Data Evaluation Report on the Bioaccumulation of Orthosulfamuron in Fish

PMRA Submission Number {.....} EPA MRID Number 46588507

Data Requirement: PMRA Data Code:
EPA DP Barcode: D320283
OECD Data Point:
EPA Guideline: 165-4 (OPPTS 850.1730)

Test material:

Common name:	Orthosulfamuron.
Chemical name:	
IUPAC name:	1-(4,6-Dimethoxypyrimidin-2-yl)-3-[2-(dimethylcarbamoyl)phenyl-sulfamoyl]urea.
CAS name:	2-[[[[[(4,6-Dimethoxy-2-pyrimidinyl)amino]carbonyl]amino]-sulfonyl]amino]-N,N-dimethylbenzamide.
CAS No.:	213464-77-8.
Synonyms	IR5878.
Smiles string:	CN(C(=O)c1ccccc1NS(=O)(=O)NC(=O)Nc1nc(cc(n1)OC)OC)C (ISIS v2.3/Universal SMILES). No EPI Suite, v3.12 SMILES String found as of 2/21/06.

Primary Reviewer: Kindra Bozicevich
Cambridge Environmental
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Company Code:
Active Code:
Use Site Category:
EPA PC Code: 108209

CITATION: van Dijk, A. 2004. Bioconcentration: Flow-through fish test with IR5878 in bluegill sunfish (*Lepomis macrochirus*). Unpublished study performed by RCC Ltd., Itingen, Switzerland; sponsored and submitted by ISAGRO S.p.A., Milano, Italy. RCC Study No. 849257. Experiment initiated July 28, 2003, and completed February 10, 2004. Final report issued May 11, 2004. 68 pp.

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ABSTRACT:

Laboratory Accumulation- Fish

The bioaccumulation of [^{14}C-5-pyrimidinyl]-labeled 1-(4,6-dimethoxypyrimidin-2-yl)-3-[2-(dimethylcarbamoyl)phenylsulfamoyl]urea (orthosulfamuron; IR5878) was studied in bluegill sunfish (*Lepomis macrochirus*) at nominal concentrations of 142 µg/L (low-dose) and 1420 µg/L (high-dose) under flow-through conditions. The experiment was conducted in accordance with OECD Guidelines No. 305 and OPPTS Guideline 850.1730, and in compliance with the Swiss Ordinance relating to Good Laboratory Practice. The test system consisted of three 75-L aerated and temperature-controlled aquaria maintained at an average temperature of 20-25°C. Two aquaria were treated with [^{14}C-5-pyrimidinyl]orthosulfamuron, and the third served as the solvent control. Fish were exposed for 14 days under a 16 hour-light/8-hour dark photoperiod, followed by a 14-day depuration period. During exposure and depuration, the water temperature was maintained at 21-23°C, dissolved oxygen concentrations were 4.5-9.7 mg/L, and pH values were 7.5-8.0. Total water hardness measured on day 6 of exposure was 196 mg $CaCO_3$/L. Total organic carbon for the treated aquaria were 32.8-40.9 mg/L during the exposure period, and 6.1-6.5 mg/L during the depuration period.

During exposure, 6 fish were collected from each treated aquarium on days 4, 7, 10, 12, and 14; an additional 8 fish were collected on days 10 and 14 and stored frozen for subsequent analysis. Six control fish were collected at the beginning and end of the exposure period. Ten control fish were collected on days 7 and 14 for lipid determination of edible and nonedible tissues. Observations of fish mortality were conducted throughout the study. Duplicate water samples were collected from the low-dose and high-dose aquaria on days 4, 7, 10, 12, and 14. Additional duplicate water samples collected on the same sampling days were stored for future analysis. Duplicate control water samples were collected at the beginning, middle, and end of exposure.

Following the 14-day exposure period, fish from each aquarium were transferred to a flow-through system with untreated tap water for a 14-day depuration period. During depuration, 6 fish from each treated aquarium were collected on days 4, 7, 11, and 14. Six control fish were collected on day 14, and duplicate control water samples were collected on days 4, 7, 11, and 14 of the depuration period.

Duplicate aliquots of the aquarium water and portions of solubilized edible and nonedible fish tissues were analyzed for total radioactivity using LSC. Additional water samples were acidified to *ca.* pH 4, partitioned twice with methylene chloride (4:1, v:v), and analyzed using LSC.

