



## DOLT-3

### *Dogfish Liver Certified Reference Material for Trace Metals*

The following table shows those elements for which certified values have been established for this dogfish (*Squalus acanthias*) liver reference material. Certified values are based on unweighted mean results from data submitted by laboratories participating in an annual intercomparison. The expanded uncertainty ( $U_{CRM}$ ) in the certified value is equal to  $U = k u_c$  where  $u_c$  is the combined standard uncertainty calculated according to the ISO Guide [1] and  $k$  is the coverage factor. The value of  $u_c$  is determined from the combined uncertainties of the various methods ( $u_{char}$ ) as well as uncertainties associated with homogeneity ( $u_{hom}$ ).

It is intended that  $U_{CRM}$  encompasses every aspect that reasonably contributes to the uncertainty of the measurand [2,3]. A coverage factor of 2 was applied for all elements. The table below lists certified values for DOLT-3.

#### TRACE ELEMENTS (milligram/kilogram)

Arsenic (d,g,h)	10.2	±	0.5
Cadmium (d,f,g,i,p)	19.4	±	0.6
Copper (d,i,p)	31.2	±	1.0
Iron (d,f,i)	1484	±	57
Lead (d,g,p)	0.32	±	0.05
Mercury (d,c,p)	3.37	±	0.14
Nickel (g,i,p)	2.72	±	0.35
Selenium (g,h,j)	7.06	±	0.48
Silver (f,g,i,p)	1.20	±	0.07
Zinc (d,i,p)	86.6	±	2.4
CH <sub>3</sub> Hg (as Hg)(s,t)	1.59	±	0.12

#### Coding

The coding refers only to the instrumental method of analyte determination.

c - Cold vapour atomic absorption spectrometry.	i - Inductively coupled plasma atomic emission spectrometry.
d - Inductively coupled plasma mass spectrometry	j - Hydride generation atomic fluorescence spectrometry.
f - Flame atomic absorption spectrometry.	p - Isotope dilution inductively coupled plasma mass spectrometry (ID-ICPMS)
g - Electrothermal vaporization atomic absorption spectrometry (ETAAS).	s - Solid phase microextraction (SPME) isotope dilution gas chromatography ICPMS
h - Hydride generation atomic absorption spectrometry.	t - Ethylation cold vapor atomic fluorescence spectrometry

## Intended Use

This reference material is primarily intended for use in the calibration of procedures and the development of methods used for the analysis of marine fauna and materials with a similar matrix.

## Storage and Sampling

This material should be kept tightly closed in the original bottle and should be stored in a cool location, away from any significant radiation sources such as ultraviolet lamps and sunlight. The bottle should be well mixed by rotation and shaking prior to use, and tightly closed immediately thereafter.

## Instructions for Drying

DOLT-3 can be dried to constant weight by:

(1) drying at reduced pressure (e.g., 50 mm Hg) at room temperature in a vacuum desiccator over magnesium perchlorate for 24 hours;

(2) vacuum drying (about 0.5 mm Hg) at room temperature for 24 hours.

## Stability

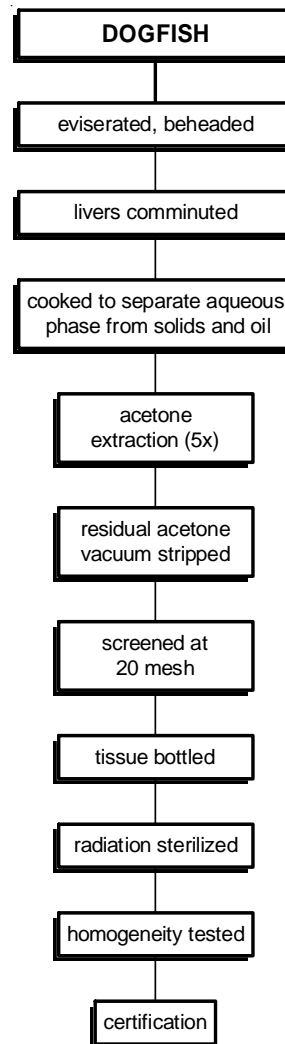
The predecessor CRM, DOLT-2, has been periodically analyzed for more than nine years and found to be both physically and chemically stable over this time interval. We expect similar behaviour from DOLT-3. The stability of this CRM will continue to be monitored and customers will be notified if any significant irregularity occurs prior to the expiry date.

