**Data Evaluation Record on the anaerobic biotransformation of oryzalin in soil**

PMRA Submission Number {.....} EPA MRID Number 41322802

| | | |
|---|---|---|
| Data Requirement: | PMRA Data Code: | |
| | EPA DP Barcode: | 378627 |
| | OECD Data Point: | |
| | EPA Guideline: | 835.4200 |

**Test material:**

| | |
|---|---|
| Common name: | Oryzalin. |
| Chemical name: | |
| IUPAC name: | 3,5-Dinitro-4-(dipropylamino)benzenesulfonamide.<br>3,5-Dinitro-$N^4$,$N^4$-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
**Cambridge Environmental**
**Signature:**
**Date:** 10/25/10

**QC/QA Manager:** Joan Gaidos
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**Date:** 19 MAY 2011

**Final Reviewer:** Cheryl Sutton, Ph.D.
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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. Anaerobic metabolism of $^{14}C$ oryzalin in sandy loam soil. Unpublished study performed, submitted and sponsored by DowElanco (now Dow AgroChemicals), Greenfield, Indiana. Laboratory Project ID.: ABC-0435. 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-$^{14}$C]-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 for 60 days under anaerobic conditions (flooding, nitrogen atmosphere) in darkness at 28°C following 23 days of incubation under aerobic conditions (24°C, 75% of 0.33 bar moisture). The water:soil ratio was 2:3 (10 mL:15 g). The experiment was submitted under USEPA Pesticide Assessment Guidelines, Subdivision N §162-2, and was conducted prior to the effective date of FIFRA GLP standards. The sandy loam soil used in the study was collected from an outdoor bin maintained at the laboratory. The test system consisted of glass vials containing treated soil (15 g) that were placed in a desiccator that was covered in aluminum foil and attached to a volatile trapping system. Humidified nitrogen 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). Test systems were maintained at 24°C and 0.33 bar moisture capacity for 23 days. After 23 days of aerobic incubation (time 0), the systems were converted to anaerobic conditions by adding 10 mL of water to the vials. Duplicate samples were collected for analysis prior to flooding (time 0) and at 30 and 60 days postflooding. The water and soil phases were not separated for analysis. The samples were extracted once with methanol by shaking at room temperature. The extracts were diluted with water, acidified (pH 1) and partitioned with ethyl acetate, and then adjusted to pH 12 and again partitioned with ethyl acetate. Aliquots of the extracts were analyzed for total radioactivity using liquid scintillation (LSC). The organic extracts were combined, concentrated, and analyzed using two-dimensional thin layer chromatography (TLC). Identifications were made by comparison to reference standards of oryzalin and ten 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 by LSC.

Incubation temperatures were reported to be 24°C during the aerobic incubation (through 23 days posttreatment) and 28°C following the introduction of anaerobic conditions; no supporting data were provided. The pH of the test systems was not measured. Redox potentials and dissolved oxygen levels were not measured. The viability of the soil was not determined.

Overall recoveries averaged 97.2 ± 2.4% (range 95.6-100.0%) of the total radioactivity at time 0 (immediate preflood). There was no significant loss of [$^{14}$C]residues from the test systems over time.

Using first-order linear regression analysis, the study authors calculated a half-life of 10 days (p. 13). However, because of the high amount of nonextractable residues, the apparent inadequacy of the extraction method to remove all identifiable [$^{14}$C]residues, and because there were only three sampling intervals, with a decrease of greater than 50% occurred between the first and second sampling intervals, half-lives were not estimated by the reviewer. Oryzalin decreased from 62.3% of the total radioactivity at time 0 to 2.4% at 30 days postflooding and 1.0% at 60 days. Seven transformation products were identified:

- 3,5-dinitro-4-(propylamino)benzenesulfonamide (OR-2);
- 3,5-diamino-4-(dipropylamino)benzenesulfonamide (OR-7);
- 2-ethyl-7-nitro-1-propyl-1H-benzimidazole-5-sulfonamide (OR-13);
- 7-amino-2-ethyl-1-propyl-1H-benzimidazole-5-sulfonamide (OR-14);
- 2-ethyl-7-nitro-1H-benzimidazole-5-sulfonamide (OR-15);
- 7-amino-2-ethyl-1H-benzimidazole-5-sulfonamide (OR-16); and
- 4-hydroxy-3,5-dinitro-benzenesulfonamide (OR-20).

In addition, two transformation products co-eluted:

- 3-amino-4-(dipropylamino)-5-nitrobenzenesulfonamide (OR-4) and
- 4-[(2-hydroxypropyl)propylamino]-3,5-dinitro-benzenesulfonamide (OR-41).