Orthosulfamuron and its transformation products in the water were identified via TLC and HPLC analyses, using reference standards of 1-(4,6-dimethoxypyrimidin-2-yl)-3-[2-(dimethylcarbamoyl)phenyl-sulfamoyl]urea (orthosulfamuron; purity 98.5%) and N-(4,6-dimethoxypyrimidin-2-yl)urea (DOP urea; purity 99.3%).

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Following 14 days of exposure: In **low-dose** fish samples, [^{14}C]residues averaged 39 ± 13 µg-equivalents/kg in the edible tissues, 46 ± 6 µg-equivalents/kg in the nonedible tissues, and 43 ± 4 µg-equivalents/kg in whole fish. Calculated bioconcentration factors (BCFs), based on the average amount of residual radioactivity in fish during the entire exposure period, were **0.29**, **0.34**, and **0.32** for edible tissues, nonedible tissues, and whole fish tissues, respectively. Lipid content in control fish samples was 11.3-11.8 mg/g in edible tissues, 20.0-21.7 mg/g in nonedible tissues, and 16.7-17.7 mg/g in whole fish. Average lipid-BCF values ranged from 16-29.

In **high-dose** fish samples, [^{14}C]residues averaged 938 ± 235 µg-equivalents/kg in the edible tissues, 1494 ± 180 µg-equivalents/kg in the nonedible tissues, and 1272 ± 125 µg-equivalents/kg in whole fish. Calculated BCFs were **0.72**, **1.14**, and **0.97** for edible tissues, nonedible tissues, and whole fish, respectively. Average lipid-BCF values ranged from 52-68.

In the low-dose aquarium water during the 14-day exposure period, total [^{14}C]residues in the water averaged 135.2 ± 5.5 µg-equivalents/L. Following partitioning of the low-dose aquarium water, [^{14}C-5-pyrimidinyl]orthosulfamuron (W1) accounted for a mean of 83.0 ± 7.0% (112 ppb) using TLC analysis, and 81.3 ± 6.0% (110 ppb) of the TRR using HPLC analysis. A major transformation product, identified as N-(4,6-dimethoxypyrimidin-2-yl)urea (DOP urea; W3), was detected at a mean of 16.6 ± 7.6% (22 ppb) using TLC analysis, and 18.8 ± 6.0% (25 ppb) of the TRR using HPLC analyses. A minor transformation product, identified as W2, was only detected on day 4 of exposure comprised 1.8% (2 ppb) of the TRR, using TLC analysis.

In high-dose water during the 14-day exposure period, total [^{14}C]residues averaged 1311.6 ± 55.4 µg-equivalents/L. Following partitioning of the high-dose aquarium water, [^{14}C-5-pyrimidinyl]orthosulfamuron accounted for a mean of 85.8 ± 2.2% (1125 ppb) using TLC analysis, and 81.3 ± 2.4% (1066 ppb) of the TRR using HPLC analysis. Major transformation product DOP urea was detected at a mean of 13.9 ± 2.7% (182 ppb) using TLC analysis and 18.7 ± 2.4% (245 ppb) using HPLC analysis. Minor transformation product W2 was only detected on day 4 of exposure and comprised 1.5% (20 ppb) of the TRR, using TLC analysis.

Following 14 days of depuration: In the **low-dose** samples, [^{14}C]residues were <LOD (32 µg-equivalents/kg) in the edible tissues, 9-20 µg-equivalents/kg in the non-edible tissues, and <LOD (13-18 µg-equivalents/kg) in whole fish. In the **high-dose** samples, [^{14}C]residues were 429-648 µg-equivalents/kg in the edible tissues, 487-841 µg-equivalents/kg in the nonedible tissues, and 552-718 µg-equivalents/kg in whole fish.

Total [^{14}C]residues in the depuration water decreased rapidly within 4 days to 0.2% of the respective average exposure level, and were below the detection limits of the low-dose (0.44 µg/L) and high-dose (4.39 µg/L) aquaria water.

During the exposure period, 1 control, 4 low-dose, and 2 high-dose fish died. No fish died during the depuration period.

Study Acceptability: This study is scientifically valid and is classified as **acceptable**.