## Expiry

Based on sample stability noted above, the certified values for DOLT-3 are considered valid until September, 2011, provided the CRM is handled and stored in accordance with instructions herein.

## Preparation of DOLT-3

This reference material was processed at the Guelph Food Technology Center, Guelph Ontario. The preparation sequence is illustrated below.



## Information values

Due to the scatter of results, a certified value for Al and Cr were not calculated. A lack of independent values precluded the certification of Sn. Information values for these analytes are given below.

Al	25	mg/kg
Cr	3.5	mg/kg
Sn	0.4	mg/kg

## Certified value

DOLT-3 was provided as an unknown sample to a group of laboratories participating in an annual intercomparison for trace metals in marine samples sponsored by NRC [4]. The predecessor CRM DOLT-2 was provided as a quality control sample. Data generated at NRC was also included in the pool of intercomparison results.

Laboratories were requested to provide triplicate dry weight values using an analytical method of choice based on total digestion of the sample. Various dissolution procedures were utilized including closed vessel with microwave heating and open vessel digestion on a hot plate. Laboratories used HNO<sub>3</sub> or a combination of H<sub>2</sub>O<sub>2</sub> and HNO<sub>3</sub>, although several participants included HF or HCl in the acid mixture.

Data was returned to NRC for evaluation. The results from a select sub-group of participants were used for the certification of DOLT-3. These laboratories were selected based on their performance history in previous intercomparisons. As well, if a laboratory did not submit satisfactory results for DOLT-2 their data for DOLT-3 were rejected.

The certified values were calculated from the unweighted means of the results of the participating laboratories. [5,6] Data were first examined for outliers using the Grubb's Test; no outliers of means were detected.

New guidelines for CRM producers suggest all sources relevant to the user of the material should contribute to the uncertainty of the certified value [2-4]. Included in the overall uncertainty estimate are uncertainties in the batch characterisation ( $u_{char}$ ), uncertainties related to possible between-bottle variation ( $u_{hom}$ ) as well as instability derived from effects relating to long-term storage and transport ( $u_{stab}$ ). Expressed as standard uncertainties these components can be combined as:

$$u_{c(CRM)}^2 = u_{char}^2 + u_{hom}^2 + u_{stab}^2 \quad (1)$$

Results for the various statistics used to calculate the certified values are shown in Table 2.

## Characterisation

The characterisation uncertainties ( $u_{char}$ ) were calculated in accordance with equation 2, where  $s$  is the standard deviation of the means and  $p$  is the number of mean results included in the calculation [6].

$$u_{char} = \frac{s}{\sqrt{p}} \quad (2)$$

## Homogeneity

The homogeneity components of the uncertainty in the certified values were derived according to the recommendation of an international study group [7]. The material was tested for homogeneity at NRC using ETAAS. Results from triplicate sub-samples (0.150 g) from ten bottles were evaluated using ANOVA.

For Cd, Ni, Al, Pb and Zn, the inhomogeneity contribution to uncertainty,  $u_{hom}$ , was set to the experimentally determined between-unit standard deviation ( $s_{between}$ ), as the best estimate of the uncertainty due to between-unit heterogeneity. However, for As, Cu, Fe, Hg, Se and Ag, the situation depicted in equation 3 occurred:

$$s_{between}^2 < \frac{s_{meas}^2}{n} \quad (3)$$

where  $s_{meas}$  is the repeatability standard deviation for the method used in the homogeneity assessment and  $n$  is the number of replicates per unit.

In these cases,  $u_{hom}$  was calculated according to:

$$u_{hom} = \sqrt{\frac{s_{meas}^2}{n}} \quad (4)$$

It is recognized that this is not an ideal situation, as it represents a worst case scenario by suggesting the homogeneity could be as poor as the precision of the measurement technique selected for homogeneity assessment.