The only major transformation product, OR-14, was 0.1% of the time 0 radioactivity and increased to 10.3% at 30 and 60 days postflooding. All other transformation products were minor. OR-16 and OR-20 were maximums of 5.3% and 4.3% at time 30 days and 0 days, respectively. OR-2, OR-4/41, OR-7, OR-13, and OR-15 were each ≤0.8% of the time 0 radioactivity. Two isolated unidentified compounds (UN-1 and UN-2), were 1.0-1.2% of the time 0 radioactivity, but were 0% throughout the remainder of the study. [$^{14}$C]Residues remaining at the origin or not associated with a discrete area totaled a maximum of 6.1% of the time 0 radioactivity. Because samples were not separated into soil and water phases for analysis, it is unknown whether the compounds were present predominantly in the soil or the water.

Extractable [$^{14}$C]residues decreased from 73.7% of the total radioactivity at time 0 to 25.2% at 60 days postflooding, while nonextractable [$^{14}$C]residues increased to 70.6%. In the 60 day sample, nonextractable [$^{14}$C]residues were associated with 18.4% humic acids, 7.6% β humus, 28.9% fulvic acids and 15.7% humin. During the 60 day posttreatment period, $CO_2$ and volatile [$^{14}$C]organics totaled 0.26% and 0.04% of the total radioactivity at time 0, respectively.

A transformation pathway was provided by the study authors. Under anaerobic conditions, oryzalin degrades via reduction of the nitro group to form OR-4 which in turn forms OR-7 which forms the benzimidazole OR-14. OR-14 is demethylated to form OR-16. Other compounds identified in the experiment were not considered to be part of the anaerobic degradation process. Study authors concluded that the transformation products are ultimately converted to polar compounds, bound residues and minor amounts of $CO_2$. 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.**
Major transformation products:
7-Amino-2-ethyl-1-propyl-1H-benzimidazole-5-sulfonamide (OR-14).
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).
3,5-Diamino-4-(dipropylamino)benzenesulfonamide (OR-7).
2-Ethyl-7-nitro-1-propyl-1H-benzimidazole-5-sulfonamide (OR-13).
2-Ethyl-7-nitro-1H-benzimidazole-5-sulfonamide (OR-15).
7-Amino-2-ethyl-1H-benzimidazole-5-sulfonamide (OR-16).
4-Hydroxy-3,5-dinitro-benzenesulfonamide (OR-20).
$CO_2$.

**Study Acceptability:** This study is classified as **not acceptable**. Sampling intervals were too infrequent to adequately assess the rate of dissipation. The water and soil were not analyzed separately. The extraction procedure was not adequate to remove all identifiable [$^{14}$C]residues from the soil. Redox potentials and dissolved oxygen levels were not measured, so it was uncertain if an anaerobic environment was actually achieved.

**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:** The guidelines used to design the study were not identified; the study was submitted under USEPA Pesticide Assessment Guidelines, Subdivision N §162-2 (p. 1). Significant deviations from the objectives of Subdivision N guidelines were noted:

Samples were collected too infrequently to accurately assess the pattern of dissipation of oryzalin. Between the 0 and 30 day postflooding intervals (first and second sampling intervals), the concentration of oryzalin decreased from 62.3% to 2.4% of the total radioactivity at time 0.

The water and soil phases were not analyzed separately, only concentrations for the total system was reported.

The extraction procedure, shaking with methanol at room temperature, appears to have been inadequate in removing all identifiable [$^{14}$C]residues from the soil. At 60 days postflooding, nonextractable [$^{14}$C]residues totaled 70.6% of time 0.

Measurements such as redox potentials and dissolved oxygen content were not taken, so the anaerobicity of the test system was unknown.

The viability of the test soil was not determined.

The water used in the experiment was not characterized.

It was not reported if samples were stored prior to analysis.

**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).

**A. MATERIALS:**

**1. Test Material** [Phenyl-U-$^{14}$C]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 | $C_{12}H_{18}N_4O_6S$ | |
| 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.

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. |
| Soil 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) | | 83 days (23 days of aerobic incubation followed by 60 days of anaerobic incubation). |
| Soil condition: (Air dried/fresh) | | The soil had been incubated under aerobic conditions (24°C, 75% of 0.33 bar moisture content) for 23 days prior to flooding. |
| Soil (g/replicate) | | 15 g (Subsamples of the bulk treated sample used in an aerobic metabolism study were transferred to individual sample vials). |
| Application rates [1] | Nominal | 6.0 mg a.i./kg soil (26,640 dpm/g soil). |
| | Actual | *ca*. 5.2 mg a.i./kg (23,060 dpm/g from time 0 aerobic) |
| Control conditions, if used | | Sterile controls were not used. |
| No. of | Controls, if used | Sterile controls were not used. |

