

CCQM-K55.b (Aldrin)
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**CCQM-K55.b Key Comparison on the
Characterization of Organic Substances for Chemical Purity**

Coordinating laboratory:

Steven Westwood*, Ralf Josephs, Tiphaine Choteau, Adeline Daireaux, Charline Mesquida and Robert Wielgosz
Bureau International des Poids et Mesures (BIPM, Coordinating laboratory)
Sèvres, France

With contributions from:

Adriana Rosso and Mariana Ruiz de Arechavaleta
Instituto Nacional de Tecnologia Industrial (INTI)
Buenos Aires, Argentina

Stephen Davies and Hongjie Wang
National Measurement Institute (Australia) (NMIA)
Pymble, NSW, Australia

Eliane Cristina Pires do Rego, Janaína Marques Rodrigues, Evelyn de Freitas Guimarães, Marcus Vinicius Barreto Sousa, Tânia Maria Monteiro, Laura Alves das Neves Valente, Fernando Gustavo Marques Violante, Renato Rubim Ribeiro Almeida, Maria Cristina Baptista Quaresma and Raquel Nogueira
Instituto Nacional de Metrologia, Qualidade e Tecnologia (INMETRO)
Xerem, Brazil

Anthony Windust
Institute for National Measurement Standards - National Research Council Canada (NRC-INMS)
Ottawa, Canada

Xinhua Dai, Xiaomin Li, Wei Zhang, Ming Li, Mingwu Shao, Chao Wei
National Institute of Metrology (China) (NIM)
Beijing, China

Siu-kay Wong
Government Laboratory of Hong Kong SAR (GLHK)
Kowloon, Hong Kong SAR

Julie Cabillic, Fanny Gantois
Laboratoire National de Métrologie et d'Essais (LNE)
Paris, France

Rosemarie Philipp, Dietmar Pfeifer, Sebastian Hein, Urszula-Anna Klyk-Seitz
Bundesanstalt für Materialforschung (BAM)
Berlin, Germany

Keiichiro Ishikawa
National Metrology Institute of Japan (NMIJ)
Tsukuba, Japan

Esther Castro and Norma Gonzalez
Centro Nacional de Metrologia (CENAM)
Querétaro, Mexico

Anatoly Krylov
D.I. Mendeleev Scientific and Research Institute for Metrology (VNIIM)
St Petersburg, Russia

Teo Tang Lin and Lee Tong Kooi
Health Sciences Authority (HSA)
Singapore

M. Fernandes-Whaley, D. Prévoo, M. Archer, R. Visser, N. Nlhapo and B. de Vos
National Metrology Institute of South Africa (NMISA)
Pretoria, South Africa

Seonghee Ahn
Korea Research Institute of Standards and Science (KRISS)
Daejeon, South Korea

Preeyaporn Pookrod, Kanjana Wiangnon, Nittaya Sudsiri, Kittiya Muaksang and Chainarong Cherdchu
National Institute of Metrology (Thailand) (NIMT)
Bangkok, Thailand

Ahmet Ceyhan Gören and Mine Bilsel
National Metrology Institute of Turkey (UME)
Gebze-Kocaeli, Turkey

Thierry LeGoff
LGC Limited (LGC)
Teddington, United Kingdom

Dan Bearden, Mary Bedner, David Duewer, Diane Hancock, Brian Lang, Katrice Lippa, Michele Schantz
and John Sieber
National Institute of Standards and Technology (NIST)
Gaithersburg, MD, USA

Comparison coordinator: Steven Westwood, BIPM (steven.westwood@bipm.org)

Introduction

The CCQM-K55 comparison was undertaken by the CCQM Organic Analysis Working Group (OAWG) for National Measurement Institutes (NMIs) and Designated Institutes (DIs) which provide measurement services in organic analysis under the CIPM Mutual Recognition Arrangement (MRA). The ability to perform suitable purity assessment on the materials that an NMI either makes available to external users as pure substance reference materials or that are used by an NMI as their primary calibrators for the assignment of the property values either of solution or matrix reference materials or for their reference measurement services is a core technical competency for the provision of measurement results in organic analysis that are traceable to the SI. The purity property value (generally reported for applications in organic analysis as the mass fraction^a of the main component) assigned to the primary calibrator in a measurement hierarchy underpins the traceability chain for all results linked to that calibrator. All NMIs with ongoing programs in organic analysis were encouraged to participate in this series of comparisons.

The comparisons allow NMIs and DIs to demonstrate that their procedure(s) for assignment of a purity property value and its associated uncertainty are fit for purpose for their intended application(s).

Summary of Previous Studies

The CCQM-P20 multi-round pilot study on purity determination was completed prior to the CCQM-K55 comparison. Studies were undertaken on the purity assessment of tributyl tin chloride (CCQM-P20.a), xylene (CCQM-P20.b), atrazine (CCQM-P20.c), chlorpyrifos (CCQM-P20.d), theophylline (CCQM-P20.e)¹ and digoxin (CCQM-P20.f)².

The “mass balance” or “summation of impurities” method for purity assessment, which aims to identify and quantify on a mass fraction (mg/g) basis all the orthogonal classes of impurity present in the material and by subtraction provides a measure of the mass fraction of the main component, was the most widely used approach by participants in the CCQM-P20 pilot studies. However the use of quantitative nuclear magnetic resonance (qNMR) approaches to obtain a direct measure of the content of the main component were also increasingly being used.

The BIPM coordinated the final two rounds of the CCQM-P20 pilot study and developed a “molecular weight v. polarity” model to map the analytical space for comparisons in this area. This model provided the criteria for the selection of the measurands for each of the four consecutive rounds – respectively CCQM-K55.a, CCQM-K55.b, CCQM-K55.c and CCQM-K55.d – that make up the initial CCQM-K55 key comparison. The relation based on this model between the proposed CCQM-K55 comparison materials, potential future measurands and major areas of calibration and measurement capability (CMC) claims for the provision of primary calibrators and calibration solutions for organic analysis currently accepted or under consideration under the CIPM Mutual Recognition Arrangement is shown in Annex A. The OAWG meeting at Sèvres in April 2008 accepted this overall strategy for the comparison as well as the specific measurand, 17 β -estradiol, proposed for the first comparison round, CCQM-K55.a. A pilot study, CCQM-P117.a, was undertaken in parallel with the key comparison. The CCQM-K55.a comparison was completed in 2009 and the Final Report was published in September 2012 in Appendix B of the BIPM Key Comparison Database.³

The BIPM proposed aldrin as the measurand for CCQM-K55.b. The proposal was discussed at several OAWG meetings and approved at the April 2009 meeting at Sèvres. The comparison samples were distributed in May 2010. The individual results were communicated to the comparison coordinator in August 2010 and the results were first discussed at the November 2010 meeting of the CCQM OAWG in

^a For the purposes of this comparison, the mass fraction of both the main component and associated impurities are expressed in units of mg/g. The upper limit value of 1000 mg/g would correspond to a “100 %” pure material.

Singapore. Further studies were subsequently undertaken to resolve a disparity between the results obtained by mass balance approaches and those obtained by qNMR. This difference, which was finally ascribed to the presence of a significant non-volatile organic impurity (ca. 1% on a relative mass fraction basis) which was not detected by the majority of participants was further discussed at the April 2011 OAWG meeting at Sèvres and an assignment of a KCRV proposal for aldrin in CCQM-K55.b based on combination of separate KCRV estimates for contributing orthogonal impurity classes was accepted.

Aldrin

Aldrin was selected as the measurand for the second round of the comparison because it:

- provides an analytical challenge representative of a laboratory's capability for the purity assignment of organic compounds of medium structural complexity and low polarity (see "How Far The Light Shines" statement);
- represents a sector for general CMC claims on the "analysis space" model (Annex A) which is distinct from the area already covered by the CCQM-K55.a measurand, estradiol.
- is a chlorinated pesticide for which there are a number of specific CMC claims in Appendix C of the BIPM Key Comparison Database;
- is classified as a Persistent Organic Pollutant (POP) and an important analyte in its own right in the areas of environmental and food residue analysis
- is safe and stable for transport in the amounts involved for the comparison and was available in sufficient amount to allow the preparation of a relatively large batch of the comparison sample.

The structure of aldrin (**1**) is shown in Figure 1 with the conventional ring numbering in bold. The structure of other compounds related to aldrin and referred to in this report are given in Annex B.

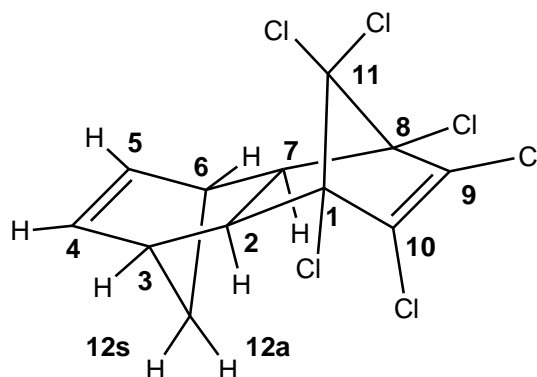


Figure 1 – Structure and ring-numbering of Aldrin

Pure aldrin is a colourless solid with a reported melting point of 104 °C. It is insoluble in water, moderately soluble in alcohols and generally soluble in non-polar organic solvents. CMC claims for the measurement of aldrin, disseminated as both pure substance and standard solution CRMs, are listed in the BIPM Key Comparison Database (KCDB) Appendix C.

KEY COMPARISON – MATERIAL AND CONDUCT OF STUDY

For the preparation of the study material for the CCQM-K55.b comparison and the parallel pilot study CCQM-P117.b, a bulk sample of technical grade aldrin donated by the NMIA was used as the source material. This material was a dark, aggregated solid. It contained less than 90% aldrin as determined by relative GC-FID response. A number of related structure or residual starting material impurities were present at significant levels in the material and which were identified by GC-MS.

Trial purification of the material by recrystallisation was investigated using various organic solvent combinations. The most suitable solvent for scaling up the production of the source material was determined to be aqueous methanol. Three successive recrystallisations, which included some recombination of crops obtained from earlier recrystallisation rounds, were required to produce a bulk material having aldrin content greater than 95% by relative GC-FID response.

This material was dried under vacuum at room temperature, ground in a planetary ball mill to give a free running grey powder and redried to constant weight under vacuum at 40 °C.

The ground, dried bulk material was subdivided into a batch of 150 individual units given the BIPM identifier OGP.014. Each unit of BIPM OGP.014 contained a minimum of 500 mg of aldrin in a glass storage vial (5 mL capacity) fitted with a rubber insert and crimped with an aluminium cap.

The batch of candidate material vials were evaluated for impurity profile, homogeneity and stability at the BIPM. The mass fraction of aldrin in the comparison material was assessed by the BIPM to be greater than 950 mg/g while the homogeneity and stability of the aldrin and the associated impurity components were suitable for the purposes of the comparison.

A summary of the results for aldrin content and for characterization of the material's impurity profile reported by the study participants are contained in this report.

“How Far The Light Shines” Statement for CCQM-K55.b

The comparison is intended to demonstrate a laboratory's performance in determining the mass fraction of the main component in a relatively pure organic material. The measurement results should be indicative of the performance of a laboratory's measurement capability for the purity assignment of organic compounds of medium structural complexity (relative molecular mass range 300-500) and low polarity ($pK_{OW} < -2$) where K_{OW} is the octanol-water partition coefficient⁴ and is representative of compounds for which related structure impurities are generally quantified by capillary gas-phase chromatography.

The expected overall outcome of the rounds making up the CCQM-K55 comparison is to evaluate through a series of strategically planned exercises the scope, applicability, limitations and appropriateness of the various procedures used to assign mass fraction property values to organic materials.

Characterisation study

The methods used to investigate, assign and confirm the quantitative composition of the CCQM-K55.b candidate material by the BIPM are summarised below.

Characterization studies

Related structure impurity content was evaluated by:

- a. GC-FID
- b. GC-MS
- c. LC-UV
- d. ^1H and ^{13}C NMR

Water content was evaluated by:

- a. coulometric Karl Fischer titration with oven transfer of water from the sample
- b. coulometric Karl Fischer titration using direct addition of the sample
- c. thermogravimetric analysis (TGA) as a consistency check for the assigned value
- d. microanalysis (% C,H content) as a consistency check for assigned value

Residual solvent content was evaluated by:

- a. GC-MS by direct injection
- b. Headspace GC-MS
- c. ^1H NMR
- d. Thermogravimetric analysis as a consistency check for the assigned value
- e. Microanalysis (% C,H content) as a consistency check for the assigned value

Non-volatile/ inorganics content :

- a. ICP-MS for common elements (Na, K, Ca, Mg, Si, Fe, Al)
- b. Microanalysis (% C, H content) as a consistency check for the assigned values

Main component (Aldrin) content

- a. qNMR
- b. Differential scanning calorimetry (DSC)

Homogeneity studies

i. Related structure components

The homogeneity of minor components related in structure to aldrin were assessed by sampling ten sub-units selected from across the candidate material batch with analysis by GC-FID and separately by LC-UV with detection at 220 nm. The minimum sample size used to prepare each analysis sample was 2.5 mg.

ii. Water

The homogeneity of the material relative to water content was assessed by coulometric Karl Fischer titration using oven transfer and a minimum sample size of 50 mg per analysis on five sub-units representative of the candidate material batch

iii. Residual solvent

The homogeneity of the material relative to methanol content was assessed by direct injection GC-MS analysis using a minimum sample size of 10 mg per analysis on five sub-units representative of the candidate material batch.

iv. Inorganics content

Three units selected from across the production batch were analysed by ICP-MS and by elemental microanalysis for carbon and hydrogen. All gave metal content levels below the detection limits (25 ppm) for each element. Results for % C, H content were in accord with the molecular formula of aldrin

v. Aldrin

As a consistency check, the homogeneity of the aldrin content in the material was assessed using the ten sub-units selected for the related structure impurity study by the same GC-FID and LC-UV methods developed for the related structure impurity characterisation. In addition a limited qNMR study was undertaken (two samples from two units of CCQM-K55.b), using dimethyl terephthalate as the internal standard, which gave a value of 958 mg/g with standard uncertainty 2 mg/g for the aldrin content of the sample

The uncertainty contributions due to the inhomogeneity of each related structure impurity component were evaluated by ANOVA. This provided an estimate of the variation due to inhomogeneity of each impurity at a stated sampling size both between and within sample units.

