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Validation of Pyrithiobac Sodium (Staple® Herbicide) ELISA for Australian Cotton Soils

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ABSTRACT

An enzyme-linked immunosorbent assay (ELISA) for pyrithiobac-sodium (Staple®) produced by DuPont was validated in Australian soils. This pyrithiobac-sodium ELISA was shown to be highly sensitive with the limit of detection of 4–5 ppt. Soil samples were extracted either in PBS buffer by shaking or by accelerated solvent extraction (ASE). While pyrithiobac sodium can be analyzed directly by ELISA after ASE extraction with 1/10 or more dilutions, the analysis of PBS extract required filtration and dilution 1/20 or more depending on the concentration. Immunoassay results compared favorably with GC-MS results for both ASE and PBS extract of incurred residue of pyrithiobac sodium in soil samples, indicating that this ELISA can be an inexpensive and reliable alternative to conventional residue analysis methods for quantification of pyrithiobac-sodium. This validation provided the basis for applying the ELISA to a field study of pyrithiobac-sodium.

Key Words: ELISA; Validation; Soil; Pyrithiobac sodium.

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INTRODUCTION

With the introduction of transgenic cotton and a reduced reliance on the organochlorine insecticide endosulfan in Australian cotton production systems, it is anticipated there will be a greater industry reliance on herbicides to control weeds. Pyrithiobac sodium (sodium 2-chloro-6-[(4,6-dimethoxypyrimidin-2-yl)thio]benzoate (Figure 1), the active ingredient of Staple® Herbicide (DuPont, Wilmington, DE), is one of the new herbicides being introduced to the Australian cotton industry. As a post-emergence weed control herbicide it has shown promise in controlling obstinate broadleaf weeds in cotton at low application rates[1] with no effect on seed cotton yield.[2] Staple® is an attractive herbicide since it can be applied to the mature cotton crop reducing the need for labor-intensive ''chipping'' of weeds in cotton fields.

One concern about the use of post emergent pesticides, is that they may reach the soil surface following application to foliage[3] where they would be subject to various transport, retention and transformation processes possibly creating an environmental risk. In the absence of published studies on the environmental fate of pyrithiobac sodium under Australian field conditions, a study was conducted to assess environmental risk.[4]

Several analytical techniques may be used to follow the movement and dissipation of pyrithiobac sodium in soil. Of the many techniques possible, high performance liquid chromatography (HPLC) and gas chromatography in combination with mass spectrometry (GC/MS) have traditionally been used to measure pyrithiobac sodium.[5,6] However, these methods are often time consuming, expensive and laborious generating high analysis costs for environmental fate studies.

In recent years there has been an increased demand for the regular monitoring of soil resources for the presence of pesticides. This has led to alternative analytical techniques being sought such as immunoassay, which provides simple, fast, and cost-effective means of residue analysis. A sensitive enzyme-linked immunosorbent assay (ELISA) for the detection of pyrithiobac sodium was developed by DuPont (Strahan, personal communication). This ELISA required validation with Australian soils before being applied to environmental fate studies and monitoring of common transport routes of this herbicide in the Australian cotton production systems.

This study describes that validation. Validation steps included the following: 1) determination of sensitivity, specificity and matrix interference with this ELISA, 2) development of extraction/cleanup methods for pyrithiobac sodium in soil(s) common to

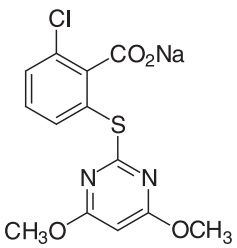

Figure 1. Chemical structure of pyrithiobac sodium.

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Australian cotton growing areas, suitable for use in ELISA, 3) determination of pyrithiobac sodium in spiked soils and field samples with incurred residues by immunoassay and instrumental method, 4) correlation of ELISA results with GC/MSD data, and 5) confirmation of ELISA by mass spectroscopy, using both positive soils and negatives.