| Parameter | | Details |
|---|---|---|
| Replications | Treatment | At time 0, two subsamples (2 x 15 g) were collected from the bulk treated aerobic soil. Ten vials were collected at 30 days and again at 60 days postflooding. Two vials were analyzed at each interval and the remaining vials were stored frozen. |
| Test apparatus | Type/material/volume | The test system consisted of glass vials containing treated soil (15 g) flooded with water (10 mL). The vials were placed in a glass desiccator that was sealed, covered with aluminum foil, and connected to a volatile trapping system. The test apparatus is illustrated in Figure 1, p. 24 of the study report. |
| | Details of traps for $CO_2$ and organic volatiles, if any | Humidified nitrogen gas was passed (flow rate not reported) 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. 24 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, *ca*. 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 30 minutes. |
| | Is the co-solvent evaporated? | Not reported. |
| Any indication of the test material adsorbing to the walls of the test apparatus? | | Adsorption to the sample vials could not be determined from the data provided. Some residues (0.08% of applied) were recovered when the desiccator was rinsed at study termination. |
| 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 during the aerobic phase, 28°C during the anaerobic phase, ranges not reported. |
| | Continuous darkness: | Yes. |
| | Moisture content (%): | Aerobic phase: Reported to be 75% of the maximum water holding capacity of 0.33 bar.<br>Anaerobic phase: 15 g soil samples were flooded with 10 mL of water. |
| | Moisture maintenance method: | Not reported. |
| Other details, if any | | None. |

Data obtained from pp. 8-9, 11-12; and Figure 1, p. 24 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 [$^{14}$C]oryzalin plus 16.059 mg of unlabeled oryzalin to 3,000 g soil. The actual application rate at the start of the aerobic incubation (time 0), was obtained from MRID 41322801 (Table 3, p. 23).

**3. Aerobic conditions:** No determinations were made to determine the aerobicity or anaerobicity of the samples during the experiment. During the aerobic phase of the study, treated soil was moistened to 75% of 0.33 bar and maintained under air (p. 9, MRID 41322801, p. 8). During the

anaerobic phase of the study, the soil was saturated with water and maintained under a nitrogen atmosphere (p. 8).

**4. Supplementary experiments:** Additional soil (2,000 g) was treated at 10x the rate used in the definitive experiment. A portion of the 10x treated soil was incubated as described in the definitive experiment. At the time the definitive experiment was established, portions (30 g) of the 1x and 10x treated soils were transferred to glass vials and flooded with water (25 mL; pp. 8-9). The vials were capped and the samples incubated in the dark at 28°C (p. 10). Samples were collected at 30 and 60 days posttreatment.

**5. Sampling:**

Table 4: Sampling details.

| Criteria | Details |
|---|---|
| Sampling intervals | 0, 30 and 60 days. |
| Sampling method | The time 0 sample appears to have been subsamples from the bulk treated aerobic soil. Ten vials were collected at 30 days and again at 60 days postflooding. Two vials were analyzed at each interval and the remaining vials were stored frozen. |
| Method of collection of $CO_2$ and organic volatile compounds | The volatile traps were collected at 14, 30, and 60 days. |
| Sampling intervals/times for: | |
| Sterility check, if sterile controls are used: | Sterile controls were not used. |
| Moisture content: | Not reported. |
| Redox potential, other: | Redox potentials and other parameters were not measured. |
| Sample storage before analysis | Not reported. |
| Other observation, if any | None. |

Data obtained from p. 9 of the study report.

## C. ANALYTICAL METHODS:

**Extraction/clean up/concentration methods:** The samples (water plus soil) 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 (25 mL). The methanol extract was 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). The aqueous fraction was then adjusted to pH 12 with concentrated NaOH and again partitioned three times with ethyl acetate (3 x 75 mL). Aliquots of the aqueous and organic fractions were analyzed using LSC. The ethyl acetate fractions were filtered through sodium sulfate, then combined and concentrated to 2 mL prior to analysis using TLC.

The desiccator holding the sample vials was rinsed at 60 days, and aliquots of the rinsate were analyzed using LSC (Table 2, p. 20).

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

[$^{14}$C]Residues remaining in the extracted soils collected at 60 days postflooding were fractionated into fulvic acids, β humus, humic acids, and humin (pp. 11-12). 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 extracted soil (humin) was allowed to air dry and aliquots were assayed for radioactive content by combustion. 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 (p. 12).

**Determination of volatile residues:** The charcoal trap was analyzed for total radioactivity using LSC following combustion (p. 10). Aliquots of the NaOH trapping solutions were analyzed by LSC.

**Total $^{14}$C measurement:** For time 0, total [$^{14}$C]residues were determined using LSC following combustion (p. 11). For 30 and 60 days postflooding, total [$^{14}$C]residues were determined by summing the concentrations of residues measured in the soil extracts, extracted soil, volatile traps, and desiccator rinse (Table 2, p. 20).

**Derivatization method, if used:** A derivatization method was not employed.

**Identification and quantification of parent compound:** Aliquots of the concentrated soil organic extracts were analyzed by two-dimensional TLC on Merck Silica Gel 60 plates (0.25 mm thick) with fluorescent indicators that were developed in one direction with chloroform:methanol:acetic acid (90:10:1, v:v:v; SS1) and in the second direction with toluene:acetone:acetic acid (70:30:1, v:v:v; SS2; pp. 10-11). 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, adding a toluene-based scintillator, and assaying by LSC. Samples were cochromatographed with an unlabeled reference standard of oryzalin that was visualized under UV light (Rf 0.67-SS1, 0.73-SS2; Figure 2, p. 25).