The uncertainty contributions due to the inhomogeneity of the major related structure components detected by GC-FID ($u_{bb(rel)}$) were evaluated by ANOVA. This provided an estimate of the variation due to inhomogeneity of related structure impurities at the stated sampling size both between and within sample units. Acceptable uncertainty contributions due to inhomogeneity were observed for each of the resolved impurities present in the sample. Table 1 shows the estimated content, $u_{bb(rel)}$ and $u_{bb(abs)}$ for each of the resolved related structure impurities, and a combined value for the overall uncertainty contribution from between unit inhomogeneity (u_{bb}) of the related structure impurities content of the material. This was calculated as 0.42 mg/g by quadratic combination of the absolute inhomogeneity uncertainties for each impurity.

Impurity	Content (mg/g) from homogeneity study*	$u_{bb(rel)}$ (%)	$u_{bb(abs)}$ (mg/g)
Isodrin	27	1.36	0.37
Dieldrin	3.0	1.08	0.03
Dechlorane	4.0	5.0	0.20
Minor impurities	1.5	2.0	0.03
Combined related structure impurities	35.5	1.2	0.42

* From relative GC-FID peak area – uncorrected for differential response factors or other impurities

Table 1: Homogeneity assessment for related structure impurities in CCQM-K55.b

Karl Fischer titration was used to assess variations in water content of the material. As the level of water in the material was below the limit of quantification of our method, it was not possible to estimate the uncertainty of between unit inhomogeneity of the water content of the material in a rigorous manner. There was however no evidence of significant inter-sample differences.

The contribution to the overall uncertainty from between unit inhomogeneity of the methanol content of the material was estimated at $u_{bb(\text{MeOH})} = 0.1 \text{ mg/g}$ from comparison of the within unit and between unit repeatability of the analysis of two 10 mg replicates taken from each of five units of the candidate material. The within unit repeatability (s_r) under these conditions was 0.1 mg/g.

Stability studies

An isochronous stability study was performed using a reference storage temperature of $-20 \text{ }^\circ\text{C}$ and test temperatures of $4 \text{ }^\circ\text{C}$, $22 \text{ }^\circ\text{C}$ and $40 \text{ }^\circ\text{C}$. A set of units from the production batch were stored at each selected temperature over 8 weeks, with units transferred to reference temperature storage at 2-week intervals.

Trend analysis of the data obtained by GC-FID analysis of the stability test samples under repeatability conditions indicated no significant change in the relative composition of aldrin or of the related structure components over this time at any of the test temperatures.

No significant changes in water content, which in any case were all below the level of quantification of our method, were observed after storage at $4 \text{ }^\circ\text{C}$ or $22 \text{ }^\circ\text{C}$. There was some evidence of minor uptake of water but only after prolonged storage at $40 \text{ }^\circ\text{C}$. The methanol content of the material was stable on storage at $4 \text{ }^\circ\text{C}$ and $22 \text{ }^\circ\text{C}$ but did decrease significantly after storage beyond 6 weeks at $22 \text{ }^\circ\text{C}$ or 2 weeks at $40 \text{ }^\circ\text{C}$.

The effect of storage temperature on methanol content of the comparison material is shown in Figure 2.

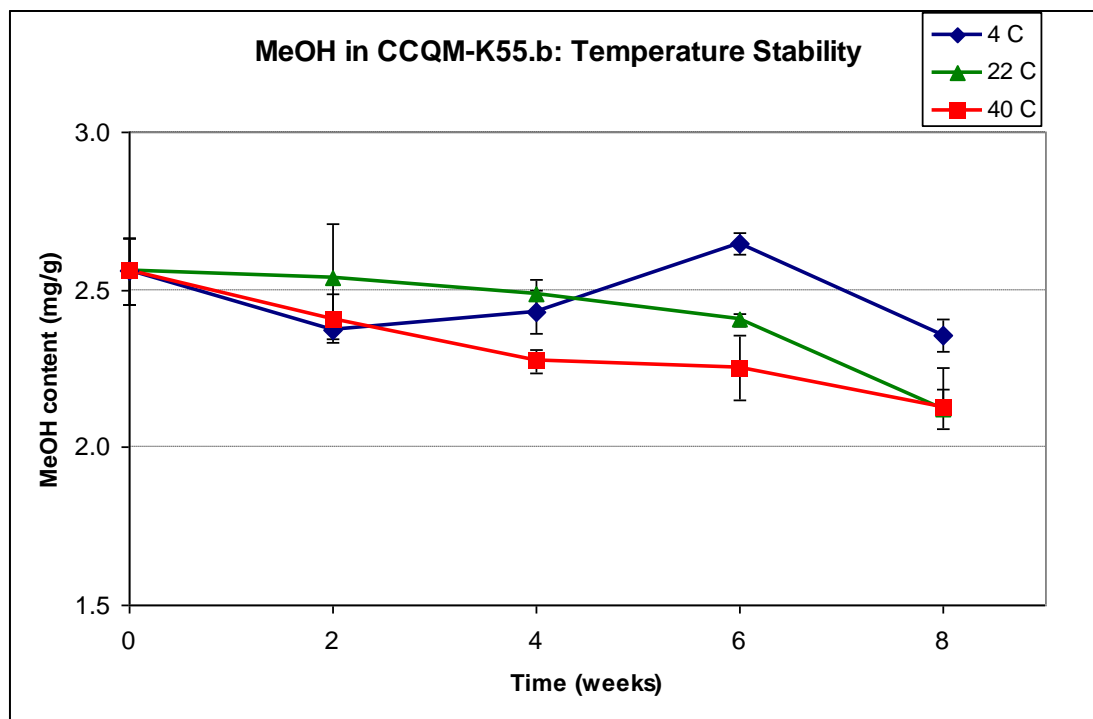


Figure 2 Effect of storage temperature on methanol content of comparison material

On the basis of these studies it was concluded that for the purposes of the comparison the material was suitably stable for short-term transport at ambient temperature, provided it was not exposed to temperatures significantly in excess of 40 °C, and for longer term storage at 4 °C.

To minimise the potential for changes in the material composition due to loss of methanol, participants were instructed to store the material at 4 °C.

Sample distribution

Two units of the study sample, each containing a minimum of 500 mg of material, were distributed to each participant. Participants were asked to return a form acknowledging receipt of the samples and to advise the co-ordinator if any obvious damage had occurred to the vials during shipping. Recipients were asked to confirm that a monitoring strip inside the shipping container had not registered a temperature in excess of 37 °C during the transport process.

The monitor strips indicated that the units originally supplied to Singapore (HSA) were exposed to temperatures in excess of 40 °C during shipping. A replacement set was provided in this case which was delivered without incident. There was also a prolonged delay in delivery of the original sample sent to CENAM due to customs clearance problems. A replacement sample set was issued, which was delivered without difficulties. Otherwise all samples were delivered to the comparison participants without incident. Every registered participant in the CCQM-K55.b comparison provided a result for their sample.

Quantities and Units

Participants were required to report the mass fraction of aldrin, the major component of the comparison sample, in one of the two units supplied to them. The additional unit was provided for method development and trial studies.

In addition all participants who used a mass balance (summation of impurities) procedure to determine the aldrin content were required to report the combined mass fraction assignment and associated uncertainty for some or all of the following sub-classes of impurity.

- i. total related structure organic substances
- ii. water
- iii. total residual organic solvent / volatile organic compounds (VOCs)
- iv. total non-volatile organics & inorganics

Participants were encouraged to also provide mass fraction estimates for the main impurity components they identified in the comparison sample.

Reported Mass Fraction of Aldrin in CCQM-K55.b

The values reported by participants for aldrin content in CCQM-K55.b are given in Table 2 with a summary plot in Figure 3.

Participant	Aldrin (mg/g)	Standard Uncertainty (mg/g)	Coverage factor	Expanded Uncertainty (mg/g)
NRC-INMS	947.8	3.0	2	6.1
NMIJ	950.1	3.2 ₃	2	6.5
HSA	950.4	1.3	2	2.6
BAM	953	4	2	8.0
NIMT	958.5	1.4	2	2.8
NMISA	960.2	1.9	2	3.7
CENAM *	960.7	3.5	2	7.0
LGC	960.9	1.9	2	3.8
NIST	961	NA	NA	+1.6 / -1.8
KRISS	961.6	0.9	2	1.8
UME	961.7	1.0	2	2.1
LNE	962.0	0.47	2	0.93
NMIA	962.1	2.4	2.07	4.9
BIPM	962.6	+0.65 / -0.75	2	+1.3 / -1.5
GLHK	963.1	1.7	2	3.4
INTI	963.7	9.5	2	19
NIM	964.6	1.2	2	2.4
INMETRO	966.8	0.57	2.45	1.4
VNIIM	972.6	0.9	2	1.8

Table 2 : Aldrin content for participants in CCQM-K55.b

* Revised value from initially reported result, received prior to circulation of all results

Aldrin content in CCQM-K55.b

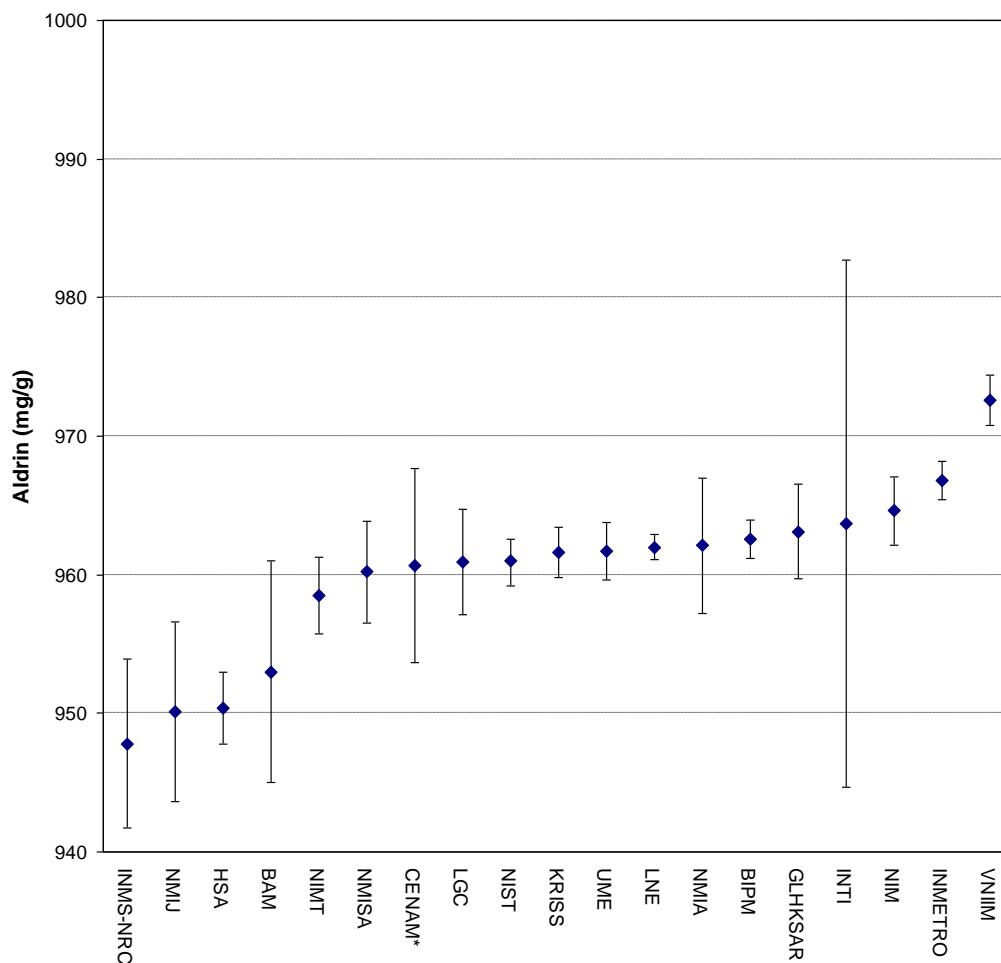


Figure 3 Aldrin content reported by participants in CCQM-K55.b (plotted with 95% confidence interval expanded uncertainties)

* Revision of initially reported result, received prior to the circulation of all results

Disparity between Mass Balance and qNMR estimates for Aldrin in CCQM-K55.b

Inspection of the reported values for aldrin content in CCQM-K55.b shows that the comparison results can be divided into two main groups – one having a consensus value for aldrin in excess of 960 mg/g and a second where the assigned value is in the vicinity of 950 mg/g.

The higher estimate was obtained by participants using a mass balance approach in which hyphenated chromatography - and qNMR in the case of NIST - was used to determine the total related structure impurity levels with contributions from other potential classes of impurities obtained by complimentary techniques. The aldrin content of CCQM-K55.b was then assigned by subtraction of the combined impurity estimate from the theoretical maximum of 1000 mg/g.

