

Data Evaluation Record on the aerobic biotransformation of oryzalin in soil

PMRA Submission Number {.....}

EPA MRID Number 41322801

Data Requirement: PMRA Data Code:
EPA DP Barcode: 378627
OECD Data Point:
EPA Guideline: 835.4100

Test material:

Common name: Oryzalin.
Chemical name:
IUPAC name: 3,5-Dinitro-4-(dipropylamino)benzenesulfonamide.
3,5-Dinitro-N⁴,N⁴-dipropylsulfanilamide.
CAS name: 4-(Dipropylamino)-3,5-dinitrobenzenesulfonamide.
CAS No: 19044-88-3.
Synonyms: OR-1; EL-119
SMILES string: C1C(S(=O)(=O)N)=CC(N(O)O)=C(N(CCC)CCC)C=1N(O)O (EpiSuite version 4.0).

Primary Reviewer: Dana Worcester
Cambridge Environmental

Signature:
Date: 10/25/10


Secondary Reviewer: Kathleen Ferguson
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Final Reviewer: Chuck Peck
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Date: 5/19/11

Company Code:
Active Code:
Use Site Category:
EPA PC Code: 104201

CITATION: Graper, L.K. and D.P. Rainey. 1989. Aerobic metabolism of ¹⁴C oryzalin in sandy loam soil. Unpublished study performed, submitted and sponsored by DowElanco (now Dow AgroChemicals), Greenfield, Indiana. Laboratory Project ID.: ABC-0434. Experiment start and termination dates were not reported. Final report issued October 11, 1989.

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EXECUTIVE SUMMARY

The biotransformation of [phenyl-U-¹⁴C]-labeled 3,5-dinitro-4-(dipropylamino)benzenesulfonamide (oryzalin; radiochemical purity 98.2%), applied at *ca.* 5.2 mg a.i./kg, was studied in a sandy loam soil (pH 7.1, organic matter 1.5%) from Indiana that was incubated under aerobic conditions for 6.1 months in the dark at 24°C and a moisture content of 75% of 0.33 bar. The experiment was submitted under USEPA Pesticide Assessment Guidelines, Subdivision N §162-1, and was conducted prior to the effective date of FIFRA GLP standards. The test system consisted of a glass crystallizing dish containing moist treated soil (1,075 g dry wt) that was placed in a glass desiccator. The desiccator was covered in aluminum foil and attached to a volatile trapping system. Humidified CO₂-free air was passed through the desiccator (flow rate not reported) for 1 hour every 12 hours; exiting gasses passed through a charcoal trap and a 1N NaOH solution (200 mL). Duplicate subsamples were collected at 0, 0.1, 0.2, 0.5, 0.7, 1.0, 1.4, 1.8, 3.0, 3.9 and 6.1 months posttreatment. The soil was extracted once with methanol by shaking at room temperature. The extracts were acidified (pH <2) and partitioned with either methylene chloride or ethyl acetate. Aliquots of the extracts were analyzed for total radioactivity using LSC. The organic extracts were combined, concentrated, and analyzed using one- and two-dimensional TLC. Identifications were made by comparison to reference standards of oryzalin and seven potential transformation products that were cochromatographed with the samples; identifications were not confirmed using a second method. Portions of the extracted soil and charcoal trap were analyzed for total radioactivity using LSC following combustion. Aliquots of the NaOH trapping solutions were analyzed using LSC, and the presence of CO₂ was confirmed by barium chloride precipitation.

During the study, the incubation temperature was reportedly 24°C and the soil moisture content was 75% of 0.33 bar; no supporting data were provided. The soil aerobicity was reportedly maintained throughout the experiment; no supporting data were provided. The viability of the soil used in the study was not determined.

Overall [¹⁴C]residue recoveries averaged 95.9 ± 4.1% (range 87.9-100.9%) of the applied. Study authors indicated that there was no significant loss of [¹⁴C]residues from the test system over time. However, overall [¹⁴C]residue recoveries consistently declined from 96.7% at 1.4 months to 87.9% at 6.1 months.

Based on nonlinear regression analysis (Sigma Plot v. 9.0, exponential decay/one compartment, two parameter), oryzalin dissipated with a reviewer-calculated half-life of 1.5 months. Oryzalin decreased from 92.2% of the applied at time 0 to 51.3% at 1 month posttreatment, 42.4% at 1.4 months, 25.7% at 3 months, and 13.1% at 6.1 months.

As the extraction method resulted in a large amount of nonextractable residues, half-lives were also estimated as if the nonextractable residues were oryzalin. Based on nonlinear regression analysis (Sigma Plot v. 9.0, exponential decay/one compartment, two parameter), oryzalin and nonextractable residues dissipated with a reviewer-calculated half-life of 18 months. The first order log/linear (Excel 2003) half-life was 19 months. The observed DT50 value was >6.1 months.

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No major transformation products were isolated. Nine minor transformation products were identified:

- 3,5-dinitro-4-(propylamino)benzenesulfonamide (OR-2);
- 3-amino-4-(dipropylamino)-5-nitrobenzenesulfonamide (OR-4);
- 3,4,5-triaminobenzene-sulfonamide (OR-9);
- 2-ethyl-7-nitro-1-propyl-1H-benzimidazole-5-sulfonamide (OR-13);
- 2-ethyl-7-nitro-1H-benzimidazole-5-sulfonamide (OR-15);
- 4-hydroxy-3,5-dinitro-benzenesulfonamide (OR-20);
- 4-[(2-hydroxypropyl)propylamino]-3,5-dinitro-benzenesulfonamide (OR-41);
- cis- and trans- isomers of 3,3'-azoxybis[4-(propylamino)-5-nitro]benzenesulfonamide (UN-1);
and
- 2-ethyl-7-nitro-1-propyl-1H-benzimidazole-5-sulfonamide, 3-oxide (UN-2).

OR-20 was the predominant transformation product at a maximum of 4.7% of the applied. OR-2, OR-4/41, OR-9, OR-13, and OR-15 were each $\leq 1.2\%$ of the applied. UN-1 and UN-2 were maximums of 1.4% and 2.3% of the applied, respectively. [^{14}C]Residues remaining at the origin or not associated with a discrete area totaled a maximum of 4.5% of the applied.