**Identification and quantification of transformation products:** Transformation products were separated, identified and quantified using two-dimensional TLC as described for the parent compound (pp. 10-11; Figure 2, p. 25). The following reference standards were available for use:

| Code Name (Compound) | CAS Name | Lot number | Purity (%) | TLC $R_f$ | |
|---|---|---|---|---|---|
| | | | | SS1 | SS2 |
| OR-2 | 3,5-Dinitro-4-(propylamino)benzenesulfonamide | -- | -- | 0.63 | 0.69 |
| OR-4[1] | 3-Amino-4-(dipropylamino)-5-nitrobenzenesulfonamide | -- | -- | 0.60 | 0.52 |
| OR-7 | 3,5-Diamino-4-(dipropylamino)benzenesulfonamide | -- | -- | 0.54 | 0.42 |
| OR-9 | 3,4,5-Triaminobenzene-sulfonamide | -- | -- | 0.07 | 0.09 |
| OR-13 | 2-Ethyl-7-nitro-1-propyl-1H-benzimidazole-5-sulfonamide | -- | -- | 0.60 | 0.36 |
| OR-14 | 7-Amino-2-ethyl-1-propyl-1H-benzimidazole-5-sulfonamide | -- | -- | 0.28 | 0.11 |
| OR-15 | 2-Ethyl-7-nitro-1H-benzimidazole-5-sulfonamide | -- | -- | 0.36 | 0.23 |
| OR-16 | 7-Amino-2-ethyl-1H-benzimidazole-5-sulfonamide | -- | -- | 0.12 | 0.05 |
| OR-20 | 4-Hydroxy-3,5-dinitro-benzenesulfonamide | -- | -- | 0.07 | 0.11 |
| OR-41[1] | 4-[(2-Hydroxypropyl)propylamino]-3,5-dinitro-benzenesulfonamide | -- | -- | 0.60 | 0.52 |

Data obtained from Table 1, pp. 17-19 and Figure 2, p. 25 in the study report.
1 The solvent systems were not able to separate OR-4 from OR-41.

**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.

## II. RESULTS AND DISCUSSION

**A. TEST CONDITIONS:** Incubation temperature were reported to be 24°C during the aerobic incubation (through 23 days posttreatment) and 28°C following the introduction of anaerobic conditions; no supporting data were provided (pp. 5, 9). The pH of the test systems was not measured. Redox potentials and dissolved oxygen levels were not measured. The viability of the soil microflora was not determined either prior to or after treatment.

**B. MATERIAL BALANCE:** Overall [$^{14}$C]residue recoveries averaged 97.2 ± 2.4% (range 95.6-100.0%) of time 0 (Table 3, p. 21, DER Attachment 2). There was no significant loss of [$^{14}$C]residues from the test system over time.

Table 5: Biotransformation of [$^{14}$C]oryzalin, expressed as percentage of total radioactivity at time 0 (immediately preflooding), in anaerobic sandy loam soil systems.

| Compound | Sampling Intervals (days) | | |
|---|---|---|---|
| | 0 (immediate preflood) | 30 postflooding | 60 postflooding |
| Oryzalin | 62.3 | 2.4 | 1.0 |
| OR-14 | 0.1 | 10.3 | 10.3 |
| OR-16 | 0.2 | 5.3 | 5.0 |
| OR-20 | 4.3 | 0.0 | 0.0 |
| OR-2 | 0.4 | 0.0 | 0.0 |
| OR-4/41 | 0.3 | 0.3 | 0.2 |
| OR-7 | 0.1 | 0.8 | 0.5 |
| OR-13 | 0.6 | 0.2 | 0.1 |
| OR-15 | 0.6 | 0.1 | 0.1 |
| UN-1 | 1.0 | 0.0 | 0.0 |
| UN-2 | 1.2 | 0.0 | 0.0 |
| Others[1] | 1.5 | 6.1 | 6.1 |
| Organic extractable residues[2] | 72.6 | 25.5 | 23.3 |
| Aqueous extractable residues | 1.1 | 1.6 | 2.4 |
| Total extractable residues[3] | 73.7 | 27.1 | 25.2 |
| Nonextractable residues | 26.3 | 68.4 | 70.6 |
| Desiccator wash | -- | -- | 0.08 |
| Carbon dioxide | -- | 0.08 | 0.12 |
| Organic volatiles | -- | 0.01 | 0.02 |
| Total recovery | 100 | 95.6 | 96.0 |

Most data obtained from Table 3, pp. 21-23 of the study report. Desiccator wash, carbon dioxide, and volatile compounds in terms of percent of time 0 obtained from Table 2, p. 20. Although two samples were collected at each sampling interval, only averaged data were reported by the study authors.
1. "Others" 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).
2. "Organic extractable residues" is the sum of the percent of oryzalin and its metabolites.
3. "Total extractable residues" was calculated by the reviewer by summing the concentrations of [$^{14}$C]residues in the organic and aqueous fractions of the sample extracts (DER Attachment 2).

