Hepditch, Birceanu and Wilkie
Supplementary Materials

Tables S1. Measured concentrations of lampricides in water.

Nominal TFM	Measured TFM (mg/L)	Average TFM (mg/L)	Nominal Nic	Measured Nic (mg/L)	Average Nic (mg/L)	Nominal TFM/Nic (mg/L)	Measured TFM/Nic (mg/L)	Average TFM/Nic (mg/L)
Control	0.2	0.2	Control	0	0.0	Control	0.2/0	0.0
2.5	2.4	2.6 ± 0.1	0.05	0.043	0.032 ± 0.006	2.5/0.025	2.4/0.02	2.5 ± 0.2/
	2.6			0.03			2.9/0.008	0.013 ± 0.004
	2.6			0.023			2.2/0.011	
5.0	5.2	4.9 ± 0.2	0.1	0.05	0.077 ± 0.032	5.0/0.05	5.2/0.024	5.2 ± 0.0/
	4.7			0.04			5.20/025	0.025 ± 0.000
	4.7			0.14			5.1/0.025	
7.5	8.1	7.8 ± 0.4	0.25	0.22	0.26 ± 0.035	7.5/0.075	7.9/0.044	7.9 ± 0.0/
	8.3			0.33			8.0/0.046	0.049 ± 0.004
	7.1			0.23			7.9/0.056	
10.0	9.9	10.2 ± 0.2	0.5	0.41	0.407 ± 0.026	10.0/0.10	10/0.073	10.1 ± 0.2/
	10.6			0.45			10.4/0.07	0.072 ± 0.001
	10.2			0.36			9.8/0.073	
12.0	12.8	12.2 ± 0.4	1.0	0.80	0.817 ± 0.012	15.0/0.15	16.4/0.106	16.0 ± 0.2/
	11.4			0.84			16.0/0.13	0.119 ± 0.007
	12.5			0.81			15.7/0.12	
15.0	16.3	16.9 ± 0.7				25/0.25	25.6/0.196	26.6 ± 0.5/
	18.3						27.3/0.22	0.208 ± 0.007
	16.3						27.0/0.209	
25.0	26.1	26.7 ± 0.4						
	27.3							
	26.6							

Tables S2. TFM toxicity test comparisons (concentrations in mg L^{-1}).
Significance values are shown from pairwise comparisons using a Bonferroni-corrected threshold value of significance, defined here as p < 0.001785 (8 groups, number of comparisons = 28, p value of 0.05)

TFM TFM	Control	2.5	5.0	7.5	10.0	12.5	15.0	25.0
Control		0.9990	0.1513	0.9990	0.3035	0.0366	0.0082	0.0001
2.5			0.1513	0.9999	0.3035	0.0363	0.0082	0.0001
5.0				0.1513	0.5862	0.0383	0.1160	0.0001
7.5					0.3035	0.0363	0.0082	0.0001
10.0						0.1771	0.0477	0.0001
12.5							0.4407	0.0001
15.0								0.0001
25.0								

Table S3. Niclosamide toxicity test comparisons (concentrations in mg L^{-1})
Significance values are shown from pairwise comparisons using a Bonferroni-corrected threshold value of significance, defined here as p < 0.0033333 (6 groups, number of comparisons = 15, p value of 0.05)

NIC NIC	Control	0.05	0.1	0.25	0.5	1.0
Control		0.9999	0.0174	0.0001	0.0001	0.0001
0.05			0.0174	0.0001	0.0001	0.0001
0.1				0.0001	0.0001	0.0001
0.25					0.3304	0.3304
0.5						0.9999
1.0						

Tables S4. TFM/1% mixtures (MIX) toxicity test comparisons (TFM/Nic concentrations in mg L^{-1})
Significance values are shown from pairwise comparisons using a Bonferroni-corrected threshold value of significance, defined here as $p < 0.0023809$ *(7 groups, number of comparisons = 21, p value of 0.05)*

MIX MIX	Control	2.5/0.025	5.0/0.05	7.5/0.075	10.0/0.10	15.0/0.15	25.0/0.25
Control		0.9999	0.0177	0.0001	0.0001	0.0001	0.0001
2.5/0.025			0.0174	0.0001	0.0001	0.0001	0.0001
5.0/0.05				0.0060	0.0001	0.0001	0.0001
7.5/0.075					0.0006	0.0001	0.0001
10.0/0.10						0.0001	0.0001
15.0/0.15							0.9999
25.0/0.25							

Table S5. TFM vs. TFM/1% mixtures (MIX) toxicity test comparisons (TFM/Nic concentrations in mg L^{-1})
Significance values are shown from pairwise comparisons using a Bonferroni-corrected threshold value of significance, defined here as p < 0.0006 (13 groups, number of comparisons = 78, p value of 0.05)

MIX TFM	Control	2.5/0.025	5.0/0.05	7.5/0.075	10.0/0.10	15.0/0.15	25.0/0.25
Control							
2.5		0.9999					
5.0			0.2124				
7.5				0.0001			
10.0					0.0001		
15.0						0.0001	
25.0							0.0001

Table S6. NIC vs. TFM/1% mixtures (MIX) toxicity test comparisons (TFM/Nic concentrations in mg L^{-1})
Significance values are shown from pairwise comparisons using a Bonferroni-corrected threshold value of significance, defined here as p < 0.0083 (4 groups, number of comparisons = 6, p value of 0.05)

MIX NIC	Control	5.0/0.05	10.0/0.10	25.0/0.25
Control				
0.05		0.0308		
0.10			0.0001	
0.25				0.3384

R code used to calculate LCs for TFM, Nic and mixtures. Please refer to the raw data Excel sheet as well for the code and its associated output. Ecotox R package was used for the analysis.

```
CODE FOR TFM
data<-
TFM.12h.Data

attach(data)

m <- LC_probit((Death / Total) ~ log10(Concentration), p = c(10,25,50,99),

          weights = Total,

          data = TFM.12h.Data[TFM.12h.Data$Concentration  != 0, ])

detach(data)



CODE FOR Nic

data<-Niclosamide.Data

attach(data)

m <- LC_probit((Deaths / Total) ~ log10(Concentration), p = c(10,25,50,99),

          weights = Total,

          data = Niclosamide.Data[Niclosamide.Data$Concentration  != 0, ])

detach(data)



CODE FOR TFM LCs in combo with Nic

data<-TFM1Nic12h.Data

attach(data)

m <- LC_probit((Deaths / Total) ~ log10(Concentration), p = c(10,25,50,99),

          weights = Total,

          data = TFM1Nic12h.Data[TFM1Nic12h.Data$Concentration  != 0, ])

detach(data)



CODE FOR Nic LCs in combo with Nic

data<-TFM1Nic12h.Data.for.Nic.LCs
data

attach(data)

m <- LC_probit((Deaths / Total) ~ log10(Concentration), p = c(10,25,50,99),

          weights = Total,

          data = TFM1Nic12h.Data.for.Nic.LCs[TFM1Nic12h.Data.for.Nic.LCs$Concentration  != 0, ])

detach(data)
```

List of SOPs cited in this manuscript

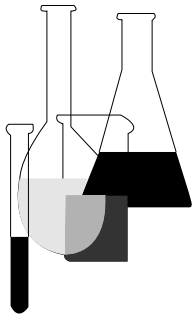
United States
Environmental Protection
Agency

Prevention, Pesticides
and Toxic Substances
(7101)

EPA 712–C–96–010
August 1996

Product Properties Test Guidelines

OPPTS 830.1670 Discussion of Formation of Impurities



INTRODUCTION

This guideline is one of a series of test guidelines that have been developed by the Office of Prevention, Pesticides and Toxic Substances, United States Environmental Protection Agency for use in the testing of pesticides and toxic substances, and the development of test data that must be submitted to the Agency for review under Federal regulations.

The Office of Prevention, Pesticides and Toxic Substances (OPPTS) has developed this guideline through a process of harmonization that blended the testing guidance and requirements that existed in the Office of Pollution Prevention and Toxics (OPPT) and appeared in Title 40, Chapter I, Subchapter R of the Code of Federal Regulations (CFR), the Office of Pesticide Programs (OPP) which appeared in publications of the National Technical Information Service (NTIS) and the guidelines published by the Organization for Economic Cooperation and Development (OECD).

The purpose of harmonizing these guidelines into a single set of OPPTS guidelines is to minimize variations among the testing procedures that must be performed to meet the data requirements of the U. S. Environmental Protection Agency under the Toxic Substances Control Act (15 U.S.C. 2601) and the Federal Insecticide, Fungicide and Rodenticide Act (7 U.S.C. 136, *et seq.*).

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OPPTS 830.1670 Discussion of formation of impurities.

(a) **Scope**—(1) **Applicability.** This guideline is intended to meet testing requirements of both the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA) (7 U.S.C. 136, *et seq.*) and the Toxic Substances Control Act (TSCA) (15 U.S.C. 2601).

(2) **Background.** The source material used in developing this harmonized OPPTS test guideline is 40 CFR 158.167 Discussion of formulation of impurities.