The group of results with the lower assigned value for aldrin were based on qNMR techniques to directly determine the aldrin content either as the stand-alone approach or undertaken in conjunction with a mass balance approach.

The direct assignment of aldrin content by qNMR was achieved by integration of the signal due to the equivalent olefinic H4- and H5-protons (Fig. 1), which are distinct from interfering signals due to the main impurities - isodrin, dieldrin, dechlorane and methanol. Representative ¹H NMR spectra for CCQM-K55.b including an expansion of the main region containing signals due to the various impurities are reproduced in Annex C.

The NMIJ and the HSA were the only participants to report a mass balance assignment below 955 mg/g. In each case they obtained direct qNMR values for aldrin in the vicinity of 950 mg/g. Their mass balance estimates were in agreement within their stated uncertainties with this value, and were markedly lower than those reported by other participants, because they reported a significant level (ca. 10 mg/g) for non-volatile organic impurity, which was not identified by other participants. It should be noted that the NRC-INMS in CCQM-K55.b and the SIRIM, who participated in the parallel CCQM-P117.b pilot study, both using qNMR as a stand alone technique, also assigned the aldrin content at a level consistent with those reported by the NMIJ and HSA.

The situation however was not simply a choice between a low qNMR estimate and (generally) a higher mass balance value for aldrin. The NMIA, BIPM and UME all obtained qNMR assignments for aldrin content that were in excess of 955 mg/g and were consistent within their associated expanded uncertainty estimates with their independent mass balance values.

A combined comparison plot of aldrin content obtained by participants by direct qNMR compared with the mass balance value obtained by the same participant is shown in Figure 4. In several cases the qNMR data shown in Figure 4 was not included in the participant's report but was made available to the study coordinator subsequent to the circulation of the comparison results and the initial discussion at the November 2010 OAWG meeting. The additional information on qNMR conditions and results, with comparison with the mass balance value reported where available, are summarised in Table 3.

Note that in Figure 4 results are plotted with their standard uncertainties or the standard deviation of the qNMR estimate rather than the expanded uncertainties as shown in other data graphs.

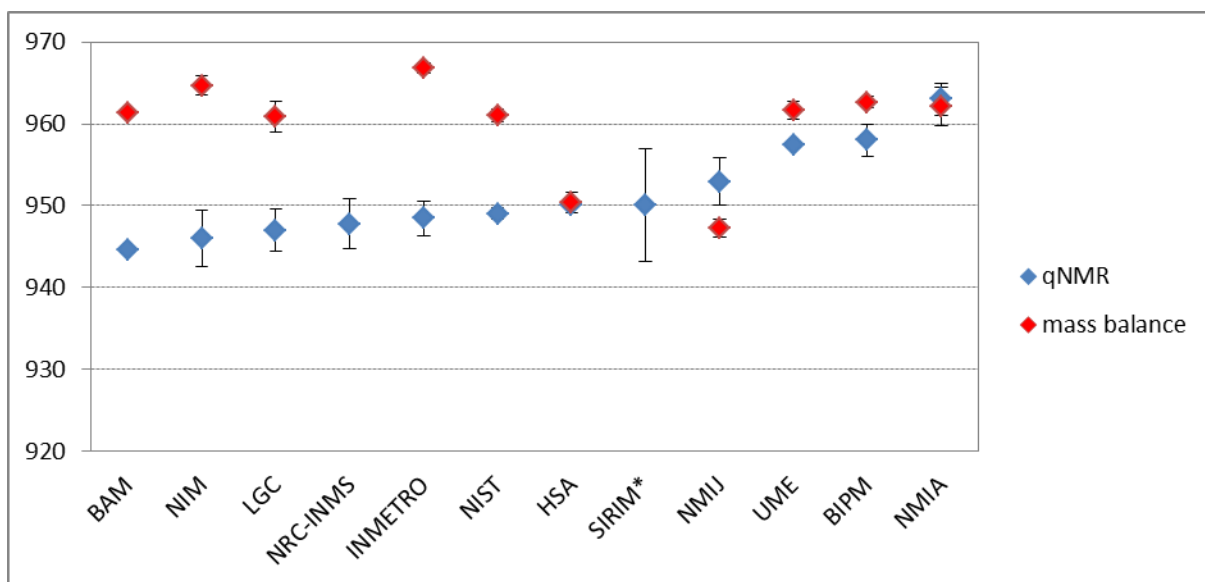


Figure 4 Comparison of direct qNMR and mass balance values ($\pm u_c$ or std. devn.) provided by the same participant for aldrin content of CCQM-K55.b (*CCQM-P117.b for SIRIM).

Participant	Solvent(s)	Quantification Standard	Aldrin (mg/g) by “direct” qNMR	Aldrin (mg/g) by “mass balance”
BAM	CDCl ₃	Benzoic acid	944.6	961.4
LGC	CDCl ₃	Trioxane	947 ($\sigma = 2.6$)	960.9 ($u_c = 1.9$)
NRC-INMS	CDCl ₃ , CD ₃ OD	Benzoic acid	947.8 ($u_c = 3$)	
INMETRO	CDCl ₃	Benzoic acid	948.5 ($\sigma = 2.1$)	966.8 ($u_c = 0.6$)
NIST	CD ₃ OD	Benzoic acid	949 ($u_c = 0.7$)	961 ($u_c = 0.8$)
NMIJ	CD ₂ Cl ₂	Benzoic acid	952.9 ($u_c = 2.9$)	947.2 ($u_c = 1.1$)
UME	d ₆ -Acetone	1,4-DCB	957.4 ($u_c = 0.2$)	961.7 ($u_c = 1.1$)
BIPM	d ₆ -DMSO	DMTP	958 ($\sigma = 2$)	962.6 ($u_c = 0.7$)
NMIA	d ₆ -DMSO, CD ₃ OD, CDCl ₃	DMTP	963 ($\sigma = 1.9$)	962.1 ($u_c = 2.4$)
HSA	CDCl ₃	Benzoic acid	950	950.4 ($u_c = 1.3$)
NIM	CDCl ₃	Benzoic acid	946 ($u_c = 3.5$)	964.7 ($u_c = 1.2$)
SIRIM*	CDCl ₃	Chlorpyrifos	950.1 ($\sigma = 6.9$)	

**Table 3 – qNMR and mass balance estimates for aldrin in CCQM-K55.b
(1,4-DCB = 1,4-Dichlorobenzene ; DMTP = Dimethyl terephthalate)**

At the OAWG discussions in November 2010, the NMIJ presented supporting data from a GC-FID external calibration using a certified sample of aldrin as the primary calibrant. Their value for aldrin in CCQM-K55.b obtained in this manner was consistent with their reported value for the comparison.

There was considerable discussion on possible reasons for the discrepancy between mass balance and qNMR results at the November 2010 OAWG meeting. To attempt to resolve the issue additional studies were requested in the following areas:

- homogeneity of aldrin in CCQM-K55.b by qNMR
- influence of solvent and internal standard on qNMR result for aldrin in CCQM-K55.b
- additional evidence for the presence of a significant non-volatile organic impurity

At the April 2011 OAWG meeting the results of qNMR studies on five samples of CCQM-K55.b selected from across the production batch and undertaken by the NRC-INMS were reported. They prepared triplicate samples from each unit and determined the aldrin content of solutions in CD₃OD with benzoic acid as the internal quantification standard. The results obtained, which also include estimates for the associated impurities isodrin and methanol, are summarised in Table 4 and are consistent with an aldrin content of CCQM-K55.b of 950 mg/g with no evidence of significant inhomogeneity between the individual units. In addition, no effect on qNMR values due to use of different solvent (comparing CD₃OD with d₆-DMSO) or internal standard (benzoic acid v. dimethyl terephthalate) was found.

Bottle	Aldrin	Isodrin	MeOH
003-A	950.5	25.5	2.2
003-B	948.0	26.3	2.1
003-C	951.6	26.4	2.0
030-A	953.0	26.5	2.0
030-B	948.2	25.5	1.9
030-C	951.9	26.3	2.1
059-A	951.9	26.8	2.0
059-B	-	-	-
059-C	952.5	26.4	2.3
090-A	952.0	26.5	1.8
090-B	951.7	26.4	1.9
090-C	946.9	25.2	2.1
125-A	952.2	26.6	2.0
125-B	952.8	26.5	2.0
125-C	945.4	25.9	2.2
Mean	950.6	26.2	2.0
sd	2.5	0.48	0.1
rsd	0.3%	1.8%	5%

Table 4 – qNMR results (mg/g) for homogeneity check of aldrin, isodrin and methanol in CCQM-K55.b (data provided by NRC-INMS for solutions in CDCl₃ using benzoic acid as quantification standard)

The NMIJ reported size-exclusion chromatography studies that indicated the presence of high molecular weight impurities equivalent to approximately 1.2% of the peak area response by refractive index detection relative to the response of the aldrin component. Peaks due to higher molecular weight species were not observed when higher purity reference standards of aldrin were analysed under the same conditions. A representative chromatogram is shown in Figure 5.

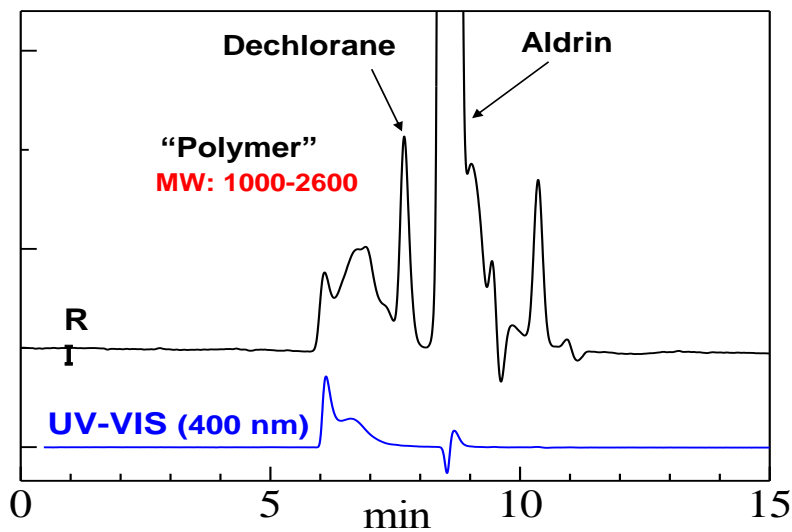


Figure 5
Gel permeation chromatogram of CCQM-K55.b (SHODEX KF-401HQ column), elution with 100% THF; detection by refractive index (black) and UV (blue) response. - reproduced from data provided by the NMIJ

The NMII also reported that cycles of dissolution and selective precipitation could be used to furnish purified samples of the non-volatile impurity.

After consideration of the additional data the study participants agreed that the presence of a non-volatile organic impurity that was not detected by the majority of mass balance approaches had been established and that this resolved the apparent difference between the aldrin values assigned by the two approaches.

The cases where higher values for aldrin were originally obtained by qNMR were explained as arising from software processing issues – in particular the applicability or otherwise of automated baseline correction algorithms commonly used in processing the raw FID data. The NMIA reported that when they used the automatic baseline correction option with their software they obtained a value for aldrin above 960 mg/g, but when this option was not used the result was 948 mg/g.

No further information is currently available as to the nature and structure of the impurity. Despite being present at appreciable levels it has still not been observed directly by MS or NMR methods and did not perturb the elemental analysis data for CCQM-K55.b significantly. This indicates that it is likely to be a polychlorinated compound structurally related to aldrin or its synthetic precursors. One suggested possibility is a mixture of oligo- or poly-(hexachlorocyclopentadiene), potentially formed as a side-product of technical grade aldrin synthesis from hexachlorocyclopentadiene monomer.

Impurity Profile and Key Comparison Reference Values (KCRVs) for Impurity Classes in CCQM-K55.b

All participants in CCQM-K55.b using a mass balance procedure to assign the aldrin content were required to give estimates for the mass fraction of the sub-classes of impurities they quantified to obtain their final aldrin mass fraction estimate. At the April 2011 WG meeting it was agreed that, as was done for CCQM-P20.f and CCQM-K55.a, the comparison coordinator would propose an overall KCRV for the aldrin content of CCQM-K55.b based on the combination of individual KCRVs for the mass fraction of each of the orthogonal classes of impurity in the CCQM-K55.b comparison sample.

This required the assignment of separate KCRVs for:

- structurally related impurities;
- water;
- volatile organic solvent;
- non-volatiles/inorganics.

i. KCRV for Aldrin-related impurity content

The structures of the individual impurities reported by two or more participants are shown, with their associated numbering, in Annex B. The major compounds identified by participants as present at levels above 0.1 mg/g in CCQM-K55.b were, in decreasing order of mass fraction, isodrin (2), dieldrin (3), dechlorane (4), dihydroaldrin (5) and endrin ketone (6). The detection in the sample at low levels (all below 0.1 mg/g) of hexachlorocyclopentadiene (7), octachlorocyclopentene (8) and chlordene (9) was also noted by several participants.

A GC-FID chromatogram obtained by NIST using a polar RTX-OP column was highly representative of GC chromatograms obtained by all other participants and shows the relative retention time of each of the main related structure impurities reported by participants and their structural assignment. It is reproduced

in Figure 6 below. It was noted by several participants that dihydroaldrin is not resolved from isodrin if a lower polarity column (eg DB-5) is used.

