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**CERTIFICATE OF ANALYSIS
REFERENCE MATERIAL No. IPO 121**

***o,o'*-DDE**

1,1-dichloro-2,2-bis(2-chlorophenyl)ethylene

$C_{14}H_8Cl_4$ (318.0)

Series No. 6A/11

Valid to: December 2018

Purity: $99.5 \pm 0.3\%$ (m/m)

Unit: 0.02 g of crystalline solid in a brown glass vial.

Storage: The material should be stored in the original closed vial in a refrigerator at $5\text{ }^\circ\text{C} \pm 4\text{ }^\circ\text{C}$ until it is required to use. Allow to equilibrate to ambient temperature before opening. It is intended for use as a reference material for the calibration of measuring equipment, for the evaluation of analytical procedures and for the study of biological activity

CONFIRMATION OF THE IDENTITY

The identity of the product was established by infrared spectroscopy, mass spectrometry, Carbon-13 NMR spectroscopy and proton NMR spectroscopy.

The IR spectrum (KBr disc technique, scanning from 4000 to 400 cm^{-1}) and mass spectrum (EI, 70 eV, temperature of ion source $250\text{ }^\circ\text{C}$) of sample was recorded. Proton NMR (200 MHz, $CDCl_3$) and Carbon-13 NMR (50 MHz, $CDCl_3$) spectra were also performed. The product structure was confirmed by the results of all above mentioned techniques.

DETERMINATION OF THE PURITY

Representative samples were drawn from the bulk material. The purity value was based on determinations made on these representative samples using the following methods:

- differential scanning calorimetry (DSC)
- gas chromatography (GC)
- high performance liquid chromatography (HPLC)
- thin layer chromatography (TLC)

The uncertainties quoted below are the half-width of a 95% confidence interval based on the standard deviation of the results obtained. The certified uncertainty is the combined uncertainty calculated according to the methodology described in¹ with a coverage factor $k = 2$. It corresponds to a confidence level of 95%. The thin layer chromatography (TLC) method was used as used as semiquantitative estimation of purity.

Determination by DSC

Range of temperature: 130°C to 160°C, heating rate: 2°C/min. The results indicated a purity value of 99.56 ± 0.07 mole% ($n = 4$).

Melting point of sample: 147°C.

Determination by GC

Column I: fused-silica DB-1 (30 m x 0.53 mm i.d.), film thickness 3.0 µm.

Column II: fused-silica DB-17 (30 m x 0.53 mm i.d.), film thickness 1.5 µm.

Temperature conditions:

- Column: 50°C (3 min) to 210°C (5°/min), 210°C (30 min) to 240 °C (5°/min)
- Injector: 230°C
- Detector (flame ionisation): 270 °C

Carrier gas: 5-6 ml/min.

Injection volume: 0.2 µl of sample solutions in acetone (0.6-1.4% *m/V*).

The purity was calculated by peak area normalisation of the chromatograms.

Result I: Three impurities were detected with a total concentration of $0.36 \pm 0.13\%$ (*m/m*) ($n = 4$).

Result II: Two impurities were detected with a total concentration of $0.20 \pm 0.01\%$ (*m/m*) ($n = 4$).

Determination by HPLC

- Column: Luna C₁₈, 5 µm, 250 x 4 mm i.d.
- Mobile phase: acetonitrile + water (60:40, *V/V*)
- Flow rate: 1.0 ml/min
- UV detection at 206 nm.
- Injection volume: 20 µl of sample solutions in acetonitrile (0.01-0.1% *m/V*).

The purity was determined assuming equal detector response factors for all constituents.

Two impurities were detected with a total concentration of $0.31 \pm 0.01\%$ (*m/m*) ($n = 4$).

Estimation by TLC

- Adsorbent: silica gel 60 G F₂₅₄
- Mobile phase:
 - I n-hexane
 - II n-hexane + ethyl acetate (9:1, *V/V*)
- Visualisation: UV at 254 nm; silver nitrate + UV irradiation
- Sample loading: 100; 1000 µg of sample in acetone.

Results: Two additional light spots were observed at the higher loading in both solvent systems.

CONCLUSIONS

On the basis of the results of three independent quantitative methods (DSC, GC and HPLC), the purity of this batch of *o,o'*-DDE was assessed as $99.5 \pm 0.3\%$ (*m/m*).

The analytical measurements were coordinated by:

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1. NIST Special Publication 1012. An approach to the metrologically sound traceable assessment of the chemicals purity of organic reference materials David L. Duever and others, US Department of Commerce USA, September 2004, p.22