SOME CORRECTIONS TO THE RECORD ON INSECT REPELLENTS AND ATTRACTANTS[1]

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ABSTRACT. Fifteen apparent errors of observation, reporting, interpretation, or attribution occurring in the insect repellent and attractant literature were examined. Topics discussed are the boiling point effect, solvents and solutions, repellent-treated netting, terpineol and diphenyl oxide, lactic acid, the smell and feel of deet (diethylmethylbenzamide), effective half-life, protection time, protection time of deet for men and women, McGuire's formula, "plussing out", King's classification, exorbitant doses, extrapolated doses, and extreme observations. The decay constant (1.36 hr^{-1}) and half-life (0.51 hr) of a mosquito-repellent bath oil (Skin-So-Soft®) are reported for the first time.

INTRODUCTION

One of the main ethical responsibilities of an author is to "honestly relate [his] work to that of others" (Council of Biology Editors 1983). Similarly the historians Barzun and Graff (1985) state that "The first virtue required [of a researcher] is ACCURACY." Thus in both science and history great importance is attached to matters of attribution and priority.

In this paper I will attempt to correct certain errors of attribution and priority in the literature of insect repellents and attractants. In addition, I will discuss several apparent errors of fact, including two of my own, in the hope that I can thereby contribute to a better understanding of insect repellents and attractants.

DISCUSSION

Boiling point and protection time.[2] It appears that the functional relation of boiling point and protection time has been independently discovered at least three times. Bunker and Hirschfelder (1925) reported that none of their 20 best repellents had a boiling point below 150°C, and this "boiling point effect" has been studied by several workers since that time. Rayner and Wright (1966) and Johnson et al. (1967), however, did not relate their work in this area to that of any prior author, and they apparently believed their respective contributions to be original. In the case of Johnson et al. (1967) a subsequent press release (Anonymous 1969) confirms that this was in fact the case. In addition, Johnson and his coworkers subsequently (1968–80) published a series of 12 papers in the *Journal of Medicinal Chemistry*, the *Journal of Pharmaceutical Sciences*, and *Mosquito News* under the general title "Topical Mosquito Repellents." These papers contributed greatly to our knowledge of the boiling point effect, but made no reference to the original work in this area by Bunker and Hirschfelder (1925).

Solvents and solutions. The dialkyl phthalates were among the earliest synthetic repellents to be discovered (Moore and Buc 1929), and they are still widely used in both the laboratory (Michener 1946, Hocking 1960, Avivi 1961[3]) and the field (Smith 1963, World Health Organization 1984). They are also produced in industrial amounts for use in the manufacture of adhesives, coatings, plastic films and sheets, paper, ink, textiles, safety glass, linoleum, perfumes, explosives, rocket propellents, and other products.

However, the use of the dialkyl phthalates as solvents in dermal toxicity studies of DDT (Draize et al. 1944, Smith and Stohlman 1944) and laboratory trials of solid repellents (Linduska and Morton 1947, Travis 1950) raises important questions of interpretation. Draize et al. (1944) and Smith and Stohlman (1944) treated dimethyl phthalate and dibutyl phthalate as essentially nontoxic, but today we know that they are not. The oral LD_{50} of dimethyl phthalate in the rat is 6,900 mg/kg; that of dibutyl phthalate is 12,000 mg/kg (*Registry of Toxic Effects of Chemical Substances* 1983). The toxic effects of the phthalates are confounded with those of DDT in Draize's and Smith's reports, and this is perhaps why their reports are not cited in the *Registry*.

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[1] The opinions and assertions contained herein are the private views of the author and should not be construed as reflecting the views of the Department of the Army or the Department of Defense. Use of a trade name does not imply official approval or endorsement of the product named.

[2] Since boiling point and molecular weight are related properties of materials (Lyman et al. 1982), I will include both under this heading. Bunker and Hirschfelder (1925) and Johnson et al. (1967) used the boiling point; Rayner and Wright (1966) used the molecular weight.

[3] Avivi, A. 1967. The maintenance of colonies of argasid ticks. WHO/VBC/68.57:139.