(b) **Information required.** The applicant must provide a discussion of the impurities that may be present in the product, and why they may be present. The discussion should be based on established chemical theory and on what the applicant knows about the starting materials, technical grade of active ingredient, inert ingredients, and production or formulation process. If the applicant has reasons to believe that an impurity that EPA would consider toxicologically significant may be present, the discussion must include an expanded discussion of the possible formation of the impurity and the amounts at which it might be present. The impurities which must be discussed are the following, as applicable:

(c) **Technical grade active ingredients and products produced by an integrated system.** (1) Each impurity associated with the active ingredient which was found to be present in any analysis of the product conducted by or for the applicant.

(2) Each other impurity which the applicant has reason to believe may be present in his product at any time before use at a level « 0.1 percent (1,000 ppm) by weight of the technical grade of the active ingredient, based on what he knows about the following:

(i) The composition (or composition range) of each starting material used to produce his product.

(ii) The impurities which he knows are present (or believes are likely to be present) in the starting materials, and the known or presumed level (or range of levels) of these impurities.

(iii) The intended reactions and side reactions which may occur in the production of the product, and the relative amounts of byproduct impurities produced by such reactions.

(iv) The possible degradation of the ingredients in the product after its production but prior to its use.

(v) Post-production reactions between the ingredients in the product.

(vi) The possible migration of components of packaging materials into the pesticide.

(vii) The possible carryover of contaminants from use of production equipment previously used to produce other products or substances.

(viii) The process control, purification and quality control measures used to produce the product.

(d) **Products not produced by an integrated system.** Each impurity associated with the active ingredient which the applicant has reason to believe may be present in the product at any time before use at a level ≥ 0.1 percent (1,000 ppm) by weight of the product based on what he knows about the following:

(1) The possible carryover of impurities present in any registered product which serves as the source of any of the product's active ingredients. The identity and level of impurities in the registered source need not be discussed or quantified unless known to the formulator.

(2) The possible carryover of impurities present in the inert ingredients in the product.

(3) Possible reactions occurring during the formulation of the product between any of its active ingredients, between the active ingredients and inert ingredients, or between the active ingredient and the production equipment.

(4) Post-production reactions between any of the product's active ingredients and any other component of the product or its packaging.

(5) Possible migration of packaging materials into the product.

(6) Possible contaminants resulting from earlier use of equipment to produce other products.

(e) **Expanded discussion.** On a case-by-case basis, the Agency may require an expanded discussion of information on impurities:

(1) From other possible chemical reactions.

(2) Involving other ingredients.

(3) At additional points in the production or formulation process.

IOP:015.4

High Performance Liquid Chromatograph

IOP:015.4

April 16, 2004

U.S. Fish and Wildlife Service
Marquette Biological Station
1924 Industrial Parkway
Marquette, Michigan 49855
U.S.A.

and

U.S. Fish and Wildlife Service
Ludington Biological Station
229 South Jebavy Drive
Ludington, Michigan 49431
U.S.A.

and

Department of Fisheries and Oceans
Sea Lamprey Control Centre
1 Canal Drive
Sault Ste. Marie, Ontario
Canada

INSTRUMENT OPERATING PROCEDURE

INSTRUMENT:

High Performance Liquid Chromatograph

MODEL:

Detector; model 486 or model 2487

Pump; model 515

Injector; Rheodyne 7725i

Data Module; model 54100

Temperature Control Module; model TCM

Column Heater Module; model CHM

MANUFACTURER:

Waters Corporation

SERIAL, PROPERTY, AND DEPARTMENT NOS:

Model number	Location	Serial number	Property number	Identifying number
486 (detector)	MBS	H97486669M	386738	Unit #1
486 (detector)	MBS	H97486676M	386739	Unit #2
486 (detector)	LBS	H97486662M	386737	Unit B
2487 (detector)	MBS	G02487660M	394425	Spare
2487 (detector)	LBS	D99487941M	390369	Unit A
2487 (detector)	LBS	L01487927M	391804	Spare
2487 (detector)	SLCC	M97487886N	97-24	97-24
2487 (detector)	SLCC	M00487792M	00-31	00-31
515 (Pump)	MBS	H97515919M	1154	Unit #1
515 (Pump)	MBS	H97515917M	1155	Unit #2
515 (Pump)	LBS	H97515934M	00223	Unit B
515 (Pump)	LBS	D99515395M	00326	Unit A
515 (Pump)	LBS	L01515143M	391803	Spare
515 (Pump)	SLCC	M97515522M	97-24	97-24
515 (Pump)	SLCC	M00515913M	00-31	00-31
7725i (Injector)	MBS	3097	1159	Unit #1
7725i (Injector)	MBS	3196	1027	Unit #2
7725i (Injector)	MBS	3199	1351	Spare
7725i (Injector)	LBS	3497	00222	Unit B
7725i (Injector)	LBS	1299	00325	Unit A
7725i (Injector)	LBS	1901	--	Spare
7725i (Injector)	SLCC	4897	97-24	97-24
7725i (Injector)	SLCC	3900	00-31	00-31
54100 (Data module)	MBS	057/76S-105473	1156	Spare
54100 (Data module)	MBS	076/76S-105159	1028	Unit #2
54100 (Data module)	MBS	059/76S-105839	1260	Unit #1
54100 (Data module)	MBS	071/76S-106171	1517	Spare
54100 (Data module)	LBS	047/76S-105461	00224	Unit B

Model number	Location	Serial number	Property number	Identifying number
54100 (Data module)	**LBS**	**118/76S-105818**	**00327**	**Unit A**
54100 (Data module)	**SLCC**	**057/76S-105527**	**97-24**	**97-24**
54100 (Data module)	**SLCC**	**050/76S-106082**	**00-31**	**00-31**
TCM (Temp control)	**MBS**	**G97TCM174M**	**1158**	**Unit #1**
TCM (Temp control)	**MBS**	**D97TCM106M**	**1157**	**Unit #2**
TCM (Temp control)	**LBS**	**G97TCM187M**	**00225**	**Unit B**
TCM (Temp control)	**LBS**	**B99TCM682M**	**00323**	**Unit A**
TCM (Temp control)	**SLCC**	**H97TCM214M**	**97-24**	**97-24**
TCM (Temp control)	**SLCC**	**M00TCM384M**	**00-31**	**00-31**
CHM (Col heater)	**MBS**	**F97CHM437M**	**1158**	**Unit #1**
CHM (Col heater)	**MBS**	**F97CHM440M**	**1157**	**Unit #2**
CHM (Col heater)	**LBS**	**F97CHM439M**	**00251**	**Unit B**
CHM (Col heater)	**LBS**	**B99CHM483M**	**00324**	**Unit A**
CHM (Col heater)	**SLCC**	**M97CHM733M**	**97-24**	**97-24**
CHM (Col heater)	**SLCC**	**M00CHM954M**	**00-31**	**00-31**

SAFETY:

Before operation of instrument read all MSDS for reagents used in procedures. Follow standard and accepted laboratory safety guidelines.

PROCEDURES:

I. Set-up of Waters high performance liquid chromatograph (HPLC)

A. Instrument components

1. The high performance liquid chromatograph components are modular to facilitate transport and replacement.

2. The modules include:
   a. Temperature control system including:
      (1) Temperature control module
      (2) Column heater
   b. Model 515 HPLC pump
   c. Model 486 (2487) tunable absorbance detector
   d. Model 746 single channel data module
   e. Rheodyne 7725i injector

B. Instrument placement, power requirements, and connections

1. Place the instrument modules on a counter top away from drafts, vibrations, and direct sunlight.

2. During transport, the pathway of stainless steel tubing for the carrier solution is broken at three sites. Each connection is reestablished:
   a. At the union between the pump and injector
   b. At the pre-column between the injector and column heater
   c. At the port on the face of the detector

3. Five of the six primary modules require an AC power outlet. A power strip connected to a line-power conditioner is preferred.

4. Connect the three signal cables:
   a. The cable from the back of the column heater is connected to the temperature control module at the port labeled UNIT 1.
   b. The cable from the back of the injector is connected to the back of the data module. The keyed connector is plugged into the port labeled TIME FUNCTIONS.
   c. The cable from the back of the detector is connected to the data module. The banana connector is plugged into the port labeled CH A; side with red wire into red terminal.

5. Replace the pre-column insert.

C. Temperature control system

1. This system reduces drift in retention time due to fluctuations in ambient temperature by maintaining a constant temperature for the chromatography column.

2. Column heater
   a. Turn the **POWER** on.
   b. Allow at least 30 minutes for the temperature of the unit to stabilize before making injections.

3. Temperature control module
   a. Turn **POWER** on.
   b. Enter the desired temperature for the column:
      (1) Press **SET.**
      (2) Enter 350 and **ENTER**. This sets the operating temperature at 35.0 ^{0}C. If the setting is not entered immediately the display will revert to the temperature of the column heater.
   c. Enter an upper temperature limit:
      (1) Press **OVER**
      (2) Enter 500 and **ENTER**. This sets the maximum temperature limit at 50.0 ^{0}C. This parameter prevents overheating of the column.

