原　著

Some Simplified Methods for the Evaluation of the Effectiveness of Fly Repellents in Laboratory and Outdoors. Insect Repellents and Attractants. XI. Yasunosuke IKEDA (Research Section of Yasugawa Plant, Sankyo Co., Ltd. Yasu-cho, Shiga Pref.). Received Oct. 1, 1959. *Botyu-Kagaku*, **24**, 175, 1959.

34. 屋外及び屋内に於ける蠅忌避剤の効力評価法について　忌避剤・誘引剤について　第11報

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屋外及び屋内における蠅忌避剤の効力試験法について報告した．統計的解析の結果，これらの方法が正確でそれぞれの異った試験から得られた成績がたがいに比較できることを知った．供試した忌避剤はクラグ・フライ・リペレント，MGK リペレント 11，タブトレックス，及びヂエチルトルアマイドの4種類である．

Some methods evaluating the effectiveness of certain fly repellents in laboratory and field tests were reported. The results of the statistical analyses showed that these methods were precise and reliable, so that the data from different tests could be compared with each other. The repellents adopted in the present tests were four commercial products such as Crag fly repellent, MGK repellent 11, Tabutrex, and diethyl-toluamide.

Various methods for testing the repellent or attractive properties of any material have been developed by many workers[2,7,8], while there have been few methods for evaluating the effect of certain fly repellents in practice. In this problem, it is often necessary to use intricate apparatus in those operations. Even if an instrument is an elaborate one, the method unfit to practical use will serve but little.

In the present paper, the author reports some simplified methods for evaluating the efficiencies of certain fly repellents in laboratory and outdoors, and attempts to apply probit method to the analysis of the data of repellent tests.

The author wishes to express his appreciation to Prof. O. Shinoda, Osaka University of Liberal Arts, for his kind guidance and encouragement given to him in the course of the present work. The author is also indebted to the executives of Sankyo Co., Ltd. for their helps and kind intentions.

Material

The repellents adopted in the experiments are four commercial products which have been widely investigated, viz., Crag fly repellent (butoxypolypropylene glycol), MGK repellent 11 (2, 3, 4, 5-bis (Δ^2 butylene) tetrahydrofurfural), Tabutrex (di-n-butyl succinate), and diethyltoluamide. Each of these samples is dissolved in acetone.

The material used is the adults of the common housefly, *Musca domestica vicina* Macq., which has been bred in this laboratory.

Laboratory Method I-Test with a Single Dose

There is a need often for evaluating the effectiveness of a single dose of a certain repellent. Here, the criterion of repellency is based on the amount of feeding for lactose pellets deposited on two papers, one of which is treated with test repellent while the other is untreated. The flies are confined into the cage containing such two papers. If the repellent is effective, the flies do not approach to the treated paper, consequently, no or lower consumption of the pellet is expected, whereas if the repellent is ineffective to flies, they will eat the pellets put on both treated and untreated papers.

After the exposure of 20 hours the pellets are removed and weighed. The percentage of repellency is computed by the following formula:

$$\text{Per cent repellency} = (C-T)/C \times 100,$$

where T is the feeding amount (mg) of lactose

on treated paper, and C is that of untreated one.

Experiment: An acetone solution containing a given amount of test sample was applied at a rate of 0.31cc per filter paper of 6cm in diameter. The treated paper was kept at room temperature, and, after the main part of the solvent had evaporated off, about 50mg of lactose pellet was placed in the center of the paper. At the same time, untreated paper with lactose pellet was provided. A pair of the treated and untreated papers was placed in the test cage consisted of glass dish, 13cm in diameter and 6cm in height, with a netted cover in the ceiling.

The tests were made with the separate sexes, 20 adults being used in each test. The flies tested were 3 or 4 days old, and they were starved for about 4 hours before testing, in order to secure the consumption of the lactose pellet. The tests were conducted in a dimly-lit place. The results are given in Table 1.

Laboratory Method II- Test with Multiple Doses

This method is useful not only to compare the effects of different repellents at the same time, but also to determine the order of effectiveness of several doses as well.

