**ATRAZINE**

**Collaborative Study**

Full Scale Collaborative Study
for the
Determination of Atrazine

In Technical Concentrate and Formulations
by Gas Chromatography and

Flame Ionization Detection

Report to CIPAC

by

Syngenta Crop Protection AG
in collaboration with DAPA

William Meyerhoffer

410 Swing Road

Greensboro, NC

USA

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# Participants (Listed by Name in Alphabetical Sequence)

|  |  |
| --- | --- |
|  |  |
| Canzani, Francisco (Laboratory 1)Baccino, FiorellaCarr, Denis (Laboratory 2)Garvey, Jim | Laboratorio de Formulaciones de Plaguicidas, Materias Primas y Fertilazantes; Direccion General de Servicios Agricolas, Ministerio de Ganaderia Agricultura y Pesca, Avenida Millan 4703. C.P. 11900, MontevideoUruguayThe Pesticide Control Laboratory, Backweston Laboratory Complex,Backweston,Celbridge,Co. Kildare,Ireland |
| Checa, Brenda (Laboratory 3) | Ministerio de Desarrollo AgropecuarioDirección Nacional de Sanidad VegetalLaboratorio de Control de Calidad de PlaguicidasPanama |
|  |  |
| De Ryckel, Bernard (Laboratory 4) | Walloon Agricultural Research Centre (CRA-W)Agriculture and Natural Environment Department Plant Protection Products and BiocidesCarson BuildingRue du Bordia, 115030 GemblouxBelgium |
| Förster, Rolf (Laboratory 5) | BASF SEAPR/DP - Li721Speyerer Strasse 267117 LimburgerhofGermany |
| Grecu, Cornel (Laboratory 6) | Alchimex SA63-Alexandru Constantinescu-011472Romania |
| Karasali, Elen (Laboratory 7) Marousopoulou, AnnaKing, Kevin (Laboratory 8) | Benaki Phytopathological Institute7 Stefanou Delta street14561 Kifissia Athens GreeceClarke Mosquito Control Products675 Sidwell CourtSt Charles, IllinoisUSA |
|  |  |
| Kuiyama, Yasushi (Laboratory 9) | Agricultural Chemicals Inspection Station (ACIS), Food and Agricultural Materials Inspection Center (FAMIC) 2-772, Suzuki-Cho, Kodaira-Shi, Japan |
|  |  |
| Mink, Christian (Laboratory 10)Doelensek, Daniela | Syngenta Crop Protection AGBreitenloh 54333 MünchwilenSwitzerland |
| Monisse, Isabelle (Laboratory 11)Laboratory 12 | Agence Fédérale pour la Sécurité AlimentaireRue de Visé, 4954020 Wandre04/252.01.58BelgiumResults not received in time for final reporting |
|  |  |
| Rezvani, Ahmad (Laboratory 13)Iregbu, ChuksShi, Kaiwei (Laboratory 14)Thongyord, Jirapan (Laboratory 15)Mongkhonwuttikan, PanidaWagner, Silke (Laboratory 16) | Pesticide Formulations & Residues, State Chemist SectionMaryland Department of Agriculture50 Harry S. Truman ParkwayAnnapolis, MD 21401USA National Center for Pesticide Quality Supervision and Inspection, Institute for Control of Agrochemicals, Ministry of Agriculture and Rural AffairsChinaThailand Department of AgricultureRatchadapisek Road, Huai Khwang District; Bangkok 10310ThailandBayer AGCrop Science DivisionResearch & DevelopmentProduct Chemistry AnalyticsBuilding 6510, MonheimGermany |

# Atrazine, General Information

Chemical name: 2-chloro-4-ethylamino-6-isopropylamino-s-triazine

ISO common name: Atrazine

CAS-Number: 1912-24-9

Structure:



Molecular mass: 215.7

Empirical formula: C8H14ClN5

# Samples

In December 2018, Information Sheet No. 318 was sent out by the CIPAC Secretary inviting members to participate in a collaborative study on the determination of atrazine as a technical material and in formulations by gas chromatography with flame ionization detection.