D. Waters model 515 pump

1. Formulate carrier solution according to instructions in section V, and place intake tube into the carrier solution (it is preferable to formulate the carrier solution the day before use).

2. Turn **POWER** on.

3. When FLOW MENU appears, press **EDIT/ENTER** button. The flashing cursor will appear.

4. The **EDIT/ENTER** button controls the position of the cursor. Change the display from 1.000 to 0.500 and press the **MENU** button to enter the value.

5. Press the **RUN/STOP** button to start the pump. The flow rate displayed on the bottom line will show 0.500 mL and a pressure reading will be displayed.

6. Change the flow to 0.700 and enter the value with the **MENU** button. When **MENU** is pressed the flow will change to 0.700 mL. Slowly continue to raise the flow rate in 0.2 or 0.3 mL increments with the **UP** and **DOWN** arrows and the **MENU** button until the flow is 2.000 mL for niclosamide/1.0 mL for TFM.

E. Waters model 486 tunable absorbance detector

1. Turn **POWER** on.

2. The detector will require several minutes to conduct a series of self-checks on systems and calibrations on the light source. The detector is ready to accept commands when ABSORBANCE and WAVELENGTH are displayed.

3. Confirm that the detector sensitivity is set at 1 (2 for model 2487):
   a. Press **AUFS.**
   b. A sensitivity of 1.00 will be displayed.

4. Set the operating wavelength:
   a. Press **8.**
   b. Enter the appropriate wavelength:
      (1) niclosamide: 330 and **ENTER**
      (2) TFM: 295 and **ENTER**

F. Waters model 746 single channel data module

1. Turn **POWER** on.

2. When ready the data module will display:
   128K RAM
   DATE TIME
   REPLAY SOFTWARE NOW ACTIVE

3. Press the **AUTO ZERO** On the detector.

4. Press **SHIFT, LCD STATUS** on the data module to clear the display.

5. Set the operating parameters:
   a. Set baseline offset: OF=10 **ENTER**
   b. Set attenuation: AT=2 **ENTER**
   c. Set chart speed: CS=1 **ENTER**

d. Set peak threshold: Press the **PT EVAL** function key. After about 1 to 2 minutes a threshold value (PT) will be set automatically and listed on the display. This procedure may be used as a check for background stability. If the resulting number is >100 the unit should be allowed to run until stability is attained. An alternate procedure is to set the PT value at a standard value of 30, but this should only be done when the background absorbance is stable.

e. Set retention time window: CW=0.15 **ENTER**

6. Press **SHIFT, LCD STATUS** on the data module to clear the display.

7. Inject a sample of lampricide-free stream water (see section II.B. for procedure), and plot the chromatogram for about 8 minutes. Terminate the plot by pressing **INJ/END A**. The plot of the stream water blank will aid in identification of the lampricide peak.

8. Inject a standard (see section VI. for procedure for formulation of standards; see section II.B. for injection procedure). Stop the run after the niclosamide or TFM peak has been plotted by pressing the **INJ/END A** function key. The data from the standard is stored in a numbered BIN. The number of the BIN along with information on the standard will be displayed.

9. Establish a dialog:

a. Press **DIALOG** to establish a dialog with the data module:

(1)	Display:	ENABLE BASELINE DRAWING? [Y/N](N)
	Respond:	Y **ENTER**
(2)	Display:	STORAGE MENU? [Y,N](N)
	Respond:	N **ENTER**
(3)	Display:	FUNCTION NUMBER [0-10](0)
	Respond:	**ENTER**
(4)	Display:	FILE NAME () ="
	Respond:	Appropriate stream name **ENTER** (Use up to 25 characters; this will be displayed at the top of each sample report)
(5)	Display:	TIME FUNCTION VALUE TT=
	Respond:	Time functions are entered through this table. To enter an "end run" command: TT= Check retention time of peak of interest on plot of standard and type in an end run time and **ENTER** TF= Type in **ER ENTER** (specifies END RUN function) TV= Type in **1 ENTER** (value of 1 signifies "yes") To enter an “auto zero” command (to automatically zero the baseline before each chromatogram) TT= **0.1 ENTER** TF= **AZ ENTER** TV= **1 ENTER** To enter an “integration inhibit” command (to eliminate the integration of unknown peaks preceding the peak of interest) TT= **0 ENTER** TF= **II ENTER**

TV= **1 ENTER**
TT= Type in a time about 0.5 minutes less than the retention time of the peak of interest and **ENTER**
TF= **II ENTER**
TF= **0 ENTER**
TT= **ENTER** (exit time function table)

10. Establish a method:
    a. Display: METHOD NUMBER [0,1,2,5](0) MN=
       Respond: 5 **ENTER** (This specifies the external standard method)
    b. Display: IF NV=0 THEN NO CALIB
       IF NV=1 THEN NORMAL CALIB
       IF NV>1 THEN MULTI-LEVEL CALIB
       NUMBER OF LEVELS [0-26](0) NV=
       Respond: 1 **ENTER** (this designates the type of calibration used; the number 1 indicates a single-point calibration)
    c. Display: INJECTIONS/LEVEL (1) =
       Respond: 3 **ENTER** (this indicates 3 injections of the standard)
       Display: TOTAL CALIB INJECTIONS, RC=3 (this indicates that 3 injections of standard will be made).

11. Establish a component table
    a. Display: COMPONENT TABLE...
       RET TIME CONC NAME
       RT=
       Respond: RT= type in retention time of standard **ENTER**
       CC= type in concentration of standard **ENTER**
       CN=" type in name of lampricide (Bayluscide or sp)
       RT= **ENTER** (ends input for component table)
    b. Display: EXPECTED CONC [Y,N] (N)
       Respond: **ENTER**

12. Establish a sample table.
    a. Display: RRT REF PEAK: RP(I)=
       Respond: **ENTER**
    b. Display: SAMPLE TABLE...
       ANALYST ( ) AN="
       Respond: Type in name of analyst **ENTER**
    c. Display: INJECTIONS/SAMPLE [0-254](0) RA=
       Respond: 1 **ENTER**
    d. Display: SAMPLES BETWEEN CALIB [0-254] (0) CI=
       Respond: **ENTER**
    e. Display: CONC UNITS () CU="
       Respond: Type in UG/L (for Bayluscide analyses) or MG/L (for TFM analyses) **ENTER**
    f. Display: SAM IX NAME SAM AMT SCALE
       SI=
       Respond: 1 **ENTER** (refers to sample INDEX found on each chromatogram)
    g. Display: SN="
       Respond: Sample name **ENTER** (up to 10 characters may be used to name the sample)
    h. Display: SA=

Respond: **ENTER**

I. Display: XF=
Respond: **ENTER**

j. Display: SI=
Respond: **ENTER** (ends dialog)

k. Display: END OF DIALOG
Respond: Press **LCD STATUS**

II. Calibration

A. The HPLC is calibrated with a single-point external standard method for both niclosamide and TFM analyses.

B. Load standard into the injector.

1. Fill the 500 uL syringe with standard solution. Insure that no bubbles are in the barrel of the syringe.

2. Turn the **LOAD/INJECT** handle to LOAD.

3. Insert the syringe needle into the loading port. A slight resistance is felt when the insertion is nearly complete. This results from the needle passing through the Teflon seal in the injector.

4. Dispense the standard into the injector. A volume of carrier solvent greater than the volume of the sample loading loop will be expelled from the injector sample loop through the vent tubes.

5. Move the **LOAD/INJECT** handle to INJECT, then remove the syringe.

C. Load and inject a second sample of the standard. See I.F.10.c. to determine the total number of standard injections designated for calibration. Generally, a third replicate will be injected.

1. The word CALIB will appear on each report between INDEX and BIN if the run is being analyzed as a standard.

2. The data module averages the response factors (RF) of all injections designated as standards to produce a detector RF on which calculations of concentrations of lampricide in unknowns are made.

3. Inject a sample of standard to check the calibration. The standard must be reported within $\pm$ 5% to be considered acceptable. A value $\pm$ 2% is highly desirable.

D. Recalibration

1. Additional calibrations can be performed at any time.

2. A calibration run is initiated by pressing the **CALIB** function key.
   a. Display: CALIB-
      [1=ON, 0=OFF] (0.)
      Respond: 1 **ENTER**
   b. The next run is designated as a calibration run.
   c. During the calibration run CALIB is displayed in LCD STATUS.

3. The calibration run is terminated by pressing the **CALIB** function key.
   a. Display: CALIB-
      [1=ON, 0=off] (1.)
      Respond: 0 **ENTER**
   b. The calibration run is complete.

4. Results of the calibration run replace those of the previous calibration. A new retention time and a new response factor are stored. The calculations of concentrations in subsequent unknown samples are based on the new values.

5. If response factor produced by the new calibration varies significantly from the previous response factor, repeat the calibration procedure to confirm the results.