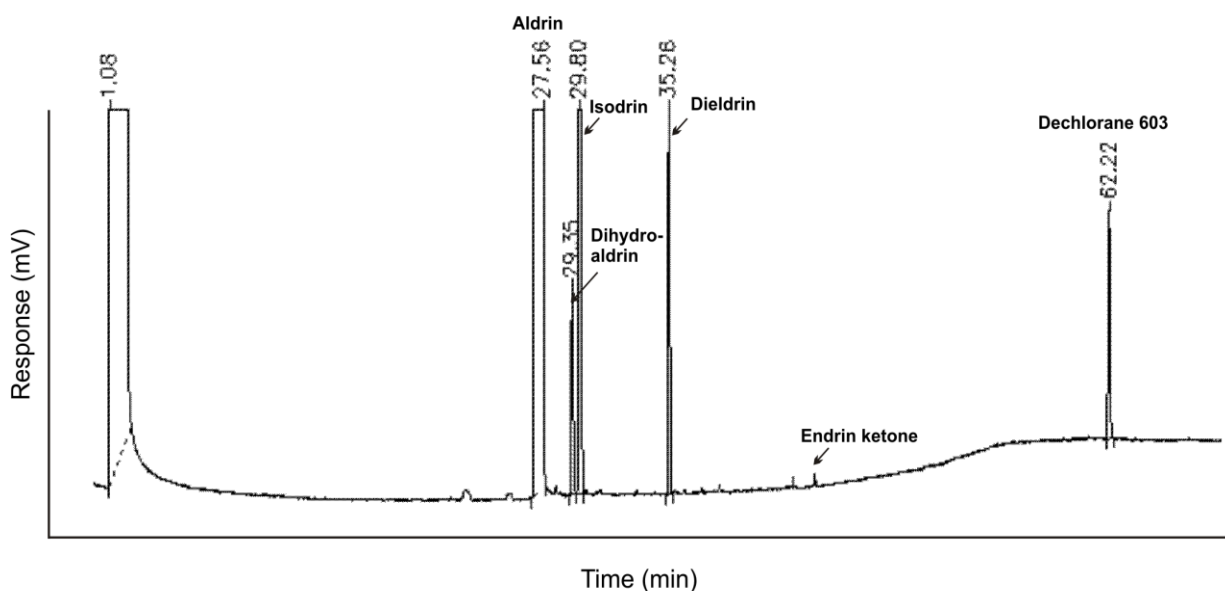


Figure 6 GC-FID chromatogram for CCQM-K55.b (reproduced from NIST report)

All participants reported and identified isodrin (2) and dieldrin (3) in the study material. The majority also reported the presence either by GC or LC or both of an impurity with a long retention time relative to aldrin, although only five participants identified it as dechlorane (4).

Many participants reported a peak corresponding to dihydroaldrin (5), eluting between aldrin and isodrin on GC using a DB-17 column or equivalent and at slightly longer retention time than aldrin by LC on reverse-phase columns. While several participants tentatively identified it as dihydroaldrin based on its mass spectrum, the NIST were the sole participant to establish the structure assignment by comparison with an independently synthesised sample. Although the reporting requirements only required a value for total related structure impurities, all but one participant also provided estimates for the major individual impurities. The reported values for individual and combined related structure impurities are listed below in Table 5.

Related substance impurities (mg/g)					
Participant	Isodrin	Dieldrin	Dechlorane	Total	u_c
INTI	21.8	2.62		24.4	0.5
VNIIM	21.3	5.2		27.3	0.9
NIM	26.47	3.4		32.5	1.1
INMETRO *	*	*	*	32.7	0.57
UME	25.62	2.94	2.68	32.85	0.047
LNE	28.38	3.53	2.03	33.94	0.46
BIPM	25.5	4.1	4.5	34.4	0.65
HSA	25.9	2.9	4.4	34.6	0.72
NIMT	28.98	3.72	2.1	34.8	1.38
NMIA	26.35	3.16	3.48	34.8	0.32
GLHKSAR	26.71	3.39	1.44	35.3	1.69
KRISS	27	3.15	3.64	35.5	0.11
NIST	26.88	3.19	4.13	36.1	0.8
BAM	26.1	3.2	3.7	36.2	0.05
LGC	28.08	4.08	3.05	36.45	1.83
NMISA	27.77	3.46	4.19	36.9	0.93
NMIJ	26.6	3.46	4.81	37	0.5
CENAM	31.9	3.6	2.65	38.9	2.6

Table 5 – Estimates of total and individual aldrin-related impurities in CCQM-K55.b
*** Presence of isodrin, dieldrin and dechlorane reported without individual estimates**

For assignment of a KCRV for total organic impurities, it is proposed to use the values reported by the fifteen participants who observed and included all three major impurities (isodrin, dieldrin and dechlorane) in their total value and exclude the three results that did not report the presence of the long retention time impurity due to dechlorane.

The mean of the selected results was chosen as the estimate of the KCRV for related structure impurity content ($KCRV_{Rel\ Subst.}$). The associated standard uncertainty of the KCRV ($u_{Rel\ Subst.}$) is the standard deviation of the mean of the selected data set.

$$\begin{aligned}
 KCRV_{Rel\ Subst.} &= 35.4 \text{ mg/g;} \\
 u_{Rel\ Subst.} &= 0.42 \text{ mg/g}
 \end{aligned}$$

The results reported by participants with their associated standard uncertainties ($k = 1$) plotted against the proposed $KCRV_{Rel\ Subst.}$ are shown in Figure 7.

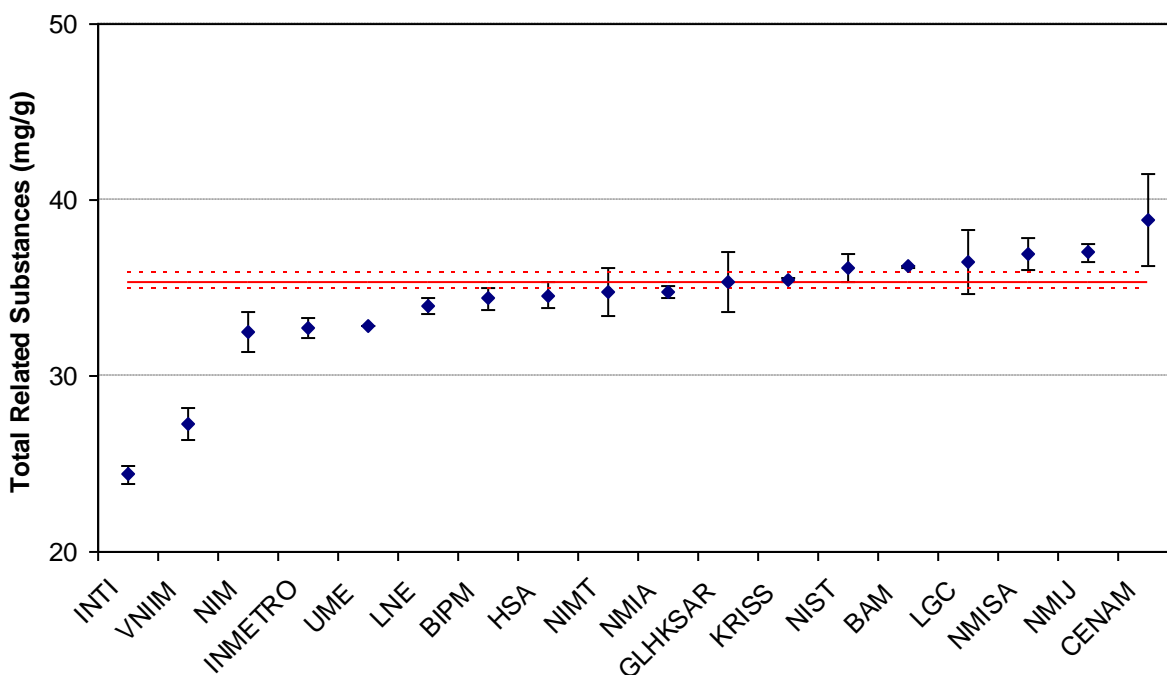


Figure 7 Estimates of total related structure impurities in CCQM-K55.b plotted with their reported standard uncertainties ($\pm u_c$, $k = 1$).
The proposed KCRV for this impurity class ($KCRV_{Rel. Subst.}$) (solid red line) is 35.4 mg/g.
Dashed red lines show the $KCRV \pm u_{Rel. Subst.}$ ($k = 1$).

ii. KCRV for water content in CCQM-K55.b

The values for water content in CCQM-K55.b provided by the participants are set out below in Table 6, along with a short description of the variant of the Karl Fischer titration method used.

Participant	Water content (mg/g)	$U_{95\%}$ (mg/g)	Karl Fischer Method outline
VNIIM	0.11	0.02	Direct addition of solution, 3 x 100 mg
NMIJ	0.31	0.04	Direct addition of solid, 3 x 55 mg
KRISS	0.32	0.14	Direct addition of solid, 3 x 20-30 mg
CENAM	0.4	0.1	Direct addition of solid, 2 x 100 mg
HSA	0.4	0.28	Direct addition of solid, 5 x 8-20 mg Oven transfer @ 170 C
BAM	0.42	0.09	Oven transfer @ 130 C, 3 x 100-150 mg
NIST	0.44	-0.09/+ 0.17	Direct addition (of soln. ?), 5 x 20-50 mg
GLHK	0.46	0.1	Oven transfer @ 160 & 230 C, 50 mg
LGC	0.47	0.98	Oven transfer @ 100 C, 3 x ?? mg
BIPM	0.5	- 0 /+ 0.58	Oven transfer @ 120 C, 3 x 50 mg
INMETRO	0.54	0.026	Direct addition of solution, 2 x 100 mg

Table 6 – Estimates of water content for CCQM-K55.b

Participant	Water content (mg/g)	$U_{95}\%$ (mg/g)	Karl Fischer Method outline
NMISA	0.6	0.22	Direct addition of solid, 3 x 50 mg
NMIA	0.91	3.6	Direct addition of solid, 3 x 20-30 mg
NRC-INMS	1	- 1 /+ 0	
NIM	1.24	0.36	Direct addition of solid, 3 x 50 mg
LNE	1.31	0.07	Oven transfer @ 100 C, 3 x 100 mg
UME	2	0.12	Direct addition of solution, 1 x 50 mg
NIMT	6.43	0.4	Oven transfer @ 120 C, 5 x 60 mg
INTI	11.9	0.2	Direct addition of solid, 25 mg

Table 6 (ctd) – Estimates of water content for CCQM-K55.b

All participants used coulometric Karl Fischer titration as the minimum method to obtain the water content estimate. A mixture of procedures including direct addition into the titration cell as a solid, direct addition as a solution and sample oven transfer to deliver the water content from a sample of CCQM-K55.b into the titration cell were used. There was generally good agreement for the presence of a small but detectable amount of water in the material. No ready explanation is available for the two results that were appreciably in excess of the median value of 0.5 mg/g. Several participants used other techniques (TGA, qNMR, elemental analysis) as confirmatory analyses or consistency checks. Because of the asymmetric spread of results, the median of the combined results for water is proposed as the KCRV for water content ($KCRV_{H_2O}$) in CCQM-K55.b. The associated standard uncertainty of the KCRV (u_{H_2O}) is assigned as the robust statistical estimate of the standard deviation of the median ($MADe/\sqrt{n}$) of the data set

$$KCRV_{H_2O} = 0.47 \text{ mg/g};$$

$$u_{H_2O} = 0.05 \text{ mg/g}$$

The results reported by participants with their associated standard uncertainties ($k = 1$) plotted against the proposed $KCRV_{H_2O}$ are shown in Figure 8.

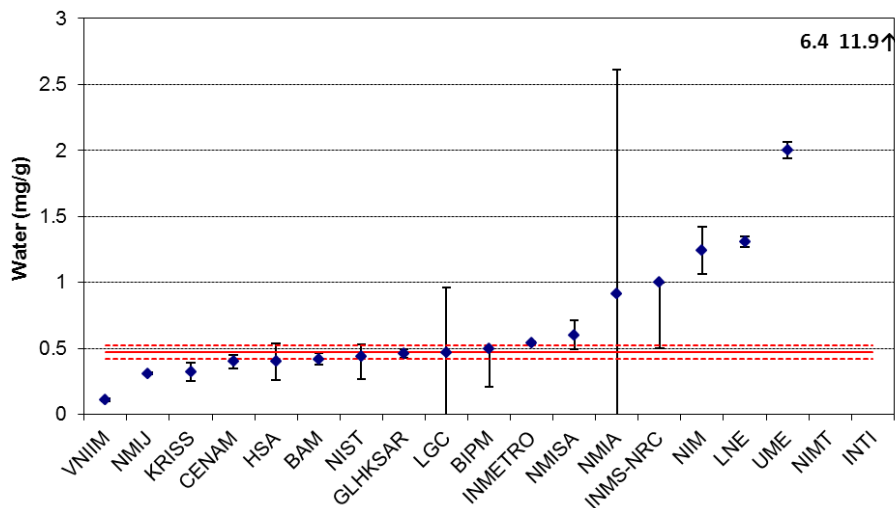


Figure 8 Estimates for water in CCQM-K55.b plotted with reported standard uncertainties ($k = 1$). The proposed KCRV for water content of CCQM-K55.b (solid red line) is 0.47 mg/g. Dashed red lines show the proposed $KCRV_{H_2O} \pm u_{H_2O}$ ($k = 1$).

iii. KCRV for VOCs in CCQM-K55.b

Fourteen of the eighteen participants provided estimates for the volatile organics content of CCQM-K55.b. Each participant who investigated residual solvent content reported a significant level of methanol in the sample.

GC approaches with detection by headspace and direct MS analysis (GC-MS) or headspace FID (, in several cases independently cross checked by NMR methods, were used to identify and quantify the methanol content of the sample. The number of participants now incorporating rigorous methods to detect residual solvent was encouraging and a significant increase compared with the earlier CCQM-P20 and CCQM-K55.a comparisons. Given the volatile nature of methanol, which makes it a challenging compound to quantify, the overall agreement with the proposed KCRV was also encouraging.

