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STUDY 3

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REVIEWED BY: E. A. Heger, M.S. Signature:
TITLE: Scientist Date:

EDITED BY: C. A. Little, Ph.D. Signature:
TITLE: Sr. Scientist/Asst. Project Manager Date:

APPROVED BY: P. H. Howard, Ph.D. Signature:
TITLE: Project Manager Date:

ORG: Syracuse Research Corp.
 Arlington, VA 22202

TEL: 703/413-9369

REVISED AND APPROVED BY: Kathryn Gallagher, Ph.D.
TITLE: Chemist
ORG: ERB II/EFED/OPP
TEL: 703-308-7149

Kathryn Gallagher
3/30/99

SIGNATURE:

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CONCLUSIONS

Metabolism - Anaerobic Aquatic

1. The portion of this study conducted with the pyridinyl-labeled diflufenzopyr is scientifically valid and provides useful information on the anaerobic aquatic metabolism of diflufenzopyr, for the low dose (0.01 ppm) treatment only. Although only single samples were utilized in the 5.3 ppm treatment rate study, adequate data were obtained in the 0.01 ppm study (in which duplicate samples were utilized) for the determination of a half-life of parent diflufenzopyr and the required residue characterization. However, no anaerobicity measurements were submitted for the 5.3 ppm pyridinyl test system, so the data for this dose of the pyridinyl label test are considered supplemental. Furthermore, the maximum concentrations of major degradates varied greatly between the low and high dose pyridinyl label experiments. The registrant needs to explain the differences in degrade concentrations and formation.

The portion of this study conducted with phenyl-labeled diflufenzopyr is not scientifically valid and does not provide useful information on the anaerobic aquatic metabolism of diflufenzopyr. Residue concentrations in the sediment of the system treated at 0.01 ppm were not determined directly, but were extrapolated from data from the 5.3 ppm treatment rate study because the analytical method was inadequate for the lower treatment rate. However, only single samples were used to obtain the data (for the 5.3 ppm study) which were used for the extrapolation. Because single samples were used in the 5.3 ppm study, those data are also not useful for the valid determination of a half-life for the parent compound (phenyl label) at that use rate. In addition to which, no anaerobicity measurements were submitted for the 5.3 ppm phenyl label test system. Furthermore, the Agency notes that all major degradates appeared at different rates and in different concentrations between the two doses for both labels, calling into question the validity of the high to low dose metabolite extrapolation for the phenyl study. The unsatisfactory nature of the phenyl label study is of particular concern because the little information obtained indicates that a metabolite of toxic concern, M2, (3, 5-difluoraniline) may be formed under anaerobic conditions in large amounts (44.5% of the applied in the 5.3 ppm study).

2. This study partially satisfies the Subdivision N Guidelines for the fulfillment of EPA data requirements on anaerobic aquatic metabolism based on the 0.01 ppm dose pyridinyl label test system. Factors which make the remaining test systems unsatisfactory for guideline 162-3 requirements include:
 - (i) the analytical method was inadequate (phenyl label, 0.01 ppm dose);
 - (ii) it could not be determined whether anaerobic conditions were assured and maintained for the systems treated at the 5.3 ppm treatment rate (pyridinyl and

phenyl label); neither pH nor redox potentials were measured throughout the study period. The registrant needs to submit data on the anaerobicity of the 5.3 ppm test systems.

3. The registrant also needs to explain the differences in degradate concentrations and formation rate among the four test systems, and the differences in half-life calculations for the pyridinyl and phenyl label studies. The inadequate phenyl label study yielded half-life equivalent estimations that were 30 to 40% longer than those determined for pyridinyl label study.

Summary

Pyridinyl ring-labeled [4,6-¹⁴C]diflufenzopyr, at nominal concentrations of 0.01 and 5.3 ppm, degraded with non-linear DT₅₀s (fifty percent dissipation times) of 20.0 and 19.6 days, respectively, in anaerobic flooded sandy loam sediment incubated in darkness at 25°C for up to 360 days. DT₅₀s were determined through least-squares exponential curve-fitting of non-transformed data. DT₉₀s determined through the exponential curve fitted to the data were 67 and 65 days for the 0.01 ppm and 5.3 ppm doses, respectively. DT₉₀s determined by visual graphical evaluation were 74 and 78 days, respectively. Linear half-lives, calculated through least squares curve-fitting to natural log-transformed data, yielded half-lives that varied depending on the number of days with parent levels below detection limits, which varied between the low and high doses. Including detection-level data in the linear regression for days 0 through 360, half-lives of 33.5 and 35.2 days were calculated ($r^2 = 0.796$ and 0.702 , respectively). As expected, the linear fit for days 0 through 360 posttreatment was significantly nonlinear based on a runs test. Using a uniform data point cut-off of 184 days, linear half-lives were determined to be 18.5 ($r^2=0.944$) and 24.2 ($r^2=0.997$) days, for the low and high pyridinyl label doses, respectively. The high dose data was found, however, to be significantly non-linear when a runs test was performed. Because of the variability in data censoring required, and the non-linearity of the regression for days 0 through 360 posttreatment, the DT₅₀s from the non-transformed data curve-fitting were selected as the data endpoints for this study.

The half-life calculated for the 5.3 ppm pyridinyl label study is of questionable validity due to the use of single samples and because anaerobic conditions were not assured. All data, designated here as percentages of the applied, were reported as percentages of the nominal application.

In the 0.01 ppm pyridinyl label test system, total parent levels were 99.5% of the applied radioactivity at 0 days posttreatment, decreased to 61% by day 14, were 36.4% by day 28, and were below the detection limit of 0.1% of the applied by 184 days posttreatment. The major degradate M1 was a maximum of 37.8% of the applied at 28 days posttreatment and was not detected by 268 days posttreatment. The major degradate M9 was a maximum of 71.3% of the applied radioactivity at 120 days posttreatment and was

65.2% at 360 days. The minor degradate M6 was detected once (in one replicate) at 0.7% of the applied radioactivity at 28 days posttreatment. Radiolabeled [^{14}C]volatiles accounted for 1.1% of the applied radioactivity, and nonextractable [^{14}C]residues were a maximum of 8.8% of the applied at 360 days posttreatment. The distribution of [^{14}C]residues between the soil and water fractions was not reported; however, the majority of the [^{14}C]residues was observed in the water phase up to 120 days posttreatment. Material balances ranged from 102.3% to 106.8% of the applied radioactivity.

