

Data Evaluation Report on the bioaccumulation of chlorothalonil in fish

PMRA Submission Number {....}

EPA MRID Number 45710224

Data Requirement: PMRA Data Code:
EPA DP Barcode: D301503
OECD Data Point:
EPA Guideline: 165-4

Common name: Chlorothalonil.
Chemical name
IUPAC: Tetrachloroisophthalonite.
CAS name: 2,4,5,6-Tetrachloro-1,3-benzenedicarbonitrile.
CAS No: 1897-45-6
Synonyms: None reported.
SMILES string: Clc1c(c(c(c1C#N)Cl)C#N)Cl)Cl

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Company Code:
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CITATION: McEwen, A.B. 1997. Chlorothalonil bioaccumulation in rainbow trout. Unpublished study performed by Huntingdon Life Sciences Ltd., Cambridgeshire, England, and sponsored and submitted by Vischim S.r.l, Milano, Italy. Study experimental start date January 15, 1996, and experimental end date August 22, 1996 (p.18). Final report issued on May 23, 1997.

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ABSTRACT

Laboratory Accumulation - Fish

The bioaccumulation of [benzotrile-U-¹⁴C]2,4,5,6-tetrachloro-1,3-benzenedicarbonitrile (chlorothalonil) was studied in rainbow trout (*Oncorhynchus mykiss*) at nominal concentrations of 0.1 and 0.5 µg/L under flow-through aquarium conditions. This experiment was conducted in accordance with EEC Council Directive 91/414/EEC, and in compliance with 40 CFR Part 160 GLP standards. The test system consisted of three 150-L glass aquaria fitted with overflows to maintain a volume of 100 L at a loading rate of 70 fish per vessel. One low-dose and one high-dose aquaria were treated. A third aquarium was untreated and served as a solvent control. The exposure period lasted 28 days, and the subsequent depuration period lasted 21 days. During 28 days of exposure, the pH of the water was 7.0-7.6, the dissolved oxygen was 6.0-10.7 mg/L, and the temperature was 9-15°C. During the 21 days of depuration, the pH of the water was 7.2-7.6, the dissolved oxygen was 9.1-10.3 mg/L, and the temperature was 12-15°C. Four fish and 600-1000 mL of water were collected on days 1, 3, 7, 14, 21, and 28 of exposure. Additional fish (number unspecified) were collected from the high-dose aquarium on day 28 for metabolite analysis. Four fish were collected from each aquarium on days 1, 3, 7, 14, and 21 of depuration. Water was extracted three times with ethyl acetate. High-dose fish were extracted three times with hexane, acetonitrile, acetonitrile:water (1:1; v:v), and water. Water and fish tissue extracts were analyzed for [¹⁴C]chlorothalonil and its transformation products using normal and reverse phase one-dimensional TLC and HPLC, and were identified by comparison to reference standards of chlorothalonil and its transformation products.

In fish exposed at 0.1 µg/L, the maximum concentration of total [¹⁴C]residues was 0.03, 0.68, and 0.36 µg/g in the edible, non-edible, and whole fish tissue, each at 28 days of exposure. The calculated bioconcentration factors (BCF) are 256, 5812, and 3077 for the edible, non-edible, and whole fish tissues, respectively. After 1 day of depuration, total [¹⁴C]residues in whole fish tissues had decreased by 31% from the 28-day exposure values. After 21 days of depuration, total [¹⁴C]residues declined to 0.008 µg/g. The calculated depuration half-lives ($t_{1/2}$) are 13, 7.7, and 8.0 days for edible, non-edible, and whole fish tissues, respectively. [¹⁴C]Residues in the fish during exposure and depuration were not characterized. The concentration of chlorothalonil in the aquarium water during exposure ranged from 33 to 92% of the recovered.

In fish exposed at 0.5 µg/L, the maximum concentration of total [¹⁴C]residues was 0.15 µg/g in edible tissue at 21 days, 2.79 µg/g in the non-edible tissue at 28 days, and 1.49 µg/g in the whole fish tissue, at 28 days of exposure. The calculated bioconcentration factors (BCF) are 306, 5694, and 3041 for the edible, non-edible, and whole fish tissues, respectively. In the edible and non-edible tissue (viscera and skeleton), [¹⁴C]chlorothalonil was not detected during the 28 days of exposure. In the edible tissue, two transformation products were identified. The triglutathione conjugate ($R_f = 0.33$) was 0.7% of the recovered (<0.001 µg/g), and the diglutathione conjugate ($R_f = 0.38$) was 3.9% of the recovered (0.004 µg/g). Four peaks were unidentified; $R_f = 0.20$ was 3.7% of the recovered (0.004 µg/g), $R_f = 0.47$ was 1.0% (0.001 µg/g), $R_f = 0.68$ was 2.9%