Extractable [^{14}C]residues decreased from 97.4% of the applied at time 0 to 23.6% at 6.1 months posttreatment, while nonextractable [^{14}C]residues increased from 2.6% at time 0 to 63.1% at 6.1 months. In the 6.1 month sample, nonextractable [^{14}C]residues were associated with 14.0% humic acid, 5.6% β humus, 25.1% fulvic acids and 18.4% humin fraction. During the 6.1 month incubation period, CO_2 and volatile [^{14}C]organics totaled 5.7% and 0.1% of the applied, respectively.

A transformation pathway was provided by the study authors. Oryzalin degrades via dealkylation, oxidation, reduction, dimerization and ring formation. Under aerobic conditions, oryzalin degrades via dealkylation, oxidation, reduction, dimerization and ring formation. Oryzalin is initially degraded to OR-20, OR-41, OR-4 or OR-2. OR-4 is degraded to OR-9 or OR-13, and OR 13 is further degraded to OR-15 and UN-2. OR-2- is transformed to UN-1. Study authors assert that the transformation products are ultimately converted to bound residues and CO_2 . However, given the uncertainty surrounding the inadequacy of the extraction method to remove all identifiable [^{14}C]residues from the soil, there is not enough information for the reviewer to support this conclusion.

Results Synopsis:

Test system used: Sandy loam soil.

Oryzalin only

Linear half-life: 2.1 months ($r^2 = 0.949$).

Nonlinear half-life: 1.5 months ($r^2 = 0.9946$).

Observed DT50: 1.0-1.4 months.

Oryzalin and nonextractable residues

Linear half-life: 19 months ($r^2 = 0.704$).

Nonlinear half-life: 18 months ($r^2 = 0.705$).

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Observed DT50: >6.1 months.

Major transformation products:

None.

Minor transformation products:

3,5-Dinitro-4-(propylamino)benzenesulfonamide (OR-2).

3-Amino-4-(dipropylamino)-5-nitrobenzenesulfonamide (OR-4) and/or 4-[(2-Hydroxypropyl)propylamino]-3,5-dinitro-benzenesulfonamide (OR-41).

3,4,5-Triaminobenzene-sulfonamide (OR-9).

2-Ethyl-7-nitro-1-propyl-1H-benzimidazole-5-sulfonamide (OR-13).

2-Ethyl-7-nitro-1H-benzimidazole-5-sulfonamide (OR-15).

4-Hydroxy-3,5-dinitro-benzenesulfonamide (OR-20).

Cis- and trans- isomers of 3,3'-azoxybis[4-(propylamino)-5-nitro]benzenesulfonamide (UN-1).

2-Ethyl-7-nitro-1-propyl-1H-benzimidazole-5-sulfonamide, 3-oxide (UN-2).

CO₂.

Study Acceptability: This study is classified as **supplemental**. No significant deviations from good scientific practices were noted. The extraction procedure (shaking with methanol) appears to have been inadequate to remove all identifiable [¹⁴C]residues from the soil, since 63% of the applied radioactivity was nonextracted at study termination.

The page numbers referenced in the DER are those that appear in the upper right on the MRID pages.

I. MATERIALS AND METHODS

GUIDELINE FOLLOWED: Guidelines used to design the study were not identified; the study was submitted under USEPA Pesticide Assessment Guidelines, Subdivision N §162-1 (p. 1). Significant deviations from the objectives of Subdivision N guidelines were noted:

The extraction procedure, shaking with methanol at room temperature, appears to have been inadequate to remove all identifiable [¹⁴C]residues from the soil. At study termination (6.1 months posttreatment), nonextractable [¹⁴C]residues totaled 63.1% of the applied

COMPLIANCE: The study was conducted prior to the effective date of FIFRA GLP standards (p. 4). Signed and dated Data Confidentiality, GLP and Quality Assurance statements were provided (pp. 2-4). A certification of the authenticity of the data and the report is included as part of the Quality Assurance Statement (p. 4).

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A. MATERIALS:

1. Test Material

[Phenyl-U-¹⁴C]oryzalin (p. 7).

Chemical Structure:

See DER Attachment 1.

Description:

Technical grade (p. 7).

Purity: Radiochemical purity:

98.2% (by TLC).

Lot No.:

553-T84-067A.

Analytical purity:

Not reported.

Specific activity:

16.3 µCi/mg.

Location of the radiolabel:

Uniformly on the phenyl ring (p. 7).

Storage conditions of

test chemical:

Storage conditions for the test substance and reference standards were not reported.

Physico-chemical properties of oryzalin.

Parameter	Value	Comment
Molecular weight (g/Mol)	Not reported.	
Molecular formula	C ₁₂ H ₁₈ N ₄ O ₆ S	
Water solubility	2.6 ± 0.1 ppm	
Vapor pressure/Volatility	Not reported.	
UV Absorption	Not reported.	
pKa	Not reported.	
K _{ow} /log K _{ow}	Not reported.	
Stability of compound at room temperature, if provided	Not reported.	

Water solubility obtained from MRID 41378401.

2. Soil Characteristics

Table 1: Description of soil collection and storage.

Description	Details
Geographic location	Not reported. Soil was collected from an outside bin at the Lilly Research Laboratories in Greenfield, Indiana.
Pesticide use history at the collection site	The soil had no known history of treatment with pesticides.
Collection date	Not reported.
Collection procedures	Not reported.
Sampling depth	Not reported.
Storage conditions	Not reported.
Storage length	Not reported.
Soil preparation	Sieved (2 mm mesh sieve).

Data obtained from p. 7 of the study report.

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Table 2: Properties of the soil.

Property	Details
Soil texture	Sandy loam
% Sand	66
% Silt	21
% Clay	13
pH	7.1
Organic carbon (%) ¹	0.9
Organic matter (%)	1.5
CEC (meq/100 g)	8.8
Moisture content at 1/3 bar (%)	12.9
Bulk density (g/mL)	1.39.
Microbial population/biomass (units)	Not reported.
Soil taxonomic classification	Not reported.
Sol mapping unit (for EPA)	Not reported.

Data obtained from p. 7 of the study report. Assuming that the particle size distribution was according the USDA soil classification system, the soil texture is correct (<http://soils.usda.gov/technical/aids/investigations/texture/>).