HSA were the only participant to report a methanol content significantly higher than the values found by other participants. They also commented that the samples they received displayed a significant level of inhomogeneity. No comments on the variability or instability in the methanol content of the sample were received from other participants.

The results obtained by the study participants of CCQM-K55.b are listed in Table 7 and shown graphically in Figure 9.

Participant	Methanol content (mg/g)	$U_{95}\%$ (mg/g)	Method(s)
GLHK	0.9	0.17	HS-GC-MS & GC-MS
NIM	1.67	0.15	HS-GC-MS & HS-GC-FID
NRC-INMS	1.9 2.5	0.6 0.3	qNMR GC-MS
BAM	2.0	0.1	HS-GC-MS & qNMR
LGC	2.14	0.46	HS-GC-MS & qNMR
NMIA	2.32	1.2	HS-GC-MS & qNMR
NIST	2.33	0.28	qNMR, check by HS-GC-MS
NMISA	2.33	0.0004	HS-GC-TOF & HS-GC-FID
KRISS	2.44	0.26	TGA
BIPM	2.5	0.1	GC-MS, check by TGA & NMR
LNE	2.74	0.18	HS-GC-FID
UME	3.0	0.12	HS-GC-MS & qNMR
NMIJ	3.49	0.08	HS-GC-MS & qNMR
HSA	5.2	0.5	HS-GC-FID

Table 7 – Estimates of residual solvent (methanol) content in CCQM-K55.b

The mean of the results is proposed as the KCRV for VOC content ($KCRV_{VOC}$) in CCQM-K55.b. The associated standard uncertainty of the KCRV (u_{VOC}) is assigned as the standard deviation of the mean of the data set

$$KCRV_{VOC} = 2.30 \text{ mg/g}$$

$$u_{VOC} = 0.16 \text{ mg/g}$$

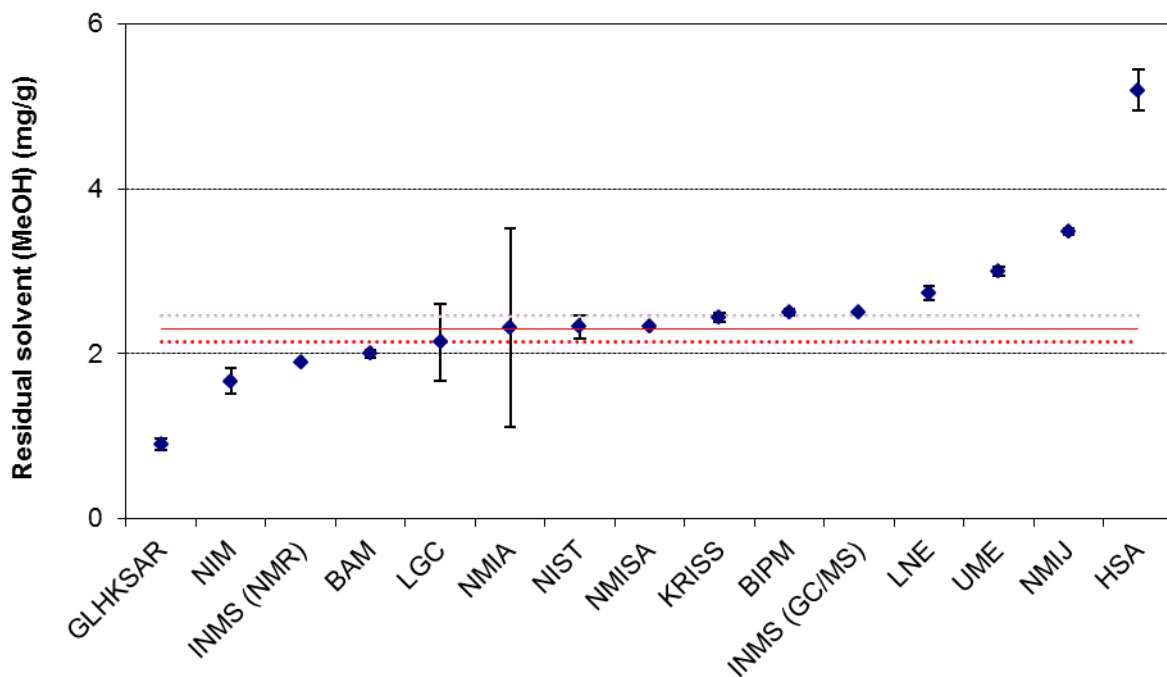


Figure 9 Estimates for methanol content reported by participants in CCQM-K55.b plotted with their associated standard uncertainties ($k = 1$). The proposed KCRV for residual solvent in CCQM-K55.b (2.30 mg/g, u_{VOC} 0.16 mg/g) is indicated by the solid red line. Dashed red lines show the $KCRV_{VOC} \pm u_{VOC}$ ($k = 1$).

iv. KCRV for non-volatile organics/inorganics content in CCQM-K55.b

The estimates reported for non-volatiles/inorganics content provided by the comparison participants are listed in Table 8. Using various methods including TGA, ICP-MS, ICP-OES or XRF spectrometry participants essentially found very low to negligible levels of inorganic material in the sample. The HSA observed a significant residue after high temperature TGA under non-oxidising conditions. NMIJ found no significant residue after high-temperature TGA but did detect a significant residue after exhaustive distillation (100 °C, 90 h, 2.5×10^{-4} Pa). The NMIJ ascribed this distillation residue to a non-volatile organic residue present in the CCQM-K55.b. As mentioned already, in further studies subsequent to the original discussion of results the NMIJ provided additional evidence through size-exclusion chromatography for the presence of an oligomeric organic-based impurity in CCQM-K55.b.

NIST reported the presence of a small level of Si-related material by XRF and GLHK reported Al-related impurity present at 0.25 mg/g by ICP-MS. GLHK noted that $AlCl_3$ is a commonly used catalyst in the manufacture of aldrin, which may account for its presence.

In general it appeared that the methods used by the other participants in this case while suitable for detecting inorganic impurities were insensitive in this case to a significant level of non-volatile organics.

Participant	Non-volatiles content (mg/g)	$U_{95\%}$ (mg/g)	Method outline
BIPM	0	0 / 0.06	ICP-MS, cross check by % C,H analysis
KRISS	0	1.8	TGA (160 - 500 °C, 1 hr)
NIMT	0	0.28	TGA (200 - 1000 °C, 20 min under O ₂)
NMIA	0	2	TGA (120 - 850 °C, 20 min [under O ₂ ?]) cross check by % C,H analysis
NMISA	0	0.22	TGA (90 - 800 °C, 10 min [under air])
VNIIM	0		ICP-MS
INMETRO	0.00008	0.000007	ICP-MS
NIM	0.038	0.04	ICP-MS
NIST	0.05	0.02	XRF
NMIJ (Inorganics)	0.12		TGA (35 - 650 °C, 1 hr under air)
LGC	0.15	0.08	ICP-MS, ICP-OES, TGA (20 - 1000 °C)
GLHK	0.25	0.28 / 0.25	ICP-MS
UME	0.5	0.007	TGA (50 - 1000 °C)
HSA	9.9	2.1	TGA (120 - 850 °C, 30 min under air)
NMIJ (Organics)	12	2	Exhaustive vacuum distillation (110 °C, 90 h, 2.5x10 ⁻⁴ Pa)

Table 8 – Estimates of non-volatiles/inorganics content for CCQM-K55.b/CCQM-P117.b

Given that the follow up studies by the NMIJ demonstrated the presence of a significant non-volatile impurity in CCQM-K55.b, the information available directly from the study is limited to the mean of the two results for non-volatile organics. It is proposed to use this value as the KCRV for non-volatiles content ($KCRV_{NV}$) in CCQM-K55.b. The associated standard uncertainty of the KCRV (u_{NV}), assigned assuming a rectangular distribution in the range between the two values used for the KCRV, is 0.58 mg/g (= half range/ $\sqrt{3}$).

$$\begin{aligned}
 KCRV_{NV} &= 11 \text{ mg/g} \\
 u_{NV} &= 0.58 \text{ mg/g}
 \end{aligned}$$

The proposed value for total non-volatiles is consistent with the data obtained subsequently by NMIJ using gel permeation chromatography.

The reported individual values for each participant are given in Table 8 and are shown graphically in Figure 10 along with the proposed KCRV.

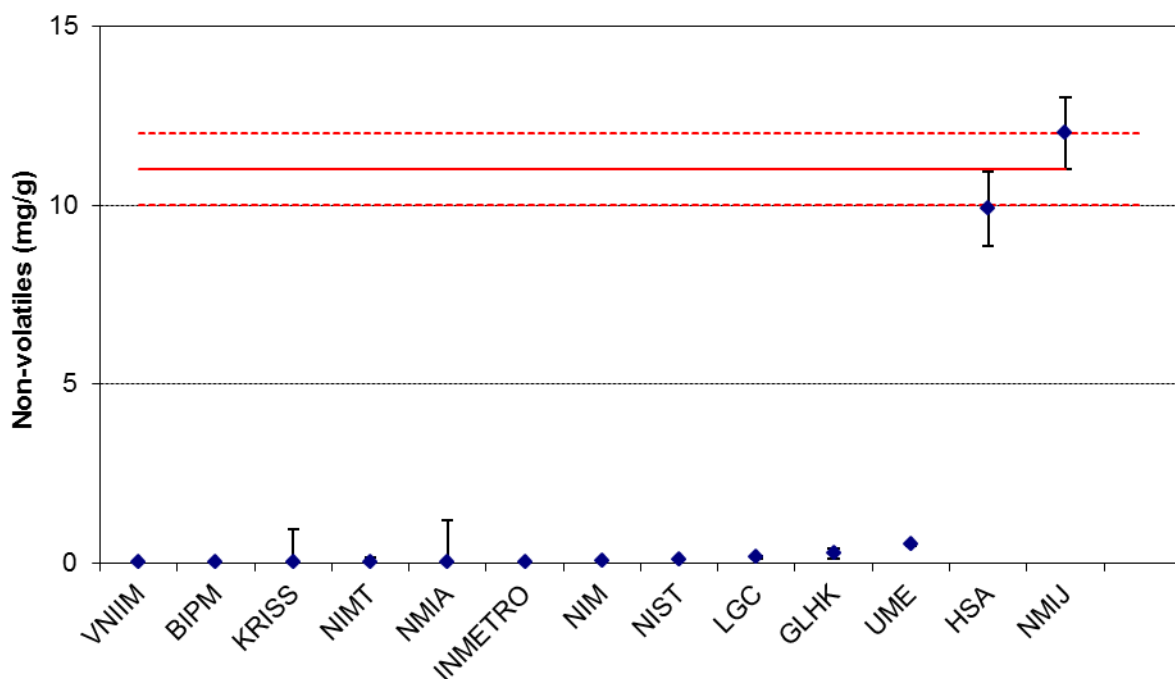


Figure 10 Estimates for combined non-volatiles/inorganics content reported by participants in CCQM-K55.b plotted with their associated standard uncertainties ($k = 1$). The proposed KCRV for non-volatiles in CCQM-K55.b (11 mg/g, $u_{NV} = 1$ mg/g) is indicated by the solid red line. Dashed red lines show the proposed $KCRV_{NV} \pm u_{NV}$ ($k = 1$).

Key Comparison Reference Values (KCRVs) for Aldrin in CCQM-K55.b

It was agreed by the participants during the discussion of results that, as for the CCQM-K55.a comparison, assignment of a KCRV for aldrin in CCQM-K55.b based solely on the combined reported results for overall aldrin content was not justified and would be misleading. After initial discussion at the November 2010 CCQM OAWG meeting, follow-on studies were undertaken to investigate the reason for the approximate 10 mg/g discrepancy between mass balance and qNMR results. Subsequent discussion of this data continued at the CCQM OAWG meeting in April 2011 where the NMIJ presented convincing additional data using in particular gel permeation chromatography to demonstrate that a high molecular weight impurity ($MW > 1000$) was present at around the 10 mg/g level in CCQM-K55.b.

The comparison coordinator was asked to follow the precedent of the approach used in the CCQM-P20.f and CCQM-K55.a comparisons to propose individual KCRVs for the mass fraction of each of the orthogonal classes of impurity present in the comparison material and to use these values to assign an overall KCRV for aldrin content.

Information on the mass fraction assignments of these major impurities was requested in the comparison protocol and reporting form from those participants using the mass balance approach to assign aldrin content.

Assignment of KCRV for Aldrin in CCQM-K55.b

The measurement equation (Eqn. 1) to assign the KCRV of aldrin in CCQM-K55.b (in mg/g) is:

$$w_{Aldrin} = 1000 - [w_{Rel.Subst} + w_{Water} + w_{VOC} + w_{NonVol} + H_{relsubst} + H_{VOC}] \quad (Eqn. 1)$$

w_{Aldrin} = KCRV for mass fraction of aldrin in CCQM-K55.b
 $w_{Rel.Subst}$ = KCRV for mass fraction of aldrin-related impurities in CCQM-K55.b
 w_{Water} = KCRV for mass fraction of water in CCQM-K55.b
 w_{VOC} = KCRV for mass fraction of volatile organic solvents in CCQM-K55.b
 w_{NonVol} = KCRV for mass fraction of non-volatile organics/inorganics in CCQM-K55.b
 $H_{Rel.Subst}$ = Correction for between unit inhomogeneity of aldrin-related impurities in the CCQM-K55.b material. Assigned 0 with associated uncertainty ($u_{H_{Rel.Subst}}$)
 H_{VOC} = Correction for between unit inhomogeneity of volatile organic content in the CCQM-K55.b material. Assigned 0 with associated uncertainty ($u_{H_{VOC}}$)

Units for reporting mass fraction (w) are mg/g throughout.

