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EVALUATION OF THE LABORATORY RABBIT MODEL FOR SCREENING TOPICAL MOSQUITO REPELLENTS[1]

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ABSTRACT. The laboratory rabbit was evaluated as a model for screening topical mosquito repellents, using data obtained in tests of deet (*N, N*-diethyl-3-methylbenzamide) against *Aedes aegypti* on humans and rabbits. Host-specific differences in the action of the test material were quantified by multiple regression analysis. The test material was less effective but more persistent in tests on rabbits, and responses of the mosquito test population were more variable.

Although rabbits have been used in studies of repellents since the 1920s (Kawamura 1926), no specific evaluation of the rabbit model has been published. Buescher et al. (1982) introduced a dose–response method for testing repellents against *Lutzomyia longipalpis* (Lutz and Neiva) (Diptera: Psychodidae) on laboratory rabbits. Adaptations of the test were subsequently used in tests against *Phlebotomus papatasi* Scopoli (Diptera: Psychodidae) (Wirtz et al. 1986), *Aedes aegypti* (Linn.) and *Anopheles albimanus* Wiedemann (Mehr et al. 1985, Rutledge et al. 1996), *Glossina morsitans* Westwood (Diptera: Glossinidae) (Wirtz et al. 1985), and *Rhodnius prolixus* Stal (Heteroptera: Reduviidae) (Buescher et al. 1985). The present report provides an evaluation of the laboratory rabbit model of Buescher et al. (1982).

Rutledge et al. (1994) determined the multiple regression of percentage of *Ae. aegypti* biting human volunteers (probit scale) on the dose of deet (*N, N*-diethyl-3-methylbenzamide) applied (logarithmic scale) and the elapsed time from the time of application. To determine the equivalent values in tests on laboratory rabbits, we used the data of Rutledge et al. (1996) for deet in ethanol (treatments) and ethanol only (control).

Doses applied in the study were calculated in units of mg/cm^2 from the stated concentrations and rates of application and transformed to the logarithmic scale for analysis. The percentage of effect of each dose was estimated by the method of Abbott (1925) from the biting rates on the treatments and controls and transformed to the probit scale for analysis. Adjusted values for 0 and 100% observations were calculated as indicated by Armitage (1971). The multiple regression of percentage of effect (probit scale) on the dose applied (logarithmic scale) and the elapsed time from the time of application was determined by standard methods (Steel and Torrie 1980).

The multiple regression equation obtained in the study was

$$y = 5.7137 + 0.8111X_1 - 0.0885X_2, \quad (1)$$

in which y is the response of the mosquito test population to deet in tests on rabbits (probits), X_1 is the applied dose (log mg/cm^2), and X_2 is the elapsed time from time of application (h). Standard errors of the y-intercept and partial regression coefficients were 0.2156, 0.3824, and 0.0170, respectively. The coefficient of multiple correlation was 0.5644. Parameters are associated with 58 degrees of freedom and are significant at the 5% level.

The multiple regression equation obtained by Rutledge et al. (1994) in tests of deet on human volunteers was

$$Y = 13.1240 + 3.3807X_1 - 1.6084X_2, \quad (2)$$

in which Y is the response of the mosquito test population to deet in tests on humans (probits). Standard errors of the Y-intercept and partial regression coefficients were 0.6236, 0.2711, and 0.1180, respectively. The coefficient of multiple correlation was 0.9868. Parameters are associated with 5 degrees of freedom and are significant at the 5% level.

Subtracting equation (1) from equation (2) gives

$$Y - y = 7.4103 + 2.5696X_1 - 1.5199X_2, \quad (3)$$

which shows that the mosquito population response in tests on laboratory rabbits differs from the mosquito population response in tests on humans by an amount equal to $7.4103 + 2.5696X_1 - 1.5199X_2$ on the probit scale.

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Values of the *Y*- and *y*-intercepts (equations 1 and 2) represent the expected response when X_1 and X_2 are 0, i.e., when a unit dose of 1 mg/cm^2 (log 1 = 0) is tested immediately after application (0 h). The difference of the *Y*- and *y*-intercepts (equation 3) is positive, indicating that the test material (deet) is less effective against *Ae. aegypti* in tests on rabbits than in tests on humans.

Values of the partial coefficients of regression on dose (equations 1 and 2) are positive, reflecting the expected increase in the percentage of mosquitoes repelled with increasing dose. The difference of the partial coefficients of regression on dose (equation 3) is positive. Because the reciprocal of the slope of the dose–response line is equal to the standard deviation of the response to the test material, this result indicates that the response of *Ae. aegypti* to deet is more variable in tests on rabbits than in tests on humans.

