**Data Evaluation Record of two Analytical Methods for Residues of Broflanilide and its Degradates in Water**

**Data Requirement:** EPA Guideline: 850.6100
**EPA PC Code:** 283200

**Test material:** Broflanilide

**DP barcode:** 450103

These studies were reviewed as part of a joint review with the Health Canada Pest Management Regulatory Agency (PMRA). This preface is an EFED supplement to the attached PMRA review of the two analytical methods in water.


**Primary Reviewer:** Charles Lee — **Date:** June 20, 2018
Evaluation Officer, EAD, PMRA

**Secondary Reviewer:** Mike Brown — **Date:** July 26, 2018
Evaluation Officer, EAD, PMRA

**Final EPA Reviewer:** Faruque Khan — Signature:
Senior Fate Scientist, EFED, OCSPP — **Date:** November 21, 2019


**Executive Summary**


Two analytical methods in water are reviewed in this DER. The analytical method, No. D1608/01 is designed for the quantitative determination of residues of broflanilide (BAS 450 I) and its metabolites DM-8007, DC-DM-8007, DC-8007 and S(PFP-OH)-8007 in surface and drinking water by LC-MS/MS. The mean recovery data were acceptable (between 80-103%) and the validated LOQ for residues of broflanilide in water are 5 ng/L for broflanilide and 25 ng/L for metabolites DM-8007, DC-DM-8007, DC-8007 and S(PFP-OH)-8007. An additional analytical method (D1705/01) was performed for the determination of broflanilide metabolites S(Br-OH)-8007), AB-Oxa, and MFBA in surface and drinking water using LC-MS/MS. The LOQ of 5 ng/L for broflanilide is less than the lowest toxicological level of concern (LOAEC of 18 ng/L for estuarine/marine invertebrate) in water. No modifications were made by the independent laboratory. This study is classified as **acceptable**.


**Table 1. Analytical Method Summary**

| Analyte(s) by Pesticide | MRID | | Matrix[1] | Method Date (dd/mm/yyyy) | Registrant | Analysis | Limit of Quantitation (LOQ) |
|---|---|---|---|---|---|---|---|
| | Environmental Chemistry Method | Independent Laboratory Validation | | | | | |
| Broflanilide and it's Degradates[2] | 50211341 50211647 | 50211648 | Surface water and Drinking water | 08/22/2018 | BASF | LC/MS/MS | 5 ng/L for broflanilide; 25 ng/L for metabolites |
| Degradates | 50211649 50211556 | 50211650 | | | | LC/MS/MS | 25 ng/L for metabolites |

[1] The surface water (lake water) sample was obtained from Lake Crabtree at 1400 Aviation Parkway, Morrisville, NC 27560 and the drinking water (well water) sample was obtained from 8026 Lowell Valley Drive, Bahama, NC 27503

[2] Chemical name, code and chemical structure for active and all major transformation products / metabolites provided in **Appendix A-1.**

**Analytical Methodology (parent compound and transformation products)**

**Reports:**

**MRID 50211341 (ECM)**
**Title:** Validation of method D1608/01: Method for the determination of BAS 450 I (Reg. No. 5672774) 5936907) and S(PFP-OH)-8007 (Reg. No. 5959598) in surface and drinking water by LC-MS/MS.

**MRID 50211647(ECM)**
**Title:** Evaluation of the limit of detection (LOD) for method D1608/01: Method for the determination of BAS 450 I (Reg. No. 5672774) and its metabolites DM-8007 (Reg. No. 5856361), DC-DM-8007 (Reg. No. 5936906), DC-8007 (Reg. No. 5936907) and S(PFP-OH)-8007 (Reg. No. 5959598) in surface and drinking water by LC-MS/MS).

**MRID 50211648 (ILV)**
**Title:** Independent laboratory validation of method D1608/01: Method for the determination of BAS 450 I (Reg. No. 5672774) and its metabolites DM-8007 (Reg. No. 5856361), DC-DM-8007 (Reg. No. 5936906), DC-8007 (Reg. No. 5936907) and S(PFP-OH)-8007 (Reg. No. 5959598) in surface and drinking water by LC-MS/MS.

**MRID 50211556 (ECM)**
**Title:** Validation of method D1705/01: Method for the determination of S(Br-OH)-8007 (Reg. No. 5959595) and AB-Oxa (Reg. No. 5959600) and MFBA (Reg. No. 6088668) in surface and drinking water by LC MS/MS.