1 Percent organic carbon was determined by the reviewer using the following formula: organic carbon (%) = organic matter (%) / 1.72.

B. EXPERIMENTAL CONDITIONS:

1. Preliminary experiments: No preliminary studies were described.

2. Experimental conditions:

Table 3: Experimental design.

Parameter	Details	
Duration of the test (days)	6.1 months.	
Soil condition: (Air dried/fresh)	Not reported.	
Soil (g/replicate)	1,075 g (dry wt. equivalent).	
Application rates ¹	Nominal	6.0 mg a.i./kg soil (26,640 dpm/g).
	Actual	ca. 5.2 mg a.i./kg (23,060 dpm/g).
Control conditions, if used	Sterile controls were not used.	
No. of Replications	Controls, if used	Sterile controls were not used.
	Treatment	At each sampling interval, two subsamples (2 x 15 g) were collected from the bulk treated soil in the desiccator.

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Parameter		Details
Test apparatus	Type/material/volume	A glass crystallizing dish containing moistened treated soil (1,075 g dry weight) was placed in a desiccator. The desiccator was sealed, covered with aluminum foil, and connected to a volatile trapping system. The test apparatus is illustrated in Figure 1, p. 28 of the study report.
	Details of traps for CO ₂ and organic volatiles, if any	Moistened CO ₂ -free air was passed through the desiccator containing the soil samples for 1 hour every 12 hours. Exiting gasses were drawn through a charcoal trap and a 1N NaOH solution (200 mL). The volatile trapping system is illustrated in Figure 1, p. 28 of the study report.
If no traps were used, is the system closed/open?		A volatile trapping system was used.
Identity and concentration of co-solvent		Methanol, <i>ca.</i> 0.03% v/w.
Test material	Volume of the test solution used/treatment:	10 mL/3,000 g.
	Application method :	The dosing solution was applied dropwise to the soil surface using a pipette. The soil was mixed on a rolling mill for 1 hour.
	Is the co-solvent evaporated?	Not reported.
Any indication of the test material adsorbing to the walls of the test apparatus?		Adsorption to the crystallizing dish containing the treated soil could not be determined from the data provided. Some residues were recovered when the desiccator holding the dish was rinsed at periodic intervals.
Microbial biomass/ population of the control (units)		Sterile controls were not used.
Microbial biomass/ population of untreated soil (units)		Not determined.
Microbial biomass/ population of treated soil (units)		Not determined.
Experimental conditions:	Temperature (°C):	24°C, range not reported.
	Continuous darkness:	Yes.
	Moisture content (%):	75% of 0.33 bar.
	Moisture maintenance method:	A bottle containing 200 mL of water was attached prior to the soil flask to supply moistened air and maintain appropriate soil moisture.
Other details, if any		None.

Data obtained from pp. 8-9; Table 3, p. 23; and Figure 1, p. 28 of the study report.

1 The nominal application rate of 6.0 mg a.i./kg is based on the application of 2.215 mg of [¹⁴C]oryzalin plus 16.059 mg of unlabeled oryzalin to 3,000 g soil (p. 8). The actual application rate at the start of the aerobic incubation (time 0) was obtained from Table 3, p. 23 in the study report.

3. Aerobic conditions: Moistened CO₂-free air was drawn through the desiccator for 1 hour every 12 hours (flow rate not reported; p. 8). No determinations were made to verify that aerobic conditions were maintained in the soil.

4. Supplementary experiments: In order to provide sufficient material for identification of transformation products, additional soil (2,000 g) was treated with 3.686 mg of [¹⁴C]oryzalin and 118.34 mg of unlabeled oryzalin in methanol (total application 60 mg a.i./kg; p. 8). The soil was mixed, transferred to aluminum pans, and moistened to 75% of 0.33 bar moisture (pp. 8-9). The

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pans were covered with aluminum foil and incubated at 24°C. At 5.5 months posttreatment, a subsample (300 g) of the soil extracted as described in the definitive experiment (pp. 12-13). The resulting ethyl acetate concentrate was dried onto dry column silica gel 60 (5 mL), and the silica gel was placed on top of a column (120 x 0.8 cm) containing the same silica gel. The column was sequentially eluted with the following solvents:

- Hexane;
- Hexane:toluene (90:10, v:v);
- Hexane:toluene (50:50, v:v);
- Toluene;
- Toluene:ethyl acetate (50:50, v:v);
- Ethyl acetate;
- Ethyl acetate:methanol:water (80:20:2, v:v:v);
- Methanol :water (95:5, v:v); and
- Methanol:glacial acetic acid (95:5, v:v).

The eluate was collected in 20-mL fractions, and aliquots were analyzed using LSC. Fractions containing the same peak or representing peaks of similar polarity were pooled. Aliquots of the eluate fractions were analyzed using one-dimensional TLC (SS1 or SS2 or ethyl acetate:methanol, 97:3, v:v). Radioactive zones were located by radioautography and scraped from the plates, [¹⁴C]residues were eluted from the silica with methylene chloride:methanol:acetic acid (80:20:1, v:v:v). Some compounds were further purified using HPLC (p. 14). [¹⁴C]Compounds were identified by NMR, MS, and/or HPLC with comparison to available reference standards.

5. Sampling:

Table 4: Sampling details.

Criteria	Details
Sampling intervals	0, 0.1, 0.2, 0.5, 0.7, 1.0, 1.4, 1.8, 3.0, 3.9 and 6.1 months.
Sampling method	At each sampling interval, two subsamples (2 x 15 g) were collected from the bulk treated soil in the desiccator.
Method of collection of CO ₂ and organic volatile compounds	The volatile traps were collected and replaced at each sampling interval.
Sampling intervals/times for: Sterility check, if sterile controls are used: Moisture content: Redox potential, other:	Sterile controls were not used. Not reported. Redox potentials and other parameters were not measured.
Sample storage before analysis	Not reported.
Other observation, if any	The desiccator was rinsed at unspecified intervals with methylene chloride and methanol. Results were reported for the 0.5 and 3 month sampling intervals.

Data obtained from pp. 9, 11 and Table 3, p. 23 of the study report.