**C. TRANSFORMATION OF PARENT COMPOUND:** In the total system (water plus soil), [$^{14}$C]oryzalin decreased from 62.3% of the total radioactivity at time 0 (immediately after flooding) to 2.4% at 30 days postflooding and 1.0% at 60 days (Table 3, p. 22).

**HALF-LIFE/DT50/DT90:** Because of the high amount of nonextractable residues, the apparent inadequacy of the extraction method to remove all identifiable [$^{14}$C]residues, and because there were only three sampling intervals, with a decrease of greater than 50% occurred between the first and second sampling intervals, half-lives were not estimated by the reviewer. The observed DT50 and DT90 values are <30 days.

Using first-order linear regression analysis, the study authors calculated a half-life of 10 days (p. 13).

**TRANSFORMATION PRODUCTS:** Seven transformation products were identified:

- 3,5-dinitro-4-(propylamino)benzenesulfonamide (OR-2);
- 3,5-diamino-4-(dipropylamino)benzenesulfonamide (OR-7);
- 2-ethyl-7-nitro-1-propyl-1H-benzimidazole-5-sulfonamide (OR-13);
- 7-amino-2-ethyl-1-propyl-1H-benzimidazole-5-sulfonamide (OR-14);
- 2-ethyl-7-nitro-1H-benzimidazole-5-sulfonamide (OR-15);
- 7-amino-2-ethyl-1H-benzimidazole-5-sulfonamide (OR-16); and
- 4-hydroxy-3,5-dinitro-benzenesulfonamide (OR-20).

In addition, two transformation products coeluted:

- 3-amino-4-(dipropylamino)-5-nitrobenzenesulfonamide (OR-4) and
- 4-[(2-hydroxypropyl)propylamino]-3,5-dinitro-benzenesulfonamide (OR-41).

The only major transformation product, OR-14, was 0.1% of the time 0 radioactivity immediately preflooding and increased to 10.3% at 30 and 60 days postflooding (Table 3, pp. 21-23). All other transformation products were minor (e.g. <10% of the time 0 concentration). OR-16 and OR-20 were maximums of 5.3% and 4.3% at time 30 days and 0 days, respectively. OR-2, OR-4/41, OR-7, OR-13, and OR-15 were each ≤0.8% of the time 0 radioactivity. Two isolated unidentified compounds, UN-1 and UN-2, were 1.0-1.2% of the time 0 radioactivity, but were 0% throughout the remainder of the study. [$^{14}$C]Residues remaining at the origin or not associated with a discrete area totaled a maximum of 6.1% of the time 0 radioactivity. 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[1] | -- | 3-Amino-4-(dipropylamino)-5-nitrobenzenesulfonamide |
| OR-7 | -- | 3,5-diamino-4-(dipropyl-amino)benzenesulfonamide |
| OR-13 | -- | 2-Ethyl-7-nitro-1-propyl-1H-benzimidazole-5-sulfonamide |
| OR-14 | -- | 7-amino-2-ethyl-1-propyl-1H-benzimidazole-5-sulfonamide |
| OR-15 | -- | 2-Ethyl-7-nitro-1H-benzimidazole-5-sulfonamide |
| OR-16 | -- | 7-amino-2-Ethyl-1H-benzimidazole-5-sulfonamide |
| OR-20 | -- | 4-Hydroxy-3,5-dinitro-benzenesulfonamide |
| OR-41[1] | -- | 4-[(2-Hydroxypropyl)propylamino]-3,5-dinitro-benzenesulfonamide |

Data obtained from Table 1, pp. 17-19 of the study report.
1 The solvent systems were not able to separate OR-4 from OR-41.

**NONEXTRACTABLE AND EXTRACTABLE RESIDUES:** Extractable [$^{14}$C]residues decreased from 73.7% of the total radioactivity at time 0 to 25.2% at 60 days postflooding, while nonextractable [$^{14}$C]residues increased to 70.6% (Table 3, p. 21, DER Attachment 2). In the 60 day

sample, nonextractable [$^{14}$C]residues were associated with 18.4% humic acids, 7.6% β humus, 28.9% fulvic acids and 15.7% humin (p. 14).

**VOLATILIZATION:** During the 60 day posttreatment period, $CO_2$ and volatile [$^{14}$C]organics totaled 0.26% and 0.04% of the total radioactivity at time 0, respectively (p. 12).