In the 5.3 ppm pyridinyl label test system, which consisted of a single replicate, the total amount of parent compound was 100.5% of the applied radioactivity at 0 days posttreatment, decreased to 58.3% of the applied by 14 days, and decreasing to a non-detectable level by 268 days posttreatment. The total amount of the major degradate M1 was a maximum of 91.8% of the applied radioactivity at 120 days posttreatment and was 74.9% at 360 days. The total amount of the major degradate M9 was a maximum of 11.1% of the applied at day 268 posttreatment, and was 10.7% on day 360. In contrast with the low dose study, M1 was the major degradate of highest concentration in this high dose phenyl study. The total amount of the minor degradate M6 was a maximum of 5.2% of the applied at day 14 posttreatment. Radiolabeled [^{14}C]volatiles accounted for a maximum of 0.1% of the applied radioactivity at day 120, and nonextractable [^{14}C]residues were a maximum of 3.2% of the applied radioactivity at 360 days posttreatment. The distribution of [^{14}C]residues between the soil and water fractions was not reported; however, the majority of the [^{14}C]residues was observed in the water phase up to 360 days posttreatment. Material balances ranged from 99.4% to 103.9% of the applied radioactivity

The half-life calculated for the 0.01 ppm phenyl study is of questionable validity because sediment data were not determined directly, but were extrapolated from the 5.3 ppm system data, due to the inadequacy of the analytical method to detect sediment Extract-I analytes (See Comment #3). Furthermore, the half-life calculated for the 5.3 ppm phenyl study is of questionable validity due to the use of single samples and because anaerobic conditions were not assured. The data is, however, provided for reference. Uniformly phenyl ring-labeled [^{14}C]diflufenzopyr, at nominal concentrations of 0.01 and 5.3 ppm, degraded with DT_{50} s of 26.2 and 28.0 days, respectively, in anaerobic flooded sandy loam sediment that was incubated in darkness at 25 °C for up to 360 days. DT_{90} s determined by graphical examination of the data were 93 and 129 days for the 0.01 ppm and 5.3 ppm doses, respectively. All data, designated here as percentages of the applied, were reported as percentages of the nominal application.

The DT_{50} s calculated for the phenyl label are longer than those determined for the pyridinyl label, by 6.2 and 8.4 days, for the low and high doses, respectively. This represents an increase of approximately 30 to 40% of the estimated half-life, over that calculated for the pyridinyl label study.

The reported amounts of parent and degradates for the 0.01 ppm phenyl label test system include extrapolated data (for sediment Extract-I compounds) from the high dose study. In the 0.01 ppm phenyl label test system, the total levels of parent compound were 100.7% of the applied radioactivity at 0 days posttreatment, decreased to 64.4% of the applied by 14 days posttreatment, was 24.6% at 56 days posttreatment, and were below detection limits by 268 days posttreatment. The total amount of the major degradate M2 was initially 1.2% of the applied radioactivity at 3 days posttreatment, was a maximum of 26.1% of the applied at 28 days posttreatment, and decreased to 2.2% by 360 days posttreatment. The minor degradate M3 was only detected at 3, 7, and 14 days posttreatment, at 0.2-0.4% of the applied radioactivity. Twenty-two unidentified degradates were each detected at $\leq 7.9\%$ of the applied radioactivity at sampling intervals up to 360 days posttreatment. Radiolabeled [^{14}C]volatiles accounted for 1.1% of the applied radioactivity at 360 days posttreatment, and nonextractable [^{14}C]residues were a maximum of 22.8% of the applied radioactivity at 184 days posttreatment. The distribution of [^{14}C]residues between the soil and water fractions was not reported; however, the majority of the [^{14}C]residues was observed in the water phase up to 28 days posttreatment. Material balances ranged from 98.8% to 104.1% of the applied radioactivity.

In the 5.3 ppm phenyl label test system only one replicate was used and anaerobic conditions were not assured through submitted data. The total levels of parent compound were 96.5% of the applied radioactivity at 0 days posttreatment, decreased to 65.1% of the applied by 14 days posttreatment, was 28.3% at 56 days posttreatment, and were equal detection limit of 0.1% of the applied by 268 days posttreatment. The total amount of the major degradate M2 was initially 1.5% of the applied radioactivity at 0 days posttreatment, was a maximum of 44.5% of the applied at 56 days posttreatment, and decreased to 1.8% by 360 days posttreatment. The minor degradate M3 was only detected at 91, 268, and 360 days posttreatment, at 0.1-0.2% of the applied radioactivity. Twenty-two unidentified degradates were each detected at $\leq 2.0\%$ of the applied radioactivity at sampling intervals up to 360 days posttreatment. Radiolabeled [^{14}C]volatiles accounted for 1.6% of the applied radioactivity at 360 days posttreatment, and nonextractable [^{14}C]residues were a maximum of 22.6% of the applied radioactivity at 268 days posttreatment. The distribution of [^{14}C]residues between the soil and water fractions was not reported; however, the majority of the [^{14}C]residues was observed in the water phase up to 56 days posttreatment. Material balances ranged from 99.1% to 104.1% of the applied radioactivity.

METHODOLOGY

This study was performed using two radiolabels (pyridinyl ring-labeled and uniformly phenyl ring-labeled diflufenzopyr) and two treatment rates; 0.01 ppm (the maximum label use rate) and 5.3 ppm (an exaggerated rate).

Samples (5 g) of sieved (2 mm) sandy loam sediment (79% sand, 5% silt, 16% clay, 1.1% organic matter, pH 7.7, CEC 11.9 meq/100 g; Table II) collected from a pond in Champaign County, IL, were placed in 40 ml glass bottles equipped with a septum for introducing hydrogen and nitrogen and for sampling volatiles. Samples were flooded with 20 ml of pre-purged (with hydrogen) pond water (pH 7.9, conductivity 0.55 mmhos/cm, 9 mg/L calcium as CaCO_3 , total dissolved solids 320 ppm; Table III) which was collected at the same time as the sediment samples; the sediment:water ratio was 1:4 (w:v). The sediment/water systems were purged with hydrogen and nitrogen, and pre-incubated anaerobically (nitrogen atmosphere) in sealed test vessels in darkness at 25 °C for 31 days. Following the pre-incubation period, the sediment/water samples were treated with pyridinyl ring-labeled [4,6- ^{14}C]diflufenzopyr (2-[1-[[[(3,5-difluorophenyl)amino]carbonyl]-hydrazono]ethyl]-3-pyridinecarboxylic acid, monosodium salt; SAN-836H; specific activity 53 mCi/mmol, radiochemical purity 98.3%) or uniformly phenyl ring-labeled [^{14}C]diflufenzopyr (monosodium salt; specific activity 56 mCi/mmol, radiochemical purity 98.2%), dissolved in sodium phosphate buffer (50 mM, pH 7.7), at nominal concentrations of 0.01 or 5.3 ppm. Treated flasks were purged with hydrogen and nitrogen, and incubated anaerobically (nitrogen atmosphere) in sealed test vessels in darkness at 25 °C for up to 360 days. Samples were removed for analysis at 0, 1, 3, 7, 14, 28, 56, 91, 120, 184, 268, and 360 days posttreatment; triplicate samples (one for pH and redox potential determinations only) were used for the 0.01 ppm treated systems and single replicates were used for the 5.3 ppm treated systems. To determine sediment viability, additional flasks were analyzed (serial dilution with plate culture) at 0, 30, 184, and 360 days posttreatment for anaerobic microbial populations in the sediment of systems treated at 0.01 ppm. Microbial populations were reported as Colony Forming Units (CFU)/g dry sediment; data indicated that both the phenyl and pyridinyl label test systems were viable at each sampling interval (Mean CFU/g = 15.6×10^5 and 16.2×10^5 , pyridinyl and phenyl labels, respectively) (Tables XIII and XIV). At each sampling interval, the headspace of each test system was flushed with "fresh air" for ten minutes and analyzed for total radioactivity by LSC; the limit of detection for [^{14}C] SAN 836H was reported as 0.2 ng.