Although Linduska and Morton (1947) and Travis (1950) recognized that the solvents they used (dimethyl phthalate and diethyl phthalate) were not inert, they did not adequately report or analyze the results they obtained. However, Linduska and Morton (1947) provided numerical data for one of the 45 solid repellents that they tested in dimethyl phthalate solution (Table 1). Assuming that the doses of dimethyl phthalate and *n*-butyl sulfone applied were roughly equivalent and that no extreme values were observed, the data of Table 1 can be interpreted in terms of a 2×2 factorial design. On this basis, *n*-butyl sulfone provided 45 min of protection in the presence of dimethyl phthalate and 426 min (7.1 hr) in its absence. The discrepancy indicates a large interaction between the solute, *n*-butyl sulfone, and the solvent, dimethyl phthalate. This interaction is estimated by the quantity $(0 + 318 - 273 - 426)/2 = -190.5$ min (-3.2 hr). Statistically, the interaction of *n*-butyl sulfone and dimethyl phthalate is symmetric, i.e., the interaction of *n*-butyl sulfone with dimethyl phthalate is the same as that of dimethyl phthalate with *n*-butyl sulfone. The interaction is a measure of the difference in the period of protection provided by *n*-butyl sulfone at the two levels of dimethyl phthalate or, conversely, the difference in the period of protection provided by dimethyl phthalate at the two levels of *n*-butyl sulfone. We cannot say whether dimethyl phthalate interferes with the action of *n*-butyl sulfone or *n*-butyl sulfone interferes with the action of dimethyl phthalate.

Note that this purely statistical conclusion agrees with Raoult's Law, which states that the vapor pressure of a substance in solution is proportional to its mole fraction. If both components of the solution are volatile, each lowers the vapor pressure of the other. If the vapor pressure of a repellent applied to the skin or to a fabric is depressed to a lower initial level, the deposit may decay to the threshold value in a shorter time, and its period of protection may be thereby curtailed. On the other hand, if the threshold level of the repellent is very low, the reduced evaporation rate can result in a longer period of decay to that level, and the period of protection provided by the repellent may be extended instead. Khan et al. (1975) reported that four perfume fixatives (ambrene, xylene musk, dinitrotriethylbutylbenzene and dibutylmethoxybenzaldehyde) significantly extended the protection time of deet but not of dimethyl phthalate, ethyl hexanediol, or Indalone. Kharitonova (1975) and Koshkina and Kharitonova (1976) reported that various solutes, including diethyl phthalate and benzyl benzoate, significantly extended the protection time of deet, dimethyl phthalate, and other repellents.

Table 1. Protection time (min) of *n*-butyl sulfone and dimethyl phthalate against *Aedes aegypti* as reported by Linduska and Morton (1947).

	Dimethyl phthalate	
n-butyl sulfone	+	−
+	318[a]	426[b]
−	273[c]	0[d]
Difference	45	426

[a] One ml of a 33% solution of *n*-butyl sulfone in dimethyl phthalate applied to the forearm; number of replications not stated.

[b] One ml of a 50% suspension of *n*-butyl sulfone in 96% water + 4% polysorbate 80 applied to the forearm; average of four replications.

[c] Native dimethyl phthalate applied to the forearm "at the same rate"; number of replications not stated.

[d] "The biting rate for *A. aegypti* was generally such that 50 to 75 bites were received on a bare untreated forearm in a 30-second exposure".

In conclusion, while the dialkyl phthalates can in principle be employed as solvents in toxicity studies and repellent trials, the design and analysis of such experiments must take into account both the activity of the solvent and its interaction with the solute. However, discussion of the older work has clarified some little-known principles of repellent action in solutions.

Extreme observations. Travis (1950) reported individual mean protection times of dimethyl phthalate against *Anopheles quadrimaculatus* Say (three test subjects), *Aedes aegypti* (Linn.) (three test subjects), and *Aedes sollicitans* (Walker) (six test subjects) and of 10 repellents, as a set, against *Aedes taeniorhynchus* (Wied.) (four test subjects).

The six means reported for dimethyl phthalate against *Ae. sollicitans* were 80, 105, 117, 147, 150, and 267 min. The mean of all six subjects was 144 min. However, Dixon's test for detection of extremes (Dixon and Massey 1969), which was not available when Travis' report was published, is significant for the mean protection time on Subject 2, 267 min:

$$r_{01} = (267-150)/(267-80) = 0.626;\ P = 0.03$$

In the absence of any indication of error this is perhaps best interpreted to mean that the population contains a small proportion of extreme individuals and that Subject 2 was one of them. Like Mr. X, whose skin was toxic and repellent to ticks (Brennan 1947), Subject 2 was evidently one apart from the ruck of test subjects. In this situation the median protection time (132 min) is perhaps a better indicator of the efficacy of dimethyl phthalate than the mean because it is not affected by extreme values.

Travis' data show that the mean protection times of dimethyl phthalate against *An. quadrimaculatus* and *Ae. aegypti* and of a set of 10 repellents against *Ae. taeniorhynchus* were always shorter for Subject 2 than for the other five test subjects, whereas the opposite was true for dimethyl phthalate against *Ae. sollicitans*. This indicates that the extreme observation (the mean for dimethyl phthalate/*Ae. sollicitans*/Subject 2) resulted, not from the repellent/host (dimethyl phthalate/Subject 2) interaction but from the vector/host (*Ae. sollicitans*/Subject 2) interaction. However, since Travis is now dead, the identity of Subject 2 is presumably lost to science, and the physiological basis of this extreme observation will probably never be known.

