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COMMISSION ON PHYTOSANITARY MEASURES

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Review of Phytosanitary Security Based on a Probit9 Treatment Standard
Agenda item 14
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I. Introduction:

1. Probit analysis is a statistical method used to calculate a dose-response relationship and is commonly used to derive the appropriate dose for a specific degree of response (Liquido et al. 1995). For the past seven decades, probit 9 treatment efficacy (99.9968% desired response) has been considered the USDA's unofficial standard for phytosanitary security. A probit 9 treatment will result in 32 survivors or less from 1,000,000 individuals treated where mortality is the desired response. Probit 9 level treatments are commonly used to achieve a conservative level of quarantine security for high risk pests (Liquido et al., 1995), but the criteria for deciding on a probit 9 level of efficacy and the management of variables associated with the research to establish probit 9 or any other level of efficacy have not been well justified or harmonized. The purpose of this document is to open such a dialogue with particular attention to:

- The meaning of probit 9
- Criteria for requiring probit 9 efficacy
- Statistical considerations
- Research considerations
- Policy considerations.

II. Background

2. Probit analysis was first proposed in the early 1930s by Chester Bliss, an entomologist at the Agricultural Experiment Station in Connecticut, in response to the problem of how to compare pesticide treatment results (Bliss, 1934). Probit analysis has been primarily used in tests where the organism has a bimodal response to the experimental stimulus; it either lives or it dies. Bliss's method required a two stage interactive calculation and interpolation from a table. In the 1950s, tables were published so calculations could be made more easily, and from these calculations computer programs were developed that have been widely used for probit analysis (Litchfield and Wilcoxon, 1949; Salsburg, 2001). Probit analysis was further developed and refined for toxicological research by David Finney (Finney; 1947, 1971). Probit analysis has been a standard method used in pharmacological testing to determine if a drug has an effect, and it is frequently used for comparing the doses at which 50% of the study organisms respond.

3. In a 1939 circular published by the USDA, Baker suggested that probit 9 level treatments would meet the phytosanitary security requirements of the United States for fruit flies (Baker, 1939). Baker's recommendation of probit 9 level treatments was based on the assumption that a lognormal transformation would relate mortality and length of exposure in a linear fashion (Baker, 1939; Liquido et al. 1995). Probit 9 was not again discussed in the literature of quarantine treatments until 1963 when it was mentioned as the standard necessary for fruit flies, citing Baker 1939 (Benschoter, 1963, Burditt et al., 1963). There are no references to suggest that Probit 9 was actually used as a standard by the USDA for quarantine treatments in the intervening period. The quarantine treatments developed and used during this period (primarily fumigations) were done using much lower sample sizes and less rigorous testing regimens than are currently required by APHIS. The methodology used would be similar, if not identical, to that used for developing any standard pesticide treatment, where mortalities of 95 to 99% are deemed adequate for control. Many of these treatments were adopted without large scale testing, or even systematic dose/mortality testing. They were adopted based on empirical evidence. An example of this type of research is the fumigation of mangoes with ethylene dibromide by Richardson (1952). The use of probit analysis for quarantine treatments and the probit 9 statistic did not become prevalent until computers and programs for probit analysis became widely available in the 1970s.

III. The meaning of Probit 9

4. In most quarantine treatments, probit analysis is used to predict the level of treatment (dose) to achieve a desired degree of mortality (response). It assumes that each organism has an innate tolerance for a certain stimulus to a specific threshold, and when the threshold is exceeded the organisms will respond with an expected and measureable result. Because an organism's tolerance is typically difficult to measure, the mean and variance from the distribution of tolerances is estimated (Chew, 1994). The response to the stimulus is dependant on the treatment and may include mortality, sterility, or cessation of maturation (Liquido et al., 1995; 1996). A regression of the probit transformation of the proportion for dose-response will be linear if the tolerance of the organism is distributed normally. If the distribution is lognormal, the probit on log (dose) will appear linear (Liquido et al. 1995).

5. Baker suggested that probit 9 level treatments would meet the phytosanitary security requirements of the U.S. for fruit flies (Baker, 1939). Baker's recommendation of probit 9 level treatments was based on the assumption that a lognormal transformation would relate mortality and length of exposure in a linear fashion (Baker, 1939; Liquido et al. 1995). During the 1960s, probit 9 level treatments became the de facto standard for quarantine security for the U.S. and many other countries.