**TRANSFORMATION PATHWAY:** A transformation pathway was provided by the study authors (Figure 3, p. 26). Under anaerobic conditions, oryzalin degrades via reduction of the nitro group to form OR-4 which in turn forms OR-7 which forms the benzimidazole OR-14. OR-14 is demethylated to form OR-16. Other compounds identified in the experiment were not considered to be part of the anaerobic degradation process (p. 15). Study authors concluded that the transformation products are ultimately converted to polar compounds, bound residues and minor amounts of $CO_2$. 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.

**D. SUPPLEMENTARY EXPERIMENT-RESULTS:** The study author stated that the open system samples were used in the identification of transformation products (p. 10). No data from these experiments were reported.

## III. STUDY DEFICIENCIES

1. Sampling intervals were too infrequent to adequately assess the rate of dissipation. Between the 0 and 30 day postflooding intervals (first and second sampling intervals), the concentration of oryzalin decreased from 62.3% to 2.4% of the total radioactivity at time 0 (Table 3, p. 22).

2. The water and soil phases were not analyzed separately, only data for the total system are reported. Based on the ratio of water to soil (10 mL/15 g), it is not certain whether there was a measurable layer of water over the soil surface (p. 9).

3. The extraction procedure appears to have been inadequate in removing all identifiable [$^{14}$C]residues from the soil. Soil samples were extracted by shaking with methanol at room temperature (p. 11). At study termination, nonextractable [$^{14}$C]residues totaled 70.6% of time 0 (Table 3, p. 21).

4. The study authors did not determine the aerobic/anaerobic conditions in the sample throughout the 60 days of incubation. Redox potentials were not measured.

5. The viability of the test soil was not determined at study initiation or any time posttreatment.

6. The water used in the experiment was not characterized.

7. It was not stated whether samples or sample extracts were stored prior to analysis.

8. The TLC analysis was not able to separate transformation products OR-4 and OR-41 (Figure 2, p. 25). The total concentration at this location was 0.2-0.3% of time 0 throughout the study.

9. Limits of Detection and Quantification were not reported.

10. It did not appear that a second analytical method was used to confirm the identification of the [$^{14}$C]compounds. The study authors refer to MS, but this appears to have only been used to confirm the identification of the reference standards (p. 12).

## IV. REVIEWER'S COMMENTS

1. No data from the aerobic incubation were provided in this MRID. However, the study author stated that the treated soils were "available for use in both ABC-0434...and ABC-0435" (p. 9). ABC-0434 corresponds to MRID 41322801, which was reviewed as part of this data package. Minor discrepancies between the reports were noted. For example, in MRID 41322801, it is reported the soils were mixed for 1 hour after treatment (p. 8). In this report (MRID 41322802), the mixing time is reported to be 30 minutes (p. 8).

2. Total residue recoveries in terms of percentage of applied for days 30 and 60 postflooding that are reported in Tables 2 and 3 (pp. 20-21) are not in agreement. The values in Table 3 are incorrect, since they do not include volatile residues.

3. There was no discussion about the identification of transformation products UN-1 and UN-2. In the companion aerobic soil metabolism experiment (MRID 41322801, p. 17), UN-1 was identified as a mixture of the cis- and trans- isomers of 3,3'-azoxybis[4-(propylamino)-5-nitro]benzenesulfonamide and UN-2 was identified as 2-ethyl-7-nitro-1-propyl-1H-benzimidazole-5-sulfonamide, 3-oxide. Since the positions of these two compounds on the TLC plates were not reported, it is not certain that the compounds identified as UN-1 and UN-2 in this study are the same as in the aerobic metabolism study.

4. Concentrations were reported in terms of percentage of radioactivity present in the soil at the time of flooding (time 0), rather than percentage of the radioactivity applied at the time of application (23 days preflooding; Table 3, p. 21).

5. 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).

6. Only averaged data were reported (Table 2, p. 20, footnote 1). It is preferred that individual sample data be reported so that between replicate variability can be assessed.

7. The β humus fraction of nonextractable residues contains [$^{14}$C]residues that are typically identified as part of the fulvic acid fraction (p. 12).

8. The study author describes the definitive experiment, in which samples were attached to a volatile trapping system, as a "closed" system (p. 9). In a supplementary experiment, flooded soil samples were incubated in sealed vials; this system is described as "open" (pp. 9-10).

9. Physico-chemical properties for oryzalin were not reported.

10. The temperature of incubation was reported to be 24°C during the aerobic portion of the experiment and 28°C during the anaerobic portion (p. 5). The minimum and maximum temperatures were not reported, and no supporting data were provided.

11. Despite the issues and deficiencies with this study, results of the study indicate that the two main degradates, OR-14 and OR-16, appear to be persistent in flooded soil, as their concentrations remained unchanged between 30 and 60 days post-flooding (OR-14 remained at approximately 10% and OR-16 at 5%).

## V. REFERENCES

1. U.S. Environmental Protection Agency. 2008. Fate, Transport and Transformation Test Guidelines, OPPTS 835.4200, Anaerobic Soil Metabolism. Office of Prevention, Pesticides and Toxic Substances, Washington, DC. EPA 712-C-08-017.