Five test samples (described below) including the Atrazine analytical reference standard (Batch 691260, 97.1% purity) and the internal standard (Dipropyl Phthalate Batch 1068214) were shipped to the participants:

A) Atrazine Technical Batch 1053185

B) Atrazine Technical Batch 1053186

C) Water Dispersible Granule (WG) Batch 914338

D) Suspension Concentrate (SC) Batch 1035793

E) Suspension Concentrate (SC) Batch 1036188

All participants with the exception of Laboratory 12 (total of 15 laboratories) sent back their results in time.

# Method

## Scope

The content of Atrazine in technical material and in formulated products (water dispersible granules, suspension concentrate)

## Principle

The Atrazine content of the samples is determined by capillary gas chromatography on a DB-225 or equivalent fused silica column, 15 m x 0.25 mm (i.d.), 0.25 μm film thickness using hydrogen carrier gas and flame ionization detection. Quantification is done by internal standard calibration.

## Procedure

Samples should be analyzed in duplicate at two different days resulting in a total of four individual test results for each sample. All test solutions should be prepared freshly on Day 2.

# Remarks of the Participants

Participants made comments about the performance of the method and noted deviations from the method. Below is a summary of specific method conditions provided by the participating laboratories.

Laboratory 1 Nitrogen, TG-1701 MS (30 m x 0.53 mm id., 1.0 µm), Solvent Acetone

Laboratory 2 Hydrogen, HP-5MS (15 m x 0.25 mm id., 0,25 µm), Solvent Acetone

Laboratory 3 Helium, DB-1701 (15 m x 0.32 mm id., 1.0 µm), Solvent Acetone

Laboratory 4 Helium, DB-225 (15 m x 0.25 mm id., 0.25 µm), Solvent Acetone, constant flow

Laboratory 5 Hydrogen, DB-225 (15 m x 0.25 mm id., 0.25 µm), Solvent Acetone

Laboratory 6 Helium, DB-225 (30 m x 0.25 mm id., 0.25 µm), Solvent Acetone, 0.7 µL injection, 2 mm ID split liner

Laboratory 7 Hydrogen, DB-1 (30 m x 1.5 mm id., 0.53 µm), Solvent Acetone

Laboratory 8 Helium, DB-1701 (30 m x 0.25 mm id., 0.25 µm), Solvent Acetone,

 Day 1 samples not filtered

Laboratory 9 Helium, DB225-MS (30m x 0.32 mm id x 0.25 µm), Solvent Acetone

Laboratory 10 Hydrogen, DB-225 (15 m x 0.25 mm id., 0.25 µm), Solvent Acetone

Laboratory 11 Hydrogen, DB-1701 (30 m x 0.25 mm id., 0.25 µm), Solvent Acetone

Laboratory 12 Results not submitted in time for final reporting in Study

Laboratory 13 Hydrogen, DB-225 (15 m x 0.25 mm id., 0.25 µm), Solvent Acetone

Laboratory 14 Nitrogen, DB-225 (30 m x 0.32 mm id., 0.25 µm), Solvent Acetone

Laboratory 15 Helium, DB-225 (30 m x 0.25 mm id., 0.25 µm), Solvent Acetone

Laboratory 16 Hydrogen, DB-225 (15 m x 0.25 mm id., 0.25 µm), Solvent Acetone

**Evaluation and Discussion**

## Evaluation of the Quality of Data and Chromatograms

The data obtained from each of the laboratories were reviewed to determine if there were any significant deviations regarding the chromatography which might affect the analysis results. Examination of provided chromatograms and raw data showed no evidence for invalid analysis results.

All other changes and observations noted by the participants were not expected to affect the analysis results significantly.

