Residues of Spinosad in Meat, Milk, and Eggs

Bonnie S. Rutherford, Robert C. Gardner, Sheldon D. West,* Cynthia K. Robb, and Sean C. Dolder

Global Environmental Chemistry Laboratory—Indianapolis Laboratory, Dow AgroSciences LLC, 9330 Zionsville Road, Indianapolis, Indiana 46268-1054

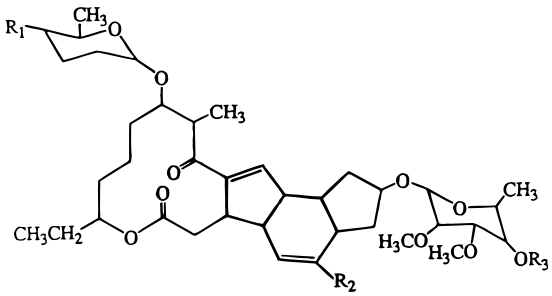
Spinosad is an insect control agent that is derived from a naturally occurring soil bacterium and has a high level of activity against insects that infest a variety of crops. Dairy and poultry feeding studies were conducted to determine the magnitude of spinosad residues in animal products that would result from the consumption of typical feed commodities containing residues of spinosad. Dairy cows were dosed for 28 days with spinosad at rates equivalent to 0, 1, 3, and 10 μg/g in the diet. Chicken hens were dosed for 42 days with spinosad at rates equivalent to 0, 0.1, 0.3, 1, and 5 μg/g in the diet. Milk, eggs, and tissue samples were analyzed by high-performance liquid chromatography and/or immunoassay methods. Spinosad residues occurred in all of the sample types but were lowest in eggs, skim milk, and lean meat and were highest in the fat.

Keywords: *Spinosad; spinosyn; residues; beef; chicken; meat; milk; eggs*

INTRODUCTION

Spinosad is the active ingredient in Tracer Naturalyte (trademark of Dow AgroSciences LLC), Success Naturalyte (trademark of Dow AgroSciences LLC), SpinTor Naturalyte (trademark of Dow AgroSciences LLC), and Conserve (trademark of Dow AgroSciences LLC) insect control products, which are registered in many countries on a variety of crops (West et al., 2000). Spinosad is produced by the soil actinomycete *Saccharopolyspora spinosa* and is a naturally occurring mixture of two active components, spinosyns A and D. The material has a high level of activity against several types of insects that infest cotton, vegetables, fruit, tree crops, ornamentals, and turf. Spinosad has a low order of toxicity to mammals, birds, and fish. The biological characteristics have been described by Sparks et al. (1995), and the field performance in cotton has been described by Thompson et al. (1995).

Prior to magnitude of residue animal feeding studies, ruminant and poultry metabolism studies were conducted to identify and characterize the residues produced in livestock consuming spinosad. A ruminant metabolism study was conducted on dairy goats dosed for 3 days with 10 μg/g ^{14}C-labeled spinosyn A or D. This study demonstrated that the major residues in tissues and milk were the parent compounds (spinosyns A and D), with spinosyn B and *N*-demethylspinosyn D being present as minor metabolites. Spinosyn K, which has been identified as a minor metabolite in plants, was not a significant metabolite in beef tissues (D. P. Rainey, J. D. Magnussen, and D. F. Berard, Dow AgroSciences, personal communication, 1994). The structures of the parent compounds and the metabolites are included in Figure 1. Lean meat, kidney, and liver tissues also contained lesser amounts of other spinosad-related metabolites, tentatively identified as hydroxylated spinosyns.



Spinosad and Metabolites

spinosyn A, $R_1 = N(CH_3)_2$, $R_2 = H$, and $R_3 = CH_3$
spinosyn D, $R_1 = N(CH_3)_2$, $R_2 = CH_3$, and $R_3 = CH_3$
spinosyn K, $R_1 = N(CH_3)_2$, $R_2 = H$, and $R_3 = H$
spinosyn B, $R_1 = NH(CH_3)$, $R_2 = H$, and $R_3 = CH_3$
N-demethyl spinosyn D, $R_1 = NH(CH_3)$, $R_2 = CH_3$, and $R_3 = CH_3$

Figure 1. Structures of spinosad and metabolites.