MATERIALS AND METHODS

Extraction

Two methods to extract pyrithiobac sodium from Australian soils were developed and compared. These methods are described below.

Soaking with Agitation Extraction

The soaking with agitation extraction procedure is based on pesticide extraction work done by Lee et al.[7] and Wang et al.,[8] in which organic compounds were extracted efficiently by soaking soil in an extractant, with some agitation. The technique has the advantages of simplicity and no requirement for sophisticated equipment. In this method, soil was thoroughly mixed, and 10 g was weighed into a vessel. Twenty mL of extractant was added and the vessel either shaken using a Braun Certomat® gyrotatory platform or tumbled end-over-end on a Creswell rotating wheel. Overnight extraction gave more consistent results than shorter extraction times (3 hr) and was the preferred method. After extraction, the supernatant was obtained by centrifuging in a bench centrifuge at 4500 g for 15 min.

Accelerated Solvent Extraction (ASE™)

This method was developed for efficient extraction of pyrithiobac sodium from soils prior to LC/UV and LC/MS analysis.[5] Before extraction, 10 g of soil was weighed into a 50-mL plastic centrifuge tube. Silica gel (7g) was weighed into the centrifuge tube and thoroughly mixed by shaking, and transferred to a 22-mL ASE™ extraction cell. The loaded cell was extracted on a DIONEX ASE™ 200 extractor using a heat step of 5 min, a static step of 10 min and a 40% extraction flush volume. Extraction of pyrithiobac sodium was carried out in purified water under pressure (2000 psi) at a temperature of 100°C. The extractor was set up with a 60-second nitrogen purge after the extraction. A solvent rinse of the ASE™ extractor lines was performed between each extraction to prevent cross contamination. The extract was collected in a capped 60-mL vial, and the extract volume was brought up to 50 mL. After extraction, the extract was cleaned-up by passing it through graphitized carbon. After clean-up, the extract could be analyzed by column-switching LC/UV and LC/MS.[5] After derivatization using diazomethane, the sample could also be analyzed by GC/MS.[6]

Analysis

Following extraction, samples were analyzed by ELISA and by GC/MSD. Some samples analyzed by ELISA were also analyzed for confirmatory purposes using a

Finnigan GCQ confirmatory method. The procedures used in these methods are summarized below.

ELISA Analysis

ELISA was performed at room temperature following the DuPont Protocol, using 96-well ELISA plates and reagents supplied from DuPont. Briefly, 0.9 mL of each sample was dispensed into tubes, and 0.1 mL of antibody solution (one tablet was hydrated in 5 mL PBS/BSA solution) was immediately added to each tube. After a 60-min incubation, 200 μL aliquots of each sample mixture were transferred to triplicate wells of the antigen-coated microwell plate and incubated a further 60 min. The plates were emptied and washed six times, before 200 μL of freshly prepared second antibody-enzyme conjugate [anti rabbit IgG (H + L)-alkaline phosphatase] was added to all wells for another 60 min incubation. After the plate incubation was completed, the plate was washed six more times and 200 μL substrate reagent (*para*-nitrophenyl phosphate) was added to each well. Absorbance values were monitored over 35 and 45 min to determine optimum colour development, aiming for a maximum of around 2.0. Absorbance was read at 405 nm (using a differential filter set at 650 nm) and each sample was applied to triplicate wells.