A different type of mid-polarity column (DB-1701; 14% cyanopropylphenyl/86% dimethylpolysiloxane) was utilized by several participating laboratories compared to the proposed Atrazine method mid-polarity stationary phase (DB-225; 50% cyanopropylphenyl/50% dimethylpolysiloxane).

For the DB-1701 capillary GC column examination of provided chromatograms showed suitable resolution obtained between Atrazine/dipropyl phthalate internal standard peaks and potential sample matrix interferences such as Atrazine technical by-product impurities. In the proposed Atrazine GC method with DB-225 column the relative retention time between Atrazine/Internal standard is approximately 1.5. For analyses using the DB-1701 column, resolution between Atrazine/internal standard was typically observed to be less than 1.5 (e.g. 1.2-1.3) but was still deemed sufficient to enable accurate Atrazine quantitation in presence of potential sample matrix interference peaks.

Therefore based on evaluation of data obtained from this collaborative study it is proposed that an alternative mid-polarity column (e.g. DB-1701, 30m x 0.25 mm, 0.25 µm) be considered for use in the Atrazine provisional CIPAC method 5215/m.

## Determination of Atrazine in Technical and Formulations

Results reported by the laboratories and the statistical evaluation of the data are listed in Tables 1-4 and displayed in Figures 1-5.

The statistical evaluation of the data was completed following the “Guidelines for CIPAC Collaborative Study Procedures for Assessment of Performance of Analytical Methods”, according to DIN ISO 5725 [Dr. Nancy Shi (Syngenta) is gratefully acknowledged for her work on statistical evaluation of the data]. The data was examined for outliers and stragglers using Mandel’s k-statistics on the within-lab variance, followed by Mandel’s h-statistics on the lab means, and iterating where necessary. The tests were performed at an alpha level of 0.01 for outlier, and 0.05 for straggler.

Mandel’s k-statistics observed stragglers (marked with \* in Table 1) and outliers (marked with \*\* in Table 1) according to Mandel’s k-statistics were observed for the WG/SC formulations as well as for the technical concentrate (TC). The Mandel’s h-statistic test identified stragglers and outliers for the technical concentrate samples and an outlier for the WG formulation (marked with \* and \*\* in Table 2).

A comparison of the RSDR of this collaborative Study with the unmodified Horwitz equation showed that the relative reproducibility standard deviation (RSDR) is below the Horwitz value in all five samples even without elimination of stragglers and outliers (see Table 3). The RSDR further improved if stragglers and outliers are eliminated (see Table 4). No more than 2 values have been removed per sample (Table 1).

The validity of the results and the suitability of the analytical method is shown. This Atrazine collaborative trial is acceptable.