III. Analysis of stream samples

A. Specify a name for each sample.

1. The name or code for each sample can contain a maximum of 10 characters.

2. Sample names are entered through the SAMPLE TABLE.
   a. Press **SHIFT, LCD STATUS** to clear the display
   b. Press **SHIFT, DIALOG**
   c. Display: ANALYST (name) AN="
      Respond: **ENTER**
   d. Display: INJECTIONS/SAMPLE [0-254](1.) RA=
      Respond: **ENTER**
   e. Display: SAMPLES BETWEEN CALIB [0-254](0.) CI=
      Respond: **ENTER**
   f. Display: CONC UNITS (UG/L) CU=
      Respond: **ENTER**
   g. Display: SAM IX NAME SAM AMT SCALE
      SI=
      Respond: SI= 1 **ENTER**
      SN= name or code for sample
      SA= **ENTER**
      XF= **ENTER**
      SI= **ENTER** (to exit SAMPLE TABLE)

3. Enter a sample name before each injection. If no sample name is entered the name of the previous sample will be repeated on the analysis report.

B. Inject the sample.

1. Fill the 250 uL syringe. Eliminate all bubbles by slowly drawing sample into the syringe and quickly expelling the sample to rinse.

2. Turn the **LOAD/INJECT** handle to LOAD.

3. Insert the syringe needle into the loading port and dispense the sample.

4. Turn the **LOAD/INJECT** handle to INJECT.

5. If an END RUN command has been entered the analysis run will end automatically. If no END RUN command has been entered the analysis run is terminated by pressing **INJ/END A.**

C. Analysis report: Each report generated after the analysis of a standard or unknown sample contains two parts, the chromatogram and data report.

1. Chromatogram
   a. A chromatogram is a graphic representation of absorbance of light through a given period of time.
   b. The primary features of interest on a chromatogram are:
      (1). Baseline absorbance: The normal response of the detector to the carrier solvent.
      (2). Absorbance peaks: The response of the detector to the absorbance of a specific wavelength of light by specific substances in a sample.
      (3). Retention times: The time in minutes between injection of a sample and absorbance maxima of a substance in the sample. The retention time is displayed along the side of a peak.
      (4). Drawn baseline: Representation of the way the data module estimates the position of the baseline under the peak during integration.

2. Data report
   a. The data report contains the numerical results of an analysis run.
   b. The primary parts of the data report are:
      (1). Header: Contains identifying and descriptive information on the sample, sample analysis, and data storage.
      (2). Results section: Contains data describing the peak of interest and the calculated concentration of lampricide in the sample (except calibration runs).

IV. Additional information and procedures

A. The data module communicates with codes. Each parameter is assigned a two-character operating code. A complete list of codes is found in the Waters 746 Data Module Operator's Manual in Appendix A Code Tables. The codes used most often follow:

1.	PW	Peak width
2.	OF	Baseline offset
3.	CS	Chart speed
4.	AT	Attenuation
5.	ER	End run
6.	PT	Peak threshold
7.	RT	Retention time
8.	RF	Response factor
9.	CW	Component window
10.	T1	Time before which peaks are not reported
11.	RA	Replicate analysis

12. II Integration inhibit

13. PH Peak height request

14. AZ Auto zero

B. Reprocessing a chromatogram

1. In certain cases the accuracy of analyses is improved by changing integration parameters. Concentrations then can be recalculated by reprocessing chromatograms.

2. Although infrequently done, chromatograms can be reprocessed by following the procedure outlined in the instrument manual.

C. Determination of concentration by peak height method

1. An interference such as a peak co-eluting with the peak of interest can produce measurement errors if complete separation cannot be attained. The use of peak height rather than peak area for quantification may produce better results.

2. Peak heights can be measured and stored if automatic parameters are used and if the PH parameter value is set at 1 or 2 prior to running the analysis.

a. When PH is set at 1 a report of peak heights is produced.
b. When PH is set at 2 a report of peak areas is produced but peak height data are saved.
c. When PH=2, peak height information can be recalled by entering PH=1 and pressing **RECALC** or **REPORT.**

3. The integrator is calibrated on 3 standard solutions when using peak heights. This minimizes the effect of a co-eluting substance.

V. Formulation of carrier

A. Preparation of acetic acid; 0.2 Molar:

1. Add about 200 mL distilled water (HPLC grade) to a 250 mL volumetric flask.

2. Dispense 2.9 mL glacial acetic acid into the volumetric flask.

3. Fill to the 250 mL mark with distilled water.

4. Label the volumetric flask.

B. Preparation of sodium acetate; 0.2 Molar:

1. Weigh 2.72 g sodium acetate (anhydrous), also known as acetic acid sodium salt, CH_3CO_2Na.

2. Add the sodium acetate to about 80 mL distilled water (HPLC grade) in a 100 mL volumetric flask.

3. Shake until the sodium acetate is dissolved, then fill the flask to the 100 mL mark with distilled water.

4. Label the volumetric flask.

C. Formulation of buffer; 0.01 Molar:

1. Add 41.0 mL of 0.2 Molar acetic acid and 9.0 mL of 0.2 Molar sodium acetate to a 1000 mL volumetric flask.

2. Fill to the 1000 mL mark with distilled water (HPLC grade).

3. Label the volumetric flask.

D. Formulation of carrier solution:

1. Add 220 mLs 0.01 Molar buffer solution to the carrier solvent reservoir.

2. Add 780 mLs methanol (HPLC grade) to the reservoir and mix (for TFM analysis the ratio will be 330 mLs 0.01 Molar buffer: 670 mLs methanol).

3. The sum of the volumes will be less than 1000 mL.

4. Larger volumes of carrier are made by adding buffer and methanol to the reservoir in the same proportion.

5. The solution should set over night with the cap open to allow degassing.

6. Label the container with % methanol, % buffer, date, and initials of formulator.

VI. Formulation of lampricide standards

A. Bayluscide standard

1. The number of niclosamide standards required for analysis is determined along with the proper concentration of each standard. The range of concentrations in the working standards includes and brackets the concentrations of niclosamide expected in the unknown water samples.
   a. A single standard may be used for analysis of stream water samples. The response for concentrations of niclosamide in the normal working range is linear.
   b. A series of three or more standards may be used as a check for precision of formulation of working standards.

2. For preparation of the working standards a sufficient volume (approximately 2000 mL) of stream water is filtered through Whatman 2V filter paper to remove solids that may cause interference during analyses (TOP:019.x).

3. A 0.50 mL sample of the 100 mg/L niclosamide field standard is dispensed into a 1000 mL volumetric flask which is filled to the volume mark with filtered stream water. An HPLC syringe may be used to dispense the standard. The precision measurement provided by the syringe will assure accuracy. Any syringe used to dispense field standard should be used ONLY to formulate standards and must be marked appropriately. The flask is stoppered and mixed. This dilution provides a 50 ug/L working standard.

4. The volumetric flask is labeled with date prepared, site from which the stream water was collected, concentration of niclosamide, and field standard code number.

B. TFM standard (50.0 ug/L)

1. Filter about 0.5 L stream water through Whatman 2V filter paper.

2. Dispense 5.0 mL of 1.0 mg/L field standard into a 100 mL volumetric flask. Fill to the 100 mL mark with filtered stream water.

3. Label the flask; include the code number of the TFM field standard used.

VII. Quality assurance and documentation

A. Each HPLC system is assigned a log book (Attachment). Every time the system is used an entry is made which documents use.

1. The date, time, stream, and initials of the operator are recorded in the OPERATION LOG.

2. The response of the instrument also is recorded in the OPERATION LOG for comparison with past and future applications. Finally, the number of injections completed each day is recorded.

3. Maintenance on any component of the system is recorded; any component substitution is noted on the COMPONENT LIST.

B. All persons who operate the instrument receive training in analytical procedures. The trained operators sign the INITIALS page in each of the HPLC log books.

C. Results of analyses are recorded on a Lampricide Analysis data form. These forms provide a permanent record of results.

MAINTENANCE:

The Waters 2487 detector requires replacement of the "keep alive" battery. If not replaced at least annually, an error screen will be displayed during the systems check. This screen cannot be by-passed. Replace the 3 volt watch battery by the following these steps:

Note: to ensure safety, ensure that the system is unplugged before opening the detector.

I. Open the detector

A. Remove the four Phillips-head screws located on the bottom edge of the detector cover (two on each side.

B. Slide the metal outer cover off from the detector.

II. Locate the battery

A. Locate on the mother board which sits upright on the right side of the detector.

B. The battery is located near the middle of the mother board and is held in place by a metal clip. The battery is silver, flat, and about the size of a quarter.

III. Replace the battery

A. Any 3-volt replacement battery (Waters catalog number WAT080443) will work. Replacements may be purchased at any electronics store.

B. Lift the clip and place the new battery into position, noting the orientation of the old battery (a + sign should be visible when properly installed.

IV. Close the unit

A. Slide the metal detector cover back in place.

B. Replace the four screws.

V. Recalibrate the unit

A. Turn the detector on and wait for the unit to warm up (about five minutes).

B. Press shift/3 (calibrate).

C. "Is the cuvette removed from its holder, and the flow cell prepared as required?" will be displayed

D. Select the "ENTER" option.

E. After the instrument completes internal checks and restorations "calibration successful" will be displayed.

F. Select the "ENTER" option.

G. The normal operating screen will appear.

H. Perform a methanol flush to the system to complete the calibration.

Additional maintenance that can be conducted in the field is limited. Refer to the company manuals for guidance in troubleshooting or routine procedures.