King's classification. King (1954) established a system of classification of insecticides and repellents in which "The least effective materials are placed in class 1 and the most effective ones in class 4." Although this system has been modified and expanded by King's successors (U.S. Department of Agriculture 1967), it still retains its essential characteristics. A source of confusion with this classification is that the scale employed runs counter to accepted usage in the English language. The ordinal of King's "class 1" is "first class," in which "first" implies "preceding all others in time, order, or importance" (*Webster's Ninth New Collegiate Dictionary* 1985). Compare "first-class seat," "first magnitude," "first string," "first sergeant," etc. with "second-class citizen," "second lieutenant," "second banana," "third-rate hotel," "fourth-class mail," etc. Logically, class 1 repellents should be the *most* effect ones, and class 4 repellents should be the *least* effective ones.

The smell and feel of deet. Several authorities, including the National Research Council (1969), Smith (1970), Khan (1977), Skinner and Johnson (1980), and the Consumers Union (1987) have stated that insect repellents in general, including deet, have an unpleasant odor and an oily, greasy, or sticky feel. However, the World Health Organization (1979) and the *Farm Chemicals Handbook* (Berg 1986) state that deet is nearly odorless, while Green (1958), Pierce (1958), Mahadevan and Varma (1967), and the Entomological Society of America (Allison 1970) state that it has a bland or agreeable, pleasant smell. In addition, Hall et al. (1957), Rosher (1957), and Mahadevan and Varma (1967) state that deet does *not* feel oily, greasy or sticky, and Rosher (1957), The Department of Agriculture (Anonymous 1957), and Pierce (1958) state that it has an agreeable, pleasant feel. Who is right?

Shambaugh and Pratt (1959)[4] reported on the responses of a panel of 18 subjects to 50, 75, and 100% deet in ethanol. All three concentrations were judged to have slight or no odor and a slightly greasy feel. Moussa (1967)[5] tested whether a panel of 7 subjects could detect the odor of 75% deet in ethanol on one or 5 persons at distances of 5 or 10 feet. The percent correct positive ranged from 0 to 37% depending on the time of day, sex of the panelist, and the size (1 or 5 persons) and distance (5 or 10 feet) of the odor source. The experiments of Shambaugh and Pratt (1959)[4] and Moussa (1967)[5] were controlled double-blind and single-blind studies, respectively.

Two additional studies relating to the smell and feel of deet were conducted by E. C. Sundberg of SRA Technologies Incorporated in 1985 and 1986.[6,7] Six sustained-release formulations of deet and a 75% solution of deet in ethanol were evaluated in 1985; two sustained-release formulations of deet and a 75% solution of deet in ethanol were evaluated in 1986. Since the additives, excipients, and structural elements of complex formulations can profoundly alter the smell and feel of the product, the results obtained with the eight sustained-release formulations will not be considered here. In each study 100 volunteers were required to evaluate the smell and feel of 75% deet immediately after application and again 10 minutes after application in a double-blind crossover (changeover) design. In addition, some volunteers were tested to determine if they could detect the odor of deet on the forearm of another person at distances of 5 (1985) or 5 and 10 (1986) feet.

In 1985, 36% of the volunteers (37% after 10 minutes) rated the smell of deet as somewhat to very pleasant, and 48% (46%) rated it as somewhat to very unpleasant. The remaining volunteers did not notice the smell. In 1986, 46% of the volunteers (46% again after 10 minutes) rated the smell of deet as somewhat to very pleasant, and 48% (44%) rated it as somewhat to very unpleasant. The remaining volunteers did not notice the smell.

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[4] Shambaugh, G. F. and J. J. Pratt. 1959. Development of insect repellents for personal use. Pesticides Section Report 1, U.S. Army Quartermaster Research and Engineering Command, Natick, MA.

[5] Moussa, M. A. 1967. Detection of deet-treated subjects under jungle conditions. *In*: Annual Progress Report of the S.E.A.T.O. Medical Research Laboratory and the S.E.A.T.O. Clinical Research Center, Bangkok. pp. 441–442.

[6] Sundberg, E. C. 1986. Evaluation of cosmetic acceptability of insect/arthropod repellent formulations: Final report. SRA Technologies, Inc., Alexandria, VA. January 7, 1986.

[7] Sundberg, E. C. 1986. Evaluation of cosmetic acceptability of insect/arthropod repellent formulations: Phase II final report. SRA Technologies, Incorporated, Alexandria, VA. October 29, 1986.

In 1985 none of 12 volunteers who had been tested to determine if they could detect the odor of deet on the forearm of another person at 5 feet were able to do so, but in 1986 one of 35 volunteers (2.9%) who had been tested to determine if they could detect the odor of deet on the forearm of another person at 5 and 10 feet reported that he could do so. However, 7 of 100 (at 5 feet) and 4 of 100 (at 10 feet) also reported that they could detect the odor of deet on the forearm of a person treated only with water (placebo effect).