Sediment/water samples were centrifuged (1500 rpm, 20 min, room temp), and the water phase was decanted and 100 or 500 μl aliquots (unspecified as to which samples were analyzed at what volume) were analyzed in triplicate for total radioactivity by LSC (See Comment #5). Aliquots of the water samples were filtered through a 45 μm membrane filter, pyridinyl-labeled samples were concentrated via evaporation under nitrogen or lyophilization (See Comment #5), and finally filtered through a 0.2 μm membrane filter. For the phenyl label experiments, the samples were not concentrated due to the volatility of M2. A large size injection loop (2ml) was used to put a larger volume (relative to the pyridinyl label) of sample onto the HPLC column.

Water samples were then analyzed by reverse-phase HPLC (Zorbax SB-C18 column) using a mobile phase gradient of water:acetonitrile (both with 0.05% trifluoroacetic acid;

100:0 to 85:15 to 65:35 to 40:60 to 25:75 to 0:100 v:v) with radioactive flow detection; detection limits were 0.01% of the injected radioactivity. Samples were co-chromatographed with non-radiolabeled reference standards which were visualized with UV (254 nm) light. Column recoveries from LSC analysis of collected water fractions ranged from 96.4% to 105.5% (pyridinyl label) and from 95.5% to 104.9% (phenyl label).

To confirm compound identities, samples were analyzed by two-dimensional TLC on silica gel plates developed perpendicularly with methylene chloride: acetonitrile: methanol:acetic acid (69:25:5:1, v:v:v:v) and ethyl acetate:methanol:ammonium hydroxide (70:25:5, v:v:v). Samples were co-chromatographed with non-radiolabeled reference standards which were visualized with UV (254 nm) detection; radiolabeled residues were quantified using radioactive imaging scanning.

Sediment samples were extracted by shaking (room temp, 20 min, orbital shaker, 250 rpm) and sonicating (5 min) with acetonitrile:water (8:2, v:v). Samples were then centrifuged (1500 rpm, 20 min, room temp) and decanted. The extraction was repeated twice and the extracts were pooled and labeled as Extract-I. Triplicate 100 or 500 μ l aliquots were analyzed for total radioactivity by LSC (unspecified as to which samples were measured at what aliquot volume, see Comment #5). The sediment was further extracted by agitating with 10 ml of 0.5 N NaOH for one hour at 80°C with a heating block shaker at a speed of 200 rpm, sonicated for five minutes, and then centrifuged as above. The extraction was repeated and the extracts were pooled, and triplicate 100 or 500 μ l aliquots (see Comment #5) were analyzed for radioactivity by LSC. To determine nonextractable residues, post-extraction soil samples were analyzed in triplicate for total radioactivity by LSC following combustion.

Aliquots of the acetonitrile:water extract from the pyridinyl label study were analyzed by HPLC and TLC as described previously. The aliquots of acetonitrile:water extract from the phenyl label study were analyzed by HPLC as described above, and by one or two dimensional TLC developed with chloroform:methanol:acetic acid (90:9:1, v:v:v) and methylene chloride:acetonitrile:methanol:acetic acid (69:25:5:1, v:v:v:v). HPLC column recoveries from LSC analysis of collected sediment Extract-I fractions ranged from 88.2% to 109.7% (pyridinyl label, low dose) and from 96.2% to 103.5% (phenyl label, high dose).

Compound identities were further confirmed in selected water phase and acetonitrile:water extracts by HPLC (as described above)/MS in the positive ion atmospheric pressure chemical ionization mode (APCI+)

Water samples (both labels and treatment rates) and acetonitrile:water sediment extracts (phenyl label) were analyzed within 24 hours of each sampling interval. Acetonitrile:water extracts from the pyridinyl label study were concentrated and stored at -20 °C (storage period not reported) prior to analysis by LSC and HPLC. The storage

period for sediments prior to the NaOH extraction varied; the length of storage of sediments prior to extraction and/or combustion was not reported. It was reported that reference substances were found to be stable throughout the course of the study.

To determine the presence of anaerobic conditions, the pH and redox potential were measured at each sampling interval in the 0.01 ppm test systems. The redox potentials were measured using a platinum electrode (E_o) and were corrected to normal hydrogen electrode potentials (E_{nhe}); data for both labels indicated that anaerobic conditions were maintained throughout the incubation in the 0.01 ppm test system. In the pyridinyl label system, redox potentials ranged from -219 to -619 mV (E_o) and from -20 mV to -420 (E_{nhe}); the pH ranged from 7.03 to 8.16 (Tables XV, XVII). In the phenyl label system, redox potentials ranged from -233 to -623 mV (E_o) and from -34 mV to -424 mV (E_{nhe}); the pH ranged from 6.98 to 8.12 throughout the incubation (Tables XVI, XVIII). Microbial population counts, redox potential, and pH were not measured at any of the sampling intervals for the 5.3 ppm test systems of either radiolabeled material.

DATA SUMMARY

Pyridinyl ring-labeled [4,6- ^{14} C]diflufenzopyr

Pyridinyl ring-labeled [4,6- ^{14} C]diflufenzopyr (radiochemical purity 98.3%), at nominal concentrations of 0.01 and 5.3 ppm, degraded with non-linear DT_{50} s (fifty percent dissipation times) of 20.0 and 19.6 days, respectively, in anaerobic flooded sandy loam sediment incubated in darkness at 25 °C for up to 360 days. DT_{50} s were determined through least-squares exponential curve-fitting of non-transformed data. R^2 s were 0.998 for both doses. DT_{90} s calculated using the exponential curve fitted to the data were 67 and 65 days for the 0.01 ppm and 5.3 ppm doses, respectively. DT_{90} s determined by visual graphical evaluation were 74 and 78 days, respectively. Linear half-lives, calculated through least squares curve-fitting to natural log-transformed data, yielded half-lives that varied depending on the number of days of data with parent levels below detection limits included in the calculation, which varied between the low and high doses. Including detection-level data in the linear regression for days 0 through 360, half-lives of 33.5 and 35.2 days were calculated ($r^2 = 0.796$ and 0.702 , respectively). As expected, the linear fit for days 0 through 360 posttreatment was significantly nonlinear based on a runs test. Using a uniform data point cut-off of 184 days, linear half-lives were determined to be 18.5 ($r^2=0.944$) and 24.2 ($r^2=0.997$) days, for the low and high pyridinyl label doses, respectively. The high dose data was found, however to be significantly non-linear when a runs test was performed. Because of the variability in data censoring required, and the non-linearity of the regression for days 0 through 360 posttreatment, the DT_{50} s from the non-transformed data curve-fitting were selected as the data end-points for this study.