In addition, a poultry metabolism study was conducted in chicken hens dosed for 5 days with 10 μg/g ^{14}C-labeled spinosyn A or D. The residues that were identified in poultry tissues and eggs were the same as those identified in goats, that is, primarily spinosyns A and D along with minor amounts of spinosyn B and *N*-demethylspinosyn D (J. D. Magnussen and S. A. Castetter, Dow AgroSciences, personal communication, 1994). After reviewing numerous spinosad metabolism and residue studies, the U.S. Environmental Protection Agency (EPA) subsequently ruled that spinosyns A and D are the residues of interest for tolerances in crops and livestock (*Fed. Regist.*, 1999).

This paper describes the results of dairy and poultry feeding studies that were conducted to determine the magnitude of spinosad residues that would result from consumption of typical animal feed commodities containing residues of spinosad. The residue data obtained from the livestock products have been used to establish residue tolerances in meat, milk, and eggs.

* Author to whom correspondence should be addressed [fax (317) 337-3255; e-mail sdwest@dowagro.com].

10.1021/jf0004457 CCC: $19.00
Published on Web 08/06/2000

EXPERIMENTAL PROCEDURES

Test Substance. Spinosad technical material was the test substance. The test substance had a purity of 88.0% and contained a mixture of spinosyns A (76.1%) and D (11.9%). Technical spinosad also contains numerous other minor spinosyns (Sparks et al., 1995). These minor components do not contribute significantly to the biological activity or the residue levels of spinosad and have not been included by the EPA as part of the total toxic residue for tolerance enforcement.

Dairy Feeding Study—Live Phase. Four groups of Holstein dairy cows were dosed for 28 days via gelatin capsules containing spinosad at rates of 0 μg/g (control), 1 μg/g (1×), 3 μg/g (3×), and 10 μg/g (10×). These rates (1×, 3×, and 10×) were selected to cover current and possible future residue levels in animal feeds resulting from residues in crops treated with spinosad as specified by EPA for animal feeding residue studies. Each group consisted of three dairy cows. Dose rates were calculated on the basis of the actual feed consumption of the cows during the acclimation period, prior to initiation of the study.

Dosing capsules were prepared by weighing the appropriate amount of test substance and transferring it into individual gelatin capsules. The dosage was corrected for the purity of the test material. Capsules were stored in sealed plastic jars in a refrigerator until used. The 12 cows were housed in individual stalls (1.2 × 2.1 m), and the dose groups were kept together. The cows were identified by unique numbers on neck tags and stall cards. All cows were 3−4 years old and in midlactation. Body weights were obtained prior to the start of dosing and again at the end of 28 days of dosing. Daily feed consumption, milk production, and the general health of the cows were monitored during the acclimation and dosing periods.

The cows were fed and milked twice a day. Water was available at all times, and stalls were cleaned daily. Natural and artificial lighting provided at least 10 h of light per day. The cows were allowed to exercise outdoors in a fenced enclosure at least two to three times per week.

The cows were dosed daily following the morning milking for 28 consecutive days. The control group was dosed first, followed by the treatment groups in order of increasing dosage. The capsules were administered using a balling gun. The cows in the control group received empty capsules.

Milk collected in the morning and evening was combined to provide a daily milk sample of ∼250 mL from each cow. The combined milk sample was proportioned on the basis of the amount of milk produced at each milking divided by the total amount produced on that day. Milk samples from different cows were not combined. Each milk sample was placed in a high-density polyethylene container and frozen.

On study days 14 and 28, additional aliquots of milk from each cow were poured into a milk separator for separation into cream and skim milk. Samples of cream (100 mL) and skim milk (250 mL) were then collected and frozen.

Representative tissue samples were collected for residue analysis within 24 h of the final dosing. The samples consisted of 1 kg of lean beef tissue (approximately equal portions of flank, loin, and leg), 500 g of fat tissue (approximately equal portions of omental, peritineal, and somatic), and 1 kg each of kidney and liver from each cow. The tissues were placed in plastic bags and were frozen within 4 h of collection. Tissues from different cows were not combined.

Poultry Feeding Study—Live Phase. Five groups of nine White Leghorn laying hens each were dosed for 42 days via gelatin capsules containing spinosad at rates of 0 μg/g (control), 0.1 μg/g (1×), 0.3 μg/g (3×), 1 μg/g (10×), and 5 μg/g (50×). These rates (1×, 3×, 10×, and 50×) were selected to cover current and possible future residue levels in animal feeds resulting from residues in crops treated with spinosad as specified by EPA for animal feeding residue studies. Dose rates were calculated on the basis of the amount of spinosad administered and the feed consumption during the 7 days prior to study initiation.