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(0.003 µg/g), "others" (not further characterized) were 3.8% (0.004 µg/g), and polar components were 28.1% of the recovered (0.028 µg/g). In the non-edible tissue (viscera), [¹⁴C]chlorothalonil was not identified after 28 days of exposure. Three transformation products were identified in the edible tissue: the triglutathione conjugate ($R_f = 0.33$) was 9.1% of the recovered (1.27 µg/g), the diglutathione conjugate ($R_f = 0.38$) was 18.8% (2.63 µg/g), and 4-hydroxy-2,5,6-trichloro-1,3-dicyanobenzene was 3.9% of the recovered (0.55 µg/g). Five peaks were unidentified, each at ≤9.9% of the recovered (1.39 µg/g). "Others" and polar components were ≤9.8% of the recovered (1.37 µg/g). Non-extractable residues were 15.2% of the recovered (2.13 µg/g). In the skeleton, two transformation products were identified. The triglutathione conjugate ($R_f = 0.33$) was 1.5% of the recovered (0.01 µg/g) and the diglutathione conjugate ($R_f = 0.38$) was 9.6% of the recovered (0.08 µg/g). Four peaks were unidentified: $R_f = 0.20$ was 5.0% of the recovered (0.04 µg/g); $R_f = 0.47$ was 3.1% (0.02 µg/g); $R_f = 0.68$ was 1.0% (<0.01 µg/g), "others" (not further characterized) were 4.5% (0.04 µg/g); and, polar components were 28.2% of the recovered (0.22 µg/g).

After 1 day of depuration, total [¹⁴C]residues in whole fish tissues had decreased by 35% from the 28-day exposure concentration. After 21 days of depuration, total [¹⁴C]residues declined to 0.051 mg/kg. The calculated depuration half-lives ($t_{1/2}$) are 9.4, 7.0, and 7.1 days for edible, non-edible, and whole fish tissues, respectively.

The concentration of chlorothalonil in the aquarium water during exposure ranged from 39-97% of the recovered.

Total lipid content in edible tissue averaged 5.4% at day 0 and ranged from 1.7-2.7% on exposure day 28 in the control and exposure aquaria.

Study Acceptability: This study is classified as **acceptable**. The study is scientifically valid, and can be used to fulfill guideline requirements for bioaccumulation in fish.

MATERIALS AND METHODS

Rainbow trout (*Oncorhynchus mykiss*, Parkwood Trout Farm, Kent, UK; ca. 1 g in weight) were acclimated in holding tanks prior to the initiation of the preliminary and definitive experiments (p.12). Descriptions of the test fish and acclimatization period were not reported in the Materials and Methods section of the study report. The experimental protocol stated that the test fish would be 8 ± 4 cm in length and <1 year of age, and would be acclimated for ≥14 days in fiberglass tanks (≥100 L) maintained at 12-15°C under a 16-hour light:8-hour dark cycle (Appendix 1, p.46). Aerated, dechlorinated laboratory tap water (hardness 150-200 mg/L as CaCO₃; pH 6.5-8.0) was supplied to the fiberglass tanks. The fish were fed commercial trout feed (pellet form) twice daily. Fish mortality was monitored for 48 hours after arrival and fish were accepted only if mortality was <5% of the population (p.12).

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A preliminary experiment was conducted to determine whether the fish could tolerate the high dose, and to determine whether "suitable" exposure concentrations could be achieved (p.12). Two aquaria were treated with [benzonitrile-U-¹⁴C]2,4,5,6-tetrachloro-1,3-benzenedicarbonitrile (chlorothalonil; radiopurity >96%; specific activity 178.6 $\mu\text{Ci}/\text{mg}$; Batch No. 1021), dissolved in methanol, at a nominal concentration of either 0.1 $\mu\text{g}/\text{L}$ or 0.5 $\mu\text{g}/\text{L}$ (pp.11-12; Appendix 8, p.82). A third aquarium was treated with methanol only to serve as the control. The stock solutions were dispensed into syringes of the test system infusion pumps, and were mixed with inflowing tank water at a rate of 1.75 mL/hour. Water samples were collected from the high-dose aquarium at regular intervals (not reported) to determine the test substance concentration. Once the required test concentrations were achieved, 30 fish were added to each aquarium. The fish were monitored for mortality over a period of 17 days (p.10). It was stated that higher mortality (number unspecified) was observed in both treatment tanks compared to the controls. The study authors concluded that the high dose was acceptable for use in the definitive experiment, since mortality did not substantially differ between the low- and high-dose aquaria.