REFERENCE:

Waters 746 Data Module Operator's Manual

Rheodyne 7725i Manual Injector Installation, Maintenance, and Troubleshooting Guide

Waters 486 Tunable Absorbance Detector Operator's Manual

Waters 515 HPLC Pump Operator's Guide

Guide to Successful Operation of Your LC System

This procedure has been reviewed and approved by the undersigned representatives of the U.S. Fish and Wildlife Service and Fisheries and Oceans Canada.

REVIEWED/APPROVED_____ DATE_____
Field Supervisor (U.S.)

REVIEWED/APPROVED_____ DATE_____
Division Manager (Canada)

ATTACHMENT

Log Book for Operation of High

Performance Liquid Chromatograph

OPERATION LOG BOOK

Including Instrument Operating Procedures

HIGH PERFORMANCE

LIQUID CHROMATOGRAPH

WATERS CORPORATION

UNIT NUMBER _____

IOP: 015.0

IOP:015.0
April 16, 1999

HPLC OPERATION LOG

HPLC use, maintenance, and response record

Date	Time	Initials	Stream/Location	Std. conc.	Response	Remarks/maintenance

IOP:015.0
April 16, 1999

LIST OF HPLC COMPONENTS

List of HPLC system components and identification numbers

Component/Model	Serial Number	Date installed/initials	Date removed/initials	Remarks

DOCUMENTATION OF TRAINED OPERATORS AND INITIALS

All persons trained in operation of this system must sign and initial

IOP:012.3
IOP:012.3B
IOP:012.2C
IOP:012.0D

Spectrophotometers

IOP:012.3

Hach DR/2000 Spectrophotometer

IOP:012.3

April 16, 2003

U.S. Fish and Wildlife Service
Marquette Biological Station
1924 Industrial Parkway
Marquette, Michigan 49855
U.S.A.

and

U.S. Fish and Wildlife Service
Ludington Biological Station
229 South Jebavy Drive
Ludington, Michigan 49431
U.S.A.

and

Department of Fisheries and Oceans
Sea Lamprey Control Centre
1 Canal Drive
Sault Ste. Marie, Ontario
Canada

INSTRUMENT OPERATING PROCEDURE

INSTRUMENT:

Spectrophotometer

MODEL:

DR/2000

MANUFACTURER:

Hach

SERIAL AND PROPERTY NOS:

Model number	Location	Serial number	Property number	Identifying number
DR/2000	MBS	960200038942	1023	1
DR/2000	MBS	960200038946	1022	2
DR/2000	LBS	940300028688	00252	1
DR/2000	LBS	950800036022	00253	2

PRECAUTIONS:

POTENTIAL INTERFERENCES:

None listed in manual

SAFETY:

No special safety precautions

PROCEDURES:

I. Background

A. The Hach DR/2000 is capable of direct readout of concentrations of TFM; however this option does not support the requirements of methods of analysis developed for line-powered spectrophotometers.

B. The procedures for analysis with the DR/2000 closely parallel those used with the Sequoia-Turner model 340 and Turner model SP-830 spectrophotometers.

1. Similarities
   a. Pre-formulated standards with concentrations of 0, 4, 8, and 12 mg/L TFM are used.
   b. The slope of the instrument response is determined through measurement of the absorbance of the TFM standards.
   c. Base/Acid measurements of background absorbance are conducted to assure accuracy of analyses.
   d. Water samples are buffered to pH ~9.0 before measurement of absorbance.
   e. Water samples are filtered before measurement of absorbance.
   f. Record keeping requirements are the same.

2. Differences
   a. The instrument wavelength setting is 400 nm versus 395 nm for dedicated, line-powered spectrophotometers.
   b. Water samples normally are not heated before analysis.

C. The accuracy of measurements depends to some degree on the comparative absorbencies of the optically-matched sample cells in each DR/2000 kit. The method of data interpretation used during TFM analyses demands that special attention is given to compensation for poor matches when they cannot be avoided.

II. Preparation

A. Set up the spectrophotometer in a shaded location. Always use the black outdoor light shield when making absorbance measurements.

B. Use line power if available, however, use of this instrument is normally reserved for situations in which line power is not available.

C. After all peripheral equipment for sample preparation is ready, press the **POWER** button. The instrument will conduct internal tests and will display **SELF-TEST**.

D. When **METHOD #** is displayed press the **SHIFT** key and then the **ABS** key. **ABS** is displayed and the instrument is then operating in the absorbance mode.

E. Adjust the wavelength control knob until the display indicates a wavelength of 400 nm. **Always approach the desired wavelength from the high side for best accuracy and repeatability**.

F. Set the unit to the **MOMENTARY** mode. This mode is used when under battery power to reduce battery use. The lamp is on only when samples are being measured if the instrument is in this mode.

1. To select this mode, press the **SHIFT** key and then the **CONFIG** key.

2. If the unit is in the **CONSTANT ON** mode press the **READ/ENTER** key to change to the **MOMENTARY** mode.

3. **MOMENTARY** will be displayed

4. Press the **CONFIG** key to exit to the **ABS** screen

G. Match the 25 mL sample cells

1. Open a new 0.0 mg/L standard and rinse and fill two factory-matched 25 mL sample cells.

2. Place the first cell in the sample cell holder with the 25 mL mark facing to the right. Press the **ZERO** key. **WAIT** will be displayed until the instrument is zero calibrated. **0.000 ABS** will appear on the display when the instrument is zero calibrated.

3. Place the second cell in the sample cell holder with the 25 mL mark facing to the right. Press the **READ/ENTER** key.

4. The sample cell should produce an absorbance $\pm$ less than 0.008 (considered an acceptable match; maximum ~ 0.05 mg/L error). If the difference is greater, clean the cell or check the absorbencies of spare sets of matched cells. If an acceptable match cannot be obtained and another matched set is not available, note the absorbance difference so absorbencies of standards can be corrected. The cell with the lesser absorbance is used as the blank.

III. Calibration–standards

A. Open the remainder of the new set of TFM standards. **Do not allow the standards to sit in sunlight** while in use and **store the standards in the dark.** If the standards are very cold (apt to fog), warm them in a water bath or in the hands.

B. Insert the cuvette that contains the blank and press zero. Wait until **0.000 ABS** is displayed.

C. Insert the 0.0 mg/L TFM standard and press **READ/ENTER**. The 0.0 standard may produce a reading other than 0.000 because of an imperfect match of cuvettes. If the cells are not suitably matched, subtract the difference due to cell mismatch (noted above) and record in log book.

D. Again insert the cuvette containing the blank and press zero. Wait until **0.000 ABS** is displayed, insert the 4.0 mg/L standard, and press the **READ/ENTER** button. Again adjust the result for differences in absorbance between blank and sample cells if

necessary. Record the corrected absorbance in the instrument log book. Repeat the procedure with 8.0 and 12.0 mg/L TFM standards

E. Divide the recorded absorbance of each standard by the concentration of TFM (mg/L; 4.0, 8.0, and 12.0). Average the results and record the mean in the log book and on the analysis data sheet.

IV. Calibration–stream water sample

A. Measure the background absorbance and B/A ratio of the stream water (TOP:018.x). **Do not interchange the cells**. Always use the same cell for the 0.0 standard (blank).

B. Prepare the TFM-free water sample for analysis

1. Add 1 mL sodium tetraborate buffer to a 250 mL stream water sample and shake.

2. Filter the sample into the cuvette with a syringe filter.

3. Adjust the sample temperature by placing the 25 mL cuvette into a water bath or by holding in the hands.

C. Insert the blank into the sample cell holder.

D. Press the **ZERO** key and wait until the instrument is zero calibrated.

E. Dry the sample cuvette with a tissue and insert into the sample cell holder.

F. Press the **READ/ENTER** key.

G. Note the measured result on the analysis form. This absorbance includes both the background absorbance of the stream water and the difference in absorbance between the blank and sample cells. **Do not correct this value for differences between cells even if it is significant.** If the difference between cells was considered significant when checked, compensation was made when producing the calibration curve.

H. Determine the B/A ratio for the stream water at the site according to procedures outlined in TOP:018.x.

V. Sample measurement

A. Collect a stream water sample containing TFM.

B. Prepare the water sample for analysis.

1. Add 1 mL sodium tetraborate buffer to a 250 mL stream water sample and shake.

2. Filter the sample into the cuvette with a syringe filter.

3. Adjust the sample temperature by placing the 25 mL cuvette into the water bath or by holding in the hands.

C. Insert the blank into the sample cell holder.

D. Press the **ZERO** key and wait until the instrument is zero calibrated.

E. Dry the sample cuvette with a tissue and insert into the sample cell holder.

F. Press the **READ/ENTER** key.

G. Record the resulting absorbance on the lampricide analysis data sheet.

H. Subtract the background absorbance (base blank on the data sheet) and record.

I. Divide the resulting absorbance by the calculated slope of the calibration curve.

J. Record the result as the concentration of TFM (mg/L) in the stream water sample.

VI. Documentation

1. Make entries into instrument log book each time instrument is used.

2. Record results of analysis on LAMPRICIDE ANALYSIS data sheet (Appendix M).

MAINTENANCE:

I. Cleaning instrument -- Methods for cleaning the spectrophotometer and sample cell are described in Section 5.1, page 43 of the instrument manual.

