3368|
|        |         | (0.8869) | (0.7954) | (0.9668) |         |         | (0.9839) | (0.9298) | (0.9441) |         |
| 4      | BF      | BF   | 6.6822 | 8.2702 | 6.2366   | 4      | BF      | 3.3030 | 3.5562 | 14.3968|
|        |         | (0.9957) | (0.9837) | (0.9973) |         |         | (1.0000) | (1.0000) | (0.7601) |         |
| 5      | BF      | BF   | 9.7264 | 11.0366| 6.8717   | 5      | BF      | 4.9822 | 4.9073 | 5.0586 |
|        |         | (0.9594) | (0.9226) | (0.9949) |         |         | (0.9994) | (0.9995) | (0.9994) |         |
| 6      | BF      | BF   | 7.9745 | 9.8577 | 6.9496   | 6      | BF      | 3.1933 | 3.9097 | 12.9433|
|        |         | (0.9869) | (0.9564) | (0.9945) |         |         | (1.0000) | (0.9999) | (0.8415) |         |
| 7      | BF      | BF   | 4.2677 | 5.3684 | 3.1086   | 7      | BF      | 6.3792 | 4.9090 | 16.4541|
|        |         | (0.9998) | (0.9990) | (1.0000) |         |         | (0.9969) | (0.9995) | (0.7004) |         |
| 8      | BF      | BF   | 4.5799 | 6.2611 | 5.3419   | 8      | BF      | 3.9876 | 5.0892 | 9.6096 |
|        |         | (0.9997) | (0.9997) | (0.9991) |         |         | (0.9999) | (0.9994) | (0.9619) |         |
| 9      | BF      | BF   | 3.7274 | 5.0256 | 2.6521   | 9      | BF      | 4.8244 | 5.5641 | 15.3288|
|        |         | (0.9999) | (0.9994) | (1.0000) |         |         | (0.9996) | (0.9988) | (0.7015) |         |
| 10     | BF      | BF   | 5.6724 | 7.3829 | 4.7328   | 10     | BF      | 7.3320 | 5.2610 | 15.3979|
|        |         | (0.9986) | (0.9919) | (0.9996) |         |         | (0.9922) | (0.9992) | (0.7046) |         |
| 1      | SF      | SF   | 5.3333 | 5.4383 | 11.1028  | 1      | SF      | 8.0300 | 10.3050| 10.7950|
|        |         | (0.9991) | (0.9990) | (0.9203) |         |         | (0.9866) | (0.9522) | (0.9305) |         |
| 2      | SF      | SF   | 1.6766 | 2.5060 | 4.3410   | 2      | SF      | 4.5714 | 5.5577 | 10.7930|
|        |         | (1.0000) | (1.0000) | (0.9999) |         |         | (0.9997) | (0.9988) | (0.9306) |         |
| 3      | SF      | SF   | 3.8297 | 5.0283 | 3.9363   | 3      | SF      | 7.0976 | 9.4034 | 11.3571|
|        |         | (0.9999) | (0.9994) | (1.0000) |         |         | (0.9937) | (0.9662) | (0.9112) |         |
| 4      | SF      | SF   | 2.3637 | 2.6843 | 3.9933   | 4      | SF      | 2.1640 | 2.2762 | 6.1370 |
|        |         | (1.0000) | (1.0000) | (0.9999) |         |         | (1.0000) | (1.0000) | (0.9976) |         |
| 5      | SF      | SF   | 2.7312 | 3.6469 | 3.4862   | 5      | SF      | 4.6195 | 6.4318 | 5.8370 |
|        |         | (1.0000) | (0.9999) | (1.0000) |         |         | (0.9997) | (0.9967) | (0.9983) |         |
| 6      | SF      | SF   | 2.7312 | 3.6469 | 3.4862   | 6      | SF      | 4.8686 | 6.0856 | 11.3985|
|        |         | (1.0000) | (0.9999) | (1.0000) |         |         | (0.9997) | (0.9977) | (0.9097) |         |
| 7      | SF      | SF   | 2.7312 | 3.6469 | 3.4862   | 7      | SF      | 6.8218 | 8.0381 | 3.4579 |
|        |         | (1.0000) | (0.9999) | (1.0000) |         |         | (0.9951) | (0.9863) | (1.0000) |         |
| 8      | SF      | SF   | 3.8297 | 5.0283 | 3.9363   | 8      | SF      | 5.9376 | 5.5777 | 19.9068|
|        |         | (0.9999) | (0.9994) | (1.0000) |         |         | (0.9981) | (0.9988) | (0.8509) |         |
| 9      | SF      | SF   | 5.3333 | 5.4383 | 11.1028  | 9      | SF      | 2.3184 | 3.8791 | 6.3330 |
|        |         | (0.9991) | (0.9990) | (1.0000) |         |         | (1.0000) | (0.9999) | (0.9970) |         |
| 10     | SF      | SF   | 1.6766 | 2.5060 | 3.6447   | 10     | SF      | 7.4209 | 9.2105 | 12.3395|
|        |         | (1.0000) | (0.9999) | (0.9999) |         |         | (0.9916) | (0.9698) | (0.8706) |         |