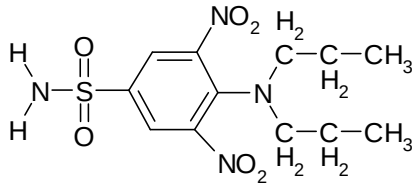
2. U.S. Environmental Protection Agency. 1982. Pesticide Assessment Guidelines, Subdivision N, Chemistry: Environmental Fate, Section 162-2, Anaerobic Soil Metabolism Studies. Office of Pesticide and Toxic Substances, Washington, DC. EPA 540/9-82-021.

## Attachment 1: Structures of Parent Compound and Transformation Products

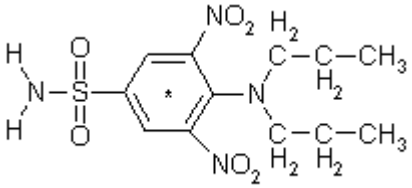
**Oryzalin [OR-1; EL-119]**

**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**

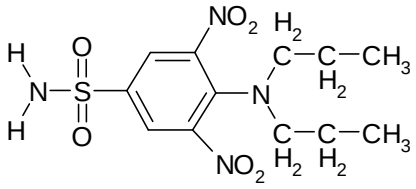


* = Location of the radiolabel.

**Identified Compounds**

**Oryzalin [OR-1; EL-119]**

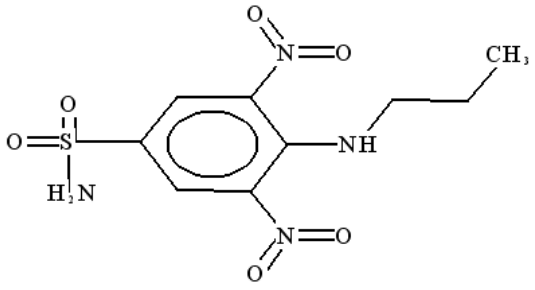
**IUPAC Name:** 3,5-Dinitro-4-(dipropylamino)benzenesulfonamide.
3,5-Dinitro-$N^4$,$N^4$-dipropylsulfanilamide.
**CAS Name:** 4-(Dipropylamino)-3,5-dinitrobenzenesulfonamide.
**CAS Number:** 19044-88-3.
**SMILES String:** C1C(S(=O)(=O)N)=CC(N(O)O)=C(N(CCC)CCC)C=1N(O)O (EpiSuite version 4.0).
**Empirical formula:** $C_{12}H_{18}N_4O_6S$ **Molecular formula:** $C_{12}H_{18}N_4O_6S$



*** structure complexity/form was sacrificed to obtain SMILES string**

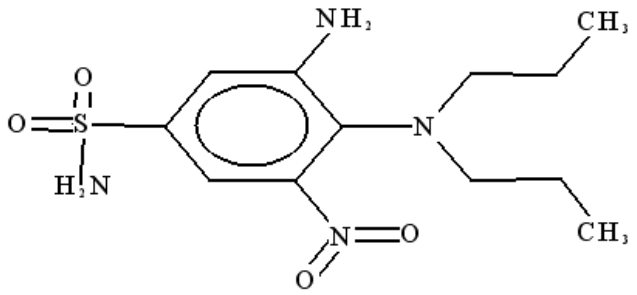
**OR-2**

**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:** $C_9H_{12}N_4O_6S$ **Molecular formula:** $C_9H_{12}N_4O_6S$



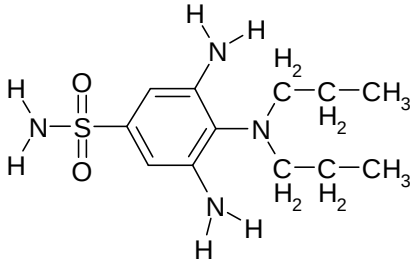
**OR-4**

**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:** $C_{12}H_{20}N_4O_4S$



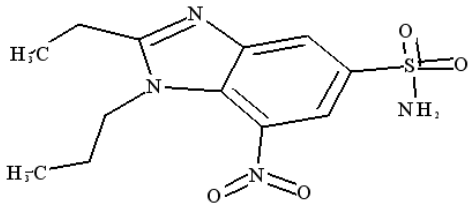
**OR-7**

**IUPAC Name:** 3,5-Diamino-4-(dipropylamino)benzenesulfonamide.
**CAS Name:** Not reported.
**CAS Number:** Not reported.
**SMILES String:** C1C(S(=O)(=O)N)=CC(N)=C(N(CCC)CCC)C=1N (EpiSuite version 4.0).
**Empirical formula:** $C_{12}H_{22}N_4O_2S$ **Molecular formula:** $C_{12}H_{22}N_4O_2S$