II. Replacing batteries -- Procedures for replacing batteries are found in Section 4.1, page 39, and Section 5.2.1, page 43 of the instrument manual.

III. Replacing Lamp -- The procedure for replacing instrument lamp is found in Section 5.2.2 on pages 43 - 44 of the instrument manual.

IV. Calibrating/adjusting lamp -- Instructions for lamp calibration are found in Section 5.3, pages 44 - 45 of the instrument manual.

V. Troubleshooting -- Section 6, page 46 - 47 of the instrument manual contains a guide for locating the cause of malfunctions in the DR/2000.

REFERENCE:

Hach DR/2000 spectrophotometer instrument manual

This procedure has been reviewed and approved by the undersigned representatives of the U.S. Fish and Wildlife Service and Fisheries and Oceans Canada.

REVIEWED/APPROVED_____ DATE_____
Field Supervisor (U.S.)

REVIEWED/APPROVED_____ DATE_____
Division Manager (Canada)

ATTACHMENTS

Log book for operation of the Hach Model DR/2000 spectrophotometer

SPECTROPHOTOMETER

HACH MODEL DR/2000

SERIAL NUMBER _____

PROPERTY NUMBER _____

UNIT NUMBER _____

LOCATION _____

Date	Time	Inits.	Stream	Std. number	mg/L	Absorb.	Slope	Remarks and maintenance

SEA LAMPREY CONTROL PROGRAM
DOCUMENTATION OF INITIALS

(All personnel trained to operate this instrument must sign and initial this form)

OPERATOR	INITIALS

IOP:012.3B

Turner SP-830 Spectrophotometer

IOP:012.3B

April 16, 2004

U.S. Fish and Wildlife Service
Marquette Biological Station
1924 Industrial Parkway
Marquette, Michigan 49855
U.S.A.

and

U.S. Fish and Wildlife Service
Ludington Biological Station
229 South Jebavy Drive
Ludington, Michigan 49431
U.S.A.

and

Department of Fisheries and Oceans
Sea Lamprey Control Centre
1 Canal Drive
Sault Ste. Marie, Ontario
Canada

INSTRUMENT OPERATING PROCEDURE

INSTRUMENT:

Spectrophotometer

MODEL:

Turner SP-830 (SM110215)

MANUFACTURER:

Barnstead/Thermolyne Corporation

SERIAL, PROPERTY, AND DEPARTMENT NOS:

Model number	Location	Serial number	Prop./Dept. number	Identifying number
SM110215	MBS	1102000211681	1404	3
SM110215	MBS	1102010980512	1514	1
SM110215	MBS	1102010980511	1520	2
SM110215	MBS	1102010221121	1521	4
SM110215	MBS	1102010328093	1513	5
SM110215	MBS	1102020107696	1575	6

Model number	Location	Serial number	Prop./Dept. number	Identifying number
SM110215	MBS	1102020107699	1576	7
SM110215	MBS	1102020107693	1577	8
SM110215	MBS	1102011087476	1578	9
SM110215	MBS	1102020107692	1579	10
SM110215	LBS	1102010221118	00367	101
SM110215	LBS	1102010221128	00368	102
SM110215	LBS	1102010758518	--	103
SM110215	LBS	1102010758511	--	104
SM110215	LBS	1102010545434	--	105
SM110215	LBS	1102010762278	--	106
SM110215	LBS	1102011087478	--	107
SM110215	SLCC	1102010868829	01-17	01-17
SM110215	SLCC	1102010868832	01-18	01-18
SM110215	SLCC	1102010868833	01-19	01-19
SM110215	SLCC	1102010221113	GLLFAS	GLLFAS
SM110215	SLCC	1102021175821	02-28	02-28
SM110215	SLCC	1102001072859	00-40	00-40

PRECAUTIONS:

POTENTIAL INTERFERENCES:

Suspended particulate matter in a sample produces an increased absorbance value.

SAFETY:

No special safety procedures are required.

PROCEDURES:

I. Installation

A. Place the instrument on a laboratory counter. Allow about two inches of space between the back panel and wall for air circulation and the power cord.

B. Place the power conditioner on the counter and plug the unit into a power outlet.

C. Connect the power cord to the instrument and to the power conditioner (voltage regulator).

D. Turn to **ON** the **POWER** switch on the left rear panel of the unit (viewed from rear). The instrument display will illuminate and show a line of four boxes.

E. Allow at least 20 minutes for instrument warm-up.

II. Controls and indicators

A. Rear panel

1. **POWER** switch: located on left rear panel (viewed from rear).

2. Fuse holders: two 1.0 amp fuses required.

3. Power socket: connection point for power cord.

B. Front panel

1. **TRANS/ABS BUTTON**: Selects between transmittance and absorbance modes.

2. **100%T/0A** button: Sets the instrument to 100% transmittance of zero absorbance.

3. **FUNCTION** button: Directs the output to RS-232C or printer interface.

4. **DISPLAY** panel: Displays %T or ABS value.

5. **WAVELENGTH** dial: sets the desired wavelength.

6. **WAVELENGTH** display: displays the wavelength.

7. Sample compartment: contains stray light filter and cuvette holder.

III. Operation

A. Complete a pre-operation lamp check.

1. Set the stray light filter to position **1**.

2. Set the wavelength to 340 nm.

3. Press the **100%T/0A** button. The display should show 100.0 in the transmittance mode and 0.000 in the absorbance mode. If so, the check is complete; enter a check mark in the **Bulb** column in the log book and proceed to III.B.

4. If the LED display shows **Err-3** or **Err-4**, the lamp has shifted during transport (see page 15 in the Operating Manual, turn the spectrophotometer off, and refer to section II. under **MAINTENANCE**).

B. The stray light filter is located in the sample compartment. Check that the wheel is set at position **2**.

C. Check the wavelength display on the face of the unit. Set the wavelength at 395 nm if different. The wavelength is set with the wavelength dial located on the right side of the unit.

D. Fill a cuvette with deionized water, dry with a tissue, and place in the cuvette holder. The mark at the top of the cuvette should face the operator. Close the sample compartment.

E. Select the **Absorbance** function by pressing the **TRANS/ABS** button. A light will be lit on the display that indicates that spectrophotometer is operating in the absorbance mode.

F. Zero the instrument by pressing the **100% T/0A** button. The display will show 0.000 when zeroed.

G. Match cuvettes that will be used in analysis. Fill a second cuvette with deionized water, wipe dry, and place in the cuvette holder with the mark at the top facing the operator. Close the sample compartment.

H. The display will indicate the sample absorbance. If the display is $\pm \leq 0.002$ units the cuvettes are matched suitably for analysis. If the difference is ≥ 0.002 units, repeat the process with additional cuvettes until a suitable match is found.

I. Conduct analyses for TFM according to TOP:018.x.

IV. Documentation

A. An instrument log book is assigned to each spectrophotometer (Attachment).

B. Each day of operation of the spectrophotometer is documented in the book.

1. Record the date, time, operator identity, and stream treatment during which the analyses will be conducted.

2. Record the identity of the set of pre-packaged TFM standards.

3. Absorbencies of the standards used for analysis are recorded.

4. The slope of the response curve is calculated and recorded.

5. The absorbance of a check standard is measured periodically during the day to confirm that instrument response has not changed.

MAINTENANCE:

I. Troubleshooting

A. A list of error codes is found on page 15 of the operating manual.

B. Maintenance procedures are found on pages 16 and 17 of the maintenance manual.

II. Realignment of the lamp

A. Set the wavelength at 330 nm and the stray light filter at position 1.

B. Press and hold down both the **TRANS/ABS** and **100%T/0A** buttons while switching on the power to the spectrophotometer. Release the buttons to test the LED display.

C. Press the **TRANS/ABS** button, F1-0 should appear on the display, followed by a 3- or 4-digit numerical value. If the value is less than 500 proceed to step D to align the lamp. If the value is greater than 500 and the error message is still being displayed the unit requires service.

D. Open the door on the right side of the spectrophotometer.

E. Move the lamp holder forward or backward to vary the position of the lamp until the highest value of light intensity is obtained. It may be necessary to rotate the lamp in the holder. When optimized the value must be >500 and may be 1000-1300.

F. Close the door and turn off the power.

G. Turn the power on again (the wavelength should still be set at 330 nm and the stray light filter at position **1**).

H. Press the **100%T/0A** button.

I. The display should show 100.0 for **TRANS** mode or 0.000 for **ABS** mode. If so, the realignment of the lamp has been successfully completed. If the error message is still displayed, replace the lamp (see page 16 of the Operating Manual). Note procedure in the **Remarks and maintenance** column of the log book and place a check in the **Bulb** column.

J. Return the unit settings to those used for lampricide analysis (see **PROCEDURES** section II.B. and II.C) and proceed with setup.