**MRID 50211649 (ECM)**
**Title:** Evaluation of the limit of detection (LOD) for method D1705/01, method for the determination of S(Br-OH)-8007 (Reg. No. 5959595), AB-Oxa (Reg. No.5959600), and MFBA (Reg. No. 6088668) in surface and drinking water by LC-MS/MS.
**MRID 50211650 (ILV)**

**Title:** Independent laboratory validation of method for the determination of S(Br-OH)-8007 (Reg. No. 5959595), AB-Oxa (Reg. No. 5959600), and MFBA (Reg. No. 6088668) in surface and drinking water by LC MS/MS (BASF method number D1705/01).

**1st analytical method for broflanilide & degradates DC-DM-8007, DC-8007, DM-8007, S(PFP-OH)-8007.**

| **Table 1. Principle of the method** | |
|---|---|
| **Items** | **Details** |
| Method Number | D1608/01 |
| Analyte | Broflanilide, DC-DM-8007, DC-8007, DM-8007, S(PFP-OH)-8007 |
| Details of sample used | Drinking (well) and surface (lake) water samples were used for the study. The samples were characterized with the amounts of calcium, sodium, magnesium, dissolved solids and organic carbon, pH, hardness, conductivity, sodium adsorption ration and turbidity. |
| Sample preparation | Add 10 mL methanol to all samples and shake/vortex for approximately 1 minute to ensure homogeneity. Syringe filter all samples using 0.45µm PTFE syringe filters directly into LC injection vials, passing the first approximately 0.2 – 0.3 mL to waste. Samples are ready for injection. For high fortification and high residue samples - further dilute with methanol-water (50:50, v/v) as necessary, to fit in the calibration curve. |
| Method for quantitative analysis of parent compound and transformation products | External standard<br>The test/reference standards were synthesized by Mitsui Chemical Agro, Inc. (MCAG, Tokyo, Japan). Japan Analytical Chemistry Consultants Co., Ltd., on behalf of MCAG, determined characterization and purity prior to use. MCI-8007, 99.67%; DC-DM-8007, 98.58%; DC-8007, 99.07%; DM-8007, 98.84%; S(PFP-OH)-8007, 99.06% |

**Table 1. Principle of the method**

| Items | Details |
|---|---|
| Method for identification of parent compound/transformation products | LC-MS/MS method<br>Column: XBridge BEH Phenyl (100 x 2.1 mm, 2.5 µm)<br>Column temperature: 50°C<br>Detector: Triple Quad Mass Spectrometer<br>Mobile phase: A – water / formic acid (1000/1, v/v)<br>B – methanol / formic acid (1000/1, v/v)<br>Gradient:* |

| Time (min) | A (%) | B (%) |
|---|---|---|
| 0.00 | 70 | 30 |
| 0.01 | 70 | 30 |
| 4.00 | 40 | 60 |
| 6.00 | 5 | 95 |
| 7.20 | 5 | 95 |
| 7.25 | 70 | 30 |
| 8.00 | 70 | 30 |

flow rate 600 µL/minute

Ionization: Electrospray ionization with positive mode

| Analyte | Retention time (min) | Quantitation [*m/z*] | Confirmation [*m/z*] |
|---|---|---|---|
| Broflanilide | 5.83 | 663→643 | 665→645 |
| DC-DM-8007 | 5.33 | 545→525 | 547→527 |
| DC-8007 | 5.65 | 559→539 | 561→541 |
| DM-8007 | 5.84 | 649→242 | 651→242 |
| S(PFP-OH)-8007 | 5.51 | 661→641 | 661→621 |

| | |
|---|---|
| Chromatograms of spiked sample, control sample, blank and standard solution | Acceptable chromatograms of the solvent blank, reference standards and fortified sample were provided. |
| Quantitation | Linear regression (1/x weighting). Direct comparison of the sample peak responses to those of external standards. |
| Criteria for setting LOD and LOQ | The limit of detection (LOD) is the lowest level of fortification tested of an analyte in the matrix with an acceptable signal to noise ratio (S/N > 3:1). The limit of quantitation (LOQ) is the lowest fortification level that can be reliably quantitated. |
| Stability of parent and transformation products at various stages of analysis | Not stated |

| **Table 1. Principle of the method** | |
|---|---|
| **Items** | **Details** |
| Special problems encountered and/or precautions to be taken during analysis/handling/storage of samples | Not stated |
| Total time for completion | Not stated |

* Gradient used by Independent Laboratory is slightly different:

| Time (min) | A (%) | B (%) |
|---|---|---|
| 0.00 | 80 | 20 |
| 0.25 | 80 | 20 |
| 2.25 | 65 | 35 |
| 4.50 | 5 | 95 |
| 5.45 | 5 | 95 |
| 5.50 | 80 | 20 |
| 6.00 | 80 | 20 |

flow rate 800 µL/minute

The method validation data for the parent compound and the metabolites are summarized in Table 2.