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C. ANALYTICAL METHODS

Extraction/clean up/concentration methods: The soil subsamples (2 x 15 g) were extracted once with methanol (1 x 75 mL) using an orbital platform shaker at room temperature for 30 minutes (p. 11). The mixture was filtered, and the filtered soil was rinsed with additional methanol (20-40 mL).

Extracts from samples collected through 2 weeks (0.5 months) posttreatment were diluted with an equal volume of water, then acidified with 20 drops of 1N HCl (p. 11). The acidified methanol:water phase (50:50, v:v) was partitioned three times with methylene chloride (3 x 150 mL). For the 2-week samples only, the methanol remaining in the aqueous phase was evaporated using a vacuum rotary evaporation in a 45°C bath. The remaining aqueous solution (*ca.* 100 mL) was acidified to a pH <2 using concentrated HCl, then was partitioned three times with ethyl acetate (3 x 100 mL).

Extracts from samples collected at and after 3 weeks (0.7 months) posttreatment were diluted with water (50 mL), then the methanol was removed from the solution by vacuum rotary evaporation in a 45°C bath (p. 11). The remaining aqueous solution was acidified to pH <2 with concentrated HCl, then partitioned three times with ethyl acetate (3 x 75 mL).

For all samples, the organic fraction (methylene chloride or ethyl acetate) was filtered through sodium sulfate, then combined and concentrated (p. 12). Aliquots of the aqueous and organic fractions were analyzed using LSC. Aliquots of the organic fraction were analyzed using TLC.

The desiccator was periodically rinsed with methylene chloride and methanol (p. 9). Aliquots of the rinsate were analyzed by LSC.

Determination of nonextractable residues: The extracted soil was analyzed for total radioactivity using LSC following combustion (p. 9).

[¹⁴C]Residues remaining in the extracted soils collected at 6.1 months posttreatment were fractionated into fulvic acids, β humus, humic acids, and humin (p. 12). The extracted soil was extracted for 16 hours with 0.5N NaOH on an orbital platform shaker. The extraction mixture was centrifuged and the supernatant decanted. The soil pellet was resuspended in water, then centrifuged again. The 0.5N NaOH extract and water rinse were combined and the resulting solution was adjusted to pH 1 with concentrated HCl. The resulting precipitate (humic acid) was removed by centrifugation. The supernatant was adjusted to pH 4.8 using 10N NaOH. The resulting precipitate (β humus) and the supernatant (fulvic acid) were separated by centrifugation. The precipitates were analyzed using LSC following combustion and the solution was analyzed using LSC. Portions of the extracted soil were air-dried and analyzed using LSC following combustion.

Determination of volatile residues: The charcoal trap was analyzed for total radioactivity using LSC following combustion (p. 9). Aliquots of the NaOH trapping solutions were analyzed by LSC (p. 10). Residues in the NaOH solutions were identified as CO₂ by precipitation with 0.5M barium chloride (p. 11).

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Total ¹⁴C measurement: Subsamples of the bulk treated soil were collected at each sampling interval and analyzed without extraction for total [¹⁴C]residues using LSC following combustion (p. 11).

Derivatization method, if used: Derivatization method was not employed.

Identification and quantification of parent compound: Aliquots of the concentrated organic portions were analyzed by two-dimensional TLC on Merck Silica Gel 60 plates (0.25 mm thick) with fluorescent indicators that were developed in chloroform:methanol:acetic acid (90:10:1, v:v:v, SS1) and toluene:acetone:acetic acid (70:30:1, v:v:v; SS2; p. 10, Table 2, p. 22). Radioactive areas on the TLC plates were located by exposure to X-ray film or by spark chamber radiograms, and were quantified by scraping the silica into vials, eluting the adsorbent with methanol or water, and assaying by LSC. Samples were cochromatographed with an unlabeled reference standard of oryzalin that was visualized under UV light (R_f 0.67-SS1, 0.73-SS2, 0.75-SS3). No confirmatory analytical method used.

Identification and quantification of transformation products: Transformation products were separated and quantified using two-dimensional TLC as described for oryzalin (p. 10). Since the two-dimensional solvent system was not able to separate transformation products OR-4 and OR-41, the organic extract from the 1 month sample was reanalyzed as described, except that chloroform:isopropanol:acetic acid (90:10:1, v:v:v; SS3) was substituted for SS2 (pp. 10, 16).

The reference standards available for use were:

Code Name (Compound)	CAS Name	Lot number	Purity (%)	TLC R _f		
				SS1	SS2	SS3
OR-2	3,5-Dinitro-4-(propylamino)benzenesulfonamide	--	--	0.63	0.69	0.74
OR-4	3-Amino-4-(dipropylamino)-5-nitrobenzenesulfonamide	--	--	0.60	0.52	0.57
OR-9	3,4,5-Triaminobenzene-sulfonamide	--	--	0.07	0.09	0.03
OR-13	2-Ethyl-7-nitro-1-propyl-1H-benzimidazole-5-sulfonamide	--	--	0.60	0.36	0.42
OR-15	2-Ethyl-7-nitro-1H-benzimidazole-5-sulfonamide	--	--	0.36	0.23	0.14
OR-20	4-Hydroxy-3,5-dinitro-benzenesulfonamide	--	--	0.07	0.11	0.04
OR-41	4-[(2-Hydroxypropyl)propylamino]-3,5-dinitro-benzenesulfonamide	--	--	0.60	0.52	0.67

Data obtained from Tables 1-2, pp. 20-22 in the study report. Although they appear in this Table 1, the study author reported that no reference standards were available for UN-1 and UN-2 (p. 17).

Detection limits (LOD, LOQ) for the parent compound: Limits of Detection and Quantification were not reported.

Detection limits (LOD, LOQ) for the transformation products: Limits of Detection and Quantification were not reported.

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II. RESULTS AND DISCUSSION

A. TEST CONDITIONS: Incubation temperatures and soil moisture content were reported to be 24°C and 75% of 0.33 bar throughout the study; no supporting data were provided (p. 9). The soil aerobicity was reportedly maintained throughout the experiment; no supporting data were provided. The viability of the soil microflora was not determined either prior to or after treatment.

