

US EPA ARCHIVE DOCUMENT

(4-22-2004)

Data Evaluation Report on the bioaccumulation of Penoxsulam in crayfish

EPA MRID Number 45831101

PMRA Submission Number {....}

Data Requirement: PMRA Data Code:
EPA DP Barcode: D288160
OECD Data Point:
EPA Guideline: 165-5

Test material:

Common name: Penoxsulam.

Chemical names:

IUPAC: 6-(2,2-Difluoroethoxy)-N-(5,8-dimethoxy-s-triazolo[1,5-c]pyrimidin-2-yl)-
 α,α,α -trifluoro-o-toluenesulfonamide;
3-(2,2-Difluoroethoxy)-N-(5,8-dimethoxy[1,2,4]triazolo[1,5-c]pyrimidin-2-yl)-
 α,α,α -trifluorotoluene-2-sulfonamide.
CAS: 2-(2,2-Difluoroethoxy)-N-(5,8-dimethoxy[1,2,4]triazolo[1,5-c]pyrimidin-2-yl)-
6-(trifluoromethyl)benzenesulfonamide.
CAS No: 219714-96-2.

Synonyms: XDE-638 (Petitioner's code).

SMILES string: n1c(nc2n1c(ncc2OC)OC)NS(=O)(=O)c3c(cccc3C(F)(F)F)OCC(F)F.

Primary Reviewer: Dana Worcester
Dynamac Corporation

Signature:
Date:

QC Reviewer: Joan Gaidos
Dynamac Corporation

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Secondary Reviewer: Lucy Shanaman
EPA Reviewer

Signature: *Lucy Shanaman*
Date: April 22, 2004

Company Code:
Active Code:
Use Site Category:
EPA PC Code: 119031

CITATION: Rick, D.L. and T.A. Marino. 2002. The bioconcentration of XDE-638 in edible tissue of the crayfish, *Procambarus clarkii*. Unpublished study performed by Toxicology & Environmental Research and Consulting, Dow Chemical Co., Midland, MI; sponsored and submitted by Dow AgroSciences, LLC, Indianapolis, IN. Study ID: 021063. Experiment initiated April 23, 2002, and completed May 16, 2002 (p.13). Final report issued October 7, 2002.

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ABSTRACT

Laboratory Accumulation - Crayfish

The bioaccumulation of [het-2-¹⁴C]-labeled 3-(2,2-difluoroethoxy)-N-(5,8-dimethoxy[1,2,4]triazolo[1,5-c]pyrimidin-2-yl)- α,α,α -trifluorotoluene-2-sulfonamide (penoxsulam; XDE-638) was studied in crayfish (*Procambarus clarkii*) using a nominal concentration of 0.494 mg/L under flow-through aquarium conditions. This experiment was conducted to meet global registration requirements and generally conformed to US EPA OPPTS 850.1730 and in compliance with 40 CFR Part 160 GLP standards. The test system consisted of two 200-L glass aquaria fitted with overflows to maintain a volume of 135 L (average flow rate of 94 mL/minute) at a loading rate of 70 crayfish per vessel. One aquaria was treated and the second was used as solvent control. The exposure period was 14 days, and the subsequent depuration period was 7 days. During 14 days of exposure, the pH of the aquaria water was 7.3-8.1, the dissolved oxygen 7.1-8.7 mg/L, and the temperature 21.4-22.5°C. During the 7 days of depuration, the pH of the aquaria water was 7.7-7.9, the dissolved oxygen 8.0-8.4 mg/L, and the temperature 21.5-22.4°C. Crayfish (3) samples were collected on days 0, 1, 3, 5, 7, 1, and 14 during exposure and after 1, 3, 5, and 7 days of depuration. Aliquots of the aquaria water and portions of solubilized crayfish tail tissue were analyzed for total radioactivity using LSC. Water (days 6 and 13) was analyzed for penoxsulam and its transformation products directly by reverse-phase HPLC. The identity of residue was established by comparison to reference standards.

The maximum concentration of total [¹⁴C]residues in crayfish tail muscle was 14.4 μ g/kg at 11 days. The average steady-state calculated bioconcentration factor (BCF) was 0.02 mL/g. [¹⁴C]Residues in the tissues were not characterized.