GC/MSD Analysis After ASE™ Extraction

Samples were cleaned-up and derivatized before GC/MSD analysis was performed. Sample purification was done by trapping pyrithiobac sodium on a 2 g graphitized carbon cartridge, followed by selective elution in a 0.1 M formic acid in 90/10 dichloromethane/methanol solution. After eluting the pyrithiobac sodium, the dichloromethane, formic acid and methanol solution was evaporated.[5] This sample was derivatized with diazomethane and the resultant methyl ester was analyzed.[6] Instrumental analysis was carried out on a Hewlett Packard 5890 Gas Chromatograph equipped with a 5791A Mass Selective Detector. A J&W DB1701 column (30m × 0.22mm × 0.25um) was used for the separation. The column temperature was ramped from 100°C (1.5 min) to 270°C at 20°C/min; the temperature was held at 270°C for 5 min. The temperatures for injector and detector were 250 and 280°C, respectively. Quantitation and confirmation of the methyl ester of pyrithiobac sodium were based on the ions at m/z = 281 and 283, respectively. The extraction and clean-up method and the derivatization methods were successfully combined and performed in a commercial laboratory (AnalChem).

Confirmation After Soaking with Agitation Extraction

Confirmation analysis of the presence of pyrithiobac sodium in soil extracts in PBS was performed. The PBS soil extract was acidified with 1 M H_2SO_4 to a pH value less than 2 and extracted with ether three times. The combined ether extracts were dried on anhydrous sodium sulfate and evaporated to dryness. The derivatization was conducted with diazomethane for 15 min, the solvent then was reduced under a stream of nitrogen and the methylated product was redissolved in toluene, and 1 μL was injected for

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analysis using a Finnigan GCQ MAT GC/MS. An RTX-5MS column (30m × 0.25 mm ID × 0.25 μm df, 5% diphenyl - 95% dimethyl polysiloxane) was used. The chromatographic conditions used are described in the above paragraph. Identification and detection of pyrithiobac methyl ester was based on the ions at *m/z* = 343, 341, 311, 283, and 281.

Matrix Effect Study

To determine the extent of interference in ELISA by co-extractants of soil, blank soils (containing non-detectable residues) were extracted by both extraction methods described. Initially a range of extractants were evaluated to assess the effect of soil coextractants on the assay. The water solubility of pyrithiobac sodium was recognized by the testing of aqueous extractants: phosphate-buffered saline (PBS) and water. In addition, methanol (90% and 9%) was also investigated as it had been used previously for efficient extraction of organophosphorus and organochlorine pesticides for soil and food products in this laboratory. Standard curves were prepared in soil extract and soil-free extractant. The aim was to achieve no significant difference in absorbance between the pyrithiobac-sodium-free soil extract and pure extractant (i.e. the two inhibition curves to be superimposed). The following methods for removing soil matrix interference were assessed: 1) Dilution of the extract from 1/10-1/100 in PBS, 2) Addition of protein- bovine serum albumin (BSA) or Telostean fish gelatin (FG) to the extract diluent, 3) Filtration of the extract through a 0.45 μm cellulose acetate filter, and 4) Accelerated solvent extraction (ASE™), without passing the sample through graphitized carbon and without diazomethane derivitization.

Three pyrithiobac-sodium-free cotton soils, obtained from cotton-grown areas of Narrabri, Wee Waa and Moree were spiked with pyrithiobac sodium (stock solution) concentrations at between 1.2 and 12 ppb in the soil. Aged field soil samples containing aged pyrithiobac sodium residues were obtained from two sources: 1) a DuPont rain simulation trial at the Darling Downs near Towoomba, Queensland, Australia in 1996 and 2) from a DuPont groundwater study conducted in Tarborro, North Carolina, USA in 1994. These soils were used to compare ASE™/GC/MSD and ASE™/ELISA results. The characteristics of these soils are shown in Table 1.

Table 1. Characteristics of soil samples from cotton fields.