## Stability

As previously discussed, based on NRC's experience with similar materials, uncertainty components for long and short term stability were considered negligible and are thus not included in the uncertainty budget.

**Table 2. Statistical Data for DOLT-3**

	components for $u_{char}$ , (mg/kg)			data sets	$u_{char}$ , (mg/kg)	$u_{hom}$ , (mg/kg)
	$S_L$	$S_w$	s of means			
As	0.62	0.26	0.64	12	0.18	0.14
Cd	0.80	0.34	0.82	16	0.21	0.23
Cu	1.6	0.78	1.6	16	0.41	0.24
Fe	59	26	61	13	17	23
Pb	0.036	0.015	0.037	12	0.011	0.020
Hg	0.15	0.090	0.16	14	0.042	0.059
Ni	0.27	0.15	0.29	13	0.079	0.077
Se	0.59	0.44	0.64	9	0.22	0.11
Ag	0.10	0.070	0.11	13	0.029	0.016
Zn	3.9	1.3	3.9	15	1.0	0.62
CH <sub>3</sub> Hg	0.070	0.050	0.052	2	0.052	0.026

$s_L$  - the between laboratory standard deviation from the laboratory intercomparison

$s_w$  - the within laboratory standard deviation from the laboratory intercomparison

### Criteria for acceptance

The property values reported in this certificate are the best estimates of the true values that can be obtained, based on the certification exercise. It is the responsibility of the analyst to assess the appropriateness of this CRM for purpose and interpret their own analytical results [8].

The user may assess laboratory bias from the difference between the calculated mean value of replicate measurements ( $\bar{x}$ ) and the certified value ( $\mu$ ):  $\bar{x} - \mu$ . According to ISO Guide 33: Uses of Certified Reference Materials, the criteria for acceptance is:

$$-a_2 - 2\sigma_D \leq \bar{x} - \mu \leq a_1 + 2\sigma_D \quad (5)$$

where  $a_1$  and  $a_2$  are adjustment values chosen by the laboratory according to economic or technical limitations or requirements and  $\sigma_D$  is the standard

deviation associated with the measurement process. The value of  $\sigma_D$  can be calculated from the user's laboratory quality control data and may be estimated from two components, as shown in equation 6:

$$\sigma_D = \sqrt{s_b^2 + \frac{s_w^2}{n}} \quad (6)$$

where  $s_b$  is the between-laboratory standard deviation component associated with the measurement process ( ideally this should include the long-term standard deviation of the user's method or, alternatively,  $s_L$  from Table 2),  $s_w$  the within-laboratory standard deviation (or repeatability standard deviation) and  $n$  the number of replicate analyses made of the reference material. Alternative methods for calculating  $s_b$  are discussed in reference 8.

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The following laboratories participated in the certification of DOLT-3:

ALS Environmental  
1988 Triumph St.  
Vancouver, B.C. V5L 1K5

Australian Institute of Marine Sciences  
Townsville Mail Center  
Queensland, 4810 , Australia  
Frank Tirendi, Cassie Payn and Stephen Boyle

Battelle Pacific Northwest  
1529 W. Sequim Bay Road  
Sequim, WA 98382  
Eric Crecelius, Laurie Niewolny, Mary Ann  
Deuth, and Brenda Lasorsa

CanTest Ltd.  
4606 Canada Way  
Burnaby, B.C. V5G 1K5  
Richard Jorntitz, Lynn Yehle, Alex Wong and  
Greg Sparrow

Massachusetts Water Resources Authority  
Central Laboratory  
190 Taft Ave.  
Winthrop, MA 02152  
Patricia Sullivan , Maria Palumbo, Jun Wang,  
Ellen C. West and Larissa Zilitinkevitch

NOAA  
National Ocean Service  
Hollings Marine Laboratory  
331 Ft. Johnson Road  
Charleston, SC 29412  
Dan Bearden, Aaron Dias

Skidaway Institute of Oceanography  
10 Ocean Science Circle  
Savannah, GA 31411  
Herb Windom, Debbie Wells and Lori Cowden