B. MATERIAL BALANCE: : Overall [¹⁴C]residue recoveries averaged $95.9 \pm 4.1\%$ (range 87.9-100.9%) of the applied (Table 3, p. 23, DER Attachment 2). Study authors indicated that there was no significant loss of [¹⁴C]residues from the test system over time. However, as seen in Table 5, overall [¹⁴C]residue recoveries consistently declined from 96.7% at 1.4 months to 87.9% at 6.1 months.

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Table 5: Biotransformation of [¹⁴C]oryzalin, expressed as percentage of the applied radioactivity (n = 1), in aerobic sandy loam soil.

Compound	Sampling Intervals (months)										
	0	0.1	0.2	0.5	0.7	1.0	1.4	1.8	3.0	3.9	6.1
Oryzalin	92.2	87.4	81.1	70.9	57.8	51.3	42.4	35.4	25.7	19.8	13.1
OR-2	1.2	0.4	0.4	0.6	0.5	0.6	0.9	0.3	1.2	0.3	0.3
OR-4/41 ¹	0.2	0.2	0.2	0.5	0.1	0.1	0.3	0.3	0.1	0.2	0.2
OR-9	--	--	--	--	--	--	0.4	0.1	0.4	--	--
OR-13	0.2	0.2	0.2	0.5	0.5	0.6	0.7	0.6	0.9	1.0	1.0
OR-15	--	--	--	0.4	0.4	0.6	0.4	0.6	0.6	0.8	0.9
OR-20	--	0.1	0.2	3.0	4.0	4.7	4.0	3.7	2.8	2.3	1.5
UN-1	0.9	0.9	0.8	1.1	1.2	1.4	1.1	1.0	1.0	0.9	0.8
UN-2	--	--	--	0.8	1.4	1.7	1.6	2.0	2.3	2.4	0.8
Other ²	2.4	2.0	1.8	2.4	2.4	2.0	4.5	3.6	0.7	3.5	3.8
Organic extractable residues ³	97.1	91.2	84.7	80.2	68.3	63.0	56.3	47.6	35.7	31.2	22.4
Aqueous extractable residues	0.3	0.4	0.9	0.3	0.5	0.7	0.9	0.8	0.6	0.7	1.2
Extractable residues	97.4	91.6	85.6	80.5	68.8	63.7	57.2	48.4	36.3	31.9	23.6
Nonextractable residues	2.6	8.7	12.4	20.3	26.9	32.5	38.7	45.4	56.1	57.4	63.1
CO ₂ ⁴	--	0.01	0.02	0.10	0.27	0.33	0.80	0.79	1.36	0.84	1.2
Organic Volatiles ⁴	--	0.00	0.00	0.00	0.00	0.01	0.01	0.02	0.03	0.02	0.01
Total recovery	100.0	100.3	98.0	100.9	96.0	96.5	96.7	94.6	93.8	90.2	87.9

Most data obtained from Table 4, pp. 24-26 of the study report. Although two samples were collected at each sampling interval, only averaged data were reported by the study authors.

1. The primary solvent systems could not differentiate between OR-4 and OR-41. An alternate solvent system used only with the 1 month posttreatment sample determined the ratio was 1:1 (pp. 16-17).

2. "Other" is residue that was bound to the origin of the TLC plate, or that streaked over the plate and gave a weak or no film response (Table 3, p. 21, footnote 5).

3. Sum of oryzalin, degradates, and other.

4. Determined by taking sum of dpm in charcoal trap and dessicator wash (organic volatiles), and base trap (CO₂) from Table 3, p. 23, and dividing by dpm initially in closed system (27,441,400).

-- = Indicates that an assay was not done for the compound at this interval because no autoradiographic response was observed in the region of interest on the TLC plate (e.g., the compound, if present, was below the limit of detection on the autoradiograph).

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C. TRANSFORMATION OF PARENT COMPOUND: [¹⁴C]Oryzalin decreased from 92.2% of the applied at time 0 to 51.3% at 1 month posttreatment, 25.7% at 3 months, and 13.1% at 6.1 months (Table 4, p. 25).

HALF-LIFE/DT50/DT90: Based on nonlinear regression analysis (Sigma Plot v. 9.0, exponential decay/one compartment, two parameter), oryzalin dissipated with a reviewer-calculated half-life of 1.5 months (DER Attachment 2). The first order log/linear (Excel 2003) half-life was 2.1 months. The observed DT50 value was 1-1.4 months.

As the extraction method resulted in a large amount of nonextractable residues, half-lives were also estimated as if the nonextractable residues were oryzalin. Based on nonlinear regression analysis (Sigma Plot v. 9.0, exponential decay/one compartment, two parameter), oryzalin and nonextractable residues dissipated with a reviewer-calculated half-life of 18 months. The first order log/linear (Excel 2003) half-life was 19 months. The observed DT50 value was >6.1 months.

Using first-order linear regression analysis, the study authors calculated a half-life of 2.1 months (p. 15).

Half-lives/DT50/DT90 of oryzalin

Compound	Half-life/DT50 ¹ (months)	Regression equation	r ²	Observed DT50 (months)	Observed DT90 (months)
Parent only					
Linear/natural log	2.1	$y = \exp(-0.3283x + 4.3549)$	0.949	1.0-1.4	>6.1
Nonlinear/normal	1.5	$y = -88.639\exp(-0.4709x)$	0.9946		
Parent + Unextractable residues					
Linear/natural log	19	$y = \exp(-0.0363x + 4.508)$	0.704	>6.1	>6.1
Nonlinear/normal	18	$y = -91.19\exp(-0.039x)$	0.705		

¹ Determined by the primary reviewer using Excel (linear) and Sigma Plot v 9.0 (nonlinear, exponential decay/one compartment, two parameter) and data obtained from Table 4, p. 25.