In 1985, 25% of the volunteers (31% after 10 minutes) rated the feel of deet as pleasant, and 14% (25%) said that they did not feel it at all. However, others reported that it had an oily (29%, 17%), greasy (9%, 5%), or sticky (2%, 4%) feel. In 1986, 54% of the volunteers (54% again after 10 minutes) rated the feel of deet as pleasant, and 13% (19%) said that they did not feel it at all. Again, others reported that it had an oily (17%, 9%), greasy (3%, 2%), or sticky (20%, 8%) feel.

It seems that sufficient data on the smell and feel of deet have been accumulated to safely conclude that it is truly objectionable to some users but not to others. The conflicting testimony cited earlier apparently reflects a genuine heterogeneity in the population in this regard. Whether this heterogeneity is biological, as in taste blindness for PTC (phenylthiocarbamide) (Snyder 1932), or cultural remains to be determined.

McGuire's formula. The balanced incomplete block design was introduced into repellent testing by F. A. Morton of the U.S. Department of Agriculture in 1945 (Wadley 1946). Two balanced incomplete block designs are employed by the Department: (1) $t = 4$, $r = 3$, $\lambda = 1$, $b = 6$, $k = 2$ and (2) $t = 6$, $r = 5$, $\lambda = 1$, $b = 15$, $k = 2$. In this notation t is the number of treatments, r is the number of replications of each treatment, λ is the number of times any two treatments occur together in a block, b is the number of blocks, and k is the number of experimental units per block. Design (2) is Morton's original design of 1945.

In repellent studies the treatments are the repellent compounds or formulations being tested, and the replications are the systematic repetitions of the test on each treatment. The meanings of "block" and "experimental unit" require further explanation. In Department of Agriculture practice each test subject tests the t repellents two at a time (on his left and right forearms) on successive days. Each test subject/test day combination is regarded as a separate block, and the two forearms of the test subject are the $k = 2$ experimental units in each block. The blocks are called "incomplete" because only two of the t repellents are tested in each block. The design is "balanced" because each repellent is tested exactly r times and each possible pair of repellents is tested together in the same block (i.e., on the forearms of the same test subject on the same test day) exactly λ times.

A special feature of designs (1) and (2) as used in repellent studies is that the number of test subjects is always equal to $t - 1$ [i.e., 3 for design (1) and 5 for design (2)], and the number of test days is always equal to t/k (i.e., 2 for design (1) and 3 for design (2)). The number of blocks is equal to the number of test subjects times the number of test days, or $t(t - 1)/k$ (i.e, 6 for design (1) and 15 for design (2)), and the treatments are assigned to the blocks in such a way that each test subject will test all t repellents, 2 ($= k$) at a time, over the period of $t/2$ ($= t/k$) days. This kind of balanced incomplete block design, in which the blocks can be arranged in sets, each set containing a complete replicate, is known as a *resolvable* balanced incomplete block design (Kempthorne 1952). It is a special case of the balanced incomplete block design, and it requires a special analysis. Although this distinction was recognized by Wadley (1946), it is not mentioned in the current "standard method" for testing repellents in the balanced incomplete block design (American Society for Testing and Materials 1983).

An important feature of incomplete block designs is that the treatment totals must be adjusted for block effects to obtain the treatment sum-of-squares needed for the variance ratio (F) test of treatment differences. Two kinds of information on the treatments are provided by the blocks: intrablock information derived from comparisons of treatments within blocks and interblock information derived from comparisons of treatments between blocks. The adjusted treatment totals can be calculated from the intrablock information only (Yates 1936) or from both the intrablock and interblock information (Yates 1940). According to Armitage (1971) the interblock information is relatively unimportant in most designs and is usually ignored in the analysis. According to Fisher and Yates (1963), however, recovery of the interblock information is usually worthwhile in experiments with ten or more degrees of freedom for blocks. Kempthorne (1952) and Cochran and Cox (1957) have discussed the differing assumptions underlying the intrablock and interblock analyses.

In 1955 Altman and Smith introduced a simple formula for calculating adjusted means in balanced incomplete block experiments. Gilbert et al. (1957, 1966) and Smith et al. (1963) subsequently attributed this formula to J. U. McGuire of the U.S. Department of Agriculture and stated that it was "modified from Kempthorne (1952)." Although the exact derivation of McGuire's formula has never been given, it can be shown to follow from Kempthorne's equation 5 (page 533), which gives the estimated treatment effect in the intrablock analysis.