Active ingredient (AI), Blood fed (BF), Complementary log-log (clog-log), d-trans-allethrin (DT) metofluthrin (MT), Sucrose fed = (SF). The values in parenthesis are the significant probability levels (p).
Fig. 1. Coefficient of variability of observed KT$_{50}$ and KT$_{95}$

The coefficient of variability (CV) of the observed KT$_{50}$ and KT$_{95}$ are shown in Fig 1. All CVs are below 5.5%, indicating that data have been generated under well controlled conditions and thus even a minor effect can be detected. The CV of the blood fed mosquitoes was relatively lower than that of the sucrose fed mosquitoes. The CV of the KT$_{95}$ is higher than that of KT$_{50}$, for a given feed type and for a given active ingredient.

Fig.2a. Box and whisker plots of medians of the estimated KT$_{50}$ and KT$_{95}$ values of the three different link functions against 0.12% w/w d–trans–allethrin for blood fed
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and sucrose fed mosquitoes considering median of the observed KT$_{50}$ and KT$_{95}$ as the control. (n=10) (P=probit, L=logit, C=complementary log–log, obs=observed)

**Fig. 2b.** Box and whisker plots of medians of the estimated KT$_{50}$ and KT$_{95}$ values of the three different link functions against 0.005% w/w metofluthrin for blood fed and sucrose fed mosquitoes considering median of the observed KT$_{50}$ and KT$_{95}$ as the control. (n=10) (P=probit, L=logit, C=complementary log–log, obs=observed)

Comparison of estimated median values of KT$_{50}$ and KT$_{95}$ using three different link functions are given in Figs. 2a and 2b. Fig. 2a corresponds to active ingredient 0.12%w/w d–trans–allethrin and Fig. 2b corresponds to active ingredient 0.005% w/w metofluthrin. Observed KT$_{50}$ and KT$_{95}$ values were considered as controls. From the Figs. 2a and 2b, it is apparent that KT$_{50}$ and KT$_{95}$ values are well separated as expected.
Table 2. Results of the mean comparison

| KT   | Active Ingredient | Fed status | p value   | F    | Link function     | Dunnett test |
|------|-------------------|------------|-----------|------|-------------------|--------------|
|      | KT<sub>50</sub>   |            |           |      |                   |              |
|      | DT                | BF         | <0.0001   | 14.96| Probit            | ***          |
|      | SF                | <0.0001    | 13.8      | Probit | Logit            | ***          |
|      |                   |            |           |      | Complementary log–log |              |
|      | MT                | BF         | 0.0005    | 7.47 | Probit            | ***          |
|      | SF                | 0.0465     | 2.07      | Probit | Logit            | ***          |
|      |                   |            |           |      | Complementary log–log |              |
|      | KT<sub>95</sub>   |            |           |      |                   |              |
|      | DT                | BF         | <0.0001   | 13.58| Probit            | ***          |
|      | SF                | <0.0001    | 13.64     | Probit | Logit            | ***          |
|      |                   |            |           |      | Complementary log–log |              |
|      | MT                | BF         | <0.0001   | 14.96| Probit            | ***          |
|      | SF                | 0.1903     | 2.11      | Probit | Logit            | ***          |
|      |                   |            |           |      | Complementary log–log |              |

*** There is a significant mean difference when compared with the observed values.
According to the Table 2, means of estimated KT\textsubscript{50} under four different conditions i.e. DT/BF, DT/SF, MT/BF, MT/SF using three different link functions are different (P<0.005) except for MT/SF (P=0.0465). Further, Dunnett mean separation revealed that mean of estimated KT\textsubscript{50} using complementary log-log link function was significantly different from the observed mean KT\textsubscript{50}.

With estimated KT\textsubscript{95} under same four conditions using three different link functions also shows that there is a significant mean difference (P<0.0001) except for condition MT/SF (P=0.1903). According to Dunnett mean separation results the means of estimated KT\textsubscript{95} using probit and logit link functions were significantly different from the observed mean KT\textsubscript{95}.

**CONCLUSION**

The estimate of KT values is comparatively an easy practice but not routinely applied for monitoring of susceptibility except in the specialized entomological laboratory testing (in the field and under insectary conditions). To achieve the recommended accuracy and precision of KT of specific vector mosquitoes, it is necessary to have both specialized entomological skills and the appropriate statistical procedures. The KT\textsubscript{95} indicates the accepted maximum tolerance limit of the target insect species against to a particular concentration of an active ingredient and it is more sensitive to the development of insecticide resistance. Therefore the accurate estimation of KT\textsubscript{95} is important in early detection of insecticide resistance. In general, KT values are estimated by fitting binary regression models with probit link function. However, from this study it can be concluded that complementary log-log link function is more appropriate to estimate KT\textsubscript{95} for *C. tritaeniorhynchus*. Both probit and logit link functions are appropriate in the estimation of KT\textsubscript{50} for the same mosquito population. However, out of the two, logit link function is recommended due to the reasons mentioned earlier. Therefore a single link function is not recommended for calculation of KT values under different conditions.

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