National Institute of Advanced Industrial Science and Technology

National Metrology Institute of Japan

Reference Material Certificate

NMIJ CRM 7408-a
No. +++

Neonicotinoid Pesticides in Artificial Urine

This certified reference material (CRM) is produced in accordance with the NMIJ's management system and is in compliance with ISO 17034 and ISO/IEC 17025. This CRM is intended for use in accuracy control of analysis and validation for analytical methods or instruments for the determination of neonicotinoid pesticides in urine.

Certified Values

The certified values of this CRM are given in the table below. The uncertainty of the certified value is the expanded uncertainty obtained by multiplying the combined standard uncertainty by a coverage factor (k) of 2, and it is the half-width of an interval estimated to have a level of confidence of approximately 95 %.

Substance	CAS No.	Certified value Mass fraction (μg/kg)	Expanded uncertainty Mass fraction (μg /kg)	Analytical Method*
Acetamiprid (N^1-[(6-chloro-3-pyridyl)methyl]-N^2-cyano-N^1-methylacetamidine)	160430-64-8	1.38	0.18	1, 2
Thiamethoxam (3-(2-chloro-1,3-thiazol-5-ylmethyl)-5-methyl-1,3,5-oxadiazinan-4-ylidene(nitro)amine)	153719-23-4	1.32	0.26	1, 2

* See **Analysis** for the applied analytical methods.

Analysis

The certified values for acetamiprid and thiamethoxam were determined based on the analytical results of these pesticides obtained by Analytical Methods 1 and 2 and isotope dilution-liquid chromatography/mass spectrometry (ID-LC/MS). The Quick, Easy, Cheap, Effective, Rugged, and Safe (QuEChERS) method was used for Analytical Method 2. Each certified value was calculated as a weighted mean of the results obtained by the following two methods:

Analytical Method 1

[Extraction and clean-up] Solid phase extraction (SPE) using polymeric cation exchange resin

[LC/MS] Column = C18; Electrospray ionization (ESI); Selected ion monitoring (SIM)

Analytical Method 2

[Extraction] Shaking with acetone

[Clean-up] Dispersive SPE (Shaking with a strong cation exchanger)

[LC/MS] Column = C18; Electrospray ionization (ESI); Selected ion monitoring (SIM)

Metrological Traceability

The certified values were determined by isotope dilution mass spectrometry (IDMS). The purity of Traceable Reference Material® (acetamiprid) and TraceSure® (thiamethoxam and thiacloprid) were evaluated by NMIJ and these were produced by FUJIFILM Wako Pure Chemical Corporation(Osaka, Japan). These were used to prepare the calibration solution for IDMS. The

certified values, therefore, are traceable to the international System of Units (SI).

Indicative Value

The indicative value of this CRM is given in the table below. The mass fraction of thiacloprid was determined based on the analytical results obtained by ID-LC/MS and Analytical Method 1. The uncertainty of the indicative value is the expanded uncertainty obtained by multiplying the combined standard uncertainty by a coverage factor (k) of 2, and it is the half-width of an interval estimated to have a level of confidence of approximately 95 %.

Substance	CAS No.	Indicative value Mass fraction (μg/kg)	Expanded uncertainty Mass fraction (μg /kg)
Thiacloprid (3-(6-chloro-3-pyridylmethyl)-1,3-thiazolidine-2-ylidenecyanamide)	111988-49-9	0.19	0.13

Expiration of Certification

This certificate is valid for three months from the date of shipment, provided that the material remained unopened and is stored in accordance with **Instructions for Storage** given in this certificate.

Description of the Material

Artificial urine (urea: 2.50×10^{-4} mg/L, sodium chloride: 0.90×10^{-4} mg/L, disodium hydrogen phosphate: 0.25×10^{-4} mg/L, ammonium chloride: 0.30×10^{-4} mg/L, dipotassium hydrogenphosphate: 0.25×10^{-4} mg/L, creatinine: 0.20×10^{-4} mg/L, sodium sulfite heptahydrate: 0.30×10^{-4} mg/L, the rest is water), was used as a raw material, and the target compounds dissolved in methanol were added to this material. This CRM is in the form of a clear and colorless liquid. The material is bottled in a glass vial (5 g for each vial).