A confusing characteristic of McGuire's mean is that it may fall outside the range of observed values for the treatment and may even be negative (American Society for Testing and Materials 1983).[8] An example of this can be found in Table 1 of Schreck et al. (1976). In this case the observed range of protection time for a repellent containing deet and 2-hydroxyethylcyclohexane-carboxylate (1:3) against the deer fly *Chrysops atlanticus* Pechuman was 6.0 to 7.8 h, while the adjusted treatment mean by McGuire's formula was only 5.9 h. Additional examples can be found in the *Quarterly* (1959 to 1981), *Semiannual* (1981 to 1983), and *Annual* (1983–1984) *Report of Entomological Research by the U.S. Department of Agriculture on Insects of Military Importance* and the annual (1984 to present) *Summary of Investigations on the Management of Insects, Ticks and Mites of Medical Importance to the Department of Defense* issued by the Department of Agriculture.

The problem of such nonsense values arises, not from the adjustment procedure itself, but from the use that is made of the adjusted values obtained. The adjustment procedure was not derived for use in estimating treatment means. It was derived as a step in the analysis of variance. It provides unbiased estimates of the *differences* between treatments, not their means. However, McGuire's formula, which was derived from the adjustment procedure, was introduced as a way "to adjust the average protection period for individual variation between hosts and testing conditions" (Altman and Smith 1955), and this use has continued to the present time.

Terpineol and diphenyl oxide. In 1960 an abstract of a paper by Andreev et al. (1958)[9] was published in *Chemical Abstracts* (54:12463e) over the byline of L. Tetzloff. This abstract states that "Oily and alc. solns. of [terpineol] protected humans from mosquitoes 73 hrs.; analogous solns. of [diphenyl oxide] protected from horsefly bites 76 hrs." Since these figures seemed incredible, I obtained a copy of the original article and had it translated. The original states that "Oil and alcohol solutions of terpineol protected [humans] from mosquitoes for at least three hours. Similar solutions of diphenyl oxide offered protection from horsefly bites for six hours and longer." Evidently the figure "7" was erroneously inserted at some point in the composition or printing of Tetzloff's abstract.

Men and women. Gilbert et al. (1966) determined the protection time of 5% deet against *Aedes aegypti* for 50 men (age range 18–51 yr) and 50 women (18–71 yr). They reported that the mean protection time for the 50 men (28.5 min) differed at the 1% level of significance from that for the 50 women (39.2 min). This conclusion was based on an analysis of variance of the test data in a randomized complete block design.[10] The actual analysis of variance was not given, and I have been unable to reconstruct it from the data provided.

Although it is obviously important, the finding of Gilbert et al. (1966) that protection time is a correlate of sex has apparently not been tested by subsequent investigators. However, internal evidence from the original report casts doubt on the validity of Gilbert's conclusion that the protection time of deet is less for men than for women.

Range: Four replicate determinations were made on each test subject. The observed range of mean protection time was 2.5 to 90 min for the men and 5.0 to 75 min for the women. Thus the overlap of the respective ranges was complete, and the midrange for men (46.25 min) was actually greater than that for women (40.0 min).

Friedman's test: Gilbert et al. (1966) reported the mean protection time for five age classes (18–19, 20–29, 30–39, 40–49, and 50–71 yr) of men and women in their Table 2. We tested the two-way table of 5 age classes × 2 sexes by Friedman's test (Steel and Torrie 1980). The mean protection times of men and women did not differ at the 5% level of significance by this test.

Test procedure: "The [treated] arm was exposed for 3 minutes immediately after treatment, in a cage of about 1,500 mosquitoes, 7 to 9 days old. Additional 3-minute test periods were begun 5, 10, 20, 30, and 40 minutes after treat-

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[8] According to the American Society for Testing and Materials (1983) McGuire's mean will fall outside the range of observed values for the treatment only if it is very low. However, it can be shown that this can also occur if it is very high.

[9] The work of Andreev et al. (1958) demonstrates the curious fact that the protection times of repellents are longer on animals (typically one or more days) than on humans (typically one or more hours). Apparently this phenomenon has never been specifically investigated.

[10] Four determinations were made on each of 50 men and 50 women. The experiment was conducted in 25 series of determinations; two men and two women were tested in each series. Each series of determinations included two subseries conducted on separate days. It is not clear how this scheme could be arranged in a randomized complete block design as stated.

ment and at 20-minute intervals thereafter. The tests were terminated when two bites were received in any 3-minute test period" (Gilbert et al. 1966). Table 2 gives a reinterpretation of the results provided by Gilbert's test procedure. In this reinterpretation each 3-minute observation period ("test period") accounts also for the preceding (intervening) period when no observations were made. In Gilbert's procedure those bites that would otherwise have occurred during the intervening periods were, in effect, postponed until the next succeeding 3-minute observation period. The time within the combined intervening plus observation period at which the end point (i.e., the second bite) would have otherwise occurred cannot be known. We have therefore chosen to suppose that it would occur with equal probability at any time within the combined intervening plus observation period. On this basis the protection time and its variance can be estimated as the mean and variance of the rectangular (uniform) distribution represented by the successive minutes of the combined intervening plus observation period. These values are shown in Table 2.