After 1 day of depuration, total [¹⁴C]residues in crayfish tissues (7.4 μ g/kg) was similar to the day 14 exposure value (7.3 μ g/kg). After 5 days of depuration, total [¹⁴C]residues were not detected in the crayfish tissue. [¹⁴C]Residues in the crayfish during depuration were not characterized.

[¹⁴C]Residues in the water were only characterized on days 6 and 13. Based on the day 6 chromatogram, only 1 peak was detectable. The peak eluted with the retention time of penoxsulam. After 6 hours of depuration, [¹⁴C]residues decreased to <2% of the exposure levels and by day 2 were below the limit of detection.

Study Acceptability: This study is classified as acceptable. The study is scientifically valid, and can be used towards fulfillment of the bioconcentration in aquatic, non-target organisms guideline, Subdivision N Guideline §165-5, data requirements for penoxsulam.

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MATERIALS AND METHODS

Red swamp crayfish (*Procambarus clarkii*, Osage Catfisheries, Inc.) were acclimated for 14 day under continuous flow conditions in water collected from Lake Huron that had been limed and flocculated with ferric chloride (p.13). Prior to use, the water was sand- and carbon-filtered, pH adjusted (gaseous CO₂) and irradiated (pH 7.1-7.6; p.14). The crayfish were fed a standard laboratory fish diet (Aquatic Diet No. 1, Harlan-Teklad; p.14). The water temperature in the holding tank was 22 ± 2°C. Diseased or unhealthy crayfish were not used in the study.

Flow-through aquatic exposure systems were prepared using two 200-L glass aquaria with plexiglass lids fitted with overflows to maintain a volume of 135 L at a loading rate of 70 crayfish per vessel (6.4 g of crayfish/L/day, p.15). Filtered, irradiated water from Lake Huron was supplied to the aquaria at a rate of approximately 1 turnover/day (94 mL/minute). One aquarium was treated with [het-2-¹⁴C]-labeled

3-(2,2-difluoroethoxy)-N-(5,8-dimethoxy[1,2,4]triazolo [1,5-c]pyrimidin-2-yl)-α,α,α-trifluorotoluene-2-sulfonamide (penoxsulam; radiopurity; 99% specific activity 30.0 mCi/mmol or 138 DPM/ng; Batch No. DE3-E1414-49; INV 1861; p.13), dissolved in DMF, at a nominal concentration of 0.470 mg/L (p.14). During the study, the mean measured concentration were 0.494 ± 0.033 mg/L (p.15). The concentration of DMF in solution was <0.1 mL/L of aquarium water at initiation. The second aquarium served as a solvent control. The aquaria were equilibrated for two days prior to exposure (p.21).

Following equilibration, 70 crayfish were transferred into each aquarium (p.15). At the initiation of exposure, the crayfish weighed an average of 12.35 g. Water quality (pH, oxygen content, and temperature) were measured at days 0, 1, 3, 5, 7, 11 and 14; temperature was measured daily (p.16, Appendix A, p.42). During 14 days of exposure, the pH of the aquaria water was 7.3-8.1, the dissolved oxygen was 7.1-8.7 mg/L, and the temperature was 21.4-22.5°C. The average turnover rate in the aquaria was 1 volume addition per 24-hour period. The crayfish were provided with 36 open-ended pipes (9 x 9 cm) for shelter; lighting conditions were not reported (p.15). Three crayfish and replicate water samples (4 mL) were collected from each aquaria at 0, 1, 3, 5, 7, 11, and 14 days (p.16), but only one crayfish per sampling interval was analyzed for total [¹⁴C]residues. Additional water (40 mL) was collected on days 6 and 13 for HPLC analysis (p.18)

Following the 14-day exposure period, both aquaria were drained and refilled with "clean" dilution water for 7 days of depuration (p.16). During the 7 days of depuration, the pH of the aquaria water was 7.7-7.9, the dissolved oxygen was 8.0-8.4 mg/L, and the temperature was 21.5-22.4°C (Appendix A, p.42). Water and three crayfish were collected from each aquarium at 1, 3, 5, and 7 days (p.16). However, only one crayfish per sampling interval was analyzed for total [¹⁴C]residues.