TRANSFORMATION PRODUCTS: No major transformation products were isolated. Nine minor transformation products were identified (pp. 16-17; Table 4, pp. 24-26):

- 3,5-dinitro-4-(propylamino)benzenesulfonamide (OR-2);
- 3-amino-4-(dipropylamino)-5-nitrobenzenesulfonamide (OR-4);
- 3,4,5-triaminobenzene-sulfonamide (OR-9);
- 2-ethyl-7-nitro-1-propyl-1H-benzimidazole-5-sulfonamide (OR-13);
- 2-ethyl-7-nitro-1H-benzimidazole-5-sulfonamide (OR-15);
- 4-hydroxy-3,5-dinitro-benzenesulfonamide (OR-20);
- 4-[(2-hydroxypropyl)propylamino]-3,5-dinitro-benzenesulfonamide (OR-41);
- cis- and trans- isomers of 3,3'-azoxybis[4-(propylamino)-5-nitro]benzenesulfonamide (UN-1);
and
- 2-ethyl-7-nitro-1-propyl-1H-benzimidazole-5-sulfonamide, 3-oxide (UN-2)

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OR-20 was the predominant transformation product at a maximum of 4.7% of the applied. OR-2, OR-4/41, OR-9, OR-13, and OR-15 were each $\leq 1.2\%$ of the applied. UN-1 and UN-2 were maximums of 1.4% and 2.4% of the applied, respectively. [^{14}C]Residues remaining at the origin or not associated with a discrete area totaled a maximum of 4.5% of the applied. Chemical names and CAS numbers for the transformation products of oryzalin are presented in Table 6. Chemical formulae, molecular weights, and SMILES strings for the transformation products are provided in Attachment 1.

Table 6: Chemical names and CAS numbers for the transformation products of oryzalin.

Applicants Code Name	CAS Number	Chemical Name
OR-2	--	3,5-Dinitro-4-(propylamino)benzenesulfonamide
OR-4	--	3-Amino-4-(dipropylamino)-5-nitrobenzenesulfonamide
OR-9	--	3,4,5-Triaminobenzene-sulfonamide
OR-13	--	2-Ethyl-7-nitro-1-propyl-1H-benzimidazole-5-sulfonamide
OR-15	--	2-Ethyl-7-nitro-1H-benzimidazole-5-sulfonamide
OR-20	--	4-Hydroxy-3,5-dinitro-benzenesulfonamide
OR-41	--	4-[(2-Hydroxypropyl)propylamino]-3,5-dinitro-benzenesulfonamide
UN-1	--	Cis- and trans- isomers of 3,3'-azoxybis[4-(propylamino)-5-nitro]benzenesulfonamide
UN-2	--	2-Ethyl-7-nitro-1-propyl-1H-benzimidazole-5-sulfonamide, 3-oxide

Data obtained from Table 1, pp. 20-21 of the study report.

NONEXTRACTABLE AND EXTRACTABLE RESIDUES: Extractable [^{14}C]residues decreased from 97.4% of the applied at time 0 to 23.6% at 6.1 months posttreatment, while nonextractable [^{14}C]residues increased from 2.6% at time 0 to 63.1% at 6.1 months (Table 4, p. 24, DER Attachment 2). In the 6.1 month sample, nonextractable [^{14}C]residues were associated with 14.0% humic acid, 5.6% β humus, 25.1% fulvic acids and 18.4% humin fractions (p. 17).

At 6.1 months posttreatment, the desiccator rinsate contained 0.0005% of the applied (p. 15).

VOLATILIZATION: During the 6.1 month incubation period, CO_2 and volatile [^{14}C]organics totaled 5.7% and 0.1% of the applied, respectively (p. 15).

TRANSFORMATION PATHWAY: A transformation pathway was provided by the study authors (p. 18; Figure 6, p. 33). Under aerobic conditions, oryzalin degrades via dealkylation, oxidation, reduction, dimerization and ring formation. Oryzalin is initially degraded to OR-20, OR-41, OR-4 or OR-2. OR-4 is degraded to OR-9 or OR-13, and OR 13 is further degraded to OR-15 and UN-2. OR-2- is transformed to UN-1. Study authors concluded that the transformation products are ultimately converted to bound residues and CO_2 . However, given the uncertainty surrounding the inadequacy of the extraction method to remove all identifiable [^{14}C]residues from the soil, there is not enough information for the reviewer to support this conclusion.

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D. SUPPLEMENTARY EXPERIMENT-RESULTS: The high dose samples were used in the identification of transformation products; the results are integrated into the definitive experiment discussion.

III. STUDY DEFICIENCIES

1. The extraction procedure appears to have been inadequate to remove all identifiable [¹⁴C]residues from the soil. Soil samples were extracted by shaking with methanol at room temperature (p. 11). At study termination, nonextractable [¹⁴C]residues totaled 63.1% of the applied (Table 4, p. 24). These nonextractable residues may be parent or may be transformation products and have the potential to be reintroduced into the environment if released from the soil. This also adds uncertainty into the estimation of a half-life of oryzalin in an aerobic environment. If nonextractable residues are included in total amount of oryzalin in the soil, the half-life would increase from approximately 2.1 months to 19 months.
2. Total recoveries for the system fell below 90% at the end of the study, with a consistent decline from 96.7% at 1.4 months to 87.9% at 6.1 months. This indicates an unaccounted steady loss of radioactivity.
3. Although the soil aerobicity was reportedly maintained throughout the experiment, no supporting data were provided. The viability of the soil microflora was not determined either prior to or after treatment.
4. Although it was stated that for each sampling period that 2 replicates of soil (15 g) were extracted and analyzed, the report only provide one result for each sampling period. It is preferred that individual sample data be reported so that between replicate variability can be assessed.
5. It was not stated whether samples or sample extracts were stored prior to analysis.
6. Limits of Detection and Quantification were not reported.
7. Although a bottle containing water was attached to the soil flask in order to supply moistened air to the system, measurements of the moisture content of the soil in the desiccator were not provided to indicate that soil moisture was monitored and maintained.

IV. REVIEWER'S COMMENTS

1. A bulk soil sample was treated and incubated (p. 9). Therefore, the reported volatile concentration is averaged based on the number of samples. It is preferred that individual sample vessels be connected to the volatile trapping system, so that more precise material balances for each sample can be calculated.