This reinterpretation of Gilbert's procedure reveals a systematic error in the protection times reported (Table 2). Since this error increases with increasing protection time, its overall effect is to exaggerate random differences in observed protection times. In addition, the variance of protection time was shown to increase with its magnitude in Gilbert's study (Table 2). This violates one of two basic assumptions of the analysis of variance (Steel and Torrie 1980) on which his claim of significance was based.

We conclude that the supposed difference in the protection provided by deet to men and women has not been proved. Gilbert et al. (1966) presented a parallel analysis of their data in which the protection times for the individual subjects were expressed as ratios to those of a standard subject who was included each time a test was done. Our remarks on the primary analysis apply more or less equally to this parallel analysis. Gilbert et al. (1966) also analyzed their data with respect to the age, weight, skin temperature, rate of transdermal moisture loss, menstrual state, and relative attractancy to mosquitoes. We have not examined these data closely.

Lactic acid. In 1968 the attraction of mosquitoes to lactic acid was reported independently in two scientific journals (Muller 1968, Acree et al. 1968). According to Acree et al. (1968) this phenomenon had been reported earlier in an "old report" written by D. M. DeLong for the Office of the Quartermaster General, Department of the Army, Washington, D.C., in 1949. Apparently neither Muller nor Acree was aware that DeLong had subsequently published his findings on lactic acid in an engineering journal (DeLong 1954). Curiously, however, all these considerations of priority turn out to be moot: The attraction of mosquitoes to lactic acid was already known in the chemical industry in 1948 (Bennett 1948).[11]

Exorbitant doses. Garson and Quintana (1969) and Quintana et al. (1970a, 1970b) reported synthesis of 18 new compounds, nine of which they tested as repellents on the forearms of volunteers at a stated dose of 20 mg/cm^2. All nine of the compounds tested were applied in solution, but the strength of the solution was reported for only two (Table 3). For those two the amount of solution applied was $20/0.50 = 40$ mg/cm^2. Assuming that the density of the solution was approximately 1, then the thickness of the resulting deposit would have been approximately 40 mm^3/100 mm^2 = 0.40 mm. A deposit of this thickness would be equivalent to four coats of a commercial latex house paint. Is it possible that the reported dose is erroneous?

Most persons applying a liquid repellent *ad libitum* will apply it at a rate of about 2 mg/cm^2 (W. G. Reifenrath, personal communication), or about a twentieth of the rate reported by Quintana and his coworkers. Although it is possible to apply more than this intentionally, a limit is eventually imposed by the inception of runoff from the skin. For most liquid repellents this limit is about 4 mg/cm^2 (W. G. Reifenrath, personal communication).

Four of the nine solutions tested were solid/liquid or solid/semisolid systems, and these could have been applied in a semisolid state (Table 3). However, the remaining five were liquid/liquid systems, and these would have been subject to the dose limitation of liquid runoff from the skin. Surprisingly little information is available on the viscosities of liquid repellents, but it is possible to show that the viscosities of the liquid/liquid solutions tested would have been within the range of reported values for commercial liquid repellents (Table 4). On the basis of this comparison, therefore, the reported dose for these five materials does indeed seem impossible.

"Plussing out." Protection time is commonly defined as the length of the period between the time of application of a repellent to the skin and the time of occurrence of a specified end point such as the first observed bite or second ob-

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[11] Bennett's work was published in 26 volumes over a period of 53 years (1933–85) under the general title *The Chemical Formulary.* I know of no better source of information on the evolution of repellents and pesticides over those years than this series.

Table 2. Reinterpretation of results provided by repellent test procedure of Gilbert et al. (1966).

Procedure (Gilbert et al. 1966)			Interpretation (Gilbert et al. 1966)		Reinterpretation (This Paper)				Estimated error	
					Period covered					
Observation	Observation period	End point	Protection time	Variance	Duration[a]	Limits[a]	Protection time[b]	Variance[b]	Average[c]	Maximum[c]
1	1st–3rd min	2nd bite	0 min	Not. detd.	3 min	1st–3rd min	2.0 min	0.8 min^2	−2.0 min	−3 min
2	5th–8th		5		5	4th–8th	6.0	1.4	−1.0	−3
3	10th–13th		10		5	9th–13th	11.0	1.4	−1.0	−3
4	20th–23rd		20		10	14th–23rd	18.5	2.9	+1.5	+6
5	30th–33rd		30		10	24th–33rd	28.5	2.9	+1.5	+6
6	40th–43rd		40		10	34th–43rd	38.5	2.9	+1.5	+6
7	60th–63rd		60		20	44th–63rd	53.5	5.8	+6.5	+16
8	80th–83rd		80		20	64th–84rd	73.5	5.8	+6.5	+16
9	100th–103rd		100		20	84th–103rd	93.5	5.8	+6.5	+16
10	120th–123rd		120		20	104th–123rd	113.5	5.8	+6.5	+16

[a] Each 3-minute observation accounts, in addition, for the preceding (intervening) period when no observations were made.