Dosing capsules were prepared by adding measured amounts of an acetone solution of spinosad test substance to each capsule. Dextrose was used as a filler and adsorbent for the acetone solution. Control capsules contained only dextrose and acetone. The capsules were stored frozen prior to use.

The hens were subgrouped in sets of three and were individually housed according to the treatment level subgroup in wire pens over a concrete floor. The hens were identified with plastic leg bands embossed with unique numbers for the study. The hens were 22 weeks old at the start of the study and ranged in weight from 1086 to 1858 g during the study. Feed and water were provided ad libitum. Fluorescent lights provided lighting for 16 h per day, and daily temperatures and humidity were recorded. Feed consumption and egg production were recorded daily, and body weights were obtained weekly. Observations were made twice daily for the general health of the chickens.

The hens were dosed daily in the morning for 42 consecutive days. The control group was dosed first, followed by the treatment groups in order of increasing dosage. The capsules were administered orally using a balling gun. The hens in the control group received the dextrose-filled placebo capsules.

Eggs were collected twice daily, and eggs from each treatment subgroup were broken and pooled as a composited sample on selected test days. The composited samples were placed in plastic containers, vigorously agitated, and placed in frozen storage. At the end of the dosing period (42 days), tissue samples were collected from half of each hen and pooled according to subgroup and tissue type. Sample weights were recorded by subgroup. The following tissue sample types were collected: (1) half of the carcass without bones; (2) the entire liver (gallbladder removed); (3) all abdominal fat; (4) all light meat (breast) and dark meat (leg, thigh, and wing) without skin, fat, or bones; and (5) subcutaneous fat. The tissue samples were placed in frozen storage after collection.

Initial Sample Preparation. Prior to analysis, frozen tissue samples were treated with liquid nitrogen, ground, and mixed. Milk samples and the composited egg samples were thawed and thoroughly mixed.

Sample Analysis by HPLC. Eggs, milk, skim milk, cream, and tissues (lean meat, kidney, liver, and fat) were analyzed by high-performance liquid chromatography (HPLC) with ultraviolet (UV) detection (West and Turner, 1998). In general, the HPLC method involved extraction of the analytes using suitable organic solvents or aqueous−organic mixtures. Initial purification of the extracts was accomplished using aqueous−organic partitioning. Further purification was achieved using silica and cyclohexyl SPE cartridges. The analytes were then separated and determined simultaneously using HPLC with UV detection at 250 nm.

The method measured the individual concentrations of the two active ingredients (spinosyns A and D) and the two minor metabolites (spinosyn B and *N*-demethylspinosyn D). The limits of detection (LOD) and quantitation (LOQ) were approximately 0.003 and 0.010 μg/g, respectively, for each analyte in all matrices except for poultry fat. For poultry fat, the LOD and LOQ were 0.01 and 0.03 μg/g, respectively. The residues of the individual analytes were determined by HPLC-UV and were then summed to provide the total spinosad residue.

The efficiency of the analytical methodology was determined by fortifying control samples with the appropriate analytes and analyzing them along with the experimental samples to determine the levels of recovery.

Immunoassay. Milk and beef tissues (lean beef, kidney, and liver) were analyzed by immunochemical analysis using the Spinosad RaPID Assay test kit (Strategic Diagnostics, Inc., Newark, DE) (Young et al., 2000). Residues of spinosad were extracted from milk with acetonitrile and from beef tissues with 80% acetonitrile/20% water. An aliquot of the extract was evaporated to dryness and reconstituted with Spinosad Sample Diluent, which was provided with the test kit. Samples were then analyzed as described on the product information insert contained within the test kit. Residues were determined by measuring the absorbance at 450 nm using an RPA-1 RaPID Analyzer (Strategic Diagnostics, Inc.). Calibration standards