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The half-life calculated for the 5.3 ppm pyridinyl label study is of questionable validity due to the use of single samples and because anaerobic conditions were not assured. All data, designated here as percentages of the applied, were reported as percentages of the nominal application.

In the 0.01 ppm pyridinyl label test system, total parent levels were 99.5% of the applied radioactivity at 0 days posttreatment, decreased to 61% by day 14, were 36.4% by day 28, and were below the detection limit of 0.1% of the applied by 184 days posttreatment (Table XLIII). The total amount of the major degradate M1 was a maximum of 37.8% of the applied at 28 days posttreatment and was below detection limits by 268 days posttreatment. The total amount of the major degradate M9 was a maximum of 71.3% of the applied radioactivity at 120 days posttreatment and was 65.2% at 360 days. The minor degradate M6 was detected once (in one replicate) at 0.7% of the applied radioactivity at 28 days posttreatment.

In the water phase of the 0.01 ppm test system, the parent compound was 96.6% of the applied radioactivity at 0 days posttreatment, decreased to 56.2% of the applied by 14 days posttreatment, was 4.0% at 91 days posttreatment, and was not detected by 184 days posttreatment (Table XXXI). The major degradate

8-methyl-5(6H)-pyrido[2,3-d]pyridazinone (M1)

was detected at 2.0% of the applied radioactivity at 1 day posttreatment, increased to a maximum of 27.9% of the applied by 28 days posttreatment, was 10.4% at 91 days posttreatment, and was not detected by 268 days posttreatment. The major degradate

8-methylpyrido[2,3-d]pyridazine-2,5(1H,6H)-dione (M9)

was initially detected at 0.4% of the applied radioactivity at 14 days posttreatment, increased to 27.1% of the applied by 56 days posttreatment, was a maximum of 45.2% at 120 days posttreatment, and was 39.1% at 360 days posttreatment. An unidentified minor degradate was detected at a maximum of 2.6% of the applied radioactivity at 268 days posttreatment.

In the acetonitrile:water sediment extract of the 0.01 ppm test system, the parent compound (pyridinyl label) was initially detected at 3.0% of the applied radioactivity at 0 days posttreatment, was a maximum of 6.8% of the applied at 1 day posttreatment, and was not detected by 91 days posttreatment (Table XXXIX). The major degradate

8-methyl-5(6H)-pyrido[2,3-d]pyridazinone (M1)

was initially present at 1.2% of the applied radioactivity at 0 days posttreatment, increased to a maximum of 11.3% of the applied by 14 days posttreatment, was 10.6% at

91 days posttreatment, and was not detected by 268 days posttreatment. The major degradate

8-methylpyrido[2,3-d]pyridazine-2,5(1H,6H)-dione (M9)

was initially detected at 0.4% of the applied radioactivity at 7 days posttreatment, was 17.9% of the applied at 91 days posttreatment, increased to a maximum of 29.4% by 268 days posttreatment, and was 26.1% of the applied radioactivity at 360 days posttreatment. The minor degradate, 2-acetylnicotinic acid (M6), was detected only at one sampling interval (28 days posttreatment) at 0.7% (one replicate only) of the applied radioactivity. The NaOH sediment extract contained a maximum of 26.1% of the applied radioactivity at 360 days posttreatment (Table XIX, see Comment #2). In the NaOH sediment extract, a maximum of 26.1% of the applied radioactivity was detected at 360 days posttreatment. Radiolabeled [^{14}C]volatiles accounted for 1.1% of the applied radioactivity, and nonextractable [^{14}C]residues were a maximum of 8.8% of the applied at 360 days posttreatment. The distribution of [^{14}C]residues between the soil and water fractions was not reported; however, the majority of the [^{14}C]residues was observed in the water phase up to 120 days posttreatment. Material balances ranged from 102.3% to 106.8% of the applied radioactivity (Table XIX).

In the 5.3 ppm pyridinyl label test system, which consisted of a single replicate, the total amount of parent compound was 100.5% of the applied radioactivity at 0 days posttreatment, decreased to 58.3% of the applied by 14 days, and decreasing to a non-detectable level by 268 days posttreatment (Table XLIV). The total amount of the major degradate M1 was a maximum of 91.8% of the applied radioactivity at 120 days posttreatment and was 74.9% at 360 days. The total amount of the major degradate M9 was a maximum of 11.1% of the applied at day 268 posttreatment, and was 10.7% on day 360. The total amount of the minor degradate M6 was a maximum of 5.2% of the applied at day 14 posttreatment.

In the water phase of the 5.3 ppm pyridinyl label test system, the parent compound was detected at 93.2% of the applied radioactivity at 0 days posttreatment, decreased to 53.5% and 16.7% of the applied by 14 and 56 days posttreatment, respectively, and was not detected by 268 days posttreatment (Table XXXIII). The major degradate

8-methyl-5(6H)-pyrido[2,3-d]pyridazinone (M1)

was initially present at 0.9% of the applied radioactivity at 0 days posttreatment, increased to 45.6% of the applied by 28 days posttreatment, was a maximum of 69.1% at 120 days posttreatment, and was 55.0% at 360 days posttreatment. The minor degradate M9 was initially present at 0.4% of the applied radioactivity at 28 days posttreatment, increased to a maximum of 8.1% by 268 days posttreatment, and was 7.9% at 360 days posttreatment. The minor degradate M6 was initially detected at 0.5% of the applied

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radioactivity at 3 days posttreatment, was a maximum of 5.2% of the applied at 14 days posttreatment, and was not detected by 184 days posttreatment. An unidentified minor degradate was detected at a maximum of 0.6% of the applied radioactivity at 184 days posttreatment.

In the acetonitrile:water sediment extract of the 5.3 ppm test system, the parent compound was 7.3% of the applied radioactivity at 0 days posttreatment, increased to a maximum of 7.5% of the applied by 3 days posttreatment, was 1.3% at 56 days posttreatment, and was not detected by 184 days posttreatment (Table XL). The major degradate

8-methyl-5(6H)-pyrido[2,3-d]pyridazinone (M1)

was initially detected at 0.2% of the applied radioactivity at 0 days posttreatment, was 19.9% of the applied at 56 days posttreatment, increased to a maximum of 23.1% of the applied by 184 days posttreatment, and was 19.9% at 360 days posttreatment. The minor degradate M6 was only detected at 7 and 28 days posttreatment, at 0.1% of the applied radioactivity. The minor degradate M9 was initially detected at 0.1% of the applied radioactivity at 14 days posttreatment, increased to a maximum of 3.0% of the applied by 268 days posttreatment, and was 2.8% at 360 days posttreatment. The NaOH sediment extract contained a maximum of 9.1% of the applied radioactivity at 360 days posttreatment (Table XXI). Radiolabeled [^{14}C]volatiles accounted for a maximum of 0.1% of the applied radioactivity at day 120, and nonextractable [^{14}C]residues were a maximum of 3.2% of the applied radioactivity at 360 days posttreatment. The distribution of [^{14}C]residues between the soil and water fractions was not reported; however, the majority of the [^{14}C]residues was observed in the water phase up to 360 days posttreatment. Material balances ranged from 99.4% to 103.9% of the applied radioactivity (Table XXI).