For the definitive experiment, flow-through aquatic exposure systems were prepared using three 150-L glass aquaria fitted with overflows to maintain a volume of 100 L (pp.11, 12). Dechlorinated tap water (hardness 100-200 mg/L as calcium carbonate) was supplied to the aquaria at a rate of approximately 7 turnovers/day. Two aquaria were treated with [benzonitrile-U-¹⁴C]2,4,5,6-tetrachloro-1,3-benzenedicarbonitrile (chlorothalonil; radiopurity >96%; specific activity 178.6 $\mu\text{Ci}/\text{mg}$; Batch No. 1021), dissolved in methanol, at a nominal concentration of either 0.1 $\mu\text{g}/\text{L}$ or 0.5 $\mu\text{g}/\text{L}$ (pp.11-12; Appendix 8, p.82). The solutions were dispensed into syringes of the test system infusion pumps (Figure 1, p.32). The third aquarium was treated with methanol and served as the control. The aquaria were equilibrated for an unspecified length of time prior to the addition of the fish.

Following the acclimatization period, 70 fish were transferred into each aquarium (p.12). At study initiation, the fish weighed approximately 1 g. Water quality (pH, oxygen content, and temperature) was measured daily (Appendix 7, pp.77-80). During 28 days of exposure, the pH of the water was 7.0-7.6, the dissolved oxygen was 6.0-10.7 mg/L, and the temperature was 9-15°C. Fish were maintained under a 16-hour light/8-hour dark cycle (p.11). During the entire study, the fish were given commercial trout feed twice daily. Four fish were collected on days 1, 3, 7, 14, 21 and 28 of exposure. Additional fish (number unspecified) were collected on day 28 for metabolite identification (p.13). Water samples (600-1000 mL) were collected on days 1, 3, 7, 14, 21, and 28 of the exposure period (Table 3, p.26). Fish were analyzed for lipid content on days 0 and 28 of the exposure period.

Following the 28-day exposure period, the remaining fish in each aquarium were transferred to corresponding "clean" flow-through aquaria for 21 days (p.12). During the 21 days of depuration, the pH of the water was 7.2-7.6, the dissolved oxygen was 9.1-10.3 mg/L, and the temperature was 12-15°C (Appendix 7, pp.77-80). Four fish were collected from each aquarium on days 1, 3, 7, 14, and 21 of depuration (p.13).

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Fish: At the time of sampling, the fish were dried, weighed, and separated into fillet/edible and viscera plus skeleton/non-edible tissues (p.13). The tissues were stored frozen for 4-6 months until processing. Samples of the edible and non-edible tissues were homogenized using scissors and total radioactivity was analyzed using LSC following combustion. The oxidizer efficiency was >95%. No correction was made for the oxidizer efficiency (p.14).

Representative pools of the high-dose fillet/edible and viscera and skeleton/non-edible tissues were extracted and fractionated in order to characterize and identify [¹⁴C]residues in the tissues (p.13). The extraction and fractionation schemes for the analysis of residues in high-dose fillet, viscera, and skeleton are presented in Figures 5a-5c of the study report (pp.36-38). The samples were extracted three times using a tissue homogenizer with hexane, acetonitrile, acetonitrile:water (1:1, v:v), and water. The extracts were centrifuged at 4000 rpm for 10 minutes, and total radioactivity was determined using LSC. All extracts except the hexane extracts were combined, concentrated by rotary evaporation, and analyzed by normal and reverse phase one-dimensional TLC. Normal phase TLC was conducted using Merck Kieselgel 60 F254 (0.25 mm) TLC plates developed in hexane:ethyl acetate:acetic acid (90:10:1, v:v:v; Solvent System 1); hexane:methylene chloride (1:1, v:v, Solvent System 2); acetonitrile:water (7:3, v:v; Solvent System 6); and, chloroform:ethyl acetate:formic acid (5:4:1, v:v:v; Solvent System 8; pp.16-17). Reverse phase TLC was conducted using Whatman LKC18F (0.2 mm) TLC plates developed in methanol:water (9:1, v:v; Solvent System 3). Radioactive areas on the plates were detected using a linear analyzer or autoradiography. The samples were cochromatographed with reference standards, which were visualized by quenching of a fluorescent indicator under UV light (wavelength not reported). Sample extracts from the high-dose fillet, viscera, and skeleton samples were also analyzed using HPLC operated under the following conditions (p.17):