Table 1. Summary of Immunoassay Residue Results for Spinosad in Milk from Dairy Cows Dosed with Spinosad for 28 Days

dosage time (day)	residues of spinosad in milk,[a] μg/mL (mean ± standard deviation)			
	control	1 μg/g	3 μg/g	10 μg/g
−2[b]	ND[c]	ND	ND	ND
−1[b]	ND	ND	ND	ND
1	ND	ND	<0.01[d]	0.018 ± 0.005
2	ND	0.026 ± 0.004	0.069 ± 0.009	0.217 ± 0.041
3	ND	0.034 ± 0.003	0.106 ± 0.024	0.371 ± 0.086
4	ND	0.038 ± 0.012	0.121 ± 0.032	0.383 ± 0.069
5	ND	0.033 ± 0.007	0.106 ± 0.010	0.434 ± 0.048
6	ND	0.041 ± 0.009	0.138 ± 0.042	0.500 ± 0.073
7	ND	0.044 ± 0.004	0.131 ± 0.031	0.558 ± 0.114
10	ND	0.050 ± 0.011	0.155 ± 0.046	0.527 ± 0.095
12	ND	0.061 ± 0.013	0.163 ± 0.040	0.535 ± 0.048
14	ND	0.071 ± 0.006	0.178 ± 0.034	0.797 ± 0.190
16	ND	0.071 ± 0.016	0.145 ± 0.040	0.619 ± 0.206
21	ND	0.043 ± 0.002	0.137 ± 0.016	0.527 ± 0.056
28	ND	0.049 ± 0.007	0.157 ± 0.036	0.506 ± 0.104

[a] Total spinosad residues measured by immunoassay (average of residues in all cows in the same dose group). [b] Days before dosing. [c] None detected at an LOD of 0.003 μg/g. [d] Value between the LOQ (0.010 μg/g) and the LOD (0.003 μg/g), i.e., a region of less certain quantitation.

were provided in the IA test kit. The LOD and LOQ for the IA method were approximately 0.003 and 0.010 μg/g, respectively.

The IA method used in this study was sensitive to several individual spinosyns as well as some metabolites and degradates. It was not capable of differentiating individual spinosyns and thus measured the total residue of spinosad. IA allowed rapid screening of the milk samples for total spinosad-related residues in order to determine when the residues had reached a plateau. In addition to detecting spinosyns A, D, and B and *N*-demethylspinosyn D, the IA method also detected trace levels of hydroxylated metabolites not measured by HPLC in lean meat, kidney, and liver tissues.

RESULTS AND DISCUSSION

Dose-Related Effects. There were no dose-related effects on general health, behavior, body weight, feed consumption, or egg/milk production of the animals in these feeding studies.

Analytical Recoveries. The efficiency of the analytical methodology was determined by fortifying control samples with the appropriate analytes and analyzing them along with the experimental samples to determine the levels of recovery. For the HPLC method, average recoveries for the various analytes ranged from 97 to 110% (whole milk, skim milk, and cream), from 72 to 90% (beef fat), from 80 to 97% (beef lean meat, liver, and kidney), from 85 to 89% (eggs), from 101 to 105% (chicken fat), and from 95 to 99% (chicken lean meat and liver). For the IA method, average recoveries were 96% for milk and 75% for beef lean meat, liver, and kidney. These recovery values compared favorably with those published previously for the HPLC method (West and Turner, 1998) and for the IA method (Young et al., 2000).

Dairy Results. Residues of spinosad in milk were determined by IA because of its shorter analysis time compared to HPLC analysis. Samples were analyzed daily for the first week and periodically thereafter. The results are summarized in Table 1. After 14 days of dosing, average residues in milk reached a plateau at 0.071 μg/mL at the 1× dosage level, 0.178 μg/mL at the 3× level, and 0.797 μg/mL at the 10× level.

Table 2. Summary of Residues of Spinosad in Milk and Tissues of Dairy Cows Dosed with Spinosad for 28 Days

tissue	assay	av residues[a] of spinosad in tissues of dairy cows			
		control	1 μg/g	3 μg/g	10 μg/g
milk	IA[b]	ND[c]	0.05	0.16	0.49
	HPLC[d]	ND	0.04	0.13	0.48
cream	IA	NA[e]	NA	NA	NA
	HPLC	ND	0.18	0.59	1.9
skim milk	IA	NA	NA	NA	NA
	HPLC	ND	<0.01[f]	0.01	0.08
lean beef	IA	ND	0.03	0.06	0.32
	HPLC	ND	0.02	0.04	0.23
kidney	IA	ND	0.08	0.32	1.0
	HPLC	ND	0.07	0.25	0.73
liver	IA	ND	0.19	0.61	2.4
	HPLC	ND	0.13	0.35	1.2
fat	IA	NA	NA	NA	NA
	HPLC	ND	0.65	1.1	5.7