The major degradate detected at the highest levels varied between the low and high dose pyridinyl studies. In contrast with the low dose study, M1 was the major degradate in the high dose pyridinyl study, reaching a maximum of 92% of the applied, and maintaining a high level through the end of the experiment (75% at 360 days). In the low dose pyridinyl study M1 reached a maximum of 38%, and was not detected at day 268 posttreatment. M9 was detected at a maximum of 11.1% of the applied in the high dose pyridinyl study, but was a maximum 71.3% of the applied at day 120 in the low dose study.

Uniformly phenyl ring-labeled [^{14}C]diflufenzopyr

The half-life calculated for the 0.01 ppm phenyl study is of questionable validity because sediment data were not determined directly, but were extrapolated from the 5.3 ppm system data, due to the inadequacy of the analytical method to detect sediment Extract-I analytes (See Comments #2 and 3). The low dose phenyl label sediment Extract-I data were extrapolated from the high dose data by multiplying the percent dose of total

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radioactivity determined in this fraction via LSC by the percent of each compound identified via HPLC in the high dose sediment extract. Furthermore, the half-life calculated for the 5.3 ppm phenyl study is of questionable validity due to the use of single samples and because anaerobic conditions were not assured. The data is, however, provided for reference. Uniformly phenyl ring-labeled [^{14}C]diflufenzopyr (radiochemical purity 98.0%), at nominal concentrations of 0.01 and 5.3 ppm, degraded with DT_{50} s of 26.2 and 28.0 days, respectively, in anaerobic flooded sandy loam sediment that was incubated in darkness at 25 °C for up to 360 days ($r^2 = 0.996$ (includes extrapolated sediment data) and 0.994, for low and high doses, respectively). DT_{90} s determined by graphical examination of the data were 93 and 129 days for the 0.01 ppm and 5.3 ppm doses, respectively. All data, designated here as percentages of the applied, were reported as percentages of the nominal application.

The DT_{50} s calculated for the phenyl label are longer than those determined for the pyridinyl label, by 6.2 and 8.4 days, for the low and high doses, respectively. This represents an increase of approximately 30 to 40% of the estimated half-life, over that calculated for the pyridinyl label study.

In the 0.01 ppm test system, the total levels of parent compound were 100.7% of the applied radioactivity at 0 days posttreatment, decreased to 64.4% of the applied by 14 days posttreatment, was 24.6% at 56 days posttreatment, and were below detection limits by 268 days posttreatment (Table XLV). These values include sediment data extrapolated from the high dose data. The total amount of the major degradate M2 was initially 1.2% of the applied radioactivity at 3 days posttreatment, was a maximum of 26.1% of the applied at 28 days posttreatment, and decreased to 2.2% by 360 days posttreatment. The minor degradate M3 was only detected at 3, 7, and 14 days posttreatment, at 0.2-0.4% of the applied radioactivity. Twenty-two unidentified degradates were each detected at $\leq 7.9\%$ of the applied radioactivity at sampling intervals up to 360 days posttreatment.

In the water phase of the 0.01 ppm test system (phenyl label), the parent compound was present at 92.6% of the applied radioactivity at 0 days posttreatment, decreased to 57.6% of the applied by 14 days posttreatment, was 22.8% at 56 days posttreatment, and was not detected by 184 days posttreatment (Table XXXII). The major degradate

3,5-difluoranyliline (M2)

was initially detected at 1.1% of the applied radioactivity at 3 days posttreatment, was a maximum of 19.6% of the applied at 28 days posttreatment, was 8.8% of the applied at 56 days posttreatment and was not detected by 120 days posttreatment. The minor degradate N,N'-bis(3,5-difluorophenyl)urea (M3) was only detected at 3, 7, and 14 days posttreatment, at 0.2-0.4% of the applied radioactivity. Twenty-two unidentified degradates were each detected at $\leq 7.9\%$ of the applied radioactivity at sampling intervals up to 360 days posttreatment.

In the acetonitrile:water sediment extract of the 0.01 ppm test system, parent and degradate concentrations were extrapolated from the 5.3 ppm system data, because levels of radioactivity fell below detectable levels on the HPLC. Data were extrapolated as described above and were reported in Table XLII. The NaOH sediment extract contained a maximum of 73.8% of the applied radioactivity at 268 days posttreatment in the 0.01 ppm test system (Table XX; see Comment #2). Radiolabeled [^{14}C]volatiles accounted for 1.1% of the applied radioactivity at 360 days posttreatment, and nonextractable [^{14}C]residues were a maximum of 22.8% of the applied radioactivity at 184 days posttreatment. The distribution of [^{14}C]residues between the soil and water fractions was not reported; however, the majority of the [^{14}C]residues was observed in the water phase up to 28 days posttreatment. Material balances ranged from 98.8% to 104.1% of the applied radioactivity (Table XX).

In the 5.3 ppm phenyl label test system only one replicate was used. Furthermore, anaerobic conditions were not assured through submitted data. The total levels of parent compound were 96.5% of the applied radioactivity at 0 days posttreatment, decreased to 65.1% of the applied by 14 days posttreatment, was 28.3% at 56 days posttreatment, and were equal detection limit of 0.1% of the applied by 268 days posttreatment (Table XLVI). The total amount of the major degradate M2 was initially 1.5% of the applied radioactivity at 0 days posttreatment, was a maximum of 44.5% of the applied at 56 days posttreatment, and decreased to 1.8% by 360 days posttreatment. The minor degradate M3 was only detected at 91, 268, and 360 days posttreatment, at 0.1 to 0.2% of the applied radioactivity. Twenty-two unidentified degradates were each detected at $\leq 2.0\%$ of the applied radioactivity at sampling intervals up to 360 days posttreatment.

In the water phase of the 5.3 ppm test system, the parent compound (phenyl label) was detected at 88.0% of the applied radioactivity at 0 days posttreatment, decreased to 58.5% of the applied by 14 days posttreatment, was 8.5% at 91 days posttreatment, and was not detected by 360 days posttreatment (Table XXXIV). The major degradate

3,5-difluoriline (M2)

was initially detected at 1.5% of the applied radioactivity at 0 days posttreatment, increased to a maximum of 36.0% of the applied by 56 days posttreatment, and was 1.8% and 0.2% at 120 and 360 days posttreatment, respectively. Twenty-two unidentified minor degradates were detected at $\leq 2.9\%$ of the applied radioactivity at sampling intervals up to 360 days posttreatment.