The principle of the method is nearly the same as that mentioned above, but this is rather compulsory feeding than the former.

Experiment: The test cage is consisted of a glass cylinder, 25cm in diameter and 15cm in height, a metallic frame with wire net in the bottom, and glass cover with a hole in the ceiling.

Round pieces of filter paper, 6cm in diameter, were soaked with 0.31cc of acetone solutions containing the test sample at the desired concentration, and after evaporating the solvent, about 50mg of lactose pellet was placed in the center of each paper. Only one untreated paper with lactose pellet was placed in the center of the cage and several treated papers with the same pellets were placed around the untreated paper at random. One hundred flies of 3 or 4 days old were used in each test. Other procedures were similar to those of Method I.

After the exposure of 20 hours the pellets were removed and weighed. The percentage of repellency was computed by the following formula:

$$\text{Per cent repellency} = (C - Ti)/C \times 100,$$

where Ti is the feeding amount (mg) of lactose on each treatment, and C is that of untreatment. The results are given in Table 2.

Assessment of the Results

It is currently inferred that the repellent effect is induced by the chemical and physical properties of the material, and a insect responds to the stimuli through a certain sensory system. Since the repellency depends upon chemical or physical response of the insect, it can readily

Table 1. Per cent repellency of four materials tested with a single dose against the houseflies. (Exposure for 20 hours at 24°—28°C, relative humidity 75—80%)

Material	Sex	Dosage (mg/900cm²)					No. of flies used
		1000	500	250	125	62.5	
Crag fly repellent	♀	82.9%	68.8%	49.3%	45.0%	15.8%	200
	♂	65.4	58.1	51.9	32.0	20.4	200
Diethyltoluamide	♀	100.0	95.4	93.4	85.0	66.0	200
	♂	100.0	96.7	84.2	74.7	72.2	200
MGK repellent 11	♀	100.0	82.3	64.4	54.2	49.3	200
	♂	100.0	73.4	62.4	56.8	50.8	200
Tabutrex	♀	100.0	84.8	73.6	69.6	47.6	200
	♂	100.0	86.5	67.8	57.9	45.3	200

Table 2. Per cent repellency of four materials tested with various dosages against the houseflies at a time. (Exposure for 20 hours at 24°—28°, relative humidity 75—80%)

Material	Sex	Dosage (mg/900cm²)					No. of flies used
		1000	500	250	125	62.5	
Crag fly repellent	♀	60.3%	53.9%	47.2%	29.4%	0.0%	200
	♂	59.9	41.4	33.0	22.6	17.5	200
Diethyltoluamide	♀	100.0	99.7	97.5	84.2	79.0	200
	♂	100.0	99.7	93.0	85.9	58.4	200
MGK repellent 11	♀	97.0	93.1	75.4	58.6	46.3	200
	♂	100.0	92.1	75.4	57.4	46.9	200
Tabutrex	♀	98.8	91.1	79.8	50.8	28.6	200
	♂	95.3	91.0	79.4	61.7	37.1	200

inferable that there may exist a reciprocal relation between dosage and response of the insect.

In the present paper, in order to determine the precision of the methods and evaluate the relative effectiveness of test repellents, the data were analyzed by probit method according to Bliss[1,6]. The reason why probit method was applied to the analysis of the data is that a high degree of rectilinear correlation nearly always existed between the response and dosages when the percentages of repellency had converted into probit and plotted against the dosages in logarithm, and, therefore this linear transformation of dosage-response curve was suited for the purpose.

The results of analyses and the median effective dose for each repellent are given in Tables 3

Table 3. Dosage-repellency regression equations of adults of the common housefly, *Musca domestica vicina* Macq., for four commercial repellents resulted from the method for evaluating a single dose.