Column	Zorbax or RX-C8 column (25 cm x 4.6 mm, particle size not reported)
Mobile phase	A: 2 mM Ammonium formate (pH 3-4) B: Acetonitrile
Gradient (A:B, v:v)	Time (min.) %A %B 0 95 5 25 0 100 30 0 100 35 95 5 40 95 5
UV detector	254 nm
Radiochemical detector	β-Ram-2A
Flow rate	1.5 mL/min

Fractions were collected and analyzed using LSC. [¹⁴C]Chlorothalonil and its transformation products were identified in the samples based on comparisons of their retention times to the

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retention time of unlabeled reference standards that were co-injected with the samples (Table 8, p.31). The limits of detection and quantification for HPLC analyses were not reported.

Due to high levels of radioactivity in the high-dose fillet and skeleton samples, these tissues were further fractionated with 0.1M NaOH overnight (p.21; Figure 5c, p.38). The extracts were reduced under a stream of nitrogen, and the base extracts were partitioned with ethyl acetate at pH 7 (fillet only) and pH 2, and analyzed by LSC.

Lipid determination: In order to determine the percent lipid during the exposure period, edible and non-edible tissues (2-10 g) from fish collected from the culture tank on days 0 and 28 of exposure were homogenized in chloroform:methanol (1:2, v:v; 10 mL), chloroform (10 mL), and water (10 mL; p.13). The samples were centrifuged and the chloroform layer was transferred to a tared glass vial. The chloroform was evaporated to dryness under nitrogen, and the weight of the dried residue (lipid) was determined.

Water: Duplicate aliquots of the aquaria water were analyzed for total radioactivity using LSC (p.14). Aliquots (ca. 250 mL) of the water were extracted three times with ethyl acetate (p.13). The extracts were concentrated using rotary evaporation and analyzed by TLC and HPLC as described for the fish samples.

RESULTS/DISCUSSION

[¹⁴C]Chlorothalonil residues accumulated in rainbow trout that were exposed to [benzonitrile-U-¹⁴C]2,4,5,6-tetrachloro-1,3-benzenedicarbonitrile at nominal concentrations of 0.1 µg/L (mean measured 0.12 µg/L) or 0.5 µg/L (mean measured 0.49 µg/L) for 28 days under flow-through aquarium conditions (pp.13, 19, 20). [¹⁴C]Chlorothalonil was stable in diluter stock solutions for the low- and high-dose treatment groups sampled on days -3, 2, 6, 10, 15, 18 and 23 of the exposure period. The radiochemical purities were ≥96.9% (Table 1, p.24).

In fish exposed at 0.1 µg/L, the maximum concentration of total [¹⁴C]residues was 0.03, 0.68, and 0.36 µg/g in the edible, non-edible, and whole fish tissues, respectively, at 28 days (Table 3, p.26). The calculated bioconcentration factors (BCF) are 256, 5812, and 3077 for the edible, non-edible, and whole fish tissues, respectively. Steady state concentrations were estimated to be 42 days for edible tissue and 25-26 days for the whole fish and non-edible tissues (Table 5, p.28). [¹⁴C]Residues in the fish tissues were not characterized. Based on TLC analyses, [¹⁴C]chlorothalonil comprised 96.9-98.9% of the applied radioactivity (0.12 µg/L) in the diluter stock solution, and 33-92% of the applied in the aquarium water (p.19; Table 1, p.24; Table 2, p.25).

After 1 day of depuration, total [¹⁴C]residues in whole fish tissues had decreased by 31% from the 28-day exposure values (Table 3, p.26). After 21 days of depuration, total [¹⁴C]residues in

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whole fish declined to 0.008 $\mu\text{g/g}$. The calculated depuration half-lives ($t_{1/2}$) are 13, 7.7, and 8.0 days for edible, non-edible and whole fish tissues, respectively (Table 5, p.28).

[^{14}C]Residues in the fish during depuration were not characterized. The concentration of chlorothalonil in the water during the depuration period was not measured.