**Table 2a. Method validation: Parent compound and converted products**

| Parameter | Parent compound | | DC-DM-8007 | | DC-8007 | | DM-8007 | | S(PFP-OH)-8007 | |
|---|---|---|---|---|---|---|---|---|---|---|
| | surface | drinking | surface | drinking | surface | drinking | surface | drinking | surface | drinking |
| % Recovery at 5 ng/L | 81-155 | 86-99 | - | - | - | - | - | - | - | - |
| % Recovery at 25 ng/L | - | - | 92-105 | 98-106 | 96-102 | 93-101 | 87-107 | 96-102 | 113-119 | 100-110 |
| % Recovery at 50 ng/L | 93-107 | 88-102 | - | - | - | - | - | - | - | - |
| % Recovery at 250 ng/L | - | - | 107-117 | 104-111 | 108-111 | 104-110 | 106-117 | 105-114 | 107-118 | 102-111 |
| Mean % recovery | 107 | 93 | 106 | 105 | 105 | 103 | 106 | 105 | 114 | 106 |
| RSD % | 15 | 5 | 7 | 3 | 5 | 6 | 7 | 6 | 4 | 3 |
| Method linearity (ng/mL) | 0.0005-0.01 | | 0.0025-0.05 | | 0.0025-0.05 | | 0.0025-0.05 | | 0.0025-0.05 | |
| Correlation coefficient (r)* | 0.9985 / 0.9980 | | 0.9980 / 0.9987 | | 0.9982 / 0.9978 | | 0.9990 / 0.9988 | | 0.9999 / 0.9981 | |
| LOD (ng/L) | 1 | | 5 | | 5 | | 5 | | 5 | |
| LOQ (ng/L) | 5 | | 25 | | 25 | | 25 | | 25 | |
| * obtained from the quantitation ion / from the confirmation ion | | | | | | | | | | |

**Table 2b. Independent laboratory validation: Parent compound and converted products**

| Parameter | Parent compound | | DC-DM-8007 | | DC-8007 | | DM-8007 | | S(PFP-OH)-8007 | |
|---|---|---|---|---|---|---|---|---|---|---|
| | surface | drinking | surface | drinking | surface | drinking | surface | drinking | surface | drinking |
| % Recovery at 5 ng/L | 93.3-129 | 88.0-108 | - | - | - | - | - | - | - | - |
| % Recovery at 25 ng/L | - | - | 70.3-94.0 | 77.0-97.3 | 84.3-95.9 | 85.4-97.0 | 82.0-114 | 94.7-113 | 87.6-102 | 78.0-108 |
| % Recovery at 50 ng/L | 88.4-100 | 88.7-95.6 | - | - | - | - | - | - | - | - |

| Table 2b. Independent laboratory validation: Parent compound and converted products | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| **Parameter** | **Parent compound** | | **DC-DM-8007** | | **DC-8007** | | **DM-8007** | | **S(PFP-OH)-8007** | |
| | **surface** | **drinking** | **surface** | **drinking** | **surface** | **drinking** | **surface** | **drinking** | **surface** | **drinking** |
| % Recovery at 250 ng/L | - | - | 69.3-86.9 | 91.6-95.9 | 82.8-91.3 | 86.6-95.7 | 87.0-96.7 | 90.8-96.2 | 75.7-99.8 | 79.5-104 |
| Mean % recovery | 103 | 95.1 | 80.2 | 90.6 | 88.9 | 91.6 | 95.6 | 97.8 | 93.1 | 92.0 |
| RSD % | 10 | 6.1 | 9.2 | 5.5 | 4.4 | 3.7 | 6.2 | 5.6 | 11 | 11.3 |
| Method linearity (ng/mL) | 0.0005-0.01 | | 0.0025-0.05 | | 0.0025-0.05 | | 0.0025-0.05 | | 0.0025-0.05 | |
| Correlation coefficient (r)* | 0.9995 / 0.9994 | | 0.9994 / 0.9990 | | 0.9997 / 0.9998 | | 0.9995 / 0.9996 | | 0.9987 / 0.9974 | |
| LOD (ng/L) | 1 | | 5 | | 5 | | 5 | | 5 | |
| LOQ (ng/L) | 5 | | 25 | | 25 | | 25 | | 25 | |
| * obtained from the quantitation ion / from the confirmation ion | | | | | | | | | | |