Texas A. & M.  
College of Veterinary Medicine  
Trace Element Research Laboratory  
College Station, TX 77843-4458  
Robert Taylor, Gerald Bratton and Bryan Brattin

Texas Parks and Wildlife  
Environmental Contaminants Laboratory  
505 Staples Road  
San Marcos, TX 78666  
Gary Steinmetz

U.S. Customs Laboratory  
214 Bourne Boulevard  
Savannah, GA 31408  
Ralph Smith

USGS  
National Water Quality Laboratory  
P.O. Box 25046  
Denver, Colorado 80225-0046

USGS-WRD  
Peachtree Business Center  
3039 Amwiler Road  
Atlanta, GA 30360-2824  
Kent Elrick, Arthur J. Horowitz and James J.  
Smetanka

WA State Dept. of Ecology  
Manchester Environmental Laboratory  
7411 Beach Drive East  
Port Orchard, Washington 98366-8204  
Randy Knox, Meredith Jones, Sally Cull and  
Karin Feddersen

## References

- [1] Guide to the Expression of Uncertainty in Measurement, ISBN 92-67-10188-9, 1st ed. ISO, Geneva, Switzerland (1993).
- [2] J. Pauwels, A. van der Veen, A. Lamberty, H. Schimmel, Evaluation of uncertainty of reference materials. *Accred Qual Assur* (2000) 5:95-99.
- [3] J. Pauwels, A. Lamberty, H. Schimmel, The determination of the uncertainty of reference materials certified by laboratory intercomparison. *Accred Qual Assur* (1998) 3:180-184.
- [4] S. Willie, Fifteenth Intercomparison for Trace Elements in Marine Sediments and Biological Tissues, NRC No. 42768, May 2002.
- [5] A. M.H.van der Veen and J. Pauwels, Uncertainty calculations in the certification of reference materials. 1.Principles of analysis of variance. *Accred Qual Assur* (2000) 5:464-469.
- [6] A. M.H. van der Veen, T. P.J. Linsinger, H. Schimmel, A. Lamberty and J. Pauwels, Uncertainty calculations in the certification of reference materials 4. Characterisation and certification. *Accred Qual Assur* (2001) 6:290-294.
- [7] S.L.R. Ellison, S.Burke, R.F.Walker, K. Heydorn, M.Månsson, J.Pauwels, W.Wegscheider, B.te Nijenhuis, Uncertainty for reference materials certified by interlaboratory study: Recommendations of an international study group. *Accred Qual Assur* (2001) 6:274-277.
- [8] R. Walker and I. Lumley, Pitfalls in terminology and use of reference materials, *Trends in Anal. Chem.*, (1999) 18: 594-616.

## Updates

It is anticipated that as more data become available, the established values may be updated and reliable values assigned to more elements.

Our web site at [http://inms-ienm.nrc-cnrc.gc.ca/calserv/chemical\\_metrology\\_e.html](http://inms-ienm.nrc-cnrc.gc.ca/calserv/chemical_metrology_e.html) will contain any new information.

Certificate issued September 2002.  
MeHg added June 2003.

*The results listed in this certificate are traceable to the SI through gravimetrically prepared standards of established purity and international measurement intercomparisons. As such, they serve as suitable reference materials for laboratory quality assurance programs, as outlined in ISO/IEC 17025. This CRM is registered at the Bureau International des Poids et Mesures (BIPM) in Appendix C of the Comité International des Poids et Mesures database listing Calibration and Measurement Capabilities accepted by signatories to the Mutual Recognition Arrangement of the Metre Convention.*

*Comments, information and inquiries should be addressed to:*

**Dr. R.E. Sturgeon**  
**National Research Council of Canada**  
**Institute for National Measurement Standards**  
**M-12, 1500 Montreal Road**  
**Ottawa, Ontario, Canada K1A 0R6**

Telephone (613) 993-2359  
Facsimile (613) 993-2451  
E-mail [crm.inms@nrc-cnrc.gc.ca](mailto:crm.inms@nrc-cnrc.gc.ca)

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