In fish exposed at 0.5 $\mu\text{g/L}$, the maximum concentrations of total [^{14}C]residues were 0.15 mg/kg at 21 days in edible tissue, 2.79 mg/kg in non-edible tissue and 1.49 mg/kg in whole fish tissue, each at 28 days (Table 4, p.27). The calculated bioconcentration factors (BCF) are 306, 5694, and 3041 for the edible, non-edible, and whole fish tissues, respectively. Steady state concentrations were estimated at 31 days for edible tissue and 23 days for the whole fish and non-edible tissues. Based on TLC analyses, [^{14}C]chlorothalonil comprised 97.9-99.3% of the applied radioactivity (0.49 $\mu\text{g/L}$) in the diluter stock solution, and 39-97% of the applied in the aquarium water (p.20; Table 1, p.24; Table 2, p.25).

In the edible and non-edible tissue and skeleton, [^{14}C]chlorothalonil was not detected during 28 days of exposure (pp.20-21; Table 6, p.29; Appendix 8, p.85). In the edible tissue, two transformation products were identified: the triglutathione conjugate ($R_f = 0.33$) was 0.7% of the recovered (<0.001 mg/kg) and the diglutathione conjugate ($R_f = 0.38$) was 3.9% (0.004 $\mu\text{g/g}$). Four peaks were unidentified; $R_f = 0.20$ was 3.7% (0.004 mg/kg), $R_f = 0.47$ was 1.0% (0.001 mg/kg), $R_f = 0.68$ was 2.9% (0.003 $\mu\text{g/g}$), "others" (not further characterized) were 3.8% (0.004 mg/kg), and polar components were 28.1% (0.028 $\mu\text{g/g}$). Following fractionation of the [^{14}C]residues in the fillet, 3.6% of the total residues (0.004 $\mu\text{g/g}$) was hexane soluble, 9.8% (0.010 $\mu\text{g/g}$) was organosoluble, and 34.9% (0.035 $\mu\text{g/g}$) was aqueous soluble. In the non-edible tissue (viscera), [^{14}C]chlorothalonil was not identified during 28 days of exposure. In the non-edible tissue, three transformation products were identified: the triglutathione conjugate ($R_f = 0.33$) was 9.1% of the recovered (1.27 $\mu\text{g/g}$), the diglutathione conjugate ($R_f = 0.38$) was 18.8% (2.63 $\mu\text{g/g}$), and 4-hydroxy-2,5,6-trichloro-1,3-dicyanobenzene was 3.9% of the recovered (0.55 $\mu\text{g/g}$). Five peaks were unidentified, each at $\leq 9.9\%$ (1.39 $\mu\text{g/g}$) of the recovered; "others" (not further characterized) and polar components were $\leq 9.8\%$ of the recovered (1.37 $\mu\text{g/g}$). Non-extractable residues were 15.2% of the recovered. In the skeleton, two transformation products were identified: the triglutathione conjugate ($R_f = 0.33$) was 1.5% of the recovered (0.01 $\mu\text{g/g}$) and the diglutathione conjugate ($R_f = 0.38$) was 9.6% (0.08 $\mu\text{g/g}$). Four peaks were unidentified; $R_f = 0.20$ was 5.0% (0.04 $\mu\text{g/g}$), $R_f = 0.47$ was 3.1% (0.02 $\mu\text{g/g}$), $R_f = 0.68$ was 1.0% (<0.01 mg/kg), "others" (not further characterized) were 4.5% (0.04 $\mu\text{g/g}$) and polar components were 28.2% (0.22 $\mu\text{g/g}$). Following fractionation of the [^{14}C]residues in the skeleton, 1.2% of the total residues (<0.01 $\mu\text{g/g}$) was hexane soluble, 8.6% (0.07 $\mu\text{g/g}$) was organosoluble and 33.3% (0.26 $\mu\text{g/g}$) was aqueous soluble.

After 1 day of depuration, total [^{14}C]residues in whole fish tissues had decreased by 35% from the 28-day exposure concentration (Table 4, p.27). After 21 days of depuration, total [^{14}C]residues in whole fish declined to 0.051 $\mu\text{g/g}$. The calculated depuration half-lives ($t_{1/2}$) are 9.4, 7.0, and 7.1 days for edible, non-edible, and whole fish tissues, respectively (Table 5, p.28).

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[¹⁴C]Residues in the fish during depuration were not characterized. The concentration of chlorothalonil in the water during the depuration period was not measured.