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Water: Aliquots of the aquaria water were analyzed for total radioactivity using LSC (pp.17-18). The LSC limit of detection was ca. 0.4 ng/mL (p.21). Aliquots (5 mL) of water from days 6 and 13 were directly analyzed by reverse-phase HPLC under the following conditions (pp.18-19):

| | | | |
|------------------------|---|----|-----|
| Column | YMC ODS-AQ (250 cm x 4.6 mm, 5 μ particle size). | | |
| Mobile phase | (A) distilled, deionized water with 1% acetic acid; (B) 50/50 acetonitrile:methanol with 1% acetic acid (v:v). | | |
| Gradient (A:B, v:v) | Time (min.) | %A | %B |
| | 0-6 | 95 | 5 |
| | 6-10 (ramped) | 0 | 100 |
| | 10-25 | 0 | 100 |
| Radiochemical detector | Berthold LB 509 | | |
| UV detector | LDC Spectromonitor 3200 (254 nm) | | |
| Flow rate | 1 mL/min | | |
| Injection volume | 5 mL | | |

In addition, the HPLC eluent fractions were collected and analyzed by LSC (p.18). [¹⁴C]Residues in the water were identified by comparison to the retention time of penoxsulam.

Crayfish: A preliminary study was conducted to determine the recovery of [¹⁴C]residues from crayfish tail muscle (p.19). Recoveries from the preliminary study were 97.4 ± 0.5%.

At the time of sampling, the crayfish were rinsed, weighed and pooled into control and treated samples. The crayfish tail muscles were separated from the exoskeleton, divided sagittally into two sections and placed in separate vials (p.20). The tissues solubilized by placing the tissue in Soluene-350 maintained in a convection oven at 40°C and analyzed for total radioactivity using LSC. Only one control crayfish at each sampling interval was analyzed for total [¹⁴C]residues. The LSC limit of detection was ca. 3 ng/g (p.21).

RESULTS/DISCUSSION

[¹⁴C]Penoxsulam residues accumulated in crayfish that were exposed to [Het-2-¹⁴C] 3-(2,2-difluoroethoxy)-N-(5,8-dimethoxy[1,2,4]triazolo[1,5-c]pyrimidin-2-yl)-α,α,α-trifluorotoluene-2-sulfonamide at a nominal concentration of 0.470 mg/L (mean measured concentration 0.494 ± 0.033 mg/L) for 14 days under flow-through aquarium conditions (pp.14, 15).

The maximum concentration of total [¹⁴C]residues in crayfish tail muscle was 14.4 μg/kg at 11 days (Table 5, p.34). The average steady-state calculated bioconcentration factor (BCF) was 0.02 mL/g (p.24). [¹⁴C]Residues in the tissues were not characterized.

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After 1 day of depuration, total [^{14}C]residues in crayfish tissues (7.4 $\mu\text{g}/\text{kg}$) was similar to the day 14 exposure value (7.3 $\mu\text{g}/\text{kg}$; Table 5, p.34). After 5 days of depuration, total [^{14}C]residues were not detected in the crayfish tissue. [^{14}C]Residues in the crayfish during depuration were not characterized.

[^{14}C]Residues in the water were only characterized on days 6 and 13. Based on the day 6 chromatogram, only 1 peak was detectable (Figure 3, p.38). The peak eluted with the retention time of penoxsulam (p.23). After 6 hours of depuration, [^{14}C]residues decreased to <2% of the exposure levels and by day 2 were below the limit of detection (p.22).

DEFICIENCIES/DEVIATIONS

1. The [^{14}C]residues in the crayfish tail tissues were not characterized. The study author reported that a threshold 50 ng/g was required under the protocol for analysis by HPLC (p.24). However, the water was analyzed by HPLC.
2. The study was conducted using crayfish. The use of the crayfish is within acceptability considering the test material is designed for aquatic environments such as rice fields, where crayfish are commonly raised as a commercial crop (p.10).
3. The age of the crayfish was not provided.
4. The lighting conditions during the study were not provided.
5. The study author noted that on day 5 the level of [^{14}C]penoxsulam declined to 0.314 mg/L due to a malfunction of the syringe pump (p.22). At 6 days the concentration had returned to 0.491 mg/L (Table 4, p.33).
6. The study was reported to have been conducted according to EPA Subdivision N guidelines 165-4 and OECD Guideline No. 305 (p.11). Signed and dated Good Laboratory Practice, Quality Assurance, and No Data Confidentiality were submitted with this study (pp.2-4).
7. Results from inorganic and organic analyses of the dilution water are presented in Tables 1 and 2 of the study report (pp.30-31).