Sources	Type	Sand %	Silt %	Clay %	Organic content %	pH
Narrabri	Grey clay	26	12	61	0.68	8.4
Wee Waa	Red sandy clay	52.5	21.3	26.2	0.7	6.7
Moree	Clay	35	14	51	1.56	7.4
Darling Downs	Clay	17–20	15	65–68	1.1	8.1
Tarborro, North Carolina, USA	Sand	88–92	4–8	4	0.6–1.2	5.4–6.4

RESULTS AND DISCUSSION

Reproducibility of Pyrithiobac Sodium Assay

The precision of the assay was studied by determining the intra-assay repeatability and between-assay reproducibility. Within plate variation (%CV = standard deviation/mean $\times 100$) of triplicates was consistently less than 10%. A representative standard curve prepared in PBS was constructed from inhibition data collected on 10 separate days (Figure 2). The percent coefficients of variation were less than 14% for all the standard curve points. Increased relative error was observed at low pyrithiobac sodium concentration for this assay, as has been discussed by Harrison et al.[9]

Sensitivity and Specificity of the Assay

The IC_{50} for pyrithiobac sodium (determined from Figure 2) was 35 ppt. The LOD for the assay was calculated as approximately 4 ppt, determined as the concentration of pyrithiobac sodium resulting in 10% inhibition of colour. This corresponds to 0.16 ppb in the soil, taking into account the extraction ratio (1 soil :2 extractant) and the minimum dilution in PBS required to remove soil matrix effects (1/20).

Assay sensitivity was influenced by the choice of diluent used to prepare the standard curve. The sensitivity was highest when PBS was used as a diluent (35 ppt), followed by water (40 ppt), 0.9% methanol in PBS (50 ppt) and 9% methanol in PBS (70 ppt). Assay colour was not significantly affected by solvent choice (less than 10% variation of absorbance in controls).

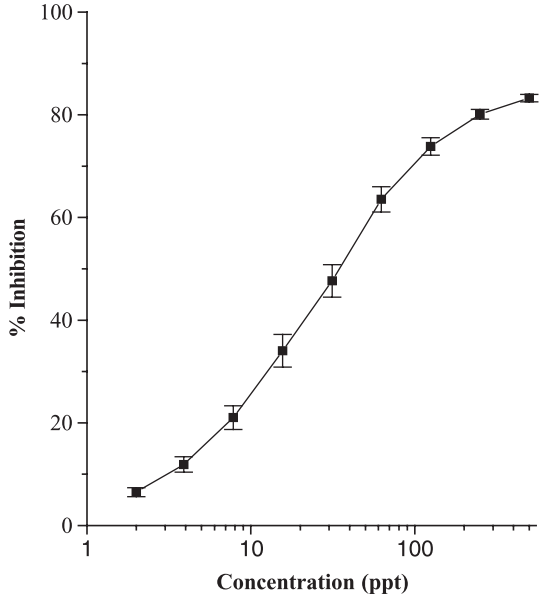

Figure 2. Standard curve for pyrithiobac sodium.

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Assay specificity was evaluated using several similarly structured aromatic herbicides (chlorobromuron, diuron, fluometuron, monolinuron, metobromuron, metoxuron, neburon, tebuthiuron, 3-(3,4-dichlorophenyl)-1-methylurea, 3,4-dichlorophenylurea) and some compounds that may reasonably be expected to be found in the Australian cotton production systems (bifenthrin, deltamethrin, lambda-cyhalothrin, DDE, DDT, endosulfan). The assay was specific for the target herbicide, with IC_{50} values for all these compounds above 10,000 ppb. The only PBS extractable metabolite of pyrithiobac sodium has been found not to show any significant cross-reactivity in the ELISA (Strahan, personal communication).

Soil Matrix Effects and their Removal

Narrabri grey clay soil from a 1997–1998 DuPont trial field site was selected as the soil for initial matrix effects studies. Standard curves prepared in water and methanol soil extracts, demonstrated a significant level of interference (the control absorbance was lowered from 2.2 to 1.4 AU) from the presence of soil co-extractants. Dilution of these extracts in PBS to 1/100 improved, but did not remove the interference completely. Using pure water as an extractant caused the soil to disperse, making it difficult to clarify the soil extract easily, even with centrifugation. It is thought possible that this contributed to the higher levels of interference observed with these extracts. PBS soil extracts were visibly clearer, possibly causing salt-induced flocculation of the suspended sediment. This may explain why PBS gave the least interference of the extractants tested.