III. Documentation

A. Note in the instrument log book all instrument malfunctions.

B. Note in the instrument log book all repairs and maintenance conducted on the spectrophotometer.

REFERENCE:

Turner Model SP-830 Spectrophotometer Operating Manual

This procedure has been reviewed and approved by the undersigned representatives of the U.S. Fish and Wildlife Service and Fisheries and Oceans Canada.

REVIEWED/APPROVED_____ DATE_____
Field Supervisor (U.S.)

REVIEWED/APPROVED_____ DATE_____
Division Manager (Canada)

ATTACHMENTS

Log book for operation of the Turner Model SP-830 spectrophotometer

SPECTROPHOTOMETER

TURNER MODEL SP-830

SERIAL NUMBER _____

PROPERTY NUMBER _____

UNIT NUMBER _____

LOCATION _____

Date	Time	Inits.	Stream	Std. number	mg/L	Absorb.	Slope	Bulb	Remarks and maintenance

SEA LAMPREY CONTROL PROGRAM
DOCUMENTATION OF INITIALS

(All personnel trained to operate this instrument must sign and initial this form)

OPERATOR	INITIALS

IOP:012.2C

Hach DR/2010 Spectrophotometer

IOP:012.2C

April 16, 2003

U.S. Fish and Wildlife Service
Marquette Biological Station
1924 Industrial Parkway
Marquette, Michigan 49855
U.S.A.

and

U.S. Fish and Wildlife Service
Ludington Biological Station
229 South Jebavy Drive
Ludington, Michigan 49431
U.S.A.

and

Department of Fisheries and Oceans
Sea Lamprey Control Centre
1 Canal Drive
Sault Ste. Marie, Ontario
Canada

INSTRUMENT OPERATING PROCEDURE

INSTRUMENT:

Spectrophotometer

MODEL:

DR/2010

MANUFACTURER:

Hach

SERIAL AND PROPERTY NOS:

Model number	Location	Serial number	Property number	Identifying number
DR/2010	MBS	000900019635	1522	#3
DR/2010	MBS	000900019687	1523	#4

PRECAUTIONS:

POTENTIAL INTERFERENCES:

None listed in manual

SAFETY:

No special safety precautions

PROCEDURES:

I. Background

A. The Hach DR/2010, the factory-suggested replacement for the DR/2000, is capable of direct readout of concentrations of TFM, however, this option does not support the requirements of methods of analysis developed for line-powered spectrophotometers.

B. The procedures for analysis with the DR/2010 closely parallel those used with the Sequoia-Turner model 340 and Turner model SP-830 spectrophotometers.

1. Similarities
   a. Pre-formulated standards with concentrations of 0, 4, 8, and 12 mg/L TFM are used.
   b. The slope of the instrument response is determined through measurement of the absorbance of the TFM standards.
   c. Base/Acid measurements of background absorbance are conducted to assure accuracy of analyses.
   d. Water samples are buffered to pH ~9.0 before measurement of absorbance.
   e. Water samples are filtered before measurement of absorbance.
   f. Record keeping requirements are the same.

2. Differences
   a. The instrument wavelength setting is 400 nm versus 395 nm for dedicated, line-powered spectrophotometers.
   b. Water samples normally are not heated before analysis.

C. The accuracy of measurements depends to some degree on the comparative absorbencies of the optically-matched sample cells in each DR/2010 kit. The method of data interpretation used during TFM analyses demands that special attention is given to compensation for poor matches when they cannot be avoided.

II. Preparation

A. Set up the spectrophotometer in a shaded location. Always use the black outdoor light shield when making absorbance measurements.

B. Use line power if available, however, use of this instrument is normally reserved for situations in which line power is not available.

C. After all peripheral equipment for sample preparation is ready, press the **POWER** button. The instrument will conduct internal tests and will display **SELF-TEST**.

D. When **ENTER PROGRAM #** is displayed press the **SHIFT** key and then the **ABS** key. **P 0 ABS** is displayed and the instrument is then operating in the absorbance mode.

E. Adjust the wavelength control knob until the display indicates a wavelength of 400 nm. **Always approach the desired wavelength from the high side for best accuracy and repeatability**.

F. Match the 25 mL sample cells

1. Open a new 0.0 mg/L standard and rinse and fill two factory-matched 25 mL sample cells.

2. Place the first cell in the sample cell holder with the 25 mL mark facing to the right. Press the **ZERO** key. **ZEROING** will be displayed (flashing) until the instrument is zero calibrated. **0.000 ABS** will appear on the display when the instrument is zero calibrated.

3. Place the second cell in the sample cell holder with the 25 mL mark facing to the right. Press the **READ** key.

4. The sample cell should produce an absorbance $\pm$ less than 0.008 (considered an acceptable match; maximum ~ 0.05 mg/L error). If the difference is greater, clean the cell or check the absorbencies of spare sets of matched cells. If an acceptable match cannot be obtained and another matched set is not available, note the absorbance difference so absorbencies of standards can be corrected. **The cell with the lesser absorbance is used as the blank.**

III. Calibration–standards

A. Open the remainder of the new set of TFM standards. Do not allow the standards to sit in sunlight while in use and store the standards in the dark. If the standards are very cold (apt to fog), warm them in a water bath or in the hands.

B. Insert the cuvette that contains the blank and press zero. Wait until **0.000 ABS** is displayed.

C. Insert the 0.0 mg/L TFM standard and press **READ**. The 0.0 standard may produce a reading other than 0.000 because of an imperfect match of cuvettes. If the cells are not suitably matched, subtract the difference due to cell mismatch (noted above) and record in log book.

D. Again insert the cuvette containing the blank and press zero. Wait until **0.000 ABS** is displayed, insert the 4.0 mg/L standard, and press the **READ** button. Again adjust the result for differences in absorbance between blank and sample cells if necessary. Record the corrected absorbance in the instrument log book. Repeat the procedure with 8.0 and 12.0 mg/L TFM standards

E. Divide the recorded absorbance of each standard by the concentration of TFM (mg/L; 4.0, 8.0, and 12.0). Average the results and record the mean in the log book and on the analysis data sheet.

IV. Calibration–stream water sample

A. Measure the background absorbance and B/A ratio of the stream water (TOP:018.x). Do not interchange the cells. Always use the same cell for the 0.0 standard (blank).

B. Prepare the TFM-free water sample for analysis

1. Add 1 mL sodium tetraborate buffer to a 250 mL stream water sample and shake.

2. Filter the sample into the cuvette with a syringe filter.

3. Adjust the sample temperature by placing the 25 mL cuvette into a water bath or by holding in the hands.

C. Insert the blank into the sample cell holder.

D. Press the **ZERO** key and wait until the instrument is zero calibrated.

E. Dry the sample cuvette with a tissue and insert into the sample cell holder.

F. Press the **READ** key.

G. Note the measured result on the analysis form. This absorbance includes both the background absorbance of the stream water and the difference in absorbance between the blank and sample cells. Do not correct this value for differences between cells even if it is significant. If the difference between cells was considered significant when checked, compensation was made when producing the calibration curve.

H. Determine the B/A ration for the stream water at the site according to procedures outlined in TOP:018.x.

V. Sample measurement

A. Collect a stream water sample containing TFM.

B. Prepare the water sample for analysis.

1. Add 1 mL sodium tetraborate buffer to a 250 mL stream water sample and shake.

2. Filter the sample into the cuvette with a syringe filter.

3. If problems with cuvette fogging occur, adjust the sample temperature by placing the 25 mL cuvette into the water bath or by holding in the hands.

C. Insert the blank into the sample cell holder.

D. Press the **ZERO** key and wait until the instrument is zero calibrated.

E. Dry the sample cuvette with a tissue and insert into the sample cell holder.

F. Press the **READ** key.

G. Record the resulting absorbance on the lampricide analysis data sheet.

H. Subtract the background absorbance (base blank on the data sheet) and record.

I. Divide the resulting absorbance by the calculated slope of the calibration curve.

J. Record the result as the concentration of TFM (mg/L) in the stream water sample.

VI. Documentation

1. Make entries into instrument log book each time instrument is used.

2. Record results of analysis on LAMPRICIDE ANALYSIS data sheet (Appendix M).

MAINTENANCE:

I. Cleaning instrument -- Methods for cleaning the spectrophotometer and sample cell are described in Section 5.1.1, page 91 of the instrument manual.

II. Replacing batteries -- Procedures for replacing batteries are found in Section 4.1, page 39, and Section 5.2.1, page 92 of the instrument manual.

III. Replacing Lamp -- The procedure for replacing instrument lamp is found in Section 5.2.2 on pages 92 - 93 of the instrument manual.

IV. Calibrating/adjusting lamp -- Instructions for lamp calibration are found in Section 5.3, page 94 of the instrument manual.

V. Troubleshooting -- Section 6, page 95 - 96 of the instrument manual contains a guide for locating the cause of malfunctions in the DR/2010.

REFERENCE:

Hach DR/2010 spectrophotometer instrument manual

This procedure has been reviewed and approved by the undersigned representatives of the U.S. Fish and Wildlife Service and Fisheries and Oceans Canada.