Values of the partial coefficients of regression on time (equations 1 and 2) are negative, reflecting the expected decrease in the percentage of mosquitoes repelled with increasing time from the time of application. The difference of the partial coefficients of regression on dose (equation 3) is negative, indicating that the test material (deet) is more persistent in tests on rabbits than in tests on humans.

Similar results were obtained by Rutledge et al. (1994) in tests on humans and laboratory mice: Test materials were less effective but more persistent in tests on rabbits, and the responses of *Ae. aegypti* to the test materials were more variable. Similarly, Andreev et al. (1958) reported that terpineol and diphenyl oxide provided 1–4 days of protection against biting Diptera in tests on horses and cattle while providing only 3–6 h protection in tests on humans.

Such host-specific differences in the action of repellents may be related to differences in pigmentation, development of the hair, presence or absence of sweat glands, and other properties of the skin (Sokolov 1982). These differences do not preclude the use of animals in repellent tests if the resulting differences in the results obtained can be quantified (equations 1–3).

This study adds the rabbit to the list of laboratory animals that have been evaluated as animal models for screening topical mosquito repellents. Previous evaluations have included the guinea pig (Kasman et al. 1953), the hairless dog (Hill et al. 1979), and the mouse (Rutledge et al. 1994).

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REFERENCES CITED

Abbott, W. S. 1925. A method for computing the effectiveness of an insecticide. J. Econ. Entomol. 18: 265–267.

Andreev, K. P., G. I. Yanovich, G. A. Kudryavtseva and R. G. Soboleva. 1958. New insect repellents for protection of humans and animals from blood-sucking flies, mosquitoes, midges, and gnats. Tr. Vses. Nauchno-Issled. Inst. Vet. Sanit. 13:152–172. [In Russian.]

Armitage, P. 1971. Statistical methods in medical research. Blackwell Scientific, Oxford, England.

Buescher, M. D., L. C. Rutledge, R. A. Wirtz and J. H. Nelson. 1985. Laboratory repellent tests against *Rhodnius prolixus* (Heteroptera: Reduviidae). J. Med. Entomol. 22:49–53.

Buescher, M. D., L. C. Rutledge, R. A. Wirtz, K. B. Glackin and M. A. Moussa. 1982. Laboratory tests of repellents against *Lutzomyia longipalpis* (Diptera: Psychodidae). J. Med. Entomol. 19:176–180.

Hill, J. A., P. B. Robinson, D. L. McVey, W. A. Akers and W. G. Reifenrath. 1979. Evaluation of mosquito repellents on the hairless dog. Mosq. News 39: 307–310.

Kasman, S., L. A. O. Roadhouse and G. F. Wright. 1953. Studies in testing insect repellents. Mosq. News 13:116–123.

Kawamura, R. 1926. Studies on tsutsugamushi disease (Japanese flood fever). Med. Bull. College Med. Univ. Cincinnati 4:1–229.

Mehr, Z. A., L. C. Rutledge, E. L. Morales, V. E. Meixsell and D. W. Korte. 1985. Laboratory evaluation of controlled-release insect repellent formulations. J. Am. Mosq. Control Assoc. 1:143–147.

Rutledge, L. C., R. K. Gupta, R. A. Wirtz and M. D. Buescher. 1994. Evaluation of the laboratory mouse model for screening topical mosquito repellents. J. Am. Mosq. Control Assoc. 10:565–571.

Rutledge, L. C., R. K. Gupta, Z. A. Mehr, M. D. Buescher and W. G. Reifenrath. 1996. Evaluation of controlled-release mosquito repellent formulations. J. Am. Mosq. Control Assoc. 12:39–44.

Sokolov, V. E. 1982. Mammal skin. Univ. of California Press, Berkeley.

Steel, R. G. D. and J. H. Torrie. 1980. Principles and procedures of statistics, 2nd ed. McGraw-Hill Book Co., New York.

Wirtz, R. A., E. D. Rowton, J. A. Hallam, P. V. Perkins and L. C. Rutledge. 1986. Laboratory testing of repellents against the sand fly *Phlebotomus papatasi* (Diptera: Psychodidae). J. Med. Entomol. 23:64–67.

Wirtz, R. A., L. W. Roberts, J. A. Hallam, L. M. Macken, D. R. Roberts, M. D. Buescher and L. C. Rutledge. 1985. Laboratory testing of repellents against the tsetse *Glossina morsitans* (Diptera: Glossinidae). J. Med. Entomol. 22:271–275.