The matrix interference could not be removed by addition of two sources of protein (BSA and FG) to the diluent. However, filtration of the extract with a 0.45μm Minisart filter (Sartorius AG, Germany), initially tested as a means to improve clarity of aqueous supernatant, was found to further decrease interference of PBS soil extracts, when they were diluted 1/100 prior to analysis. Pyrithiobac sodium showed no binding to the cellulose acetate filter, demonstrated by the closeness of the standard curves analyzed before and after filtration (data not shown).

PBS extracts were prepared from two other cotton growing soils ''Moree clay'' and ''Wee Waa red sandy clay'' using the overnight PBS shaking method, with filtration of the extract. Assay colour was not affected by dilutions of extract between 1/20 and 1/100. These two soils showed similar (or lower) matrix interference than Narrabri soil suggesting a minimum extract dilution of 1/20 may be an appropriate level of dilution for routine analysis for all three soil types. However, we have used a 1/100 or greater dilution of the field samples since residues of pyrithiobac sodium were relatively high (greater than 1 ppb in the soil) and required these dilutions to fall within the standard curve range.

The ASE™ method, without graphitized carbon clean-up, could remove interference with the ELISA caused by three pyrithiobac-sodium-free Australian soils' extracts. ASE™ extracts were clear, presumably due to filtration of the sample as it exits the ASE™ extraction cell. The ASE™ extracts gave some slight matrix interference at 1/10 dilution [assay sensitivity was unchanged, but color in the control was reduced by 20%]. The matrix interference was effectively removed at a 1/100 dilution.

There was only a slight difference in visual appearance between extracts prepared using overnight PBS extraction with filtering and those prepared by ASE™. The color

Table 2. Spike and recovery of pyrithiobac sodium from Narrabri cotton soil using soaking with agitation extraction.

	Recoveries %		
Spiked (ppb)	Fresh spiked + tumbled	1 Week aged + tumbled	1 Week aged + shaken
1.2	150 ± 6	132 ± 15	95 ± 10
2.4	108 ± 3	97 ± 5	90 ± 1
12	91 ± 3	84 ± 4	79 ± 1

of soil extract after ASE™ (variable sample extract volumes between 30–35 mL) varied from pale yellow to yellow, but following adjustment to 50 mL, the resultant color became either very pale yellow or not visible. The filtered PBS extracts were generally colorless or a pale yellow. The red soil gave stronger colored extract in both methods.

Spike and Recovery

Both methods of overnight agitation extraction gave acceptable recoveries of one-week-aged spiked residues using Narrabri soil (Table 2). The PBS shaking method was preferred for routine extractions because of its low cost and simplicity.

Lower recoveries (< 70%) at 6 – 12 ppb levels of pyrithiobac sodium (Table 3) from Moree soil indicate the overnight PBS extraction method may have a negative bias in the analysis of the Moree soil samples. However, this extraction method generated acceptable recoveries in Narrabri and Wee Waa soil at levels between 1.2 and 12 ppb. However, the higher recoveries at 1.2 ppb may cause positive bias for these soils.

Controls (pyrithiobac sodium-free soil samples) consistently showed no detection (below 10% inhibition relative to the absorbance of the PBS control) with 1/20 dilution, indicating a low probability of obtaining false positives. Spikes close to the LOD (0.12–0.24 ppb) were consistently detected with inhibition of color > 10% (Table 4). This indicates that reliable results can be obtained for residues at or above the LOD, with a low probability of false negatives.

Table 3. Spike and recovery in Moree and Wee Waa soils using soaking with agitation extraction.