REVIEWED/APPROVED_____ DATE_____
Field Supervisor (U.S.)

REVIEWED/APPROVED_____ DATE_____
Division Manager (Canada)

ATTACHMENTS

Log book for operation of the Hach Model DR/2010 spectrophotometer

SPECTROPHOTOMETER
HACH MODEL DR/2010

SERIAL NUMBER _____

PROPERTY NUMBER _____

UNIT NUMBER _____

LOCATION _____

Date	Time	Inits.	Stream	Std. number	mg/L	Absorb.	Slope	Remarks and maintenance

SEA LAMPREY CONTROL PROGRAM
DOCUMENTATION OF INITIALS

(All personnel trained to operate this instrument must sign and initial this form)

OPERATOR	INITIALS

IOP:012.0D

Hach DR/2400 Spectrophotometer

IOP:012.0D

April 16, 2003

U.S. Fish and Wildlife Service
Marquette Biological Station
1924 Industrial Parkway
Marquette, Michigan 49855
U.S.A.

and

U.S. Fish and Wildlife Service
Ludington Biological Station
229 South Jebavy Drive
Ludington, Michigan 49431
U.S.A.

and

Department of Fisheries and Oceans
Sea Lamprey Control Centre
1 Canal Drive
Sault Ste. Marie, Ontario
Canada

INSTRUMENT OPERATING PROCEDURE

INSTRUMENT:

Spectrophotometer

MODEL:

DR/2400

MANUFACTURER:

Hach

SERIAL AND PROPERTY NOS:

Model number	Location	Serial number	Property number	Identifying number
DR/2400	SLCC	030100001158	02-31	02-31

PRECAUTIONS:

POTENTIAL INTERFERENCES:

None listed in manual

SAFETY:

No special safety precautions

PROCEDURES:

I. Background

A. The Hach DR/2400, the factory-suggested replacement for the DR/2010, is capable of direct readout of concentrations of TFM, however, this option does not support the requirements of methods of analysis developed for line-powered spectrophotometers.

B. The procedures for analysis with the DR/2400 closely parallel those used with the Turner model SP-830 spectrophotometers.

1. Similarities
   a. Pre-formulated standards with concentrations of 0, 4, 8, and 12 mg/L TFM are used.
   b. The slope of the instrument response is determined through measurement of the absorbance of the TFM standards.
   c. Base/Acid measurements of background absorbance are conducted to assure accuracy of analyses.
   d. Water samples are buffered to pH ~9.0 before measurement of absorbance.
   e. Water samples are filtered before measurement of absorbance.
   f. Record keeping requirements are the same.

2. Differences
   a. The instrument wavelength setting is 400 nm versus 395 nm for dedicated, line-powered spectrophotometers.
   b. Water samples normally are not heated before analysis.

C. The accuracy of measurements depends to some degree on the comparative absorbencies of the optically-matched sample cells in each DR/2400 kit. The method of data interpretation used during TFM analyses demands that special attention is given to compensation for poor matches when they cannot be avoided.

II. Preparation

A. Set up the spectrophotometer in a shaded location. Always use the black outdoor light shield when making absorbance measurements.

B. Use line power if available, however, use of this instrument is normally reserved for situations in which line power is not available.

C. After all peripheral equipment for sample preparation is ready, press the **POWER** button. The instrument will conduct internal tests and will display **Wavelength Calibration**.

D. The **Main Menu** will automatically be display once the calibration is complete. Select the **Single Wavelength** option on the touch pad screen.

E. With the **Single Wavelength** main screen active, select the Wavelength button and enter the desired wavelength (for TFM **400** nm).

F. Match the 25 mL sample cells

1. Open a new 0.0 mg/L standard and rinse and fill two factory-matched 25 mL sample cells.

2. Place the first cell in the sample cell holder with the 25 mL mark facing to the right and close the black outdoor light shield. Press **ZERO** on the touch pad screen. **"ZEROING..."** will be displayed until the instrument is zero calibrated. **0.000 ABS** will appear on the display when the instrument is zero calibrated.

3. Place the second cell in the sample cell holder with the 25 mL mark facing to the right and close the black outdoor light shield.

4. The sample cell should produce an absorbance $\pm$ less than 0.008 (considered an acceptable match; maximum ~ 0.05 mg/L error). If the difference is greater, clean the cell or check the absorbencies of spare sets of matched cells. If an acceptable match cannot be obtained and another matched set is not available, note the absorbance difference so absorbencies of standards can be corrected. **The cell with the lesser absorbance is used as the blank.**

III. Calibration–standards

A. Open the remainder of the new set of TFM standards. Do not allow the standards to sit in sunlight while in use and store the standards in the dark. If the standards are very cold (apt to fog), warm them in a water bath or in the hands.

B. Insert the cuvette that contains the blank and press zero. Wait until **0.000 ABS** is displayed.

C. Insert the 0.0 mg/L TFM standard and close the black outdoor light shield. The 0.0 standard may produce a reading other than 0.000 because of an imperfect match of cuvettes. If the cells are not suitably matched, subtract the difference due to cell mismatch (noted above) and record in log book.

D. Again insert the cuvette containing the blank and close the black outdoor light shield. Wait until **0.000 ABS** is displayed, insert the 4.0 mg/L standard, and close the black outdoor light shield. Again adjust the result for differences in absorbance between blank and sample cells if necessary. Record the corrected absorbance in the instrument log book. Repeat the procedure with 8.0 and 12.0 mg/L TFM standards

E. Divide the recorded absorbance of each standard by the concentration of TFM (mg/L; 4.0, 8.0, and 12.0). Average the results and record the mean in the log book and on the analysis data sheet.

IV. Calibration–stream water sample

A. Measure the background absorbance and B/A ratio of the stream water (TOP:018.x). Do not interchange the cells. Always use the same cell for the 0.0 standard (blank).

B. Prepare the TFM-free water sample for analysis

1. Add 1 mL sodium tetraborate buffer to a 250 mL stream water sample and shake.

2. Filter the sample into the cuvette with a syringe filter.

3. Adjust the sample temperature by placing the 25 mL cuvette into a water bath or by holding in the hands.

C. Insert the blank into the sample cell holder.

D. Press the **ZERO** key and wait until the instrument is zero calibrated.

E. Dry the sample cuvette with a tissue and insert into the sample cell holder.

F. Close the black outdoor light shield.

G. Note the measured result on the analysis form. This absorbance includes both the background absorbance of the stream water and the difference in absorbance between the blank and sample cells. Do not correct this value for differences between cells even if it is significant. If the difference between cells was considered significant when checked, compensation was made when producing the calibration curve.

H. Determine the B/A ration for the stream water at the site according to procedures outlined in TOP:018.x.

V. Sample measurement

A. Collect a stream water sample containing TFM.

B. Prepare the water sample for analysis.

1. Add 1 mL sodium tetraborate buffer to a 250 mL stream water sample and shake.

2. Filter the sample into the cuvette with a syringe filter.

3. If problems with cuvette fogging occur, adjust the sample temperature by placing the 25 mL cuvette into the water bath or by holding in the hands.

C. Insert the blank into the sample cell holder.

D. Press the **ZERO** key and wait until the instrument is zero calibrated.

E. Dry the sample cuvette with a tissue and insert into the sample cell holder.

F. Close the black outdoor light shield.

G. Record the resulting absorbance on the lampricide analysis data sheet.

H. Subtract the background absorbance (base blank on the data sheet) and record.

I. Divide the resulting absorbance by the calculated slope of the calibration curve.

J. Record the result as the concentration of TFM (mg/L) in the stream water sample.

VI. Documentation

1. Make entries into instrument log book each time instrument is used.

2. Record results of analysis on LAMPRICIDE ANALYSIS data sheet (Appendix M).

MAINTENANCE:

I. Cleaning instrument -- see page 51, Section 8.2 in the instrument manual.

II. Replacing batteries -- See page 51, Section 8.6-8.7 in the instrument manual.

III. Replacing Lamp -- See page 51, Section 8.3 in the instrument manual.

IV. Re-certification interval–see page 52, Section 8.8 in the Instrument Manual.

REFERENCE:

Hach DR/2400 Spectrophotometer Instrument Manual

This procedure has been reviewed and approved by the undersigned representatives of the U.S. Fish and Wildlife Service and Fisheries and Oceans Canada.

REVIEWED/APPROVED_____ DATE_____
Field Supervisor (U.S.)

REVIEWED/APPROVED_____ DATE_____
Division Manager (Canada)

ATTACHMENTS

Log book for operation of the Hach
Model DR/2400 spectrophotometer

SPECTROPHOTOMETER
HACH MODEL DR/2400

SERIAL NUMBER _____

PROPERTY NUMBER _____

UNIT NUMBER _____

LOCATION _____

Date	Time	Inits.	Stream	Std. number	mg/L	Absorb.	Slope	Remarks and maintenance

SEA LAMPREY CONTROL PROGRAM
DOCUMENTATION OF INITIALS

(All personnel trained to operate this instrument must sign and initial this form)

OPERATOR	INITIALS