In the acetonitrile:water sediment extract of the 5.3 ppm test system, the parent compound was initially detected at 8.5% of the applied radioactivity at 0 days posttreatment, increased to a maximum of 9.4% of the applied by 3 days posttreatment, was 4.4% at 28 days posttreatment, and was 0.1% at 360 days posttreatment (Table XLI). The minor degradate M2 was initially detected at 0.1% of the applied radioactivity at 3 days

posttreatment, increased to a maximum of 8.5% of the applied by 56 days posttreatment, and was 1.6% at 360 days posttreatment. The minor degradate M3 was detected at $\leq 0.2\%$ of the applied radioactivity at sampling intervals up to 360 days posttreatment. Twenty-two minor degradates were detected at $\leq 0.9\%$ of the applied radioactivity at sampling intervals up to 360 days posttreatment. The NaOH sediment extract contained a maximum of 74.0% of the applied radioactivity at 268 days posttreatment (Table XXII). Radiolabeled [^{14}C]volatiles accounted for 1.6% of the applied radioactivity at 360 days posttreatment, and nonextractable [^{14}C]residues were a maximum of 22.6% of the applied radioactivity at 268 days posttreatment. The distribution of [^{14}C]residues between the soil and water fractions was not reported; however, the majority of the [^{14}C]residues was observed in the water phase up to 56 days posttreatment. Material balances ranged from 99.1% to 104.1% of the applied radioactivity (Table XXI).

Total levels of the major degradate M2 varied between the low and high dose phenyl studies. In the low dose phenyl study, the total amount of the major degradate M2 was a maximum of 26% of the applied at 28 days posttreatment, and decreased by nearly half to 14% at 56 days posttreatment. In the high dose study, the total amount of the major degradate M2 was 40% of the applied at 28 days posttreatment, and increased to a maximum of 44.5% of the applied at 56 days posttreatment.

COMMENTS

1. Only single samples were used for the 5.3 ppm test systems (pyridinyl and phenyl labels). The use of single samples is generally not considered to be good laboratory practice; at a minimum, duplicate samples are necessary for the valid determination of a half-life. For the pyridinyl label study, however, adequate data were obtained in the 0.01 ppm study (in which duplicate samples were utilized) for the valid determination of a half-life and the required residue characterization.
2. The analytical method was inadequate to characterize degradates in the 0.01 ppm phenyl label test system; radioactivity present in the caustic (NaOH) extract represented 71.2% of the applied radioactivity at 360 days posttreatment (Table XX). Following further analysis by HPLC, 22 unidentified degradates were isolated and characterized by the study author only as "polar ^{14}C -compounds" (Appendix V). Degradates 1, 2, and 14 were detected at $\geq 10\%$ of the applied radioactivity. Although both TLC and LC/MS were used in an attempt to identify NaOH extract degradates, no identities were reported. The use of a less caustic solvent would have been appropriate prior to the use of 0.5 N NaOH, to reduce compound degradation and permit more a complete identification of the radiolabeled degradates present. The report stated that the compounds were stripped from the sediment matrix "as a last resort by the harsh action of the alkali." Based on the soil aerobic metabolism study submitted by the registrant for diflufenzopyr (MRID 44170153), further organic solvent extraction procedures prior to the use of NaOH would have been likely to increase recovery of identifiable compounds from the sediment.

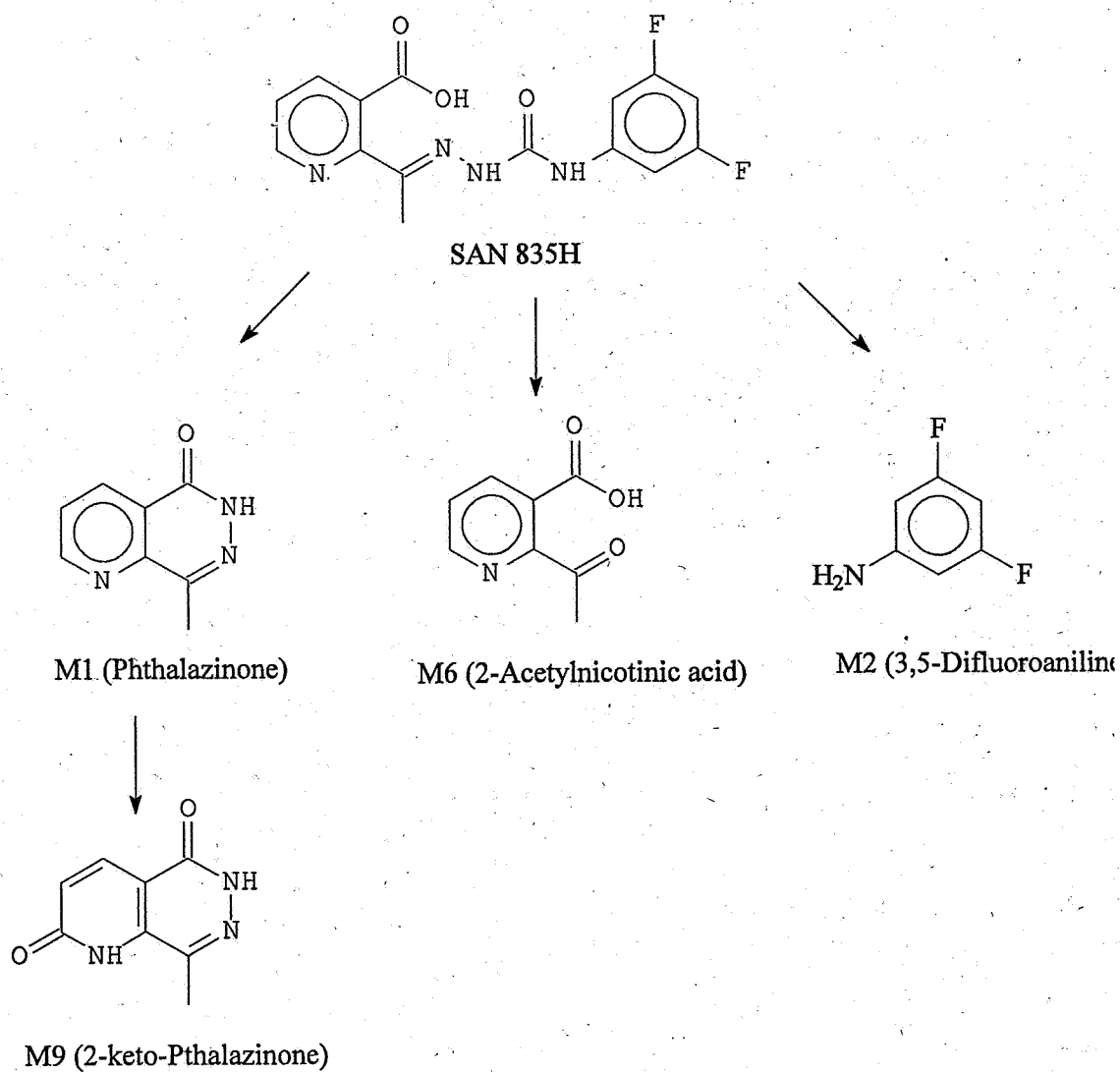
14

Additional methods development to identify these shortcomings in the analytical protocol would have resulted in improved results, and should have been undertaken.