**Table 1: Atrazine Assay in TC and Formulations (g/kg); results for each laboratory on Day 1 and Day 2**

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
|   | Atrazine SAMPLE A | Atrazine SAMPLE B | Atrazine SAMPLE C | Atrazine SAMPLE D | Atrazine SAMPLE E |
|   | Day1 | Day2 | Day1 | Day2 | Day1 | Day2 | Day1 | Day2 | Day1 | Day2 |
| Laboratory 1 | 957.1 | 971.6 | 957.2 | 971.4 | 897.3 | 892.8 | 451.3 | 450.9 | 433.2 | 433.2 |
| Laboratory 2 | 960.8 | 967.8 | 963.3 | 967.7 | 889.5 | 887.1 | 438.0 | 440.8 | 433.1 | 438.0 |
| Laboratory 3 | 976.9\*\* | 998.9\*\* |  982.3 | 979.7 | 931.7\*\* | 909.7\*\* | 450.9 | 448.9 | 444.1 | 441.0 |
| Laboratory 4 | 972.5 | 971.9 | 972.6 | 972.8 | 886.7 | 884.7 | 428.4 | 422.9 | 431.1 | 429.3 |
| Laboratory 5 | 974.1 | 973.8 | 971.9 | 971.6 | 884.4 | 891.1 | 439.8 | 444.6 | 437.3 | 442.2 |
| Laboratory 6 | 970.8 | 972.3 | 972.4 | 975.5 | 885.9 | 889.7 | 446.0 | 444.2 | 443.9 | 442.5 |
| Laboratory 7 | 968.4 | 963.4 | 976.5 | 975.9 | 891.8 | 895.7 | 430.6 | 429.2 | 431.5 | 433.6 |
| Laboratory 8 | 972.0 | 964.6 | 969.2 | 963.8 | 879.6 | 868.0 | 427.4 | 429.9 | 430.7 | 426.1 |
| Laboratory 9 | 971.0 | 972.2 | 971.6 | 968.3 | 886.3 | 883.6 | 440.7 | 433.9 | 437.2 | 433.5 |
| Laboratory 10 | 971.3 | 972.3 | 969.5 | 968.3 | 887.6 | 883.2 | 437.6 | 440.2 | 435.3 | 435.9 |
| Laboratory 11 | 969.3 | 972.8 | 968.6 | 970.9 | 895.3 | 884.2 | 439.0 | 428.8 | 433.5 | 426.3 |
| Laboratory 13 | 967.0 | 963.7 | 976.0 | 959.8 | 885.4 | 880.1 | 456.1\* | 444.9\* | 438.2 | 441.9 |
| Laboratory 14 | 972.1 | 972.5 | 969.3 | 969.6 | 893.3 | 894.2 | 435.8 | 439.1 | 434.0 | 435.2 |
| Laboratory 15 | 965.0 | 966.0 | 960.8 | 962.3 | 880.5 | 882.1 | 438.5 | 440.0 | 433.0 | 440.1 |
| Laboratory 16 | 968.7 | 968.9 | 969.0\*\* | 994.1\*\* | 885.9 | 899.1 | 442.2 | 450.0 | 437.0 | 443.7 |

**\*** Mandel’s k-statistic straggler

\*\* Mandel’s k-statistic outlier

\*\*\* Laboratory 12 did not analyze the samples

**Table 2: Mean Values**

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
|   | Atrazine SAMPLE A | Atrazine SAMPLE B | Atrazine SAMPLE C | Atrazine SAMPLE D | Atrazine SAMPLE E |
|   |   |   |   |   |   |
| Laboratory 1 | 964.4 | 964.3 | 895.1 | 451.1 | 433.2 |
| Laboratory 2 | 964.3 | 965.5 | 888.3 | 439.4 | 435.6 |
| Laboratory 3 | 987.9\*\* | 981.0 | 920.7\*\* | 449.9 | 442.6 |
| Laboratory 4 | 972.2 | 972.7 | 885.7 | 425.7 | 430.2 |
| Laboratory 5 | 974.0 | 971.8 | 887.8 | 442.2 | 440.0 |
| Laboratory 6 | 971.6 | 974.0 | 887.8 | 445.1 | 443.2 |
| Laboratory 7 | 965.9 | 976.2 | 893.8 | 429.9 | 432.6 |
| Laboratory 8 | 968.3 | 966.5 | 873.8 | 428.7 | 428.4 |
| Laboratory 9 | 971.6 | 970.0 | 885.0 | 437.3 | 435.4 |
| Laboratory 10 | 971.8 | 968.9 | 885.4 | 438.9 | 435,6 |
| Laboratory 11 | 971.1 | 969.8 | 889.8 | 433.9 | 429.9 |
| Laboratory 13 | 965.4 | 967.9 | 882.8 | 450.5 | 440.1 |
| Laboratory 14 | 972.3 | 969.5 | 893.8 | 437.5 | 434.6 |
| Laboratory 15 | 965.5 | 961.6 | 881.3 | 439.3 | 436.6 |
| Laboratory 16 | 968.8 | 981.6\* | 892.5 | 446.1 | 440.4 |