MATERIALS AND METHODS:

Bluegill sunfish (*Lepomis macrochirus*, Osage Catfisheries, Inc., Missouri) were acclimatized in the laboratory in tap water for at least 2 weeks prior to study initiation (fish age not reported; p. 19). The average fish weight at the onset of acclimatization was 0.65 g (n >10). During the acclimatization and test periods, the fish were fed once daily with a diet of known lipid and total protein content (p. 20). The diet was based on *ca.* 2% of the average fish body weight, taking into account increasing body weights and the decreasing number of fish per sampling interval.

Prior to study initiation, low-dose and high-dose stock solutions containing the parent compound were prepared (pp. 21-22). [^{14}C-5-Pyrimidinyl]orthosulfamuron (IR5878; radiochemical purity >98%; specific radioactivity 51.07 mCi/mmol, 120.3 µCi/mg, 4.452 MBq/mg, 267117 dpm/µg; Lot 208; p. 17) was dissolved in 90% aqueous ethanol (50 mL). The concentration of [^{14}C-5-pyrimidinyl]orthosulfamuron in solution was 0.511 mg/mL at a specific radioactivity of 4.4520 MBq/mg (120.32 µCi/mg; stock solution 1), based on LSC analysis. For the low-dose stock solution, with a target concentration of 142 µg/L, 746.36 mg of unlabelled test material were diluted in DMF (60 mL), ethanol (400 mL), and stock solution 1 (25 mL). The solution was alkalized by adding 6M NaOH, and brought to a final volume of 500 mL with ethanol. For the high-dose stock solution, with a target concentration of 1420 µg/L, 7459.99 mg of unlabelled test material were diluted in DMF (60 mL), ethanol (*ca.* 415 mL), and stock solution 1 (25 mL). The solution was alkalized by adding 6M NaOH. The low and high-dose test solutions were stored at -20°C prior to use.

Application solutions for low-dose and high-dose levels were prepared by diluting the stock solutions in an ethanol:1.0% alkaline purified water solution (400 mg NaOH into 100 mL 0.5M $NaHCO_3$) (25:975, v:v) to establish nominal test concentrations of 142 µg/L for the low-dose aquarium and 1420 µg/L for the high-dose aquarium (pp. 20, 22). The concentration of each test solution was confirmed to be 135.2 µg/L and 1311.6 µg/L for the low-dose and high-dose levels, respectively, using LSC analysis (pp. 14, 37). The water solubility of orthosulfamuron at 20°C was reported to be 26.2 mg/L at pH 4, 629 mg/L at pH 7, and 38,900 mg/L at pH 8.5.

Flow-through aquatic exposure systems were prepared using three 75-L aerated and temperature controlled aquaria (1 control, 2 treated) maintained at an average temperature of 20-25°C, using a 16-hour light/8-hour dark photoperiod (light intensity *ca.* 300-400 lux; 30 minute transition period; pp. 20-21; Figure 4, p. 53). Prior to test initiation, test concentrations in the exposure aquaria were adjusted by adding 300 mL of application solution to each aquarium. Throughout the test, the aquaria were equilibrated with tap water at a flow-through volume of 250 L/day. [^{14}C-5-Pyrimidinyl]orthosulfamuron was delivered at a rate of 1000 mL/24 hours (1389 µL/2 minutes; implicating 25 mL organic solvent or 0.01% of the flow-through volume of 250 L/day; *ca.* 3× the aquarium volume). The test material was delivered into a mixing flask via a Hamilton dispenser unit where the application solutions were pre-diluted with tap water. Each aquarium was equilibrated with the test substance for 3 days (*ca.* 10 aquarium volumes) prior to introduction of the test fish

Following equilibration, 45 fish were transferred into the control aquarium and 80 fish were transferred into each low and high-dose aquarium (p. 19). The fish had a mean weight of 0.7-0.8 g (sample size of 5-10 fish). The loading ratios for the control, high-dose and low-dose aquaria were 0.13, 0.26, and 0.22 g/L/day, respectively.

During exposure, 6 fish were collected from each low-dose and high-dose aquarium on days 4, 7, 10, 12, and 14 (pp. 24-25). The fish were randomly collected, rinsed with water, sacrificed in 1.5% (v:v) ethylene glycol monophenyl ether in purified water, and blotted dry. An additional 8 fish were collected on days 10 and 14 and stored at *ca.* -20 °C until further analysis. For the control aquarium, 6 fish were collected at the beginning and end of the exposure period. Ten control fish were collected on days 7 and 14 for lipid determination of edible and nonedible tissues. Observations of fish mortality were conducted throughout the study (p. 23). Duplicate 10-mL water samples were collected from each treated aquarium on days 4, 7, 10, 12, and 14. Duplicate 200-mL water samples were collected on the same schedule and stored at -20°C for future analysis. For the control aquarium, duplicate 10-mL water samples were collected at the beginning, middle, and end of exposure. Water samples and fish tissue samples were stored at *ca.* -20°C for a maximum of 3 months (p. 27).