	Recoveries %	
Spiked (ppb)	Moree soil	Wee Waa red soil
1.2	137 ± 4	145 ± 5
2.4	94 ± 6	121 ± 12
6	64 ± 4	96 ± 1
10	60 ± 1	82 ± 2
12	58 ± 6	75 ± 1

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Table 4. Detection of pyrithiobac sodium spikes in Narrabri soil at or near the LOD.

Spiked ppb in soil	Detected ppb in soil
0.12	0.12 ± 0.03
0.24	0.26 ± 0.02

Validation with Field Soil Samples

Both ASE™/GC/MSD and PBS over night soaking with agitation/ELISA results were compared (blind comparison) for a set of 28 aged, field soils collected during a DuPont rain simulation trial in 1996. All soil samples were stored at −20°C before analysis. Pyrithiobac sodium levels in the soil (as determined by GC/MSD and ELISA methods) showed high correlations (r = 0.93 and r = 0.92 respectively) for both extraction methods. A paired t-test indicated there was no significant statistical difference between ELISA and GC values. The equation of the regression line between ASE™/GC/MSD and PBS over night soaking with agitation/ELISA results for the ASE™ extraction was $y = 0.92x + 15.4$. The equation of the line in the comparison of PBS extracts ($y = 0.77x + 7.0$) showed a slope significantly less than 1, indicating the values obtained by ASE™/GC/MSD were generally higher. It was suspected that this was due to higher extraction efficiency of pyrithiobac sodium from aged soil by a rigorous ASE™ extraction.

The results in Table 3 indicate that recoveries for the 1.2 ppb fortifications were high and that the recoveries for the 12 ppb fortification were low. We believe that this is due to the dilution of the sample that was required for analysis.

When samples from ASE™ and PBS extractions were both analyzed by ELISA, a regression equation ASE™ = 1.1 × PBS + 13 was obtained with r = 0.93. The slope of 1.1 shows that the values obtained by ASE™ extraction are greater than values determined by PBS extraction by almost 10%, indicating that the ASE™ extraction method is more efficient. Although the PBS extraction method showed acceptable recoveries for one-week, laboratory ''aged'' soil, the more robust ASE™ extraction seems to give better recoveries for field weathered soil samples. Such an observation is quite common when comparing ELISA and instrumental analysis, since extraction conditions for conventional analyses are frequently harsher than those used for ELISA. This study indicates that pyrithiobac sodium has the potential to bind very tightly to some soils, requiring high temperature and pressure to extract, like the conditions used for the ASE™ extractions.

Confirmation of Pyrithiobac Sodium Soil Samples by GC/MS

Blank and positive pyrithiobac sodium soil sample extracts extracted by soaking agitation extraction were confirmed by GC/MS, operated in the positive chemical ionisation mode. The mass spectrum of pyrithiobac sodium (methylated product), which eluted at about 11 min, showed a base peak at *m/z* 341 and characteristic ions at *m/z* 343, 309, 311, and 281. It can be concluded that the compound eluting at about 11 min

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from these positive extracts was pyrithiobac-sodium. No peak was found to be pyrithiobac sodium in the negative soil extracts. MS results indicated that no detectable residues of pyrithiobac sodium were present in the blank soil, while the parent pyrithiobac sodium was found in the positive soil samples.

CONCLUSION

The results of this study indicate that the pyrithiobac sodium ELISA method described can perform as a rapid, economical screening method for soil extracts; however the PBS extraction/ELISA method should not be used without a confirmatory method. Samples where detectable levels are found should be re-analyzed by GC/MS or LC/MS analytical techniques, using the ASE™ extraction method described to confirm the presence of pyrithiobac sodium. Levels of pyrithiobac sodium extracted by the PBS extraction method may be lower than levels extracted by the ASE™ method. The levels may be significantly different, depending on the soil type. We suggest that spike and recovery experiments be carried out for each soil type when this soaking and agitation soil extraction method is applied.