The standard uncertainty associated with the mass fraction estimate was calculated from equation (2):

$$u_{w_{Aldrin}} = \sqrt{(u_{w_{Rel.Subst}})^2 + (u_{w_{Water}})^2 + (u_{w_{OrgSol}})^2 + (u_{w_{NonVol}})^2 + (u_{H_{RelSubst}})^2 + (u_{H_{OrgSol}})^2} \quad (Eqn. 2)$$

The KCRVs for the impurity classes used for calculation of a mass balance KCRV for aldrin in the CCQM-K55.b comparison are summarised in Table 9.

Input factor w	Proposed KCRV (mg/g)	n	$u(KCRV)$ (mg/g)
Related structure organics	35.4	15	0.42
Water	0.47	17	0.05
Volatile organics	2.30	14	0.16
Non-volatiles/inorganics	11.0	2	0.58
Homogeneity - related structure impurities	0.0	large	0.42
Homogeneity – volatile organics	0.0	large	0.1
Combined value	49.17		0.85

Table 9: KCRV values for impurities used for calculation of aldrin KCRV and associated combined standard uncertainty in CCQM-K55.b

When substituted into the equations (1) and (2), the KCRV for aldrin content becomes:

$$\begin{aligned}
 KCRV_{Aldrin} &= 1000 - [KCRV_{Rel.Subst} + KCRV_{Water} + KCRV_{Org.Solv.} + KCRV_{NonVol.} + H_{RelSubst} + H_{OrgSolv}] \text{ mg/g} \\
 &= 1000 - [35.4 + 0.47 + 2.3 + 11] \text{ mg/g} \\
 &= 950.8 \text{ mg/g}
 \end{aligned}$$

$$\begin{aligned}
 u_{KCRV_{Aldrin}} &= \sqrt{(u_{KCRV_{RelSubst}})^2 + (u_{KCRV_{Water}})^2 + (u_{KCRV_{OrgSolv.}})^2 + (u_{KCRV_{NonVol.}})^2 + (u_{H_{RelSubst}})^2 + (u_{H_{OrgSolv}})^2} \text{ mg/g} \\
 &= \sqrt{(0.42)^2 + (0.05)^2 + (0.16)^2 + (0.58)^2 + (0.42)^2 + (0.1)^2} \text{ mg/g} \\
 &= 0.85 \text{ mg/g}
 \end{aligned}$$

This is regarded as a conservative estimate for the standard uncertainty of the KCRV.

Figure 11 shows the participant results with their reported standard uncertainties plotted against the proposed KCRV (solid red line) and its associated standard uncertainty ($k = 1$). Figure 12 shows the same results with their expanded uncertainty and the KCRV with the corresponding expanded uncertainty for an approximately 95% coverage range (dashed red lines).

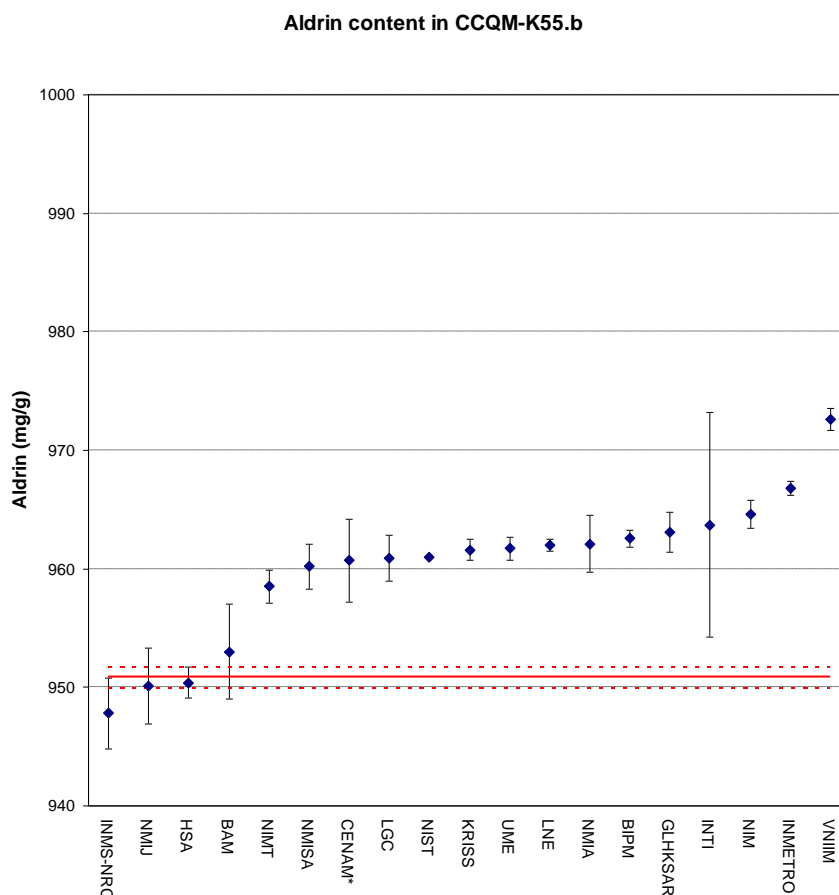


Figure 11: Mass fraction estimates by participants for aldrin in CCQM-K55.b with their reported combined standard uncertainty (u). Key Comparison Reference Value for CCQM-K55.b (solid red line) is 950.8 mg/g . The calculated combined standard uncertainty of the KCRV is 0.85 mg/g. Dashed red lines show $KCRV \pm u_c$ ($k = 1$)

Aldrin content in CCQM-K55.b

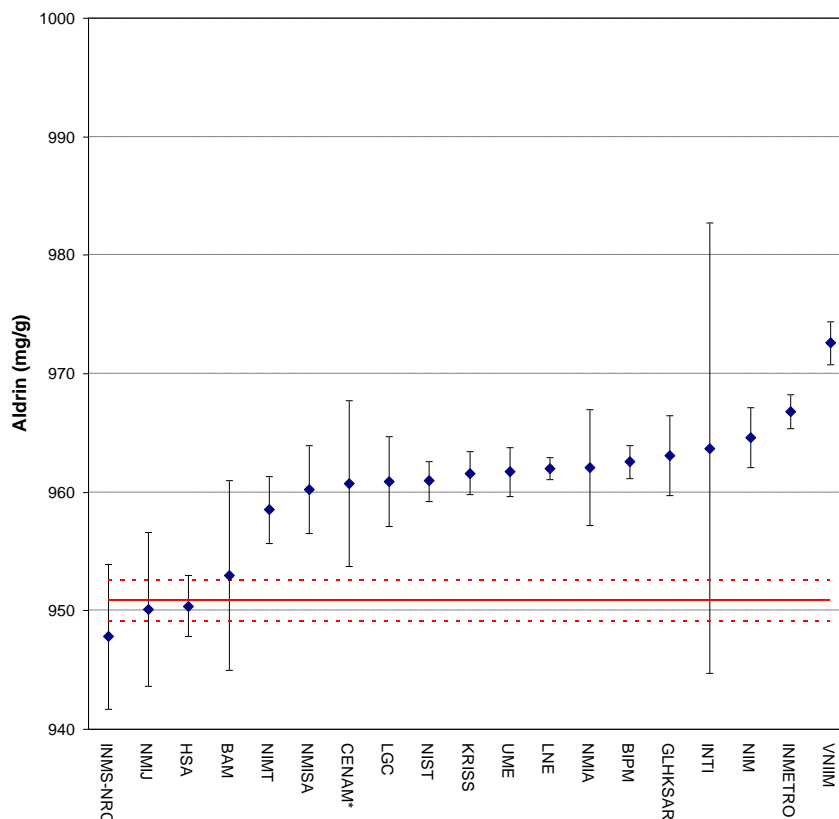


Figure 12: Mass fraction estimates by participants for aldrin in CCQM-K55.b with reported expanded uncertainty corresponding ($U_{95\%}$). Key Comparison Reference Value for CCQM-K55.b (solid red line) = 950.8 mg/g . The expanded uncertainty for 95% coverage range of the KCRV (dashed red lines) is 1.7 mg/g.

The degree of equivalence of a participant's result with the KCRV (D_i) is given by:

$$D_i = w_i - KCRV_{Aldrin}$$

The expanded uncertainty U_i at the approximately 95% coverage level associated with the D_i was calculated as:

$$U_{95\%}(D_i) = 2 * \sqrt{u(w_i)^2 + u(KCRV_{Aldrin})^2}$$

Table 10 records the degree of equivalence (D_i) of each key comparison participant's result with the proposed KCRV for aldrin. These results are shown graphically in Figure 13.

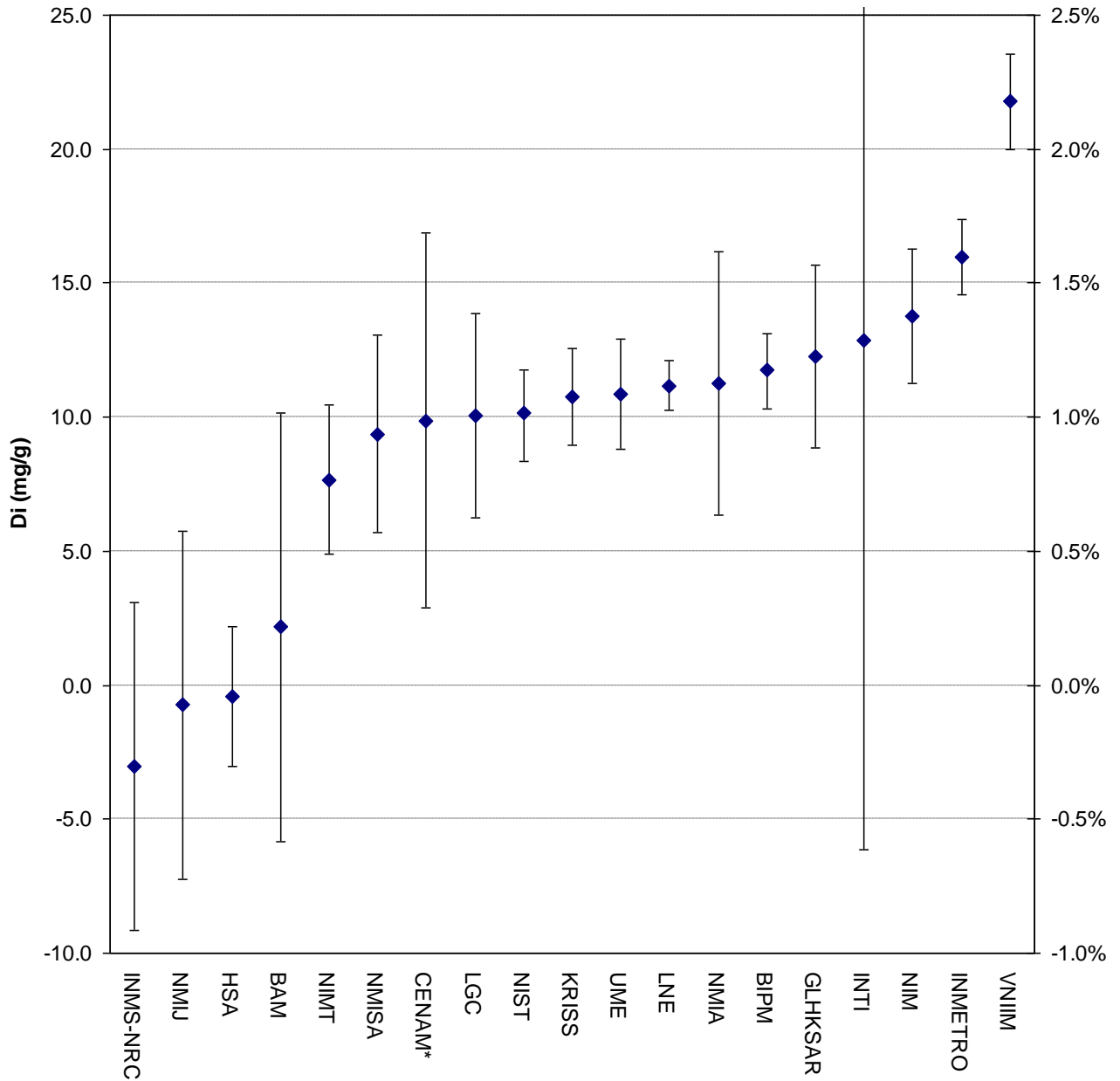


Figure 13: Degree of equivalence (absolute and relative) with the aldrin KCRV for each participant. Points are plotted with the associated expanded uncertainty in the degree of equivalence corresponding to an approximately 95% coverage range.