**2nd analytical method for broflanilide degradates S(Br-OH)-8007, AB-Oxa, and MFBA.**

| **Table 3. Principle of the method** | |
|---|---|
| **Items** | **Details** |
| Method Number | D1705/01 |
| Analyte | S(Br-OH)-8007, AB-Oxa, MFBA |
| Details of sample used | Drinking (well) and surface (lake) water samples were used for the study. The samples were characterized with the amounts of calcium, sodium, magnesium, dissolved solids and organic carbon, pH, hardness, conductivity, sodium adsorption ration and turbidity. |
| Sample preparation | S(Br-OH)-8007, AB-Oxa, and MFBA have the potential to adhere to container walls. As a result, any water samples to be analyzed (of unknown volume) must be transferred to a new container (while measuring sample volume), such as a graduated cylinder. An equal volume of methanol should be added to the original container; shake methanol in containers for 15 minutes at 300 rpm on a mechanical shaker, ensuring that solvent contacts all interior surfaces of the container. The methanol should then be transferred to the new container that is holding the sample. (Be sure the new container used has adequate capacity to contain both the sample and the methanol to be added as well as allow adequate mixing.) The diluted sample should then be mixed, filtered, and analyzed. |
| Method for quantitative analysis of parent compound and transformation products | External standard<br>The test/reference standards were synthesized by Mitsui Chemical Agro, Inc. (MCAG, Tokyo, Japan). Japan Analytical Chemistry Consultants Co., Ltd., on behalf of MCAG, determined characterization and purity prior to use. S(Br-OH)-8007, 98.38%; AB-Oxa, 98.89%; MFBA, 99.87% |

**Table 3. Principle of the method**

| Items | Details |
|---|---|
| Method for identification of parent compound/transformation products* | LC-MS/MS method<br>Column: XBridge BEH Phenyl (100 x 2.1 mm, 2.5 µm)<br>Column temperature: 50°C<br>Detector: Triple Quad Mass Spectrometer<br>Mobile phase: A – water / formic acid (1000/1, v/v)<br>B – methanol / formic acid (1000/1, v/v)<br>Gradeint: |

| Time (min) | A (%) | B (%) |
|---|---|---|
| 0.00 | 70 | 30 |
| 0.10 | 70 | 30 |
| 1.10 | 45 | 55 |
| 1.20 | 30 | 70 |
| 3.20 | 5 | 95 |
| 4.20 | 5 | 95 |
| 4.25 | 70 | 30 |
| 5.00 | 70 | 30 |

flow rate 600 µL/minute

Ionization: Electrospray ionization with positive mode

| Analyte | Retention time (min) | Quantitation [*m/z*] | Confirmation [*m/z*] |
|---|---|---|---|
| S(Br-OH)-8007 | 2.6 | 601→256 | 601→581 |
| AB-Oxa | 3.3 | 583→444 | 583→494 |
| MFBA | 1.9 | 274→254 | 274→254 |

| | |
|---|---|
| Chromatograms of spiked sample, control sample, blank and standard solution | Acceptable chromatograms of the solvent blank, reference standards and fortified sample were provided. |
| Quantitation | Linear regression (1/x weighting). Direct comparison of the sample peak responses to those of external standards. |
| Criteria for setting LOD and LOQ | The limit of detection (LOD) is the lowest level of fortification tested of an analyte in the matrix with an acceptable signal to noise ratio (S/N > 3:1). The limit of quantitation (LOQ) is the lowest fortification level that can be reliably quantitated. |
| Stability of parent and transformation products at various stages of analysis | Not stated |

**Table 3. Principle of the method**

| Items | Details |
|---|---|
| Special problems encountered and/or precautions to be taken during analysis/handling/storage of samples | S(Br-OH)-8007, AB-Oxa, and MFBA have the potential to adhere to container walls. The glassware used for the method should be thoroughly rinsed with methanol followed by acetone to prevent contamination. |
| Total time for completion | Not stated |

