



UNITED STATES ENVIRONMENTAL PROTECTION AGENCY  
WASHINGTON, D.C. 20460

OFFICE OF  
PREVENTION, PESTICIDES AND  
TOXIC SUBSTANCES

**6/19/2001**

**PESTICIDE REGISTRATION (PR) Notice 2001-4**

**NOTICE TO MANUFACTURERS, PRODUCERS, FORMULATORS,  
AND REGISTRANTS OF PESTICIDE PRODUCTS**

**ATTENTION:** Persons Responsible for Federal Registration and Reregistration of Antimicrobial Pesticide Products

**SUBJECT:** Elimination of Phenol Resistance Testing for Antimicrobial Disinfectant and Sanitizer Pesticides

This notice announces the discontinuation of phenol resistance testing as a part of efficacy testing for antimicrobial disinfectants and sanitizers. Effective immediately, the Agency will consider registering or reregistering antimicrobial disinfectant or sanitizer pesticides without supporting phenol resistance testing. All other data in support of registration or reregistration, including any required efficacy testing data, must be submitted and then accepted by the Agency.

**I. BACKGROUND**

Phenol resistance testing is a standard that has traditionally been used to estimate the intrinsic resistance or sensitivity of some test bacteria to chemical disinfectants and sanitizers. For years the Agency has been aware of the lack of standard and uniform resistance levels to phenol expressed by the test cultures used in the existing Official Methods of Analysis of the Association of Official Analytical Chemists (AOAC) test methods. Historically, the inability to maintain and propagate test cultures that express standard and uniform levels of phenol resistance has been a recognized scientific problem which has persisted for at least 70 years. Furthermore, the inability of many reputable and competent testing facilities to achieve consistent test results with the phenol resistance standard has prompted both concern and action by the Agency.

On September 10, 1997, after internal scientific deliberation, the Agency placed before the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA) Scientific Advisory Panel (SAP) the following question regarding phenol resistance:

What scientific direction should be taken regarding the lack of standard and uniform resistance levels to phenol of the test cultures used in the existing AOAC (Association of Official Analytical Chemists) efficacy test methods? Should the Agency:

- Totally eliminate the phenol resistance requirement; or
- Modify the required phenol resistance patterns to provide a broader range of acceptable resistance; or
- Replace the phenol resistance requirements with some other procedures that assure hardness and equivalence to test cultures, such as standard, quantitative inoculum level?

Briefly, the response provided to the Agency by the SAP was as follows:

“There is no current relevance to requiring the phenol resistance test; hence (the) phenol coefficient method should be eliminated and new protocols should be established for defining the conditions for culturing test microorganisms with suitable resistance levels to antimicrobials. . . .

In summary, phenol resistance does not appear to be linked to other germicidal or antibiotic (drug) resistance nor is it a good marker of genetic variability or similarity. Furthermore, levels of phenol susceptibility frequently are outside current EPA guidelines, resulting in significant expense to industry and government.

Quantitative inoculum level is a better choice, if growth and environmental conditions suggested for any new methods are considered. However, current methodologies for quantitation are not accurate, as they do not take into account inadequate removal of microorganisms from natural substrates and the ability of the organisms to become more resistant to pH change, oxidation, altered temperature, etc. Research is needed in this area. The Panel recommends that the Agency request a consensus approach of available experts who can define reproducible tests which model germicidal resistance in nature.”

## **II. PRESENT PHENOL RESISTANCE STANDARDS FOR ANTIMICROBIAL PRODUCT EFFICACY TESTING**

Subdivision G of the Pesticide Assessment Guidelines, part 91-1, describes the general product performance (efficacy) standards for disinfectants and sanitizers. Subsection (b)(3)(I) of part 91-1 refers to the AOAC standard tests that may be used to satisfy the data requirements of 40 CFR 158.640. The following AOAC tests include references to phenol resistance testing.

Phenol resistance testing procedures and standards appear as a part of AOAC Official Method 955.11, Testing Disinfectants against *Salmonella typhi*, Phenol Coefficient Method (Final Action 1964), and are referenced by several other AOAC testing methods. The Phenol Coefficient Method (PCM) provides instructions and specifies satisfactory phenol control readings for *Salmonella typhi* (Table 955.11B), for *Staphylococcus aureus* (Table 955.12), and for *Pseudomonas aeruginosa* (Table 955.13). Method 955.14, Testing Disinfectants against *Salmonella choleraesuis*, Use Dilution Method, specifies an expressed phenol resistance equivalent to that specified for *S. typhi* in the PCM, while method 955.15, Testing Disinfectants against *Staphylococcus aureus*, Use Dilution Method, specifies that expressed phenol resistance be the same as that previously specified for this organism in the PCM. There is no phenol resistance expression specified for *P. aeruginosa* for use in the Use Dilution Method. Method 955.16, Chlorine (Available) in Disinfectants, Germicidal Equivalent Concentration, specifies phenol resistance for either *S. typhi* or *S. aureus* by reference to the resistances previously specified in the PCM. Method 955.17, Fungal Activity of Disinfectants, specifies the phenol resistance for use with *Trichophyton mentagrophytes*. Method 960.09, Germicidal and Detergent Sanitizing Action of Disinfectants specifies phenol resistance of *Escherichia coli* should be equivalent to that specified for *S. typhi* in the PCM and that for *S. aureus* equivalent to that previously specified in the PCM. Finally, Method 965.12, Tuberculocidal Activity of Disinfectants, a carrier based resistance test, specifies a phenol resistance determination for the test organism, *Mycobacterium bovis*, while present on the surface of porcelain penicylinder carriers.

### **III. ELIMINATION OF THE PHENOL RESISTANCE COMPONENT FOR ANTIMICROBIAL DISINFECTANT AND SANITIZER PRODUCT EFFICACY TESTING**

The Agency has reviewed the SAP's recommendation to eliminate, modify, or redirect the use of phenol resistance testing for antimicrobials. The Agency concurs with the SAP and has engaged in considerable discussion and deliberation, internally and with members of the scientific and regulated communities, that phenol resistance expressed by such organisms as *S. typhi* (a representative enteric pathogen) and *S. aureus* (major source of wound infection), when used as part of the phenol coefficient method, has been an unsatisfactory standard for evaluating the sensitivity of certain microorganisms used during disinfectant efficacy testing. The phenol resistance assay does not mimic the test systems in the current efficacy methods.

The Agency has also considered the recommendation to modify the phenol resistance patterns currently deemed acceptable, thus changing the established growth/non-growth patterns. Although this modification would greatly facilitate meeting an acceptable phenol pattern, it would not solve other inherent problems associated with the use of the phenol resistance test (e.g. technique sensitive assay, highly variable results, non-carrier based test, disassociation with the efficacy method.)

The Agency acknowledges that inoculum titre and quality will influence the outcome of the efficacy evaluation of an antimicrobial. The use of a quantitative inoculum level and other

parameters of inoculum quality as suitable indicators of a challenge population is recognized and should be studied further. As an interim measure, the Agency will generally expect that a minimum inoculum level of  $10^4$  cfu/carrier for all test microorganisms should be achieved when an AOAC carrier-based test method is used. The inoculum level should be reported with the efficacy data submission.

Given the inapplicability of a test organism's resistance to phenol when disinfectants or sanitizers are tested for their efficacy performance, the Agency will no longer require submission of testing to demonstrate compliance with AOAC-specified levels of expressed phenol resistance by test microorganisms during the efficacy evaluation of disinfectants or sanitizers.

#### **IV. SCOPE OF POLICY**

This PR Notice describes the requirements set forth in Agency regulations and FIFRA, and provides general guidance to EPA and to affected parties as well. While the requirements in FIFRA and Agency regulations are binding on EPA and applicants, this notice is intended to provide guidance to EPA personnel, pesticide registrants and applicants, and the public. As a guidance document, this policy is not binding on either EPA or any outside parties, and the EPA may depart from the guidance where circumstances warrant and without prior notice. Registrants and applicants may propose alternatives to the recommendations described in this notice, and the Agency will assess them for appropriateness on a case-by-case basis.

#### **V. FOR FURTHER INFORMATION**

For further information, contact Michele Wingfield at 703-308-6349 or at [wingfield.michele@epa.gov](mailto:wingfield.michele@epa.gov)

/signed by Frank T. Sanders/

Frank T. Sanders, Director  
Antimicrobials Division  
Office of Pesticide Programs