[a] Average of residues in all cows in same dose group. [b] Total spinosad residues measured by immunoassay. [c] None detected at an LOD of 0.003 μg/g. [d] Total residues of spinosyns A, D, and B, and *N*-demethylspinosyn D measured by HPLC. [e] Not applicable (not assayed) by IA. [f] Value between the LOQ (0.010 μg/g) and the LOD (0.003 μg/g), i.e., a region of less certain quantitation.

Skim milk and cream collected after 14 and 28 days of dosing were assayed by HPLC. The results indicated that the residues partition preferentially into cream (Table 2).

Residues in lean beef, kidney, and liver were analyzed by both IA and HPLC. IA detected some hydroxylated metabolites that could not be determined by HPLC due to the lack of appropriate reference standards. Lean meat, kidney, and liver were determined radiochemically to contain 24−32% of these hydroxylated metabolites in a ^{14}C metabolism study with goats (Rainey et al., personal communication, 1994). However, the hydroxylated metabolites comprised only 4% of the total radioactive residue in fat tissue residues in the metabolism study, with spinosyn A comprising 86% of the residue. Fat tissues from the feeding study were thus assayed only by the HPLC method.

A good correlation of results from the HPLC and IA methods was observed (Table 2). At each dosage level, the residues detected in lean beef, kidney, and liver by IA were slightly higher than residues detected by HPLC, suggesting the detection of additional spinosad-related metabolites other than spinosyns A, D, and B and *N*-demethylspinosyn D. The HPLC chromatograms from the study, particularly those from the 10 μg/g dose group, contained additional minor peaks suggestive of other spinosyns. It is possible that these materials might have been the hydroxylated metabolites detected in the metabolism study, although standards were not available to provide reference points for the retention times. Spinosyn A was the major residue in each matrix, constituting ∼75−80% of the residue, whereas spinosyn B constituted 15−20% of the residue in these tissues.

Spinosad residues occurred in all of the sample types but were lowest in skim milk and highest in beef fat (Table 2). Tissue residues at the 10 μg/g dose level in Table 2 were similar to those that had occurred in the radiolabeled metabolism study, in which the goat had been dosed for 3 days with 10 μg/g ^{14}C-labeled spinosyn A. The total radioactive residues in goat tissues were 3.6 μg/g in fat, 0.30 μg/g in lean meat, 0.97 μg/g in kidney, and 1.6 μg/g in liver (Rainey et al., personal communication, 1994).

Table 3. Summary of HPLC-UV Residue Results for Spinosad in Eggs from Hens Dosed with Spinosad for 42 Days

	residues of spinosad in eggs,[a] μg/g (mean ± standard deviation)				
day	control	0.1 μg/g	0.3 μg/g	1 μg/g	5 μg/g
−1[b]	ND[c]	ND	ND	ND	ND
1	ND	NA[d]	NA	NA	ND
4	ND	NA	NA	NA	0.100 ± 0.024
7	ND	NA	NA	NA	0.130 ± 0.027
10	ND	NA	NA	NA	0.207 ± 0.019
13	ND	NA	NA	NA	0.227 ± 0.097
20	ND	NA	NA	NA	0.217 ± 0.019
28	ND	ND	ND	0.014 ± 0.006	0.140 ± 0.047
35	ND	ND	ND	0.012 ± 0.003	0.177 ± 0.038
41	ND	ND	<0.01[e]	<0.01[e]	0.186 ± 0.019

[a] Total spinosad residues (average of total residues in eggs from all hens in same dose group). [b] One day before dosing. [c] None detected at an LOD of 0.003 μg/g. [d] Not applicable (sample not analyzed). [e] Value between the LOQ (0.010 μg/g) and the LOD (0.003 μg/g), i.e., a region of less certain quantitation.