Sex	Repellent tested	Regression equation y=a+b (X−x)	Degree of freedom (n)	χ^2	Range of Pr.	ED-50 (mg/900cm²)
Female	Crag fly repellent	y=5.06442+1.46897(X−2.38906)	3	2.26113	0.70−0.50	221.41
	Diethyl toluamide	y=6.04542+1.64658(X−2.15558)	2	0.90110	0.70−0.50	33.17
	MGK repellent 11	y=5.51879+1.36163(X−2.33397)	2	4.15245	0.20−0.10	89.74
	Tabutrex	y=5.65342+1.42216(X−2.29819)	2	2.74904	0.30−0.20	68.98
Male	Crag fly repellent	y=4.86875+1.02824(X−2.41062)	3	0.50786	0.95−0.90	345.35
	Diethyl toluamide	y=5.96974+1.38485(X−2.20115)	2	3.10650	0.30−0.20	31.69
	MGK repellent 11	y=5.48797+1.11851(X−2.36317)	2	5.22315	0.10−0.05	84.51
	Tabutrex	y=5.53786+1.56749(X−2.29891)	2	2.88300	0.30−0.20	90.32

Table 4. Dosage-repellency regression equations of adults of the common housefly, *Musca domestica vicina* Macq., for four commercial repellents resulted from the method for evaluating multiple doses at the same time.

Sex	Repellent tested	Regression equation $y=a+b(X-\bar{x})$	Degree of freedom (n)	χ^2	Range of Pr.	ED-50 (mg/900cm²)
Female	Crag fly repellent	y=4.82639+0.85734(X−2.41818)	2	3.51590	0.20−0.10	417.51
	Diethyl toluamide	y=6.05448+1.82300(X−1.99739)	2	9.44857	0.01<	26.24
	MGK repellent 11	y=5.55122+1.67067(X−2.24149)	3	5.60174	0.20−0.10	81.58
	Tabutrex	y=5.34729+2.24122(X−2.21497)	3	3.40015	0.50−0.30	114.81
Male	Crag fly repellent	y=4.62888+0.97650(X−2.44968)	3	2.55541	0.50−0.30	675.66
	Diethyl toluamide	y=5.78982+2.37897(X−2.02786)	2	4.54023	0.20−0.10	49.64
	MGK repellent 11	y=5.52671+1.61552(X−2.23867)	2	5.68658	0.10−0.05	81.78
	Tabutrex	y=5.50347+1.73617(X−2.24149)	3	1.92434	0.70−0.50	89.44

and 4. In these tables, X represents logarithm of the applied dosage (mg) per 900cm², and Y represents the percentage of repellency in probit. As shown in the results, the test of *chi* squares showed that the assumption of parallelism in all tests was justified. Merely the datum of diethyltoluamide in the second tests with female flies does not fit the straight line. This might be due to a higher percentage of repellency occurred in the lowest dosage.

Effects of Age

In order to ascertain the effect of age of test flies to the results of the tests, the tests with adult flies at different ages were performed by using the first method. Individual flies used in the tests were 2, 4, and 6 days old respectively. In the test, only Tabutrex was used as test sample. The method and the test conditions were the same to those mentioned above. The results are given in Table 5. There was little difference in susceptibility among the different aged groups of flies, though the recently emerged adults were slightly sensitive than the aged adults to Tabutrex.

Table 5. Relation between the repellent efficiency and age of test insects. Repellent used was Tabutrex alone, and was applied at a rate of 250mg/900cm². Exposure for 20 hours at 24° to 28°, relative humidity 75—80%. Average of five replicates.

Age of insect	Repellency %	
	Female	Male
2 days old	91.4±17.1	94.2±17.9
4 days old	72.6±17.1	86.3±17.9
6 days old	76.0±17.1	86.3±17.9
F	3.6338<	0.6904<

$F^2_{12}=3.8853$, significant at 0.05 level.

Effects of the Sexes

As shown in Table 8, both sexes of housefly reacted differently to the same repellent.

Since the effectiveness of some repellents varied with the sexes, the results of the tests are not comparable unless the tests were made either with the groups having constant sexual ratio, or with separate sexes.

Factors Influencing the Performance of Repellent Tests

Size of lactose pellet : In these methods, the size of the lactose pellets was one of the important factors which affect the results. When the pellet used was greater than 80mg, the repellent effect could not evaluate precisely, since some flies can feed on the pellet without contact with the treated paper. Suitable size of the lactose pellet was about 50mg in weight.