Following the 14-day exposure period, the fish were transferred to a flow-through system with untreated tap water for a 14-day depuration period (pp. 21-22). The exposure aquaria were cleaned and discrete areas were wiped with 0-1 dpm/cm^2 of ethanol (*ca.* 100 cm^2 of cover, various wall positions and drain-pipe) and analyzed for total radioactivity to confirm lack of contamination within the aquaria.

During depuration, 6 fish from each treated aquarium were collected on depuration days 4, 7, 11, and 14 (pp. 24-25). Fish were collected and sacrificed as previously described. Six control fish were collected at the end of the depuration period. Duplicate control 10-mL water samples were collected on depuration days 4, 7, 11, and 14. Following sampling, the water samples and fish tissue samples were stored at *ca.* -20°C for a maximum of 3 months (p. 27).

Water: Water samples were pipetted from a central area of each aquarium prior to fish feeding and immediately before fish sampling (p. 25). Water samples were also taken from the mixture chambers. Duplicate 10-mL aliquots of the water from each aquarium were analyzed for total radioactivity using LSC (pp. 25-27). The additional 200-mL aliquots of the water were adjusted to pH 8-9 and stored at -20°C. After thawing, the samples were acidified to *ca.* pH 4, and one sample per sampling interval and dose level (5 per dose level) was partitioned twice with methylene chloride (4:1, v:v). The samples were combined and concentrated to *ca.* 1.8-2.7 mL. Aliquots (1-2 mL) were analyzed for total radioactivity using LSC (p. 27). Additional aliquots of the water samples were analyzed via TLC and HPLC analyses.

One-dimensional normal phase TLC was conducted using precoated silica gel 60 F_{254} plates (5 cm × 20 cm; 0.25 mm) developed in chloroform:methanol:ammonium hydroxide (25%; 75:22:3, v:v:v; SS1), acetonitrile:water:ammonium hydroxide (25%; 80:18:2, v:v:v; SS2), and 2-propanol:water:ammonium hydroxide (25%; 80:10:10, v:v:v; SS3; p. 28). The samples were

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applied onto the surface of the TLC plates using a spraying technique (TLC linomat, type IV). The plates were developed without chamber saturation. The samples were cochromatographed with unlabeled reference standards of orthosulfamuron (purity 98.5%; Batch G009/02; p. 16) dissolved in ethanol at 2 mg/mL and N-(4,6-dimethoxypyrimidin-2-yl)urea (DOP urea; Reference item A; Batch No. FCF/T/198-01; purity 99.3%) that were visualized using UV light (254 nm). Areas of radioactivity were detected using an Automatic TLC-Linear Analyzer equipped with a Data Processing System. The retention factors for the reference standards were as follows:

Chemical	SS1	SS2	SS3
Orthosulfamuron (IR5878)	0.34-0.50	0.56-0.64	0.72-0.75
DOP urea (Reference item A)	0.82-0.88	Not analyzed.	Not analyzed.

HPLC analysis was used as a secondary method to determine the metabolite pattern in the exposure water (p. 29).

HPLC

Pre-column	Lichrospher RP18, 5 Φm (4 H 4 mm).
Column	Supelcosil LC-18 DB, 3 Φm (150 H 3 mm).
Mobile phase	A: Ammonium acetate (1mM); pH 4.5 B: Acetonitrile
Gradient	Min. %A %B 0 80 20 20 10 90 20.1-30 80 20
Flow rate	0.5 mL/minute
UV detection	254 nm

The samples were cochromatographed with unlabeled reference standards that were visualized using UV light (254 nm) and radioactive flow detection. The retention times for the reference standards were as follows:

Chemical	Lot Number	Purity (%)	Retention Time (minutes)
Orthosulfamuron (IR5878)	Not reported	Not reported	11.2-11.7
DOP urea (Reference item A)	Not reported	Not reported	6.2-6.4

Fish: The 6 fish sampled from each aquarium were separated into edible (fillet and skin) and nonedible (head, fins, viscera, and skeleton) tissues, weighed, and solubilized at *ca.* 50°C for 24-48 hours with tissue solubilizing agent Soluene 350 (100 mg/mL solubilizer; pp. 26-27).