Attachment 1
Structures of Parent and Transformation Products

Penoxsulam

IUPAC name:

3-(2,2-Difluoroethoxy)-N-(5,8-dimethoxy[1,2,4]triazolo[1,5-c]pyrimidin-2-yl)- α,α,α -trifluorotoluene-2-sulfonamide

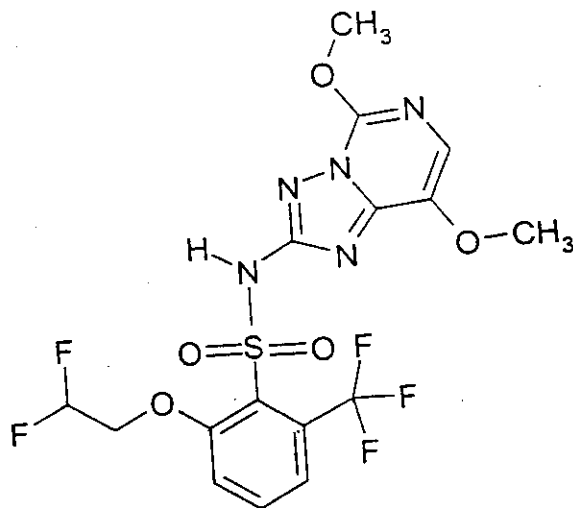
CAS name:

2-(2,2-Difluoroethoxy)-N-(5,8-dimethoxy[1,2,4]triazolo[1,5-c]pyrimidin-2-yl)-6-(trifluoromethyl)benzenesulfonamide

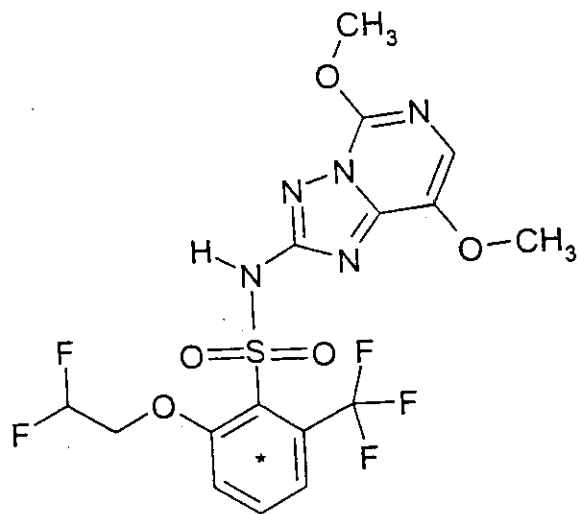
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219714-96-2

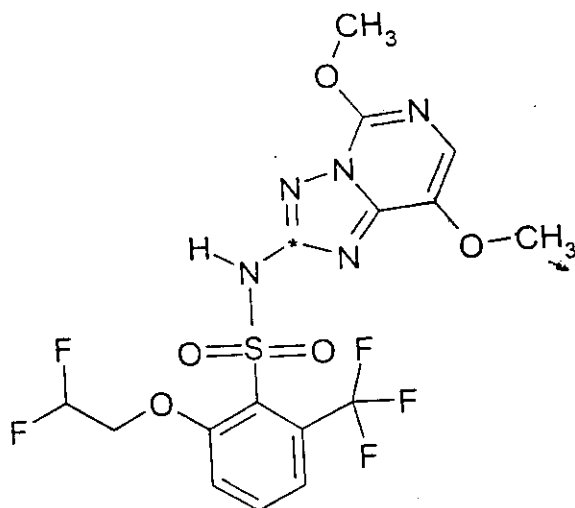
Unlabeled



[Phenyl-U-¹⁴C] label



[Triazolopyrimidine-2-¹⁴C] label



* Position of the radiolabel.