Table 4. Summary of Residues of Spinosad in Eggs and Tissues of Hens Dosed with Spinosad for 42 Days

	av total residues of spinosad in poultry tissues[a]				
tissue	control	0.1 μg/g	0.3 μg/g	1.0 μg/g	5.0 μg/g
eggs	ND[b]	ND	<0.01[c]	<0.01[c]	0.19
whole body	ND	<0.01[c]	<0.01[c]	0.02	0.17
light meat	ND	ND	ND	ND	0.04
dark meat	ND	ND	ND	ND	0.07
abdominal fat	ND	<0.03[d]	0.03	0.14	1.2
subcutaneous fat	<0.03[d]	0.03	0.04	0.16	1.1
liver	ND	ND	ND	0.01	0.09

[a] Average total residues of spinosyns A and D determined by HPLC. [b] None detected at an LOD of 0.003 μg/g. [c] Value between the LOQ (0.010 μg/g) and the LOD (0.003 μg/g), i.e., a region of less certain quantitation. [d] Value between the LOQ (0.030 μg/g) and the LOD (0.010 μg/g), i.e., a region of less certain quantitation.

Poultry Results. Spinosad residues occurred in eggs and in all of the poultry tissues but were highest in fat (Tables 3 and 4). Except for residues near the LOQ of the method in some samples after 28−41 days of dosing, residues of spinosad in eggs were usually above the LOQ only at the 5 μg/g (50×) dosage level.

At the 50× dosage level, average residues reached a plateau at 0.227 μg/g after 13 days of dosing (Table 3). After 42 days of dosing at 50×, average total residues in the whole body tissue samples were similar to residues in eggs (Table 4). Residue levels in light meat, dark meat, and liver were less than residue levels in fat tissues, and residue levels in light meat were nearly half those in dark meat. In poultry samples where significant residues of spinosad were found, spinosyn A constituted ∼78% of the total in eggs ∼73% in tissues.

The octanol/water partition coefficient (log k_{ow}) for spinosyn A has been reported to range from 2.8 at pH 5.0 to 5.2 at pH 9.0. Log k_{ow} for spinosyn D has likewise been reported to range from 3.2 to 5.2 (Thompson et al., 1995). On the basis of these log k_{ow} values, it was expected that spinosad residues would tend to be highest in cream and fat.

Spinosad residue tolerances in crops that are components of animal feed have been used by the EPA in conjunction with the residues results in beef and poultry to establish residue tolerances in livestock commodities (*Fed. Regist.*, 1999).

ACKNOWLEDGMENT

We acknowledge the technical contributions of B. H. Arnold, B. A. Blakeslee, M. W. Harris, W. F. Langer, and D. L. Young.

LITERATURE CITED

Fed. Regist. **1999**, *64* (184), 51451−51460.

Sparks, T. C.; Thompson, G. D.; Larson, L. L.; Kirst, H. A.; Jantz, O. K.; Worden, T. V.; Hertlein, M. B.; Busacca, J. D. Biological characteristics of the spinosyns: new naturally derived insect control agents. *Proc. Beltwide Cotton Conf.* **1995**, 903−907.

Thompson, G. D.; Busacca, J. D.; Jantz, O. K.; Borth, P. W.; Nolting, S. P.; Winkle, J. R.; Gantz, R. L.; Huckaba, R. M.; Nead, B. A.; Peterson, L. G.; Porteous, D. J.; Richardson, J. M. Field performance in cotton of Spinosad: a new naturally derived insect control system. *Proc. Beltwide Cotton Conf.* **1995**, 907−910.

West, S. D.; Turner, L. G. Determination of the naturally derived insect control agent spinosad and its metabolites in meat, milk, cream, and eggs by high-performance liquid chromatography with ultraviolet detection. *J. Agric. Food Chem*. **1998**, *46*, 4620−4627.

West, S. D.; Yeh, L. T.; Turner, L. G.; Schwedler, D. A.; Thomas, A. D.; Duebelbeis, D. O. Determination of spinosad and its metabolites in food and environmental matrices—Part I: HPLC with UV detection. *J. Agric. Food Chem*. **2000**, in press.

Young, D. L.; Mihaliak, C. A.; West, S. D.; Hanselman, K. A.; Collins, R. A.; Phillips, A. M.; Robb, C. K. Determination of spinosad in food and environmental matrices—Part III: Immunoassay methods. *J. Agric. Food Chem*. **2000**, in press.

Received for review April 6, 2000. Revised manuscript received June 21, 2000. Accepted June 26, 2000.

JF0004457