[b] The protection time and its variance were calculated from the rectangular (uniform) distribution of minutes in the combined intervening and observation periods.

[c] The estimated error was calculated as the difference of the reinterpreted protection time (or its farthest limit) as shown and the protection time reported by Gilbert et al. (1966).

served bite ("confirmed bite") (King 1954). However, it sometimes happens that the test is terminated before the end point is reached, and in this case the results are commonly recorded with a "+", as in 171+ minutes. This outcome is known as "plussing out."

"Plussing out" can lead to serious errors in data analysis if one is not familiar with mathematical operations involving inequalities. The report of Gilbert et al. (1970) is a good example. Gilbert et al. correctly reported the averages of series containing one or more "+" values with the "+" retained. For example the average of a series ranging from 76 to 171+ was correctly reported as "132+." On the other hand, they reported the ratios of two "+" values without qualification. For example the ratio (105+)/(132+) was reported as "0.80". They also reported least significant differences for means of series containing "+" values. Mathematically, however, such ratios and least significant differences are indeterminate. Some of these erroneous data were subsequently repeated by Smith (1970) in the same form.

Observations known only to exceed a certain value (denoted by a "+" in repellent work) are examples of "censored" data, and there is an extensive literature dealing with the theory and methods of analysis of such data. When the observations are times to an event, as in protection times, the methods of analysis are known as "survival data analysis" (Lee 1980).

Repellent-treated netting. In 1972 Grothaus and Adams announced the discovery of repellent-treated netting as "an innovation in mosquito-borne disease protection." These authors were apparently unaware that this method of protection had been described earlier by investigators in the United States (Knipling 1949), Canada (Twinn 1950), England (Christophers 1947), Scotland (Cameron 1947), the Soviet Union (Pavlovskiy and Pervomaiskiy 1940), and South Africa (Afridi and Arthur 1945). In addition, Dowling (1955) reported that British troops operating in malarious areas in World War II were issued individual "anti-mosquito outfits" consisting of small wallets containing ¼ inch mesh nets impregnated with dimethyl phthalate.

Protection period. According to Schreck (1977), Bacot and Talbot (1919) determined the "protection periods" of their experimental materials by exposing the treated forearm in a cage of mosquitoes "at 2-, 3-, and 5-hr intervals after treatment." However, review of Bacot and Talbot's paper shows that they did not determine

Table 3. Materials tested by Garson and Quintana (1969) and Quintana et al. (1970a, 1970b) at 20 mg/cm^2 (AI).

Solute		Solvent		Solution	
Name	Physical state[a]	Name	Physical state	Strength	Physical state[c]
1. Dihydroxyacetone monopropionate	liquid	ethanol	liquid	Not stated	liquid
2. Dihydroxyacetone monopentanoate	liquid	ethanol	liquid	Not stated	liquid
3. Dihydroxyacetone monohexanoate	liquid	ethanol	liquid	Not stated	liquid
4. Dihydroxyacetone monoheptanoate	liquid	ethanol	liquid	Not stated	liquid
5. Dihydroxyacetone monooctanoate	liquid	ethanol	liquid	Not stated	liquid
6. Dihydroxyacetone monoundecanoate	solid	ethanol	liquid	Not stated	uncertain
7. Dihydroxyacetone monobenzoate	solid	ethanol	liquid	Not stated	uncertain
8. Trimethyl[β-(undecanoyloxy)ethyl]-ammonium iodide	solid	polyethylene glycol	semisolid[b]	50%	uncertain
9. Dodecyldimethyl[β,γ-bis(undecanoyloxy)-propyl]ammonium iodide	solid	polyethylene glycol	semisolid[b]	50%	uncertain

[a] Compounds for which the boiling point was reported are assumed to be liquids; those for which the melting point was reported are assumed to be solids.

[b] Solvent was reported as "polyethylene glycol ointment (USP)". Polyethylene glycol is manufactured in several grades designated by average molecular weights, which range from 200 to 20,000. The physical state ranges from clear, viscous liquid to hard, white, waxy solid, depending on grade. In pharmacology an ointment is, by definition, semisolid.

[c] Inferred by the author from the data shown.