5-OH-XDE-638

IUPAC name:

6-(2,2-Difluoroethoxy)-N-(5,6-dihydro-8-methoxy-5-oxo-s-triazolo[1,5-c]pyrimidin-2-yl)- α,α,α -trifluoro-o-toluenesulfonamide

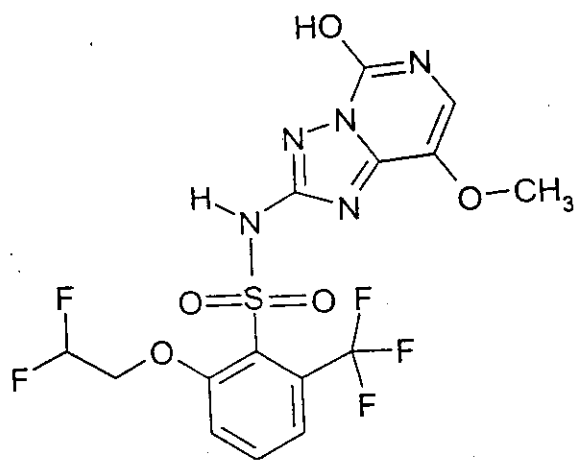
CAS name:

2-(2,2-Difluoroethoxy)-N-(5,6-dihydro-8-methoxy-5-oxo[1,2,4]triazolo[1,5-c]pyrimidin-2-yl)-6-(trifluoromethyl)benzenesulfonamide

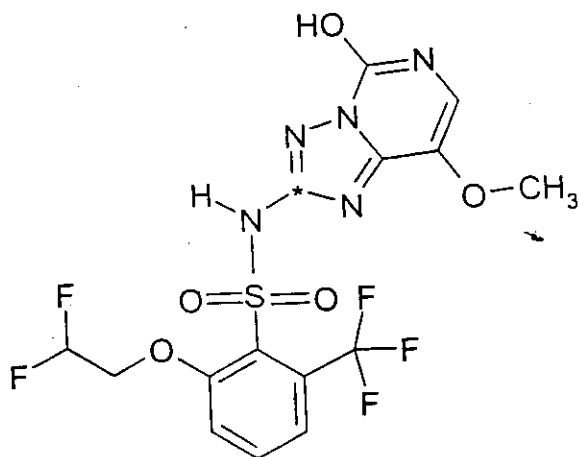
CAS No:

NA

Unlabeled



[Triazolopyrimidine-2-¹⁴C] label



* Position of the radiolabel.

BSTCA

IUPAC name:

3-[6-(2,2-Difluoroethoxy)- α,α,α -(trifluoro-*o*-toluenesulfonylamido)-s-triazole-5-carboxylic acid

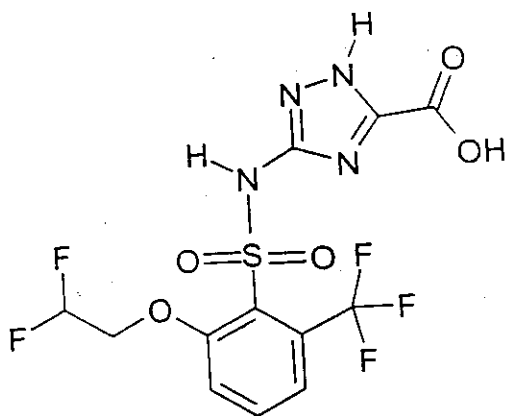
CAS name:

3-[[[2-(2,2-Difluoroethoxy)-6-(trifluoromethyl)phenyl]-sulfonyl]amino]-1H-1,2,4-triazole-5-carboxylic acid

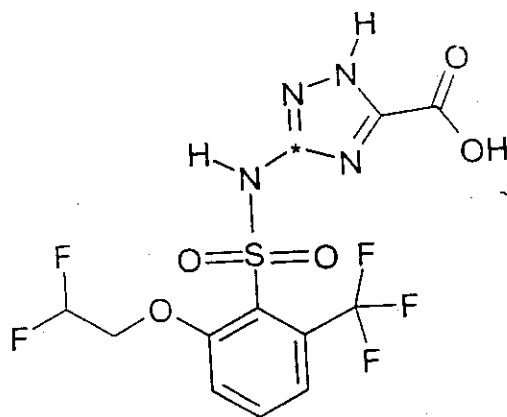
CAS No:

NA

Unlabeled



[Triazolopyrimidine-2-¹⁴C] label



* Position of the radiolabel.