Participant	D_i (mg/g)	U_D (mg/g)
NRC-INMS	-3.0	6.2
NMIJ	-0.7	6.7
HSA	-0.4	3.1
BAM	2.2	8.2
NIMT	7.7	3.3
NMISA	9.4	4.2
CENAM	9.9	7.2
LGC	10.1	4.2
NIST	10.2	1.7
KRISS	10.8	2.5
UME	10.9	2.6
LNE	11.2	1.9
NMIA	11.3	5.1
BIPM	11.8	2.1
GLHK	12.3	3.8
INTI	12.9	19.1
NIM	13.8	2.9
INMETRO	16.0	2.0
VNIIM	21.8	2.5

Table 10: Degrees of equivalence D_i and expanded uncertainties U_D at approximately 95% coverage range in mg/g for aldrin in CCQM-K55.b

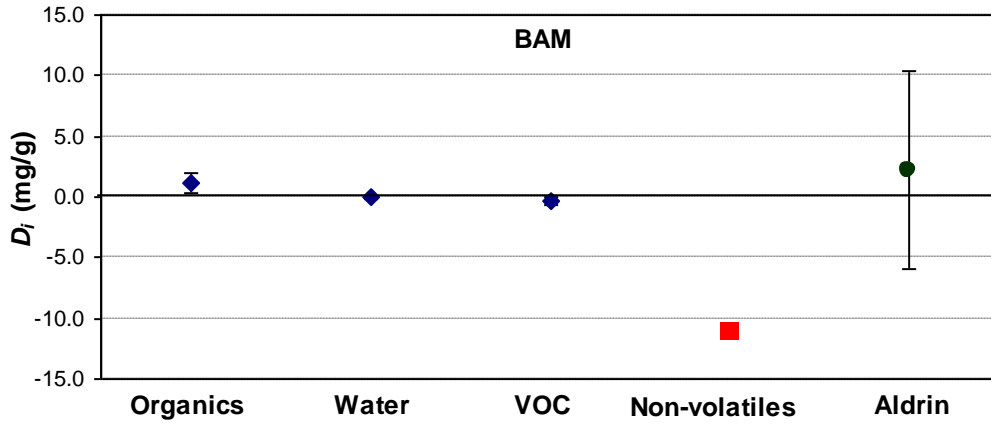
Degree of equivalence plots for impurity KCRVs in CCQM-K55.b

The motivation for assigning KCRVs for the contributing impurity classes in CCQM-K55.b was to assess the fitness of the individual mass balance methods and to confirm that an overall value for the main component in agreement with the KCRV for aldrin did not arise simply through cancellation of errors in the contributing impurity assignments. The derived DoE plots also allow for a visualization of specific problem areas in cases where agreement with the KCRV for aldrin was not achieved.

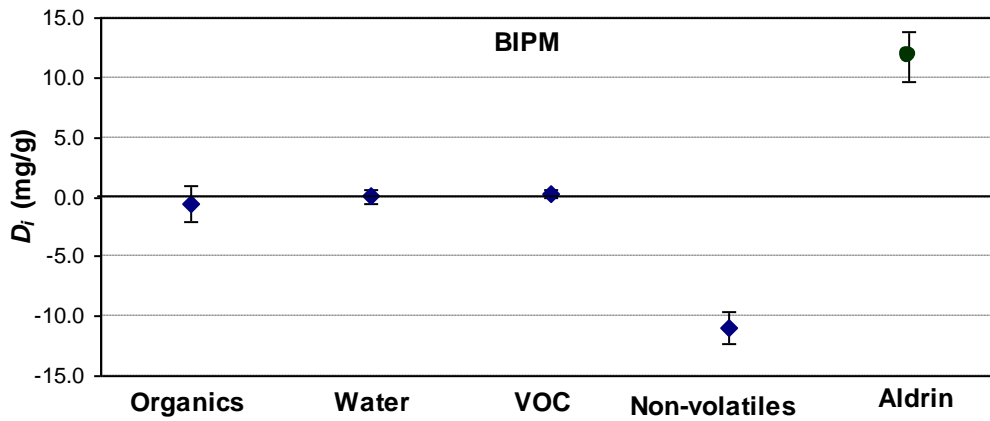
The combined DoE plots by participant for each impurity class quantified are shown on the following pages. To aid in assessment and comparison, the DoE of the overall result for aldrin (cf Figure 13) is plotted at the right (green data point). Where a participant used a mass balance approach but provided no information on a particular class of impurities a “pseudo” DoE is shown in this case as a red data point. This provides information on the validity of the participant’s implicit assumption that the particular impurity component does not make a significant contribution to the overall purity.

Mass Balance KCRV DoEs by Participant:

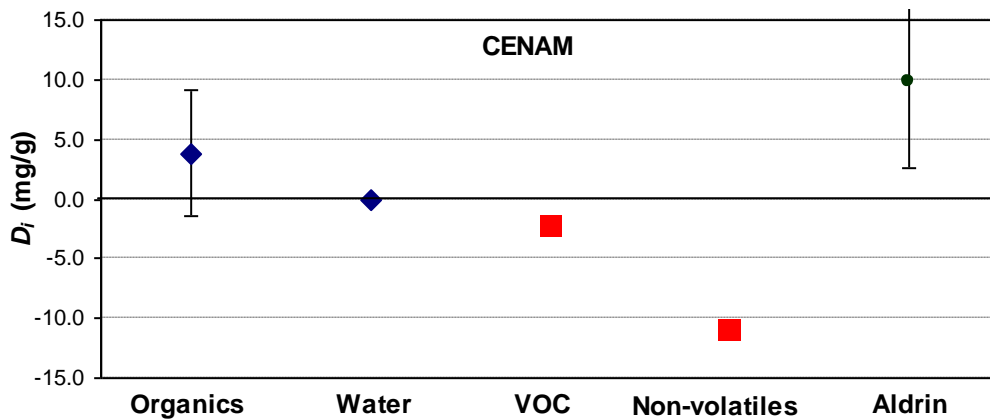
BAM



BIPM

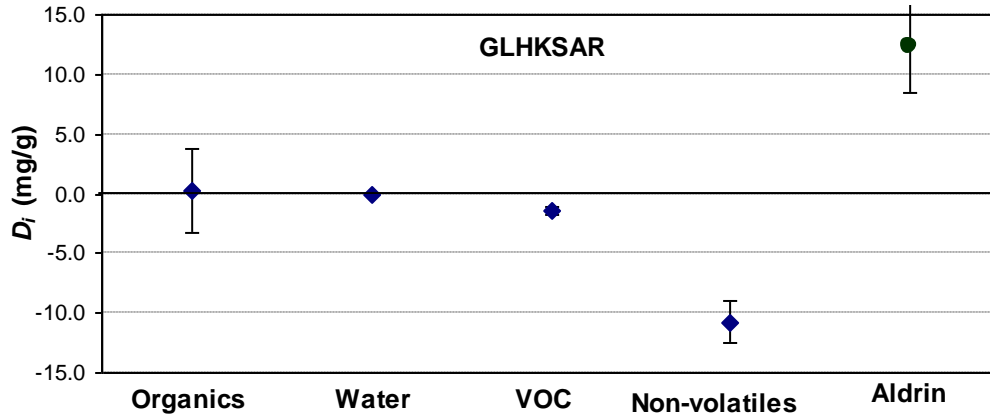


CENAM

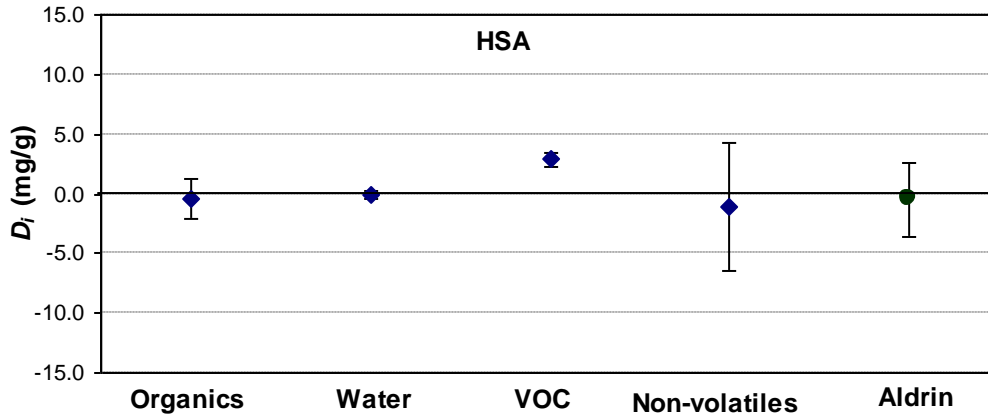


Key: \blacklozenge = DoE for reported impurity; \blacksquare = "DoE" when no value reported;
 \bullet = DoE for aldrin in CCQM-K55.b

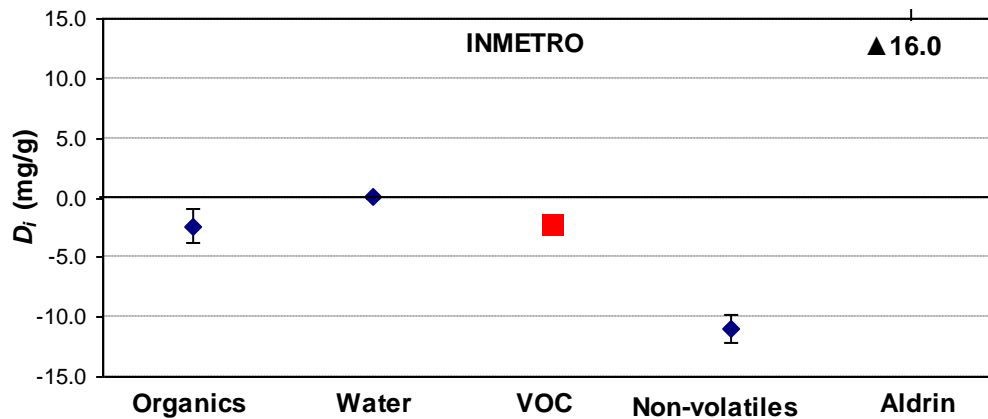
GLHK



HSA

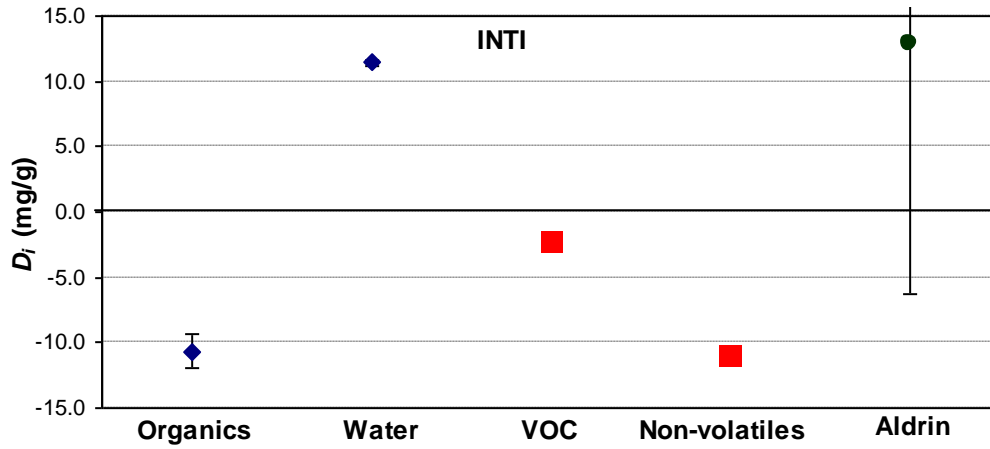


INMETRO

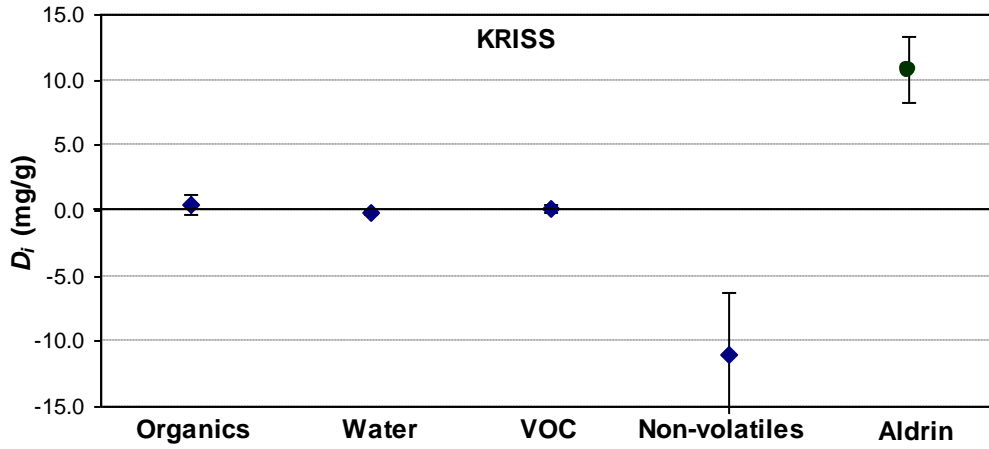


Key: **◆** = DoE for reported impurity; **■** = "DoE" when no value reported;
■ = DoE for aldrin in CCQM-K55.b