* Confirmatory Instrumentation and Conditions for MFBA:
Column: Acquity UPLC BEH C18 (50 x 2.1 mm, 1.7 µm)
Column temperature: 50°C
Detector: Triple Quad Mass Spectrometer
Mobile phase: A – water / formic acid (1000/1, v/v)
B – methanol / formic acid (1000/1, v/v)
Gradeint:

| Time (min) | A (%) | B (%) |
|---|---|---|
| 0.00 | 90 | 10 |
| 0.10 | 70 | 30 |
| 1.10 | 45 | 55 |
| 1.30 | 5 | 95 |
| 1.90 | 5 | 95 |
| 2.00 | 90 | 10 |
| 2.50 | 90 | 10 |

flow rate 600 µL/minute

Ionization: Electrospray ionization with positive mode
Ion monitored [*m/z*]: 274→254

The method validation data for the parent compound and the metabolites are summarized in Table 4.

**Table 4a. Method validation: Parent compound and converted products**

| Parameter | S(Br-OH)-8007 | | AB-Oxa | | MFBA | |
|---|---|---|---|---|---|---|
| | surface | drinking | surface | drinking | surface | drinking |
| % Recovery at 25 ng/L | 71-86 | 70-90 | 77-91 | 77-91 | 97-114 | 93-107 |
| % Recovery at 250 ng/L | 91-106 | 87-109 | 70-88 | 82-95 | 95-113 | 95-109 |
| Mean % recovery | 88 | 86 | 83 | 87 | 106 | 102 |
| RSD % | 11 | 17 | 6 | 6 | 5 | 5 |
| Method linearity (ng/mL) | 0.0025-0.05 | | 0.0025-0.05 | | 0.0025-0.05 | |
| Correlation coefficient (r)* | 0.9997 / 0.9989 | | 0.9993 / 0.9978 | | 0.9992 / 0.9996 | |
| LOD (ng/L) | 5 | | 5 | | 5 | |
| LOQ (ng/L) | 25 | | 25 | | 25 | |
| * obtained from the quantitation ion / from the confirmation ion | | | | | | |

| Table 4b. Independent laboratory validation: Parent compound and converted products | | | | | | |
|---|---|---|---|---|---|---|
| **Parameter** | **S(Br-OH)-8007** | | **AB-Oxa** | | **MFBA** | |
| | **surface** | **drinking** | **surface** | **drinking** | **surface** | **drinking** |
| % Recovery at 25.0 ng/L | 84-107 | 84.5-104 | 60.8-97.9 | 74.7-101 | 68.7-83.4 | 82.5-101 |
| % Recovery at 250.0 ng/L | 98.0-122 | 88.4-96.6 | 70.5-90.9 | 77.5-90.6 | 102-119 | 104-112 |
| Mean % recovery | 103 | 94.8 | 84.2 | 87.5 | 92.2 | 100.4 |
| RSD % | 9.2 | 4.8 | 9.4 | 10.3 | 22 | 9.4 |
| Method linearity (ng/mL) | 0.0025-0.25 | | 0.0025-0.25 | | 0.0025-0.25 | |
| Correlation coefficient (r)* | 0.9987 / 0.9980 | | 0.9978 / 0.9974 | | 0.9994 / 0.9999 | |
| LOD (ng/L) | 5 | | 5 | | 5 | |
| LOQ (ng/L) | 25 | | 25 | | 25 | |
| * obtained from the quantitation ion / from the confirmation ion | | | | | | |