Instructions for Storage

This CRM should be stored at temperatures of −20 °C to −30 °C under dark conditions.

Instructions for Use

The CRM is for laboratory use only and not for *in vivo* use. About two hours prior to use, the CRM should be taken out from a freezer and left at room temperature (20 °C to 25 °C) until it thaws. Do not heat the CRM. The vial should be gently turned upside down several times to ensure homogenization. The CRM, after thawing, should be used up immediately. More than 2.0 g of the material should be used. Storing the thawed material may result in changes in the pesticide concentration. Once thawed, the CRM cannot be frozen and stored again.

Precautions for Handling

A mask, gloves, and other personal protective equipment should be used when this CRM is handled. NMIJ CRM 7408-a IS INTENDED FOR IN VITRO LABORATORYUSE ONLY. This CRM should be disposed of in accordance with applicable laws and regulations. Refer to the safety data sheet (SDS) on this CRM before use.

Preparation

A solution containing target pesticides was spiked to the artificial urine material (ISEKYU Corporation, JIS T3214). This mixture was completely homogenized and bottled in glass vials by FUJIFILM Wako Pure Chemical Corporation.

Technical Information

The concentrations of imidacloprid (1-(6-chloro-3-pyridylmethyl)-*N*-nitroimidazolidin-2-ylideneamine), clothianidin ((*E*)-1-(2-chloro-1,3-thiazol-5-ylmethyl)-3-methyl-2-nitroguanidine), dinotefuran (1-methyl-2-nitro-3-(tetrahydro-3-furylmethyl) guanidine), and acetamiprid-*N*-desmethyl (N^1-(6-chloro-3-pyridylmethyl)-N^2-cyanoacetamidine) obtained by Analytical Method 1 described in the Analysis section were 1.47 μg/kg, 1.34 μg/kg, 12.66 μg/kg, and 1.26 μg/kg, respectively. Dinotefuran, clothianidin, and acetamiprid-*N*-desmethyl were gradually degraded under the storage conditions. The concentration of creatinine

obtained by ID-LC/MS was 1.9 g/kg. The densities of this CRM are 1.0139 g/cm^3 at 20 °C and 1.0125 g/cm^3 at 25 °C.
The concentration of nitenpyram ((*E*)-*N*-(6-chloro-3-pyridylmethyl)-*N*-ethyl-*N'*-methyl-2-nitrovinylidenediamine) was 0.75 µg/kg measured by the National Institute for Environmental Studies Japan using SPE (hydrophilic-lipophilic balanced reversed-phase sorbent) and ID-LC/MS/MS.

NMIJ Analysts

The technical manager for this CRM is HANARI N., the production manager and the analyst are OTAKE T.

Information

If substantive technical changes occur that affect the certification before the expiration of this certificate, NMIJ will notify the registered customers. Customer registration on the NMIJ Website (given below) will facilitate notification. Technical reports regarding this CRM can be obtained from the contact details given below.

Reproduction of Certificate

In reproducing this certificate, it should be clearly indicated that the document is a copy.

Note

This CRM was developed in the collaborative study with the National Institute for Environmental Studies Japan.

April 1, 2020

ISHIMURA Kazuhiko
President
National Institute of Advanced Industrial Science and Technology

If you have any questions about this CRM, please contact:
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National Metrology Institute of Japan,
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Revision history

December 24, 2020: The expanded uncertainties were reevaluated and changed for Clothianidin based on the results of stability assessment after certification.

September 30, 2021: The expanded uncertainties for Acetamiprid and Thiamethoxam were reevaluated and the certified value for Thiacloprid and Clothianidin were eliminated based on the results of stability assessment after certification. The value for Thiacloprid was provided as Indicative value. The value of Clothianidin at the time of the certification was added in Technical Information.

September 28, 2023: The expanded uncertainties for Acetamiprid and Thiacloprid were reevaluated based on the results of stability assessment after certification.