6. In 1986, Couey and Chew published formulas for estimating the number of pests that must be treated for a given number of survivors at a given confidence level and treatment efficacy. From this,

a probit 9 study with 95% confidence level and no survivors would require that 93,613 insects be tested. If control mortality is considered, this number would be adjusted higher (Follett and Nevin, 2006). In the last three decades, the requirements for developing quarantine treatments have become so stringent, that developing a new treatment may take three to five years of research. More recently, many national and international research publications have questioned the legitimacy of a probit 9 standard, identifying flaws and proposing alternatives (e.g. Liquido et al. (1995); Baker et al. (1990); Mangan et al. (1997); Vail et al. (1993); and Landolt et al. (1984). Haack et al., (2011) identified problems associated with using probit 9 as a standard for developing new treatments for wood packing materials. To date, all such studies have focused on insect pests, leaving open the question of treatment metrics for other pests such as pathogens.

7. APHIS has been flexible in some cases in approving the use of treatments that have not had large numbers of insects treated. This has mainly been where raising large numbers of organisms for the work is difficult or impossible. Examples include approval of 400 gy irradiation as a generic dose for many species of insects, and treatment of limes with hot water for surface pests (Gould and McGuire, 2000; Hallman, 2004).

8. APHIS also makes a distinction between treatments approved for ongoing use and those needed for provisional or emergency purposes. The application of probit 9 requirements, including attendant research and policy aspects of provisional and emergency treatments, usually involves greater uncertainty and requires greater flexibility to the detriment of consistency.

IV. Criteria for requiring probit 9 efficacy

9. Probit 9 level mortality treatments are typically applied to cases that meet the following criteria:

- the host is suitable;
- the infestation rate is high;
- the host is easily infested;
- distribution of the pest is highly clumped; and
- the pest is internal or difficult to detect (Liquido et al. 1995; 1996).

10. In other words, probit 9 level treatments would seem to be synonymous with high risk situations. This level of treatment in most instances is employed without the need for detailed or time consuming data collection on the prevalence of the pest, the level of infestation, or the likelihood of establishment. Probit 9 level treatments are an attractive option for pest risk management because they provide a conservative level of quarantine security, although they may not provide the desired level of consistency in all situations (Liquido et al. 1995; 1996).

11. Probit 9 level treatments are convenient mainly due to the relative speed in which they can be developed, tested, and implemented for tolerant hosts (Liquido et al. 1996). As a result, a number of precedent-setting treatments have been established that come to be viewed as risk management standards when they are actually based on bilateral agreement for a measure that is believed to result in substantial “overkill” in order to facilitate trade. Such treatments are difficult to link to consistent policies for a threshold level of risk or arguments of equivalency, and are more likely to violate the principle of “least restrictive measure”. The legitimacy of such treatments comes from the fact that they are bilaterally agreed, not that they are technically justified.

12. From a risk standpoint, it is not mortality which is important but rather survivors. Linking the efficacy of the treatment to some level of risk, even if it is only a threshold, requires a good estimate of the anticipated infestation level in order to estimate survivorship. Beyond this, there are biological and other important variables to consider for estimating the likelihood of establishment if the treatment is to have a rational relationship to the risk. From this standpoint, the technical criteria listed above should be considered in light of a pest risk analysis (PRA), which is often not the case.

V. Statistical considerations

13. The use of probit 9 level treatments as a standard for phytosanitary security is not based on any specific scientific data. Statistical distributions other than probit may often provide a better fit to data (Haack et al., 2011). It has been argued that probit 9 level treatments actually provide an insufficient level of security in many instances (Landolt et al. 1984; Robertson et al. 1994a; Liquido et al. 1995; 1996; Chew, 1996). With fluctuations in distribution and population levels, probit 9 values are often inaccurate. Based on normal probit 9 conditions, with a 95% confidence limit, the surviving individuals may range from 29 to 136 individuals per 1,000,000 organisms treated per shipment (Paull and Armstrong, 1994). The fluctuation of surviving organisms under normal conditions, confounded with other variables that may change infestation rate, will drastically alter the risks associated with introduction and establishment of surviving individuals (Landolt, 1984). In other words, the efficacy of the probit 9 treatment will vary with infestation rate and distribution, leaving many commodities over- or under-treated. In addition, probit 9 treatments ignore several risk factors that may increase the probability of introduction, such as:

- the actual infestation rate of the volume of treated fruit;
- the potential for culling out infested fruit (changing the pest distribution pattern);
- the infestation rate of good hosts vs. poor hosts; and
- the probability of a mating pair arriving at the same time in the same market (Paull and Armstrong, 1994).