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2. Total residue recoveries in terms of percentage of applied that are reported in Tables 3 and 4 (pp. 23-24) are not in agreement. The values in Table 4 are incorrect, since they do not include volatile residues.
3. The study authors reported that the soil was obtained from an outdoor bin maintained at Greenfield, Indiana (p. 7). They did not specify where the soil had been collected or how long it was stored in this bin. In addition, the storage conditions of the bin were not reported (e.g., covered).
4. The study author stated that the change from methylene chloride to ethyl acetate as the partitioning solvent was necessary to extract polar compounds from the aqueous phase (p. 12).
5. The primary solvent systems could not differentiate between OR-4 and OR-41. An alternate solvent system used only with the 1 month posttreatment sample determined the ratio was 1:1 (pp. 16-17). The study author stated that since the concentration of OR-4/41 was $\leq 0.5\%$ of the applied at all sampling intervals, differentiation of the two compounds at other intervals was not considered necessary.
6. The β humus fraction of nonextractable residues contains [^{14}C]residues that are typically identified as part of the fulvic acid fraction (p. 12).
7. Physical and chemical properties for oryzalin were not reported.
8. The temperature of incubation was reported to be 24°C (p. 9). The minimum and maximum temperatures were not reported, and no supporting data were provided.

V. REFERENCES

1. U.S. Environmental Protection Agency. 2008. Fate, Transport and Transformation Test Guidelines, OPPTS 835.4100, Aerobic Soil Metabolism. Office of Prevention, Pesticides and Toxic Substances, Washington, DC. EPA 712-C-08-016.
2. U.S. Environmental Protection Agency. 1982. Pesticide Assessment Guidelines, Subdivision N, Chemistry: Environmental Fate, Section 162-1, Aerobic Soil Metabolism Studies. Office of Pesticide and Toxic Substances, Washington, DC. EPA 540/9-82-021.

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Attachment 1: Structures of Parent Compound and Transformation Products

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Oryzalin [OR-1; EL-119]

IUPAC Name: 3,5-Dinitro-4-(dipropylamino)benzenesulfonamide.

3,5-Dinitro- N^4,N^4 -dipropylsulfanilamide.

CAS Name: 4-(Dipropylamino)-3,5-dinitrobenzenesulfonamide.

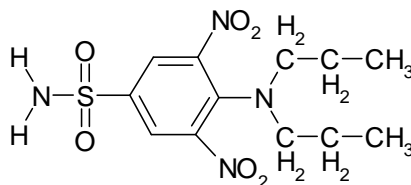
CAS Number: 19044-88-3.

SMILES String: C1C(S(=O)(=O)N)=CC(N(O)O)=C(N(CCC)CCC)C=1N(O)O (EpiSuite version 4.0).

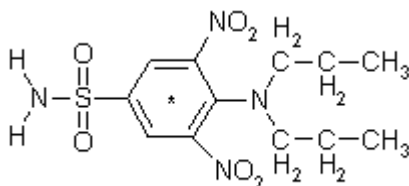
Empirical formula: $C_{12}H_{18}N_4O_6S$

Molecular formula: $C_{12}H_{18}N_4O_6S$

Unlabeled



* structure complexity/form was sacrificed to
obtain SMILES string
[ring-UL- ^{14}C]Oryzalin
[benzene-U- ^{14}C]Oryzalin
[^{14}C]Oryzalin



* = Location of the radiolabel.

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Identified Compounds

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Oryzalin [OR-1; EL-119]

IUPAC Name: 3,5-Dinitro-4-(dipropylamino)benzenesulfonamide.
3,5-Dinitro-N⁴,N⁴-dipropylsulfanilamide.

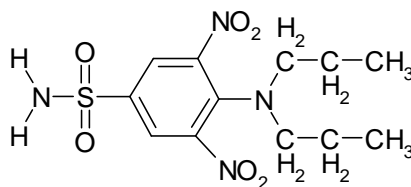
CAS Name: 4-(Dipropylamino)-3,5-dinitrobenzenesulfonamide.

CAS Number: 19044-88-3.

SMILES String: C1C(S(=O)(=O)N)=CC(N(O)O)=C(N(CCC)CCC)C=1N(O)O (EpiSuite version 4.0).

Empirical formula: C₁₂H₁₈N₄O₆S

Molecular formula: C₁₂H₁₈N₄O₆S



* structure complexity/form was sacrificed to obtain SMILES string

OR-2

IUPAC Name: 3,5-Dinitro-4-(propylamino)benzenesulfonamide.

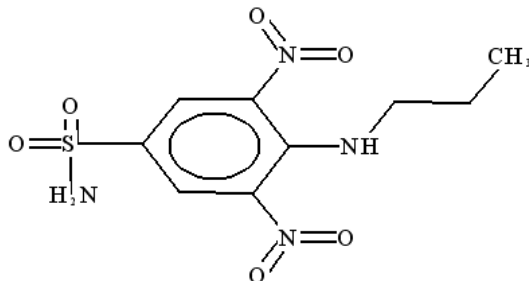
CAS Name: Not reported.

CAS Number: Not reported.

SMILES String: N(CCC)c1c(cc(cc1N(=O)=O)S(N)(=O)=O)N(=O)=O (EpiSuite version 4.0).

Empirical formula: C₉H₁₂N₄O₆S

Molecular formula: C₉H₁₂N₄O₆S



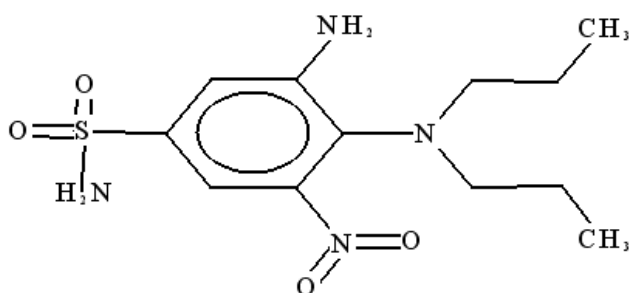
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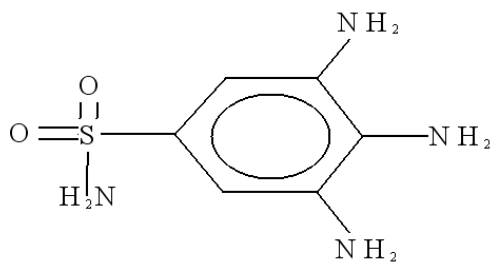
OR-4

IUPAC Name: 3-Amino-4-(dipropylamino)-5-nitrobenzenesulfonamide.
CAS Name: Not reported.
CAS Number: Not reported.
SMILES String: CCCN(CCC)c1c(cc(cc1N)S(N)(=O)=O)N(=O)=O (EpiSuite version 4.0).
Empirical formula: $C_{12}H_{20}N_4O_4S$ **Molecular formula:** $C_{12}H_{20}N_4O_4S$



OR-9

IUPAC Name: 3,4,5-Triaminobenzenesulfonamide.
CAS Name: Not reported.
CAS Number: Not reported.
SMILES String: Nc1c(cc(cc1N)S(N)(=O)=O)N (EpiSuite version 4.0).
Empirical formula: $C_6H_{10}N_4O_2S$ **Molecular formula:** $C_6H_{10}N_4O_2S$



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OR-13

IUPAC Name: 2-Ethyl-7-nitro-1-propyl-1H-benzimidazole-5-sulfonamide.