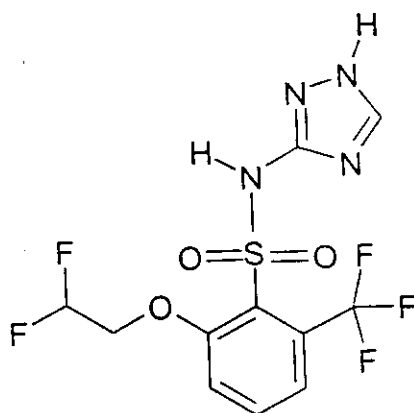
BST

IUPAC name: 6-(2,2-Difluoroethoxy)- α,α,α -trifluoro-N-s-triazol-3-yl-o-toluenesulfonamide

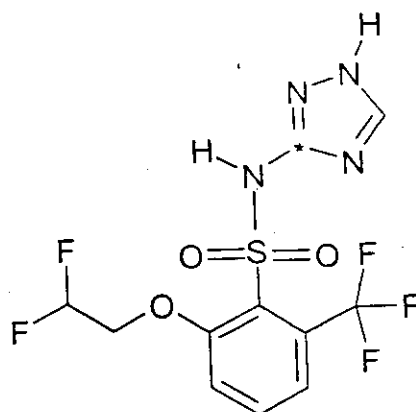
CAS name: 2-(2,2-Difluoroethoxy)-N-1H-1,2,4-triazole-3-yl-6-(trifluoromethyl)benzenesulfonamide

CAS No: NA

Unlabeled



[Triazolopyrimidine-2-¹⁴C] label



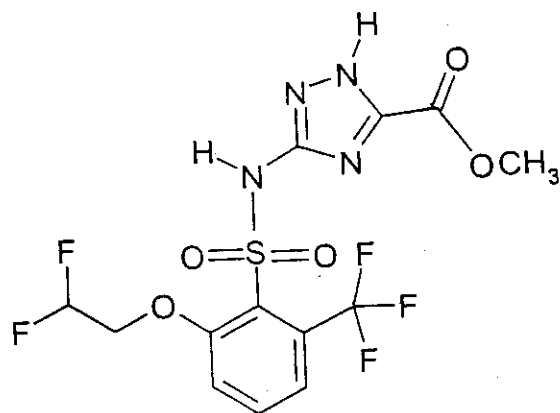
* Position of the radiolabel.

BSTCA-methyl

IUPAC name: Methyl 3-[6-(2,2-difluoroethoxy)- α,α,α -trifluoro-*o*-toluenesulfonamido]-s-triazole-5-carboxylate

CAS name: Methyl 3-[[[2-(2,2-difluoroethoxy)-6-(trifluoromethyl)phenyl]sulfonyl]amino]-1H-1,2,4-triazole-5-carboxylate

CAS No: NA

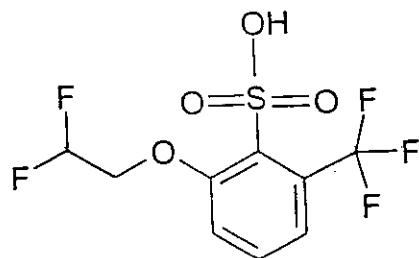


BSA

IUPAC name: 6-(2,2-Difluoroethoxy)- α,α,α -trifluoro-*o*-toluenesulfonic acid

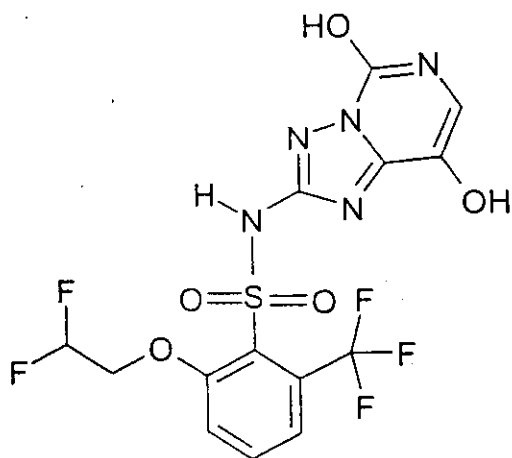
CAS name: 2-(2,2-Difluoroethoxy)-6-(trifluoromethyl)benzenesulfonic acid