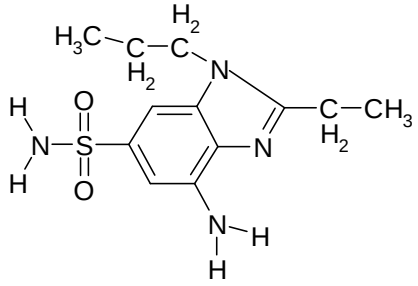
**OR-13**

**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:** $C_{12}H_{16}N_4O_4S$ **Molecular formula:** $C_{12}H_{16}N_4O_4S$



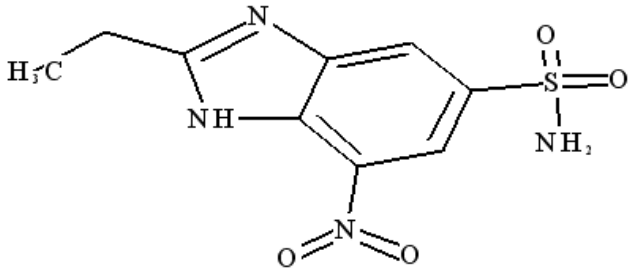
**OR-14**

**IUPAC Name:** 7-Amino-2-ethyl-1-propyl-1H-benzimidazole-5-sulfonamide..
**CAS Name:** Not reported.
**CAS Number:** Not reported.
**SMILES String:** C1(S(=O)(=O)N)=CC(N)=C2N=C(CC)N(CCC)C2=C1 (EpiSuite version 4.0).
**Empirical formula:** $C_{12}H_{18}N_4O_2S$ **Molecular formula:** $C_{12}H_{18}N_4O_2S$



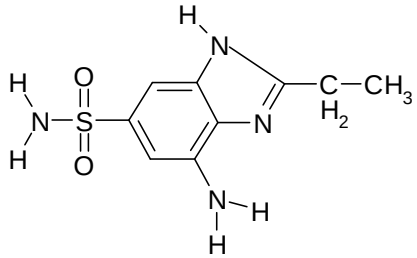
**OR-15**

**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:** $C_9H_{10}N_4O_4S$ **Molecular formula:** $C_9H_{10}N_4O_4S$



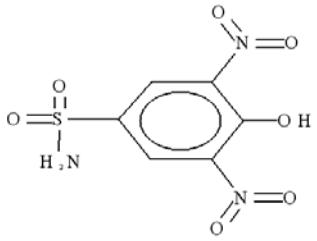
**OR-16**

**IUPAC Name:** 7-Amino-2-ethyl-1H-benzimidazole-5-sulfonamide..
**CAS Name:** Not reported.
**CAS Number:** Not reported.
**SMILES String:** C1(S(=O)(=O)N)=CC(N)=C2N=C(CC)NC2=C1(EpiSuite version 4.0).
**Empirical formula:** $C_9H_{12}N_4O_2S$ **Molecular formula:** $C_9H_{12}N_4O_2S$



**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).
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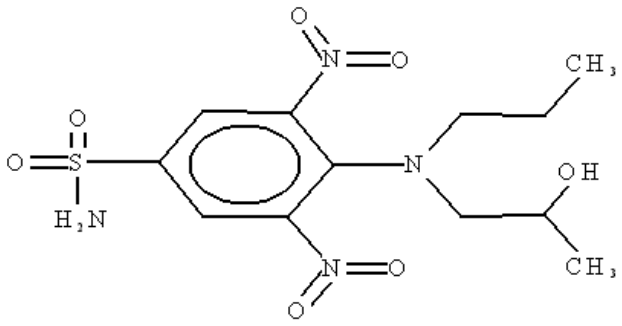


**Carbon Dioxide**

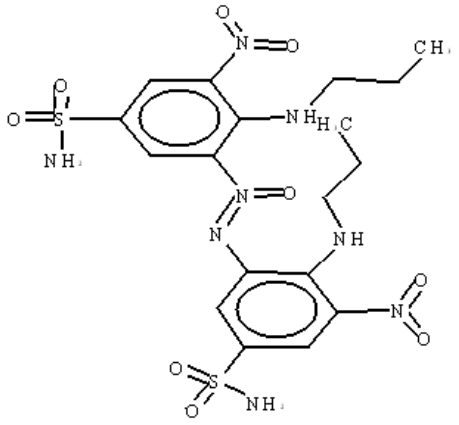
| | | | |
|---|---|---|---|
| **IUPAC Name:** | Carbon dioxide. | | |
| **CAS Name:** | Carbon dioxide. | | |
| **CAS Number:** | 124-38-9. | | |
| **SMILES String:** | C(=O)(=O) (EpiSuite version 4.0). | | |
| **Empirical formula:** | $CO_2$ | **Molecular formula:** | $CO_2$ |

O=C=O

**Unidentified Reference Compounds**

**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:** $C_{18}H_{24}N_8O_9S_2$ **Molecular formula:** $C_{18}H_{24}N_8O_9S_2$



**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:** $C_{11}H_{16}N_5O_5S$