3. The application rate in the 0.01 ppm study (phenyl label) was not consistent with the sensitivity of the analytical method. The maximum label use rate of diflufenzopyr is 0.2 lb a.i./A; the final concentration (used for this study) of 0.01 ppm was determined assuming the maximum application rate diluted in 2 meters of standing water over one acre. The concentration of parent and degradates in the 0.01 ppm acetonitrile:water sediment extracts were not determined directly, but were extrapolated from the 5.3 ppm system data. The low dose phenyl label sediment Extract-I data were extrapolated from the high dose data by multiplying the percent dose of total radioactivity determined in this fraction via LSC by the percent of each compound identified via HPLC in the high dose sediment extract. Because degradation rates may vary with concentration, the use of extrapolated data precludes the valid determination of a half-life at the low treatment rate. Additionally, only single replicates were utilized for the 5.3 ppm test systems and anaerobic conditions were not assured in that study (see Comment #4), making the extrapolated data even less reliable.
4. Anaerobic conditions were not assured for the systems treated at 5.3 ppm (pyridinyl label and phenyl label); neither pH nor redox potentials were measured. The EPA requires such data to confirm that anaerobic conditions were assured and maintained throughout the incubation period.
5. Either evaporation or lyophilization was used to concentrate the water and acetonitrile:water extracts from the pyridinyl label test system. Data were not separated according to concentration method so that the reviewer could evaluate the effect these two methods may have had on the results. Additionally, it was stated in the results that either "100 or 500 µl aliquots" were analyzed in several instances, without specifying which samples were analyzed at the noted volumes. Application of consistent laboratory methods during experimentation is considered good laboratory practice and is necessary in order to reduce the number of variables which can affect the results of the study.
6. It was unclear whether sediment samples used for the NaOH extractions (both phenyl and pyridinyl label studies) were stored for less than 30 days prior to extraction. Clarification by the registrant is necessary. In the event that samples were stored for longer than 30 days prior to analysis, storage stability data are required. Storage stability studies should be conducted using water and sediment samples collected from the test sites that have been fortified separately with the compound and its degradates and stored for a duration equal to the longest storage interval for the test samples.
7. The reported water solubility of the parent compound is 5850 ppm in pH 6.8 phosphate buffer and 63 ppm in deionized water (no temperature reported).

8. Sterilized controls, maintained in a manner similar to test samples, were not used in the study (for either label).
9. Degradates were reported as M1 through M9, and structures were provided. Chemical names were not reported in study, but were reported in the photodegradation on soil study (MRID 44307409); all degradate names used in this study were obtained from MRID 44307409.
10. The registrant only reported residue data in the form of percentages of the applied or injected radioactivity. In future studies submitted to the EPA, it is necessary that residue data also be submitted in units of concentration, such as ppm.

Proposed Metabolic Pathways of SAN 835H in Anaerobic Aquatic Metabolism Studies



Diffuzenzopyr
Anaerobic Aquatic Metabolism Study: Data Analysis

PYRIDINYL LABEL

LOW DOSE

LINEAR REGRESSION (Days 0-360)

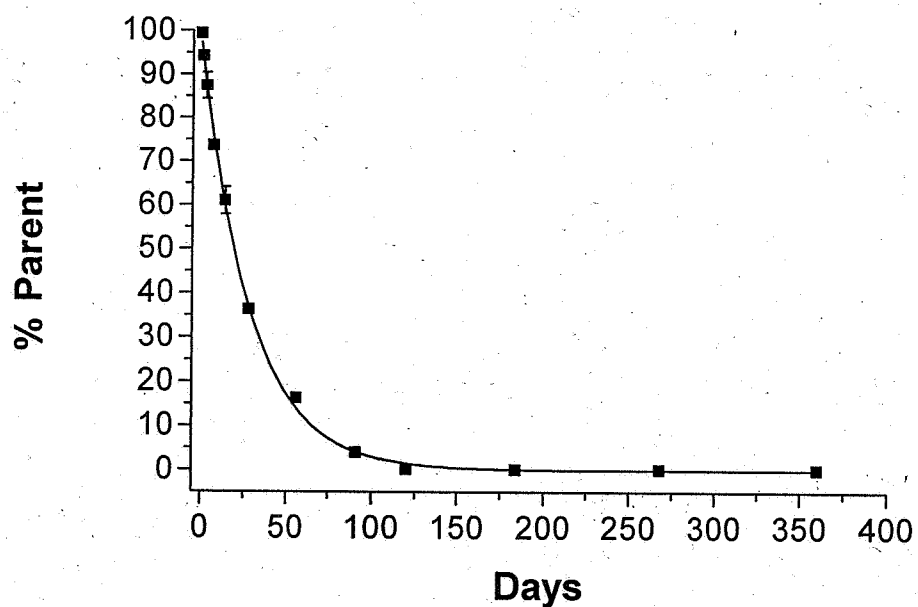
Parameter	Value	Half-Life Calculation (days)
Slope	-0.0207 +/- 0.0022	0.693/-k= 33.5
95% Confidence Interval	-0.0253 to -0.0160	27 to 43
Goodness of Fit r^2	0.796	
Is Slope Significantly Nonzero? P value Deviation from Zero	<0.0001 Significant	
Runs Test P value Significantly Nonlinear?	<0.0001 Significant	