Total lipid content in edible tissue averaged 5.4% at day 0, and ranged from 1.7-2.7% on exposure day 28 in the control and exposure aquaria (p.22).

DEFICIENCIES/DEVIATIONS

1. The study author reported that tissue samples were stored frozen and extracted and analyzed within 4-6 months of sampling (p.13). Storage stability data were not included in this study, and it is unknown if chlorothalonil is stable when stored frozen in fish tissues.
2. The parent compound did not appear to be stable in the test water during the study (Table 2, p.25 of the study report). In the low-dose aquarium, the concentration of chlorothalonil decreased from 83-92% of the applied on days 1-7 to 33-78% on days 14-28 of the exposure period (Table 2, p.25). In the high-dose aquarium, it was 95-97% of the applied on days 1-2, 39% on day 3 and 64-93% on days 7-28 of the exposure period. No explanation for the low recoveries was provided by the study author, with two exceptions. The author explained that the low recovery of parent in the water sample collected from the high-dose aquarium at 3 days (39%) was the result of the fact that the sample was stored frozen prior to analysis (p.25). The author explained that the low recovery of parent in the water sample collected from the low-dose aquarium at 14 days (33%) may have occurred as a result of additional washes that were required following concentration of the sample. Low extractability was also attributed to adsorption of the test compound to particulate matter in the tank water samples. It was not stated whether all water samples were stored frozen prior to analyses. Storage stability data for water samples were not included in the study report.
3. No fish were collected for the analysis of whole fish tissue samples. Instead, the study author calculated the levels of components present in the fish from the data for the individual fish based on a mass ratio basis of fillet:skeleton:viscera of 0.42:0.48:0.1 (p.21). The author obtained this ratio from the tissue pools at the high-dose treatment day 28 sampling interval. It is preferred that samples of whole fish tissues be analyzed separately. Summed residue data from edible and nonedible tissues may then be used as a check against concentrations found in whole fish tissue samples.
4. The 96-hour LC₅₀ for rainbow trout exposed to chlorothalonil was not reported.
5. The mortality of the test fish during the acclimatization, exposure, and depuration phases of the study were not reported.
6. Results for inorganic and organic analyses of the dilution water were not included in the study report.

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7. A biotransformation pathway for chlorothalonil in rainbow trout was proposed by the study author (Appendix 9, p.86). Chlorothalonil degraded to 4-hydroxy-2,5,6-trichloro-1,3-dicyanobenzene or the digluthathione conjugate, which further degraded to the triglutathione conjugate.
8. A 16-hour light:8-hour dark cycle was employed (p.11). ASTM recommends a photoperiod of 12 hours light/12 hours dark or 14 hours light/10 hours dark.
9. The reviewer noted that the chemical structure for the following pairs of reference compounds was identical: 1) 2,5-dichloro-4,6-dithioisophthalonitrile (Reference 13) and 2,5-dichloro-4,6-dimercaptoisophthalonitrile (Reference 14) and 2) 2,4,5-trichloro-6-thioisophthalonitrile (Reference 9) and 2,4,5-trichloro-6-mercaptoisophthalonitrile (Reference 15). The reviewer also noted that the chemical structure for 5-chloro-2,4,6-tri(methylthio)isophthalonitrile (Reference 10) was incorrectly illustrated. One of the three methylthio groups was presented as H₃S, instead of CH₃S.
10. Limits of detection and quantitation were not reported for LSC, TLC, and HPLC analyses of fish tissue and water samples. It is necessary that both limits of quantitation and detection be reported to allow the reviewer to evaluate the adequacy of the test method for the determination of the parent compound and its transformation products.
11. The study author noted that the reduction in total residues at days 14 and 21 of the exposure period may be the result of an infusion pump failure overnight during those days (p.20).
12. Section 9.3 of the experimental protocol refers to storage of "soil extracts and residues", rather than fish or water samples (p.50).
13. It was reported that subsamples of combined acetonitrile:water extracts from viscera were treated with 0.1M sodium hydroxide, neutralized, and analyzed by TLC/HPLC (p.13). However, Figure 5b of the study report indicates that the combined extracts were directly analyzed by HPLC and TLC without being neutralized prior treatment (p.37).
14. It was reported that the test water was maintained at 10-15°C on page 13 of the study report. However, based on the water quality characteristics table provided in Appendix 7, the water temperature was 9-15°C during the exposure period (pp.77-78).