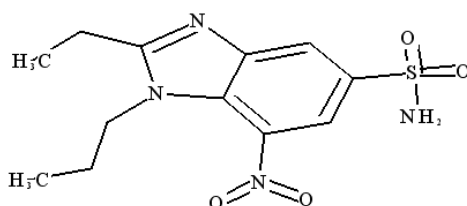
CAS Name: Not reported.

CAS Number: Not reported.

SMILES String: C1(S(=O)(=O)N)=CC(N(=O)=O)=C2N(CCC)C(CC)=NC2=C1 (EpiSuite version 4.0).

Empirical formula: C₁₂H₁₆N₄O₄S

Molecular formula: C₁₂H₁₆N₄O₄S



OR-15

IUPAC Name: 2-Ethyl-7-nitro-1H-benzimidazole-5-sulfonamide.

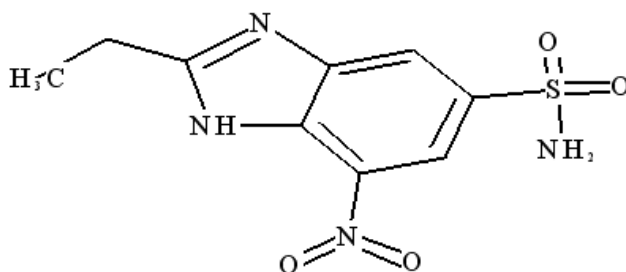
CAS Name: Not reported.

CAS Number: Not reported.

SMILES String: C1(S(=O)(=O)N)=CC(N(=O)=O)=C2NC(CC)=NC2=C1 (EpiSuite version 4.0).

Empirical formula: C₉H₁₀N₄O₄S

Molecular formula: C₉H₁₀N₄O₄S



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OR-20

IUPAC Name: 4-Hydroxy-3,5-dinitro-benzenesulfonamide.

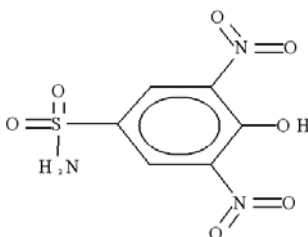
CAS Name: Not reported.

CAS Number: Not reported.

SMILES String: Oc1c(cc(cc1N(=O)=O)S(N)(=O)=O)N(=O)=O (EpiSuite version 4.0).

Empirical formula: $C_6H_5N_3O_7S$

Molecular formula: $C_6H_5N_3O_7S$



OR-41

IUPAC Name: 4-[(2-Hydroxypropyl)propylamino]-3,5-dinitro-benzenesulfonamide.

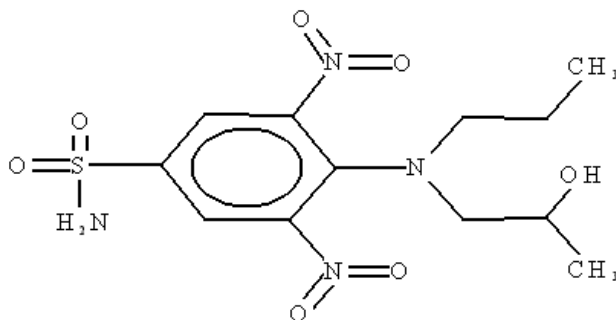
CAS Name: Not reported.

CAS Number: Not reported.

SMILES String: Nc1c(cc(cc1N)S(N)(=O)=O)N (EpiSuite version 4.0).

Empirical formula: $C_{12}H_{18}N_4O_7S$

Molecular formula: $C_{12}H_{18}N_4O_7S$



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Carbon Dioxide

IUPAC Name: Carbon dioxide.

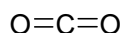
CAS Name: Carbon dioxide.

CAS Number: 124-38-9.

SMILES String: C(=O)=O (EpiSuite version 4.0).

Empirical formula: CO₂

Molecular formula: CO₂



UN-1

IUPAC Name: 3,3'-Azoxybis[4-(propylamino)-5-nitro]benzenesulfonamide.

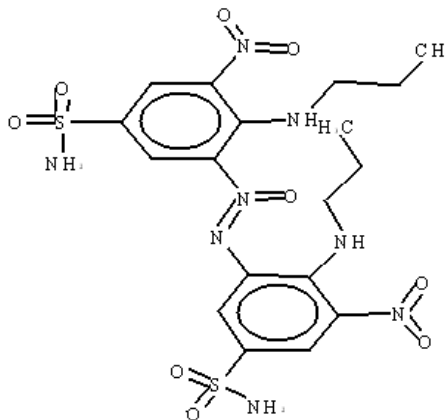
CAS Name: Not reported.

CAS Number: Not reported.

SMILES String: CCCNc1c(cc(cc1N(=O)=O)S(N)(=O)=O)N(=O)=Nc2c(NCCC)c(N(=O)=O)cc(S(N)(=O)=O)c2 (EpiSuite version 4.0).

Empirical formula: C₁₈H₂₄N₈O₉S₂

Molecular formula: C₁₈H₂₄N₈O₉S₂



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UN-2

IUPAC Name: 2-Ethyl-7-nitro-1-propyl-1H-benzimidazole-5-sulfonamide, 3-oxide.

CAS Name: Not reported.

CAS Number: Not reported.

SMILES String: C1(S(=O)(=O)N)=CC(N(=O)(=O))=C2N(=O)=N(CC)N(CCC)C2=C1
(EpiSuite version 4.0).

**Empirical
formula:**

$C_{11}H_{16}N_5O_5S$

Molecular formula:

$C_{11}H_{16}N_5O_5S$