14. In quarantine treatments, the estimation of a true survivor proportion of pests in a specific treatment is crucial to ensure quarantine security, but is often difficult to estimate. Concentrating on the upper distribution limit will aid in determining the true survival proportion (Chew 1994). However, assessing treatment efficacy on the narrow criteria of 99.9968% mortality may be too limited to evaluate properly. The upper extreme of the probability distribution is difficult to estimate with any degree of certainty, making it difficult to accurately estimate probit 9 level mortality (Robertson et al. 1994a, Liquido et al. 1995; 1996).

15. A study by Couey and Chew (1986) examining the relationship between confidence intervals and sample size in quarantine treatments showed that sample size drastically affects the level of security achieved. They demonstrated that approximate probit 9 (or 1/35,000 or 29 per million) level treatment with a sample size of 35,000 with one surviving individual and a confidence interval of 95% would result in 0.7 to 159 survivors per million. The inability to accurately predict survival following a probit 9 treatment in shipments of different sample sizes greatly lowers the level of security achieved with a probit 9 treatment.

16. Accurately estimating quarantine security increases in difficulty due to several factors beyond sample size. Under abnormal or fluctuating conditions the degree of security achieved may fluctuate greatly. Several factors have been identified that may compromise the level of phytosanitary security achieved with probit 9 treatments. These factors include, but are not limited to:

- per treatment in-field infestation rate;
- the size of the shipment;
- pre-shipment cultural practices;
- survival and reproductive capacity of the organism;
- packaging and shipping conditions;
- seasonality of shipments; and
- distribution of the commodity (Chew, 1996).

17. The above factors may drastically alter the distribution pattern of the pests within the treated shipment. Probit analysis assumes normal distribution, but with changing ecological and physiological factors, the distribution patterns may in fact be non-random, skewed, or clustered (Mangan). If the distribution is not normal, the data may be a poor fit for this statistical model (Haack et al., 2011; Robertson et al., 1994b). Thus, a probit analysis may not be the appropriate statistical method for all situations.

18. The tests required to prove probit 9 level efficiency for new treatments, while relatively quick and straight-forward for some commodities, often prove difficult to perform on other commodities and for a variety of pests. Probit 9 quarantine treatments typically require large-scale testing (~100,000 insects) of the most tolerant life stage to validate probit 9 estimates (Liquido et al.1996). For some commodities and pests, the required number of fruit and pests are not available for these tests due to natural small production or low prevalence. In addition, when treatment levels are determined for probit 9 efficacy, the treatments are often too rigorous and unfeasible due to phytotoxicity (Liquido et al. 1996). For example probit 9 methyl bromide treatments in cucurbits may cause serious injury to watermelon deeming them unfit for human consumption (Cowley et al. 1991). Cowley et al. (1991) performed efficacy trials and determined that a lower probit level of 8.4 did not cause serious injury to the fruit and still provided adequate quarantine security.

19. In cases of low infestation, the use of Probit 9 may be wasteful and unnecessary, while lower probit treatments would be more economically sound and aid in preserving the quality of the commodity (Liquido et al. 1995). Thus, probit 9 quarantine treatments are not appropriate for all situations and alternate probit levels or even alternate treatments may be more effective in providing quarantine security.

20. Many poor hosts for fruit flies are rarely infested and survival in these poor hosts is often very low (Mangan). Probit 9 level treatments are unnecessarily applied in many situations without consideration of the:

- rate of infestation;
- gregariousness of the pest;
- survival and reproductive capacity of the pest;
- inherent hardiness of the pest to environmental stress;
- packaging and shipping conditions;
- seasonality and distribution of commodity (Liquido et al., 1995; 1996).