NONLINEAR REGRESSION BASED ON FIRST ORDER EXPONENTIAL DECAY WITH UNTRANSFORMED DATA Days 0-360: PYRIDINYL LABEL LOW DOSE

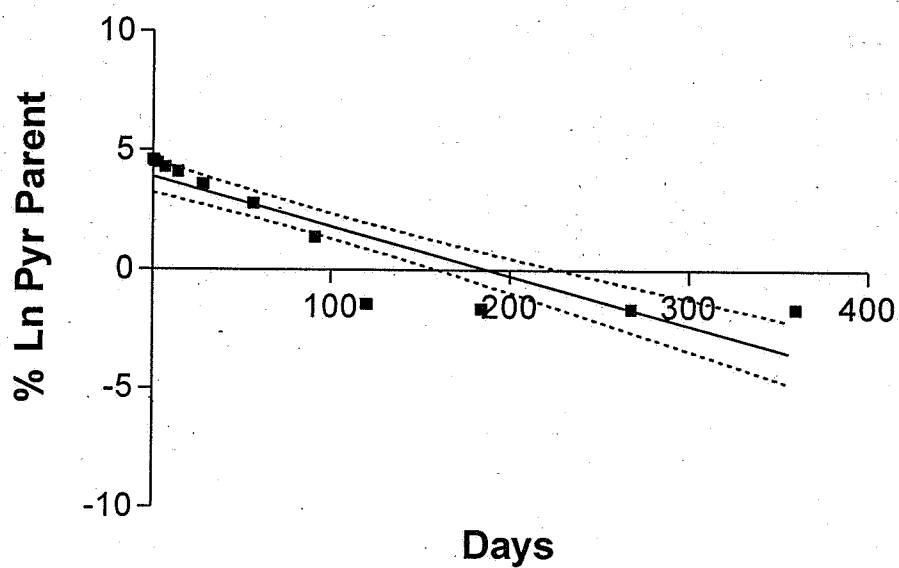
Parameter	Value	DT ₅₀ Calculation (days)
K	-0.0346	0.693/-K= 20.0
95% Confidence Interval of K	-0.0365 to -0.0326	19 to 21
Std error of K	0.0009	
Goodness of Fit r^2	0.998	
Runs Test P value Deviation from Model DT ₉₀ extrapolation from model DT ₉₀ Graphical Evaluation	0.0856 Not Significant	[ln (10/100)]/-0.0346=66.5 74 days

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Diflufenzopyr
Anaerobic Aquatic Metabolism
(Pyridinyl label, Low Dose)



Diflufenzopyr
Anaerobic Aquatic Metabolism
(Pyridinyl label, Low Dose)
Linear regression



Diffuzenzopyr
Anaerobic Aquatic Metabolism Study: Data Analysis

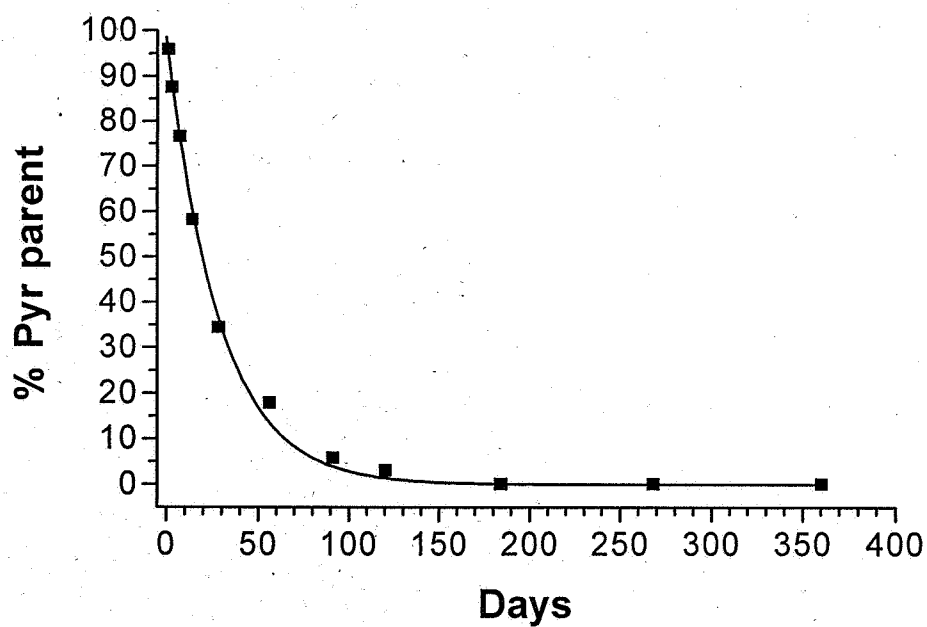
HIGH DOSE , PYRIDINYL LABEL
LINEAR REGRESSION (Days 0-360)

Parameter	Value	Half-Life Calculation (days)
Slope	-0.0197 +/- 0.0018	0.693/-k= 35.2
95% Confidence Interval	-0.0237 to -0.0158	29 to 44
Goodness of Fit r^2	0.924	
Is Slope Significantly Nonzero? P value Deviation from Zero	<0.0001 Significant	
Runs Test P value Significantly Nonlinear?	<0.013 Significant	

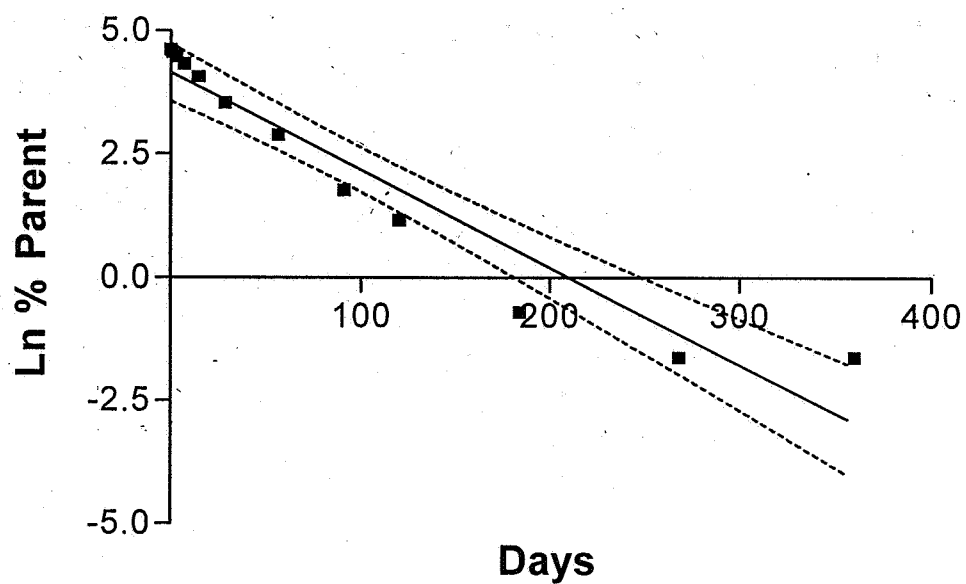
NONLINEAR REGRESSION BASED ON FIRST ORDER EXPONENTIAL DECAY WITH
UNTRANSFORMED DATA Days 0-360: PYRIDINYL LABEL HIGH DOSE

Parameter	Value	DT ₅₀ Calculation (days)
K	-0.0353	0.693/-K= 19.6
95% Confidence Interval of K	-0.0384 to -0.0322	18 to 22
Std error of K	0.0014	
Goodness of Fit r^2	0.998	
Runs Test P value Deviation from Model DT ₉₀ extrapolation from model DT ₉₀ Graphical Evaluation	0.0242 Significant	 [ln (10/100)]/-0.0353=65 78 days

**Diflufenzopyr Anaerobic Aquatic
(Pyridinyl High Dose)**



**Diflufenzopyr Anaerobic Aquatic
(Pyridinyl High Dose)
Linear regression**



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Pages 22 through 57 are not included in this copy.

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