The results of the two methods of soil extraction need particular comment. The ASE™ method performed satisfactorily in extracting pyrithiobac sodium from field aged soils and removing soil matrix interference from several Australian soil types. The PBS method (which did not require sophisticated equipment or cleanup) involved extracting soil overnight in PBS buffer with agitation. The extract was centrifuged, filtered through a 0.45 μm filter and diluted at least 1/20 before analysis. ASE™ and soaking with agitation overnight extraction methods correlated well with each other (using ELISA, $R = 0.93$) and also with instrumental analysis using GC results ($R = 0.93$ and 0.92, respectively). Although the soaking and agitation extraction method is not as good as ASE™ extraction for removing Pyrithiobac-sodium™ from field aged soil samples, soaking and agitation extraction still provides a rapid, low cost screening method without the need for specialized equipment. This PBS extraction method is ideal for using in conjunction with ELISA as a screening technique. Of the four Australian soil types examined, three provided very satisfactory results using the soaking and agitation extraction method.

The advantages of the pyrithiobac sodium ELISA have been demonstrated in a field study. These include high sample throughput, simplicity, speed and cost effectiveness. The validated method was used in a field study focusing on the environmental fate of Staple® in Australian cotton production systems. This study demonstrated the usefulness of ELISA as an analytical tool for detecting pyrithiobac sodium and the results are described elsewhere.[4]

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REFERENCES

1. Jordan, D.L.; Frans, R.E.; McClelland, M.R. Total postemergence programs in cotton with sethoxydim and DPX-PE350. Weed Technol. **1993**, *7*, 196–201.
2. Jordan, D.L.; Frans, R.E.; McClelland, M.R. Cotton (*Gossypium hirsutum*) response to DPX-PE350 applied postemergence. Weed Technol. **1993**, *7*, 159–162.
3. Reddy, K.N.; Locke, M.A.; Bryson, C.T. Foliar washoff and runoff losses of lactofen, norflurazon, and fluometuron under simulated rainfall. J. Agric. Food Chem. **1994**, *42*, 2338–2343.
4. Mitchell, G.; Kennedy, I.R.; Sanchez-Bayo, F. *Field Soil Dissipation of Pyrithiobac Sodium Following Application of Staple® Herbicide*; Report to DuPont, 1999.
5. Sumpter, S.R.; Peterson, B.A.; Ledeker, K.W.; Mulderig, L.J. *Analytical Method for Determination of Pyrithiobac Sodium in Soil Using Subcritical Extraction, Graphitized Carbon Cleaned-up, and Column-Switching LC/UV Analysis with Confirmation by LC/MS*; DuPont Report No. AMR 2745-93, DuPont Crop Protection, E.I. du Pont de Nemours and Company: Wilmington, DE, 1993.
6. Sumpter, S.R.; Class, R.; Bacher, R. *Analytical Method for the Determination of Pyrithiobac Sodium in Whole Milk, Beef Tissue, and Whole Chicken Egg Samples*; DuPont Report No. AMR 4838-97, DuPont Crop Protection, E.I. du Pont de Nemours and Company: Wilmington, DE, 1998.
7. Lee, N.J.; Beasley, H.L.; Kimber, S.W.L.; Silburn, M.; Woods, N.; Skerritt, J.H.; Kennedy, I.R. Application of immunoassays to studies of the environmental fate of endosulfan. J. Agric. Food Chem. **1997**, *45*, 4147–4155.
8. Wang, S.; Allan, R.D.; Skerritt, J.H.; Kennedy, I.R. Development of a class-specific competitive ELISA for the benzoylphenylurea insecticides. J. Agric. Food Chem. **1998**, *46*, 3330–3338.
9. Harrison, R.O.; Braun, A.L.; Gee, S.J.; O'Brien, D.J.; Hammock, B.D. (1989) Evaluation of an enzyme-linked immunosorbent assay (ELISA) for the direct analysis of molinate (Ordram®) in rice field water. Food Agric. Immunol. **1989**, *1*, 37–52.

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