**Comments/Deficiency**

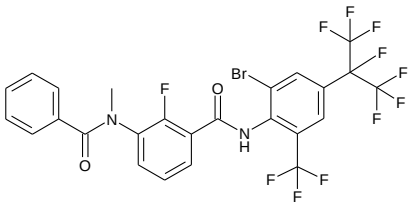
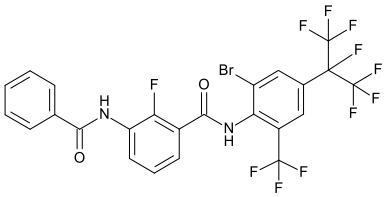
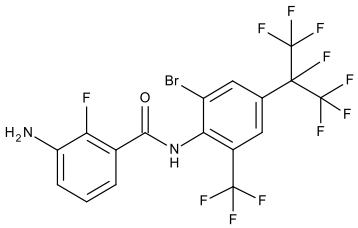
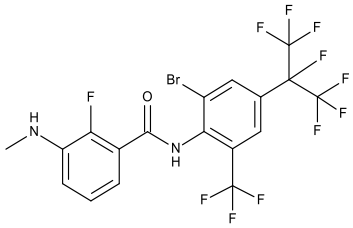
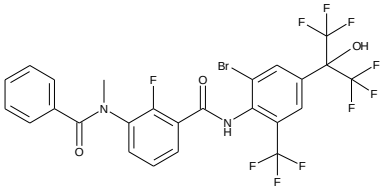
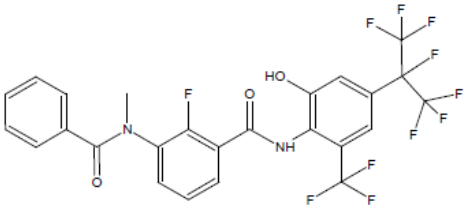
The submitted studies have provided analytical methods for the determination of broflanilide and metabolites in surface and drinking water. No deficiencies have yet been identified but please note that any transformation products/metabolites present at levels greater than 10% of the initial concentration of the pesticide at any time during the study, as well as those products that have not attained 10% (e.g., 8-9%) but show a continuous increase in concentration up until the termination of the study, are considered to be major. (Also, transformation products/metabolites that are of toxicological concern are considered to be major, even if their maximum concentrations are less than 10% of the initial parent concentration). In the ILV, the RSD was high (22%) for MFBA recoveries in surface water, but not for MFBA recoveries in drinking water (9.4%).

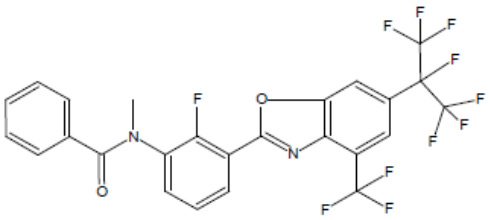
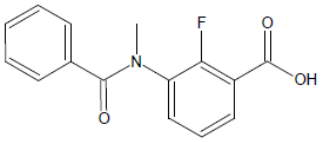
**Conclusion**

Two LC-MS/MS methods were developed for the determination of broflanilide and its metabolites in water and were validated in both drinking and surface water. The recovery data were acceptable (between 70-120%), and the LOQs were determined to be 5 ng/L for broflanilide and 25 ng/L for the metabolites respectively. These methods are **acceptable** for use as post-registration monitoring methods.

## Appendix A. Chemical structures:

**Table A-1. Chemical name, code and chemical structure for active and all major transformation products / metabolites**

| Chemical name | Code | Chemical structure |
|---|---|---|
| *N* -[2-bromo-4-(perfluoropropan-2-yl)-6-(trifluoromethyl)phenyl]-2-fluoro-3-(*N* -methylbenzamido)benzamide | MLP-8607, BAS 450 I, MCI-8007, Reg. No. 5672774 [broflanilide] |  |
| 3-benzamido-N-[2-bromo-4-(perfluoropropan-2-yl)-6-(trifluoromethyl)phenyl]-2-fluorobenzamide | DM-8007, MLP-8473, Reg. No. 5856361 |  |
| 3-amino-N-[2-bromo-4-(perfluoropropan-2-yl)-6-(trifluoromethyl)phenyl]-2-fluorobenzamide | DC-DM-8007, MLQ-2111, Reg. No. 5936906 |  |
| *N*-[2-bromo-4-(perfluoropropan-2-yl)-6-(trifluoromethyl)phenyl]-2-fluoro-3-(methylamino)benzamide | DC-8007 |  |
| *N*-[2-bromo-4-(1,1,1,3,3,3-hexafluoro-2-hydroxypropan-2-yl)-6-(trifluoromethyl)phenyl]-2-fluoro-3-(*N*-methylbenzamido)benzamide | S(PFP-OH)+8007, MLQ-2172, Reg. No. 5959598 |  |
| 2-fluoro-*N*-[2-hydroxy-4-(perfluoropropan-2-yl)-6-(trifluoromethyl)phenyl]-3-(*N*-methylbenzamido)benzamide | S(Br-OH)-8007, Reg. No. 5959595 |  |

| | | |
|---|---|---|
| *N*-{2-fluoro-3-[6-perfluoropropan-2-yl)-4-(trifluoromethyl)-1,3-benzooxazol-2-yl]phenyl}-*N*-methylbenzamide | AB-Oxa, Reg. No. 5959600 |  |
| 2-fluoro-3-(*N*-methylbenzamido) benzoic acid | MFBA, Reg. No. 6088668 |  |