21. To improve treatment efficiency, the probability of the introduction of a reproductive unit needs to be closely examined along with biological, ecological, quarantine treatment, and marketing data to determine the quantitative risk (Liquido et al., 1995). The probability of importing a mating pair is far more likely than an actual introduction (Landolt et al., 1984), and therefore, treating to prevent a mating pair would provide a conservative level of phytosanitary security. In situations where the infestation can be reliably estimated the intensity of the inspection or mitigation can be determined based on the probability of a founding pair present in a given area (Liquido et al., 1995) using the maximum pest limit model (Baker et al., 1990). An appropriate level of treatment can be determined by relating percentage infestation, mean larval density per fruit, and treatment efficacy for various values of allowable numbers of pests per load (Liquido et al., 1995). For example, for a shipment of 36,000 fruits with 1% infestation, probit 9 mortality would produce 0.012 survivors, which is an impractical requirement. A probit 7.77 level treatment is more practical, which would allow 1 surviving individual (Chew, 1996). Table 1 shows the number of survivors from a population of 1 million for different probit levels.

Table 1. Probit level survivors for a population of one million (95% confidence)

	Number of Survivors in a Million
Probit	**Sum**
5	500000.000
5.2	420740.291
5.4	344578.258
5.6	274253.118
5.8	211855.399
6	158655.254
6.2	115069.670
6.4	80756.659
6.6	54799.292
6.8	35930.319
7	22750.132
7.2	13903.448
7.4	8197.536
7.6	4661.188
7.8	2555.130
8	1349.898
8.2	687.138
8.4	336.929
8.6	159.109
8.8	72.348
9	31.671
9.2	13.346
9.4	5.413
9.6	2.112
9.8	0.793
10	0.287
10.2	0.100
10.4	0.033
10.6	0.011
10.8	0.003
11	0.001

22. Probit treatment levels should be tailored for the specific commodity and pest of interest. Tailoring probit levels to specific commodities would ensure that the NPPO is in accordance with Article 5 of the SPS Agreement using the “least trade restrictive” measure. Treatment levels below probit 9 efficacies may only apply to commodities with low infestation rates (Liquido et al., 1995), and detailed risk assessments would need to be developed to ensure phytosanitary security requirements are met.

23. When a treatment is necessary to permit movement of commodities, and halting the movement causes major economic hardship, it should be possible to use the best scientific evidence available to provide a treatment for a short term period. This would allow time for research to take place to provide a more precise estimate of the treatment necessary to give the desired level of quarantine security. At the culmination of the research, the treatment being used could be adjusted, if necessary. This flexible policy would allow safe commerce while providing for adjustment while new information is accumulated.

VI. Conclusion

24. Probit 9 level quarantine treatments are often used for high risk organisms that pose a considerable threat for introduction. The efficacy of probit 9 treatments ensures a conservative level of security for most situations when infestation rates are known and the pests respond in a normal distribution pattern. Under fluctuating conditions, (variations in infestation rate and distribution patterns) probit 9 level treatments may be insufficient to guarantee quarantine security. For poorly infested commodities, probit 9 treatments are often excessive, leading to phytotoxic damage. Despite these shortcomings, probit 9 level treatments continue to be viewed and used as the unofficial standard for phytosanitary security in the U.S. and elsewhere.

25. Ensuring phytosanitary security can be achieved through several different methods, including Pest Free Areas, Systems Approaches, treatments based on maximum pest limit, alternative probit level treatments, combination treatments and others. As methyl bromide and other chemical treatments become less desirable, the popularity and necessity of the above quarantine methods continues to increase. A considerable amount of biological and ecological data on the pest and commodity is needed to establish any of the above quarantine programs; however, when employed properly these measures may provide the most balanced and efficient alternatives. These measures, while often labor intensive in the establishment and data collecting phases, are tailored to the specific commodity and pest to ensure an appropriate level of quarantine security.

26. The use of probit 9 level treatments remain an effective tool in quarantine treatments, however, the application of probit 9 level treatments should be justified through treatment models, and not exercised as a standard in all quarantine situations. Customizing treatments to specific commodities and pest complexes will demonstrate that all quarantine treatments are justified and based on scientifically sound data. The establishment of such a process would justify and harmonize safeguarding measures across trading partners and provide compliance with the SPS agreement, while allowing the flexibility desired for specific pests and pathways.

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