The length of exposure : When the length of exposure was prolonged, the consumption of lactose pellet was clearly increased. In such a case, there was no difference in the rate of feeding on the treated and untreated pellets. It is undesirable that the length of the exposure is over a period of 24 hours, since the repellent matetial is more or less volatile. To secure the consumption of the lactose pellet, it is desirable to starve the flies for several hours before the start of experiment.

Illumination : Since housefly is very phototropic, an uneven illumination has an effect on the result of the test. In the present work, the tests were made in a dimly-lit place in order to avoid the reaction of houseflies to light.

Method of Field Test and Its Reliability

The details of the method were described in the preceding paper[4]. The outline of the method is as follows : the trap, consisted of domed glass bottle of 20cm in diameter, with a funnel opening of 7cm in diameter at the bottom. Small amout of water is poured in the circumambient trough at the base, so that flies which enter through the basal opening are unable to escape. The trap is placed on a filter paper treated with test repellent. Bait, mashed fresh fish entrails, is placed in a small dish underneath the funnel opening of the trap. Tests are conducted in an area heavily infested with flies. The collection is continued for the whole day. The collected flies are classified into large groups, while some species considered to be important as a vector or nuisance are identified closely. The criterion of repellency is based on the number of flies caught in each bottle. The percentage of effectiveness is computed by the following formula :

$$\text{Per cent repellency} = \frac{n_c/N - n_t/N}{n_c/N} \times 100,$$

where N is the total number of flies collected in all traps, and n_c is the numher of flies collected in the check, while n_t is that of test trap. In this manner, N is considered as an index related to the density of fly population during the test period in test area.

Experiment : Flies were collected every day during 10.00 to 16.00. In these collections, various species of flies were caught in the traps, while only one species, the common house-fly, was selected as the subject of the present work. The house-flies collected were reckoned without regard to sex. The results are given in Table 6.

Table 6. Comparative effectiveness of four commercial repellents against the houseflies in outdoor using the bait trapping method.

Material	Per cent repellency Dosage mg/900cm²					No. of flies caught
	1000	500	250	125	62	
Crag fly repellent	90.0	72.5	60.0	40.0	—	215
MGK repellent 11	—	94.1	91.2	70.6	55.9	166
Diethyl toluamide	—	98.3	94.9	84.7	71.2	266
Tabutrex	—	83.3	70.8	58.3	41.7	262

In order to examine the preciseness of the estimates, the data were subjected to probit analysis. From the results of the test of *chi* squares, it was concluded that the estimates were justified. Regression equations and the median effective doses of the repellents tested are given in Table 7.

Comparison of the Data from Various Testing Methods and Discussion

From the statistical view point, it may safely be said that, if the data are compared at the median effective dose, these methods are reasonably precise and dependable, so that the data from a series of tests, either in laboratory or in outdoors, can be compared with each other. However, the data obtained from different

Table 7. Dosage-repellency regression equations of adults of the common housefly, *Musca domestica vicina* Macq., for four commercial repellents in the range of dosage from 62 to 1000mg per 900cm² in outdoor tests.

Repellent tested	No. of flies caught	Regression equation $y=a+b\cdot(X-\bar{x})$	Degree of freedom (n)	χ^2	Range of Pr.	ED-50 (mg/900cm²)
Crag fly repellent	215	$y=5.30384+1.59561(X-2.44964)$	2	0.4949	0.80—0.70	181.63
Diethyl toluamide	266	$y=5.96458+1.72903(X-2.03643)$	2	0.1031	0.95—0.90	30.10
MGK repellent 11	166	$y=5.60548+1.68779(X-2.07760)$	2	0.9279	0.70—0.50	52.34
Tabutrex	262	$y=5.27812+1.27738(X-2.16834)$	2	0.0273	0.99—0.98	89.25

methods, at any arbitrary point, are difficult to compare, since the efficiency of repellent action (*b*) varies with the testing methods. As shown in Table 8, there was little difference in the *order* of effectiveness, whereas the *degree* of each effect varied with the testing methods. These phenomena may be due to a difference in response of flies to the bait used. The bait used in the laboratory tests was a taste material viz. lactose, while odorous materials such as mashed entrails of fish were used as fly attractants in field tests. In these methods, a measure of the effectiveness of test repellent is derived from its ability to prevent an insect from feeding on baits, either natural or chemical, and, therefore, it is natural that each bait has a

Table 8. Sexual difference of susceptibility in the houseflies and comparison of the order of effectiveness of four repellents at the ED-50 resulted from various testing methods.