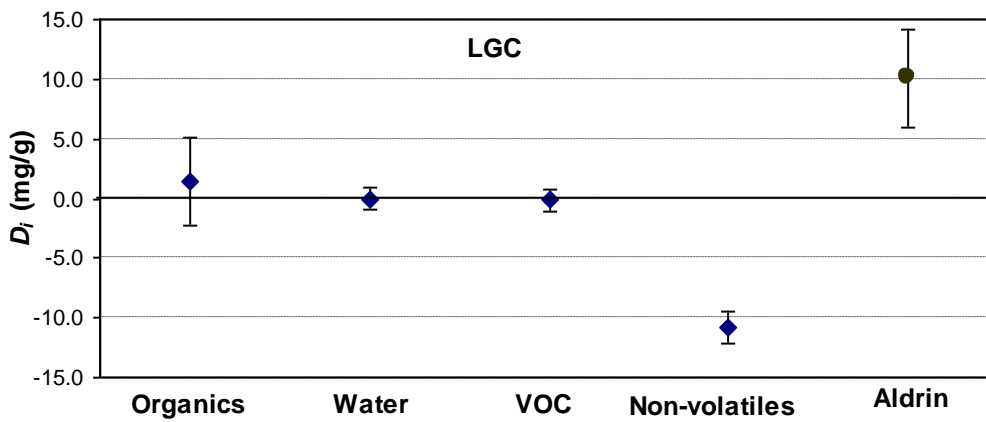
INTI



KRISS

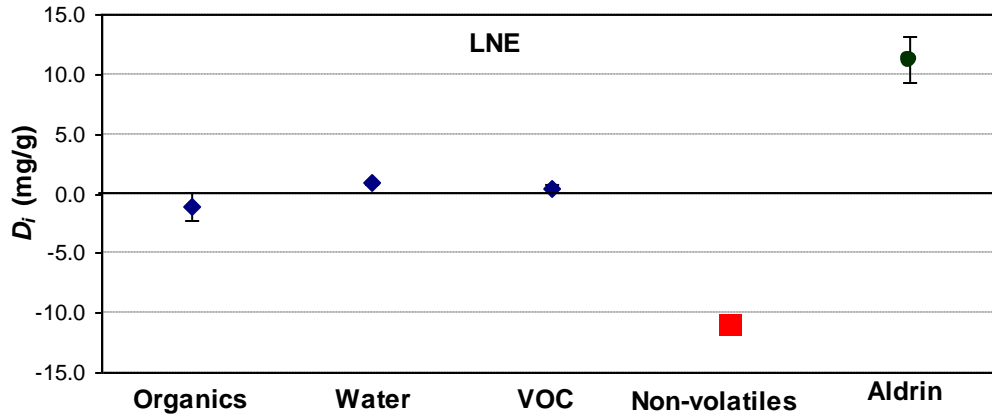


LGC

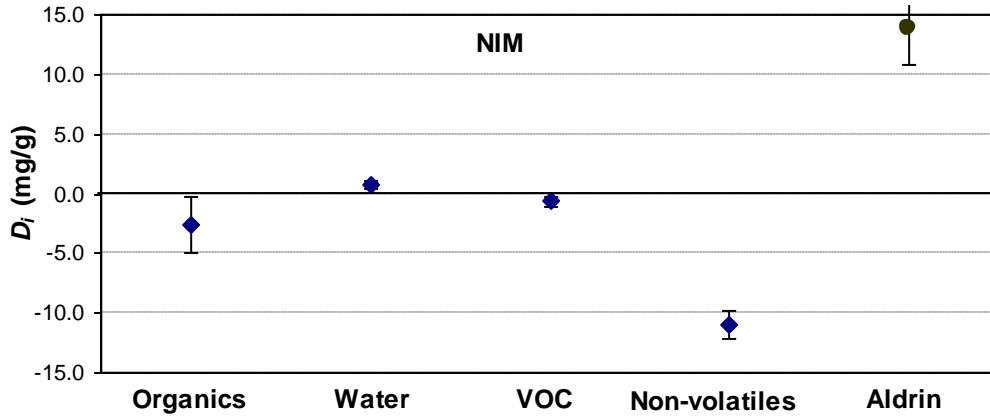


Key: ◆ = DoE for reported impurity; ■ = "DoE" when no value reported; ● = DoE for aldrin in CCQM-K55.b

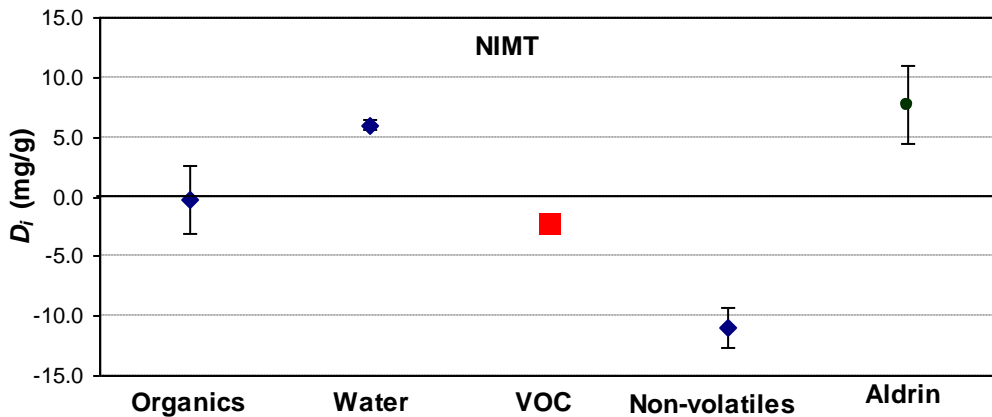
LNE



NIM

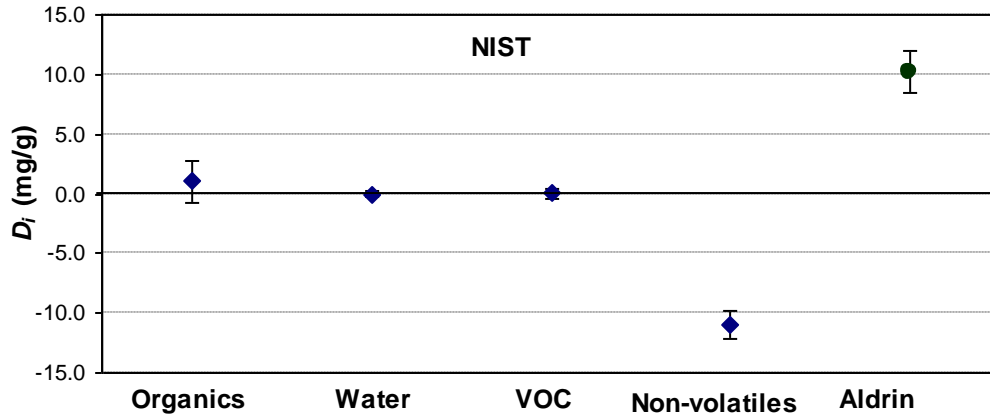


NIM

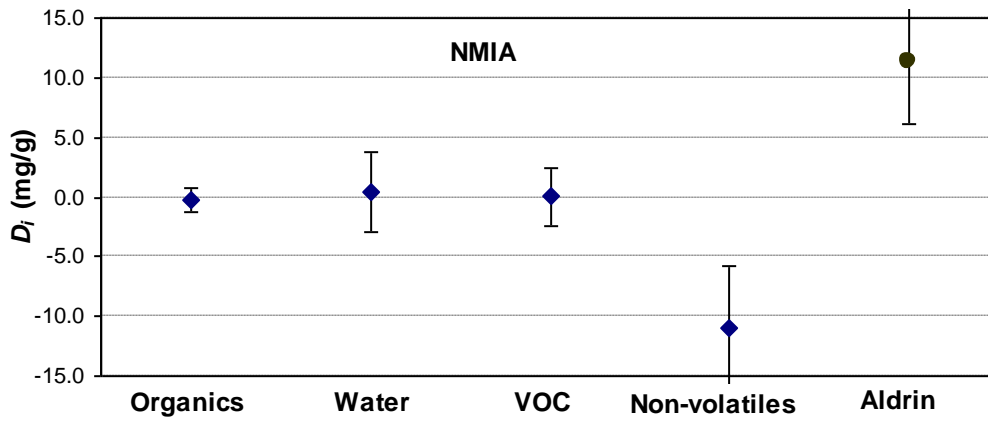


Key: ♦ = DoE for reported impurity; ■ = “DoE” when no value reported;
■ = DoE for aldrin in CCQM-K55.b

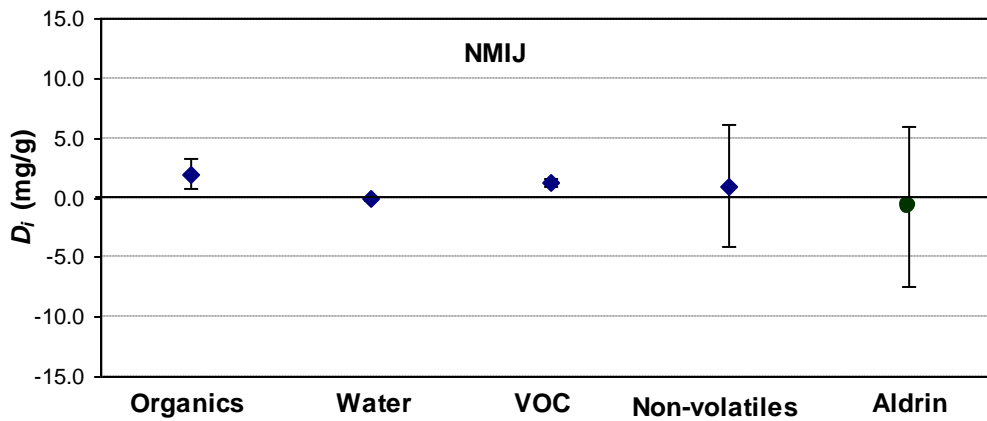
NIST



NMIA

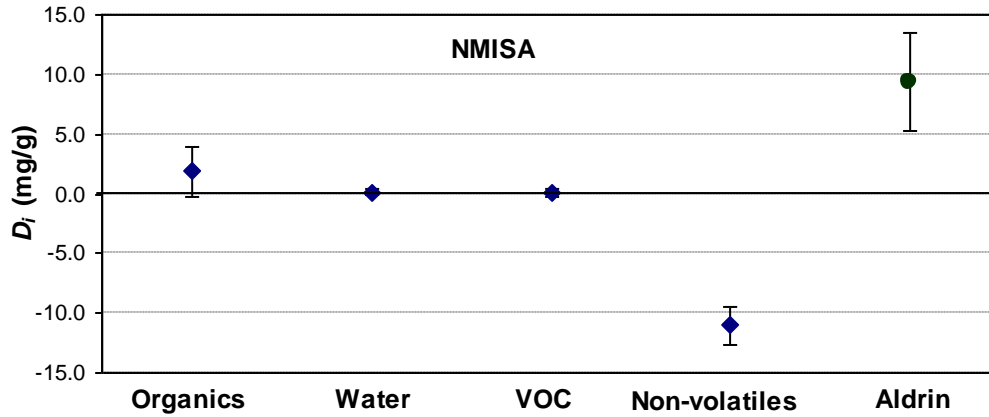


NMIJ

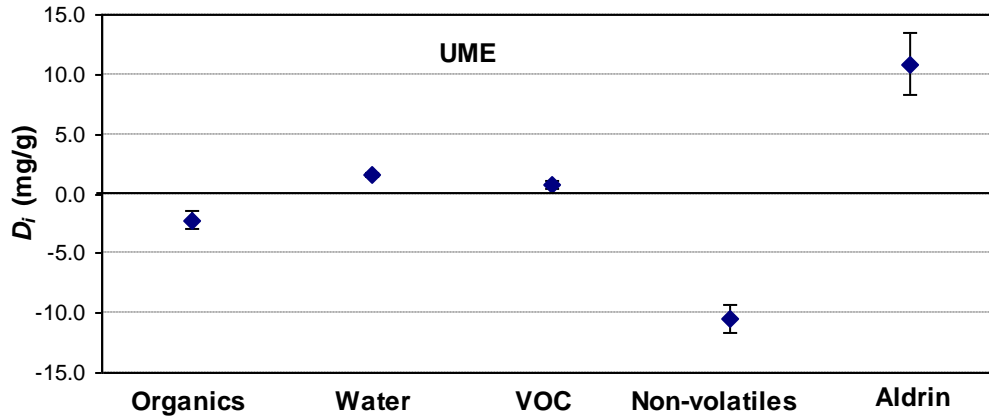


Key: \blacklozenge = DoE for reported impurity; \blacksquare = DoE for aldrin in CCQM-K55.b

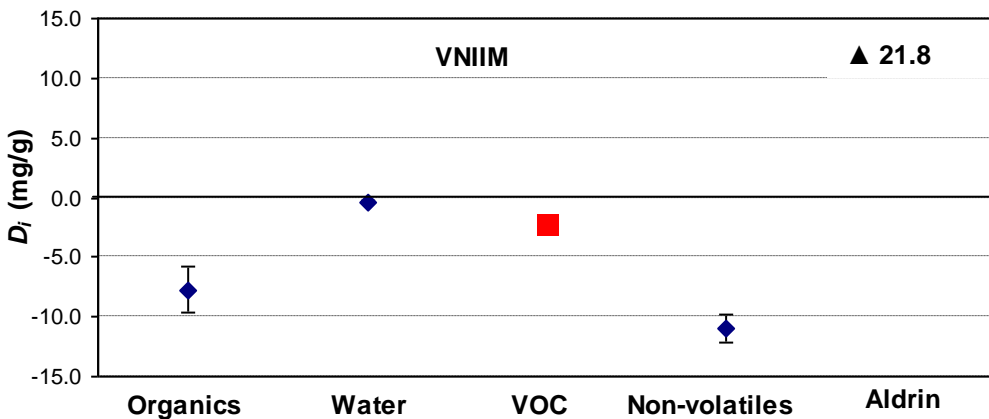
NMISA



UME



VNIIM



Key: ◆ = DoE for reported impurity; ■ = "DoE" when no value reported; ■ = DoE for aldrin in CCQM-K55.b

CONCLUSIONS AND HOW FAR THE LIGHT SHINES

Aldrin was selected to be a representative low polarity, moderately complex organic compounds capable of analysis by GC. It was anticipated to provide an analytical measurement challenge representative for the value-assignment of compounds of broadly similar structural characteristics.

For such a relatively impure material, there was excellent agreement between the majority of participants in both the identification and the quