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SUMMARY OF DOCTORAL THESIS

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Title: **Method Development of Commercially Available Fenitrothion-ELISA Kit for Soil Residue Analysis**

（フェニトロチオン ELISA キットを用いた土壌残留分析に関する研究）

Our studies demonstrated the applicability of commercially available fenitrothion-ELISA kits (HORIBA Biotechnology, SMART ASSAY SERIES) for crops in the analysis of soil samples. We have exploited two different sensitive ELISA kits and developed a method with simple clean-up and filtration. One plate has a sensitivity range at 1.5-15 ppb (low sensitivity) and the other plate (high sensitivity) has sensitivity range at 0.15-2 ppb.

Samples for recovery was spiked with fenitrothion at 0.26 μg/g soil. The low sensitive fenitrothion-ELISA kit performed well in analysis of 5 out of 10 soil samples, showed recovery above 70%, however, the results were biased high (>120%) in the other 5 soil samples compare to GC. The overestimated recovery ranges from 127-163% for mostly sandy soils. It was concluded that sandy soils contained interfering soil matrices such as divalent ions and soil organic matter. The ELISA absorbance decreased by addition of Ca^{2+} and Mg^{2+} ions, and ultrafiltration of the soil extract through a high-performance centrifugal concentrator (9 kDa) provided removal of the ELISA interferences for some sandy samples.

The effect of dilution at higher ratio was further examined in high sensitive fenitrothion-ELISA kit. Addition of synthetic zeolite was also tried to removed Ca^{2+} and Mg^{2+} ions. Dilution did not resolve the overestimation on fenitrothion recovery. The employment of synthetic zeolite during the soil extraction process had a positive effect in the removal of some contaminants. On the other hand, ultrafiltration (9kDa) of diluted soil extracts had fenitrothon recoveries in ELISA comparable to GC.

The applicability of the fenitrothion–ELISA kit was further exploited by analyzing incubated soil samples with fenitrothion pesticide. The sample, spiked with fenitrothion at 2.7 μg/g wet soil, was analyzed for residue, after 1 week of incubation, by GC, HPLC and ELISA. A slight overestimation of fenitrothion recoveries were observed in the soil samples treated with zeolite. As no cross-reactivity of 3-methyl 4-nitrophenol, a major degradation product of fenitrothion in soil was observed, it was supposed that the overestimation was caused by soil interfering matrices. In contrast, the result of fenitrothion recovery in ELISA was comparable to GC and HPLC by ultrafiltration of the soil extract through 9 kDa. It was considered, high-molecular-weight organic materials interfered the ELISA reaction. The fenitrothion ELISA kit can be used for pesticide residue monitoring of various soils, including sandy soils, with only simple ultrafiltration before analysis.

By the procedures, comparable results in soil residue analysis were achieved with the conventional GC and HPLC analyses. Estimation of the level of contamination of pesticide in the environment by simple ELISA method is one advantage to pool valuable pesticide residue information for risk assessment. In this way, further contamination could be prevented and environmental risks can be avoided.