\* Mandel’s h-statistic straggler

\*\* Mandel’s h-statistic outlier

\*\*\* Laboratory 12 did not analyze the samples

**Table 3: Summary of the Statistical Evaluation - No elimination of any
 Stragglers /Outliers**

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
|  | **Sample A** | **Sample B** | **Sample C** | **Sample D** | **Sample E** |
| **Xm** | 970.3 | 970.7 | 889.6 | 439.7 | 435.9 |
| **L** | 15 | 15 | 15 | 15 | 15 |
| **Sr** | 5.33 | 6.27 | 6.00 | 3.80 | 2.97 |
| **SL** | 4.49 | 3.57 | 9.32 | 7.50 | 4.08 |
| **SR** | 6.97 | 7.21 | 11.08 | 8.41 | 5.05 |
| **r** | 14.93 | 17.56 | 16.79 | 10.64 | 8.32 |
| **R** | 19.52 | 20.20 | 31.03 | 23.53 | 14.14 |
| **RSDr** | 0.55 | 0.65 | 0.67 | 0.86 | 0.68 |
| **RSDR** | 0.72 | 0.74 | 1.25 | 1.91 | 1.16 |
| **RSDR(Hor)** | 2.01 | 2.01 | 2.04 | 2.26 | 2.27 |

**Table 4: Summary of the Statistical Evaluation - with elimination of Mandel’s h and k
 Statistic Stragglers /Outliers**

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
|  | **Sample A** | **Sample B** | **Sample C** | **Sample D** | **Sample E** |
| **Xm** | 969.1 | 970.0 | 887.3 | 438.9 | 435.9 |
| **L** | 14 | 14 | 14 | 14 | 15 |
| **Sr** | 3.63 | 4.43 | 4.61 | 3.32 | 2.97 |
| **SL** | 2.23 | 3.92 | 4.73 | 7.29 | 4.08 |
| **SR** | 4.26 | 5.92 | 6.60 | 8.01 | 5.05 |
| **r** | 10.16 | 12.40 | 12.90 | 9.28 | 8.32 |
| **R** | 11.93 | 16.57 | 18.48 | 22.43 | 14.14 |
| **RSDr** | 0.37 | 0.46 | 0.52 | 0.76 | 0.68 |
| **RSDR** | 0.44 | 0.61 | 0.74 | 1.82 | 1.16 |
| **RSDR(Hor)** | 2.01 | 2.01 | 2.04 | 2.26 | 2.27 |

Sample A: Results of Lab 3 eliminated; Sample B: Results of Lab 16 eliminated; Sample C: Results of Lab 3 eliminated; Sample D: results of Lab 13 eliminated; Sample E: no results eliminated.

xm = overall sample mean

L = number of laboratories

sr  = repeatability standard deviation

RSDr = relative repeatability standard deviation

r = repeatability limit

sR = reproducibility standard deviation

RSDR = relative reproducibility standard deviation

R = reproducibility limit

sL = “pure” between laboratory standard deviation

RSDR(Hor) = relative reproducibility standard deviation (Horwitz equation)

**Figures 1 – 5 (All Results)**

**Figure 1:**

**Figure 2:**

**Figure 3:**

**Figure 4:**

**Figure 5:**

# Conclusions

Fifteen different laboratories participated in this collaborative study. The results from the laboratories are provided in Tables 1-2, the statistical summary is included in Tables 3-4. The results for all of the samples evaluated are illustrated in Figures 1-5.

Without elimination of any outliers or stragglers the between lab experimental Relative Reproducibility Standard Deviation (% RSDR) is below the calculated acceptable value based on the Horwitz curve calculation (% RSDR (Hor)) in all samples. The minimum number of considered results after elimination of stragglers and outliers was 14.

Taking into account the relatively high number of participating laboratories a broad basis was given even after elimination of the outliers. Therefore, we consider this Atrazine method as presented to be suitable with potential inclusion of the mid-polarity DB-1701 capillary GC column as an alternative to the proposed DB-225 column. We recommend accepting this method as a provisional CIPAC MT-method for the determination of Atrazine in both technical concentrate and its associated formulated products (WG, SC).