CAS No: NA



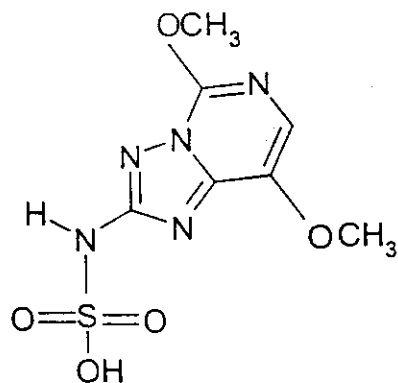
5,8-diOH

IUPAC name: NA
CAS name: 2-(2,2-Difluoroethoxy)-6-trifluoromethyl-N-(5,8-dihydroxy-
[1,2,4]triazolo[1,5-c]pyrimidin-2-yl)benzenesulfonamide
CAS No: NA



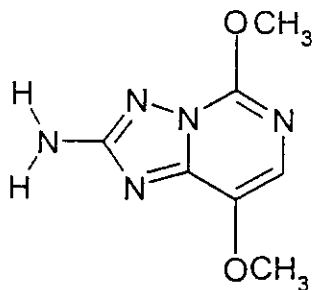
TPSA

IUPAC name: NA
CAS name: 5,8-Dimethoxy[1,2,4]triazolo-[1,5-c]pyrimidin-2-yl-sulfamic acid
CAS No: NA



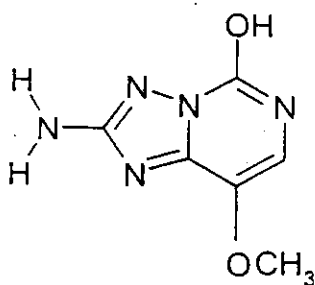
2-Amino TP

IUPAC name: 2-Amino-5,8-dimethoxy-s-triazolo[1,5-c]pyrimidine
CAS name: 5,8-Dimethoxy[1,2,4]triazolo[1,5-c]pyrimidin-2-amine
CAS No: NA



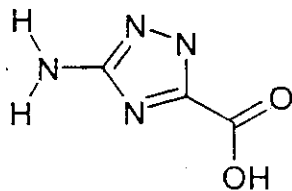
5-OH, 2-Amino TP

IUPAC name: NA
CAS name: 8-Methoxy[1,2,4]triazolo-[1,5-c]pyrimidin-5-ol-2-amine
CAS No: NA



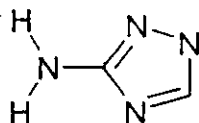
2-Amino TCA

IUPAC name: NA
CAS name: 2-Amino-1,3,4-triazole-5-carboxylic acid
CAS No: NA



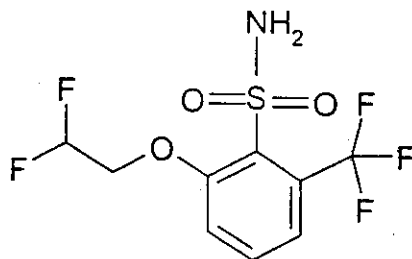
2-Amino-1,3,4-triazole

IUPAC name: NA
CAS name: 2-Amino-1,3,4-triazole
CAS No: NA



Sulfonamide

IUPAC name: 2-(2,2-Difluoroethoxy)-6-(trifluoromethyl)-benzenesulfonamide
CAS name: 2-(2,2-Difluoroethoxy)-6-(trifluoromethyl)-benzenesulfonamide
CAS No: NA



Sulfonylformamidine

IUPAC name: 2-(2,2-Difluoroethoxy)-N-[(E)iminomethyl-6-(trifluoromethyl)benzenesulfonamide
CAS name: 2-(2,2-Difluoroethoxy)-N-(iminomethyl-6-(trifluoromethyl)-benzenesulfonamide
CAS No: NA