Table 4. Viscosities of five experimental repellents tested by Quintana et al. (1970a, 1970b) at 20 mg/cm^2 (AI) compared with those of five commercial liquid repellents.[a]

Compound	Viscosity (centipoise, cp)			
	20°C	25°C	30°C[b]	35°C
	Experimental Repellents[c]			
Dihydroxyacetone monopropionate	96	73	56	43
Dihydroxyacetone monopentanoate	138	104	78	60
Dihydroxyacetone monohexanoate	166	124	93	71
Dihydroxyacetone monoheptanoate	199	148	111	84
Dihydroxyacetone monooctanoate	239	176	132	99
	(solvent)[d]			
Ethanol	1.20	1.08	1.00	0.92
	Commercial Repellents[d]			
Benzyl benzoate	—	8	—	—
Deet	—	—	13	—
Dibutyl phthalate	20	16	—	—
Dimethyl phthalate	—	17	—	9
Ethyl hexanediol	322	—	—	—

[a] The viscosities shown should be regarded as maximal, since the effect of the solvent would have been to "cut" or "thin" the solute (repellent). The values given can be interpreted in terms of a series of like values for familiar materials. In the following series a superscript indicates the temperature at which the value was determined, if different from 30°C: ether, 0.22 cp; chloroform, 0.51; isopropyl alcohol, 1.8; ethylene glycol, 14; linseed oil, 33; sebum, 86; castor oil, 450; glycerin, 1500^{20}; chlordane, 6900^{25}.

[b] Approximate temperature of the skin.

[c] Estimated from the molecular structure by Method 2 of Grain (1982). The estimated average error is 22%.

[d] Literature values.

the "protection periods" of their materials and did not use that term or any equivalent term in their report. The test interval varied from 0 to 19 hours, not from 2 to 5 hours, and tests at different test intervals were done on different days, usually by a different individual (Bacot or Talbot) using a different dose (0.75 or 1.00 g) of the test material. In view of this I believe that Rudolfs (1926, 1930) should be credited with (or blamed for) originating the concept of "protection period" and the equivalent terms "protection time" and "repellent time" that are also in common use.

Extrapolated doses. In dose-response studies the median effective dose (ED_{50}) and/or other doses of interest (such as the ED_{95} or 95% effective dose) are estimated from a regression line calculated from the observed responses of the test insects to a range of trial doses. Ordinarily the process is one of interpolation. Extrapolation beyond the range of doses tested is considered improper because it leads to serious errors, the most obvious of which are extreme and inordinate estimates. Oddly, however, this stricture seems to apply only to the point estimate; upper and lower confidence limits that fall outside the range of trial doses are seldom criticized.

In six of my papers (Skinner et al. 1979, 1980; Buescher et al. 1983, 1984, 1985, 1987) ED_{90}s and ED_{95}s in excess of 10 mg/cm^2 were reported for several commercial and experimental repellents tested against assassin bugs, mosquitoes, and chiggers. Such doses are impossible for the reason discussed in a preceding section, that the repellent would run off the surface of the treated area. The figures given may have some value for comparison or emphasis, but like the ideal gas, the perfect vacuum, and absolute zero they have no basis in physical reality. This point was not always made clear when the papers in question were published.

Effective half-life. In 1982 Rutledge et al. published a figure purporting to show the loss of effectiveness (percent of mosquitoes repelled) of a proprietary bath oil, Skin-So-Soft®, against *Ae. aegypti* with time (hours after application) as a "first order decay process," implying that the logarithm of percent effectiveness is inversely proportional to time (Rutledge et al. 1982, Fig. 2). From this figure the effective half-life[12] of the bath oil was inferred to be 1.6 hours. Subsequently it was found that this model of the decay process is not correct (Rutledge et al. 1985, equation 4), and Fig. 2 of Rutledge et al. (1982) and the effective half-life inferred from it are therefore erroneous.

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[12] The term "effective half-life" can be defined as the time required for the *effectiveness* (percent of mosquitoes repelled) of the repellent to fall to half of its original value. This is not the same as the half-life, which is defined as the time required for the *amount* (mg/cm^2) of repellent present on the skin to fall to half its original value.

As an alternative I have determined the half-life[12] and the decay constant of the bath oil by the method of Rutledge et al. (1985) using the data of Rutledge et al. (1982, Fig. 1 and 2). These values are 0.51 hr (95% confidence limits 0.14–0.86) and 1.36 hr^{-1} (95% confidence limits 0.80–4.86), respectively. This reanalysis confirms our original conclusion (Rutledge et al. 1982) that although the bath oil is effective against *Ae. aegypti*, it is not as persistent as deet, which has a half-life of 0.67 hr (Rutledge et al. 1985). It will provide effective protection if applied as frequently as needed, but it has not been registered for this use by the Environmental Protection Agency, and no application for registration has been submitted by the manufacturer.

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