Material tested	Method	Index of the order of effectiveness (log ED-50)		Median effective dose (mg/900cm²) (antilog)	
		Female	Male	Female	Male
Crag fly repellent	I	2.34521	2.53826	221.41	345.35
	II	2.62067	2.82973	417.51	675.66
	III	2.25920		181.63	
Diethyl toluamide	I	1.52068	1.50090	33.17	31.69
	II	1.41896	1.69586	26.24	49.64
	III	1.47856		30.10	
MGK repellent 11	I	1.95297	1.92691	89.74	84.51
	II	1.91156	1.91264	81.58	81.78
	III	1.71886		52.34	
Tabutrex	I	1.83874	1.95578	68.98	90.32
	II	2.06002	1.95151	114.81	89.44
	III	1.95062		89.25	

Method I: Test with a single dose. II: Test with multiple doses. III: Bait trapping in outdoor, irrespective of sex.

different influence upon the results.

Although it is difficult to indicate the repellent efficiencies in quantitative expression, there are some reasons to express the repellent effects in quantitative terms. In strict sense, if a repellent allows only one insect to alights on a surface which has been treated with it, it will be concluded that its effect has disappeared, or that it is not a repellent. Practically, however, if a large number of biting insects attack an untreated animal, whereas the treated animal has only a few, it will be concluded that it is efficacious material, bacause the repellent brings a greater comfort on the animal. The degree of repellent effect must be expressed in quantitative terms as in the estimation of an insecticidal effect.

The methods reported in the present paper may not be sufficient to solve the general problem of testing repellents, but they seem to be useful to evaluate the effect of some repellents in practice.

Finally, a convenient method, termed Sandwich-Method, for testing some fly repellents[3,5] is added.

Résumé

Some methods for evaluating the effectiveness of certain repellents against the common housefly, *Musca domestica vicina* Macq., were reported.

The data from various testing methods were fitted to provisional regression lines when the percentages of repellency had converted into probit and plotted against the dosages in logarithm. From the results of the analysis, it was concluded that these methods were so reliable that the data from a series of tests in laboratory or outdoors could be compared each other. However, the results of the tests were not directly comparable unless the comparison could be made at the median effective dose, since the slope of curve in each repellent varied with the testing methods. It is obvious that the bait used as fly attractants had some influences upon the difference of the results in each method.

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Synthesis of BHC-1-C^{14} and Separation of its Isomers. Mode of Action of BHC. I. Shoziro Ishii, Chisato Hirano (National Institute of Agricultural Sciences, Nishigahara, Tokyo) and Yoshio Tamaki (Tokyo University of Agriculture and Technology, Fuchu, Tokyo) Received Oct. 4, 1959. *Botyu-Kagaku*, **24**, 181, 1959 (with English résumé, 184).

35. BHC-1-C^{14} の合成並びに各異性体の分離*

BHCの作用に関する研究 I** 石井象二郎・平野千里（農林省農業技術研究所），玉木佳男***（東京農工大学農学部）

34. 10. 4 受理

BHC の植物，昆虫に対する作用を研究するため，C^{14} 標識 BHC を C^{14} 標識ベンゼンより合成し，放射性 α, β, γ, δ 異性体を分離し，その確認を行った．

緒　　言

BHC はわが国で最も多量に使われる殺虫剤であるが，作物，昆虫に対する作用は，DDT やパラチオンなどに比べて研究されていない．また BHC には各種の異性体があり，分子構造の違いによって著るしく昆

* 要旨を1959年4月日本応用動物昆虫学会大会で講演発表した．
** 文部省科学研究費による研究の1部である．
*** 現在農林省東海近畿農業試験場茶業部．