Duplicate 0.3-1.0 mL samples (containing *ca.* 30-100 mg tissue) were analyzed for total radioactivity using LSC.

For lipid determination, the 10 control fish sampled on days 7 and 14 were separated into edible and nonedible tissues, pooled, weighed, and stored at -20°C (p. 26). The samples were thawed, homogenized with dry ice using a blender, and extracted by gently shaking overnight at room temperature with methanol:methylene chloride (1:1, v:v). The fish:extraction solvent ratio was 1:8 (w:v). A second extraction was conducted by shaking for 30 minutes at room temperature with methanol:methylene chloride (1:1, v:v) using a fish:extraction solvent ratio of 1:2 (w:v). The extracts were filtered, combined, dried with sodium sulphate, and concentrated to dryness under reduced pressure. The fish lipid residue was determined after drying at 105°C and subsequent cooling in an exicator to constant weight.

The additional 8 fish sampled from each low-dose and high-dose aquarium on exposure days 10 and 14 were separated into edible and nonedible tissues, weighed, and stored at -20°C (p. 26). These fish tissues were not used for further analysis since the average BCF value was below 1000.

RESULTS AND DISCUSSION:

Water quality parameters were reportedly monitored and maintained throughout the study period (pp. 23, 32; Tables 1-2, pp. 38-39). During the exposure phase, the temperature was maintained at 22-23°C, dissolved oxygen concentrations ranged from 4.5-7.7 mg/L, and pH values ranged from 7.8-7.9. During the depuration phase, the temperature was maintained at 21-22°C, dissolved oxygen concentrations were 7.9-9.7 mg/L, and pH values were 7.5-8.0. Total water hardness was measured once on day 6 and was 11 degrees or 196 mg $CaCO_3$/L. Total organic carbon (TOC) values during pre-equilibrium and exposure ranged from 32.8-40.9 mg/L for the low-dose and high-dose aquaria (Table 2B, p. 39). TOC values for the control water ranged from 31.7-42.1 mg/L. During depuration, TOC values for the treated and control aquaria were 6.1-6.5 mg/L.

Following 14 days of exposure: In the **low-dose** samples, [^{14}C]residues accounted for an average of 39 ± 13 µg-equivalents/kg (equivalent to ppb) in edible tissues, 46 ± 6 µg-equivalents/kg in the nonedible tissues, and 43 ± 4 µg-equivalents/kg in whole fish (p. 34; Table 5A, p. 42). Bioconcentration factors (BCFs), calculated based on the average amount of residual radioactivity in fish during the entire exposure period, for edible tissues, nonedible tissues, and whole fish, were **0.29**, **0.34**, and **0.32**, respectively (p. 35; Table 5B, p. 42). Lipid content in control fish sampled at 7 and 14 days was 11.3-11.8 mg/g in edible tissues, 20.0-21.7 mg/g in nonedible tissues, and 16.7-17.7 mg/g in whole fish (Table 8, p. 45). Average lipid-BCF values ranged from 16-29.

In the **high-dose** samples, [^{14}C]residues accounted for an average of 938 ± 235 µg-equivalents/kg in edible tissues, 1494 ± 180 µg-equivalents/kg in nonedible tissues, and 1272 ±

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125 µg-equivalents/kg in whole fish (Table 6A, p. 43). Calculated bioconcentration factors (BCFs) for edible tissues, nonedible tissues, and whole fish were **0.72**, **1.14**, and **0.97**, respectively (p. 35; Table 6B, p. 43). Average lipid-BCF values ranged from 52-68.

In the low-dose aquarium water, total [^{14}C]residues ranged from 120-201 µg-equivalents/L during the 3-day pre-equilibrium phase and averaged 135.2 ± 5.5 µg-equivalents/L during the 14-day exposure period (p. 33; Table 3A, p. 40). In the high-dose aquarium water, total [^{14}C]residues ranged from 1391-1550 µg-equivalents/L during the 3-day pre-equilibrium phase and averaged 1311.6 ± 55.4 µg-equivalents/L during the 14-day exposure period.

In the low-dose and high-dose aquarium water, [^{14}C]residues partitioning into methylene chloride accounted for an average of 99.1 ± 0.34% of the TRR and an average of 0.9 ± 0.34% of the TRR remained in the aqueous phase (normalized data for comparison of various time points; p. 36; Tables 9-10, pp. 46-47).

Following partitioning of the low-dose aquarium water, TLC and HPLC analyses showed that [^{14}C-5-pyrimidinyl]orthosulfamuron (W1) accounted for a mean of 83.0 ± 7.0% (112 ppb; calculated as % of the TRR × actual test concentration, *i.e.* 83.0% × 135.2 µg/L = 112 ppb) and 81.3 ± 6.0% (110 ppb) of the TRR, respectively (p. 36; Tables 11A-11B, p. 48; Figures 5-6, pp. 54-55). A major transformation product, identified as N-(4,6-dimethoxypyrimidin-2-yl)urea (DOP urea; W3), was detected at a mean of 16.6 ± 7.6% (22 ppb) and 18.8 ± 6.0% (25 ppb) of the TRR using TLC and HPLC analyses, respectively. A minor transformation product, identified as W2, was detected on day 4 of exposure only, and comprised 1.8% (2 ppb) of the TRR, based on TLC analysis.

Following partitioning of the high-dose aquarium water, TLC and HPLC analyses showed that [^{14}C-5-pyrimidinyl]orthosulfamuron (W1) accounted for a mean of 85.8 ± 2.2% (1125 ppb) and 81.3 ± 2.4% (1066 ppb) of the TRR, respectively (p. 36; Tables 12A-12B, p. 49; Figures 7-9, pp. 56-58). Major transformation product DOP urea was detected at a mean of 13.9 ± 2.7% (182 ppb) and 18.7 ± 2.4% (245 ppb) of the TRR using TLC and HPLC analyses, respectively. Minor transformation product W2, detected on day 4 of exposure only, comprised 1.5% (20 ppb) of the TRR, based on TLC analysis.

Following 14 days of depuration: In the **low-dose** samples, [^{14}C]residues accounted for <LOD (32 µg-equivalents/kg) in the edible tissues, 9-20 µg-equivalents/kg in the non-edible tissues, and <LOD (13-18 µg-equivalents/kg) in whole fish (LOD, 13 µg/kg; p. 34; Table 5A, p. 42; Table 7, p. 44). In the **high-dose** samples, [^{14}C]residues accounted for 429-648 µg-equivalents/kg in the edible tissues, 487-841 µg-equivalents/kg in the nonedible tissues, and 552-718 µg-equivalents/kg in whole fish (Table 6A, p. 43; Table 7, p. 44).

During depuration, total [^{14}C]residues decreased rapidly within 4 days to 0.2% of the respective average exposure level and below the detection limits of the low-dose (0.44 µg/L) and high-dose (4.39 µg/L) aquaria (p. 33; Table 4A, p. 41). Total [^{14}C]residues in the mixing chambers at low-dose and high-doses on days 0, 1, 3, and 9 averaged 130.4 ± 7.9 µg-equivalents/L and 1327.8 ±

73.2 µg-equivalents/L, respectively (Table 3B, p. 40). Total [^{14}C]residues in the control aquarium water were negligible, in general, during the study (one sample was assumed to be contaminated; Table 4B, p. 41). Based on these results, it was determined that the Hamilton dispenser system used to deliver the test material was sufficient, with average dose levels reflecting 95.2% and 92.4% of the target concentrations for the low-dose and high-dose aquaria, respectively (p. 33).

During the 14-day exposure period, 1 control fish (2.2%), 4 low-dose fish (5.0%), and 2 high-dose fish (2.5%) died. No test fish died during the 14-day depuration period.

DEFICIENCIES/DEVIATIONS:

1. The radiochemical purity of [^{14}C-5-pyrimidinyl]orthosulfamuron in the high-dose stock solution was determined to be 95.5% (SS1) using TLC analysis (p. 18; Figure 1A, p. 50). The radiochemical purity of SS1 after 2 days of stirring during the study was 95.4% (SS1; Figure 2, p. 51). The radiochemical purity of [^{14}C-5-pyrimidinyl]orthosulfamuron in a pre-test high-dose stock solution after 1 day of stirring at room temperature was determined to be 94.8% (SS1), 94.1% (SS2), and 96.1% (SS3); and after 3 and 4 days of stirring, was 93.4% (SS1) and 92.3% (SS1), respectively. These data do not confirm stability of the test compound in the application solution at test conditions.

2. The dissolved oxygen concentration of the water in the control and low-dose aquaria dropped to slightly below 60% saturation due to a temporarily low air supply that occurred at night on days 8-14 of exposure (p. 31; Table 2, p. 39).

REVIEWER'S COMMENTS:

1. The study was conducted according to OECD Guideline No. 305 and OPPTS Guideline 850.1730 and in compliance with the Swiss Ordinance relating to Good Laboratory Practice, which is based on OECD Principles of Good Laboratory Practice (pp. 13, 19). Signed and dated Data Confidentiality, GLP, and Quality Assurance statements were provided (pp. 2-7).

2. The storage stability of [^{14}C-5-pyrimidinyl]orthosulfamuron in the stock solution was determined using high-dose stock solution after 27 and 39 days of storage at -20°C (p. 18). An aliquot of the stored stock solution was analyzed using TLC (SS1; 95.5% parent; Figure 1B, p. 50) and HPLC (96.4% parent; Figure 3, p. 52) analyses.

3. No accumulation plateau was reached due to lack of bioaccumulation.

4. The exposure concentrations of the test substance were <10% of the 96-hour LC_{50} concentration for bluegill, as required by Subdivision N guidelines. The LC_{50} for orthosulfamuron in bluegill sunfish was reported to be >142 mg/L (p. 17).

5. During the study, uneaten food and fish excreta were siphoned from the aquaria *ca.* 1 hour after feeding (p. 23).

6. The reported mortality for the low-dose (4 fish) and high-dose (2 fish) aquaria includes 1 fish from each treated aquarium that died when it was accidentally siphoned into the hose due to its small size (p. 32).

7. In the Allocation table presented on study report page 19, the low- and high-dose test concentrations were reported in the wrong columns.

Attachment 1: Structures of Parent Compound and Transformation Products

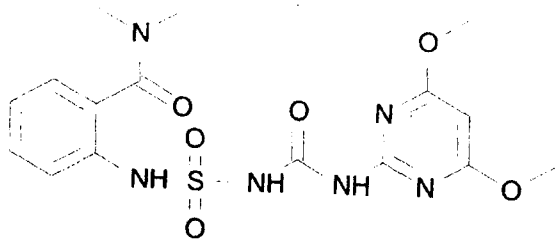
Orthosulfamuron [IR5878]

IUPAC Name: 1-(4,6-Dimethoxypyrimidin-2-yl)-3-[2-(dimethylcarbamoyl)phenylsulfamoyl]urea.

CAS Name: 2-[[[[[(4,6-Dimethoxy-2-pyrimidinyl)amino]carbonyl]amino]sulfonyl]amino]-N,N-dimethylbenzamide.

CAS Number: 213464-77-8.

SMILES String: CN(C(=O)c1ccccc1NS(=O)(=O)NC(=O)Nc1nc(cc(n1)OC)OC)C (ISIS v2.3/Universal SMILES).
No EPI Suite, v3.12 SMILES String found as of 2/21/06.



The position of the radiolabel was not reported.

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Identified Compounds

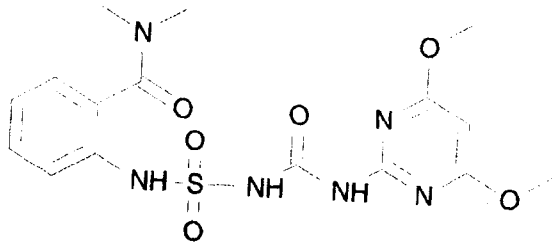
Orthosulfamuron [IR5878]

IUPAC Name: 1-(4,6-Dimethoxypyrimidin-2-yl)-3-[2-(dimethylcarbamoyl)phenylsulfamoyl]urea.

CAS Name: 2-[[[[[(4,6-Dimethoxy-2-pyrimidinyl)amino]carbonyl]amino]sulfonyl]amino]-N,N-dimethylbenzamide.

CAS Number: 213464-77-8.

SMILES String: CN(C(=O)c1ccccc1NS(=O)(=O)NC(=O)Nc1nc(cc(n1)OC)OC)C (ISIS v2.3/Universal SMILES).
No EPI Suite, v3.12 SMILES String found as of 2/21/06.



DOP-urea

IUPAC Name: N-(4,6-Dimethoxypyrimidin-2-yl)urea.

CAS Name: Not reported.

CAS Number: Not reported.