J. Pestic. Sci. 28, 215–216 (2003)

Society Awards 2003

(on prominent achievement)

Assessing the Side-Effects of Pesticides on the Microbial Ecosystem in the Soil Environment*

Hiroki YAMAMOTO**

Faculty of Life and Environmental Science, Shimane University, Matsue, Shimane 690-8504, Japan

(Accepted March 4, 2003)

Key words: soil environment, microbial flora, microbial activity, ecological risk assessment, rice paddy.

Introduction

Pesticides by definition are biologically active compounds. Therefore, they have the potential to be active in any organisms of the environment in which they occur. While every effort is made to ensure that the activity is confined to the intended target, absolute specificity has not been achieved and non-target organisms may be at risk. Microorganisms are of prime importance in the recycling of key elements essential for biological processes and thus for the maintenance of soil fertility. Since current agricultural practices apply biologically active compounds to soil as a part of pest control, it is important to carefully understand the effects of these compounds on the soil microbial community. In the late 1960's, concern developed that the continuous input of pesticides into soils might affect the soil microflora and so impair soil fertility. SETAC, OECD and other international organizations have developed regulatory guidelines.

In this paper, our proposal for recommended guidelines for testing pesticide effects is introduced, and then an outline of our studies assessing the side effects of pesticides on the soil microbial community are described.

A Proposal for Recommended Test Guidelines

In the early 1970's, there were indications that regulatory authorities in Europe were considering the introduction of a requirement for tests of pesticide effects on soil microflora. After several international workshops in Europe, recommendations on testing the side-effects were drawn up by the participants and published. The US EPA was similarly interested in this issue. Also in Japan, similar meetings have been organized over the past 20 years to discuss the issue and to produce arguments for the most meaningful test, and a draft proposal for the testing was published. The aims were to recommend environmentally relevant tests and to optimize the basis for understanding and evaluating data on interactions between the soil microflora and pesticides.

1. Which Processes and Organisms Are of Major Importance?

There is inadequate knowledge of what a soil ecosystem is and which processes and organisms are of major importance in maintaining soil quality. The philosophical basis for the tests was discussed at the beginning, and several governing principles were adopted:

1) The parameters concerning soil fertility should be considered.
2) Microbial biomass should be tested rather than plate count analysis.
3) Parameters and test methods should be selected so that a researcher can get reasonably reproducible results.
4) The impact of pesticides on the structure of microbial community is also important.
5) The importance of rice crop in world agriculture argued for inclusion of specific tests of microbial functions known to be important in rice paddies.

The parameters below were accepted as recommended tests.

Tests under upland field conditions

Microbial biomass, Nitrification and Mineralization of organic matter

Tests under paddy field conditions

Microbial biomass, Nitrification, Mineralization of organic matter, Algal growth and Nonsymbiotic nitrogen fixation

2. Interpretation and Evaluation of Data

The problem of evaluating the significance of differences between treated and untreated samples is fundamental to side-effects testing. Is a statistically significant difference

---

* See Part II for the full Japanese article.

** To whom correspondence should be addressed.
E-mail: yamahiro@life.shimane-u.ac.jp

regarded as an environmentally significant difference?

Laboratory-derived data should be compared to known data on changes in soil microflora or soil microbial activities which result from acceptable or unavoidable natural perturbations such as fluctuations of temperature, changes in water potentials, physical disturbance of soil, nutrient supply etc. There is a continued need to develop scientifically valid means of assessing data obtained from the recommended tests.

Recovery from the Inhibition

Bensulfuron methyl was the first sulfonylurea herbicide commercialized for use in rice, and it remains the dominant sulfonylurea for broadleaf and sedge weed control in this crop. Side-effects of the herbicide on microflora were assessed according to the recommendation stated above.

BSM had no significant effect on the air-drying effect on ammonification when applied at the $1\times(0.3\ mg/cm^2)$ and $10\times(3\ mg/cm^2)$ recommended field application rate ($1\times$ RFAR and $10\times$ RFAR, respectively). BSM significantly ($P<0.01$) reduced the rate of nitrification immediately after the application. However, the activity recovered completely within 28 days in samples treated with the herbicide ($1\times$ RFAR), and within 56 days in samples at $10\times$RFAR. No significant effects on microbial biomass were detected following herbicide application at $1\times$RFAR and $10\times$RFAR. Although some differences are statistically significant, there is no pattern that seems related to the herbicide treatment.

This case study is a good example that consideration of recovery from damage is essential to assess the effects of pesticides on a dynamic ecosystem.

Natural Fluctuations of Microbial Activity in Rice Paddy Soils

To develop a reference for conducting a risk assessment of the effects of pesticides on soil ecosystem from a perspective considering natural fluctuations, seasonal fluctuations of microbial parameters in actual rice paddy fields were investigated. The results shown in this study will provide useful information as a reference to conduct risk assessments on the side-effects of pesticides since they include possible rice herbicide effects in the context of effects due to natural stress.

Fluctuations of various parameters for soil microbial activities in paddy field soil were monitored over 20 months for possible use as a basis for assessing pesticide effects. To avoid the possibility that prior pesticide or chemical fertilizer treatment may have altered soil microflora, the reference paddy was one which had not received chemical treatments for at least five years. Both no-chemical and conventional paddies were monitored for microbial parameters including nitrification, ammonification, microbial biomass etc. Although the range of fluctuations was different for each parameter, the coefficients of variation (CVs) for most were 20 to 50%, which reflects the fluctuation caused by natural stress as well as anthropologic stress. No trends in fluctuation were apparent for any of the parameters. Similarly, no differences between no-chemical and conventional paddy were apparent. Many studies have reported the effects of pesticides based only on statistical differences between tests and controls derived from laboratory testing. The results of this study, however, emphasize the importance of understanding natural fluctuations in microbial parameters when interpreting and assessing the risk to microbial populations of pesticide exposure.

Effects of Repeated Application

Effects of long term, repeated application of chlorothalonil fungicide on cellulose decomposition in soil and soil microflora were investigated. Test plots were drenched with chlorothalonil twice a year for six years at $1\times$RFAR and $5\times$RFAR. Cellulose decomposition was suppressed only in winter in the $1\times$RFAR plot, and such seasonal variation was also observed in the $5\times$RFAR plot with incomplete recovery in summer. The population of total and Cx-cellulase producing fungi remained unchanged while the population of fungi which vigorously decomposed filter paper decreased with the application rates. The relative dominance and frequency of a fungal species, *Rhizoctonia solani*, on the cellulose sheets at low temperature were high in the control soil but extremely low in the applied soil. The suppressive effects of the chlorothalonil on cellulose decomposition in soils at low temperature seem to be attributable to a reduction in the dominance of *R. solani* on cellulose sheets. Since the species is one of the target fungi for drenching with chlorothalonil, the suppressive effect should be defined as a side-effect with respect to phenomenon, but as an objective effect with respect to mechanism. These results indicate the importance of research on the structure of microflora and consideration of environmental conditions such as temperature, as well as continuous monitoring, in assessing the side-effects of pesticides on soil ecosystem.

Conclusion

Even though there are numerous reports on the effect of pesticides on soil microorganisms, it is very difficult to assess the effects from those results. This is, in part, due to a lack of standardization in experimental design and methods. We proposed a recommended guideline for environmentally relevant tests to optimize the basis for understanding and evaluating data on interactions between the soil microflora and pesticides. Also, we pointed out matters that demand special attention for testing pesticide effects on microbial ecosystems. Many more studies based on this proposal are strongly expected to accumulate data by scientists who are concerned with pesticide effects on the soil environment. The accumulation of data is necessary to make it possible to develop a means of interpreting results obtained in laboratory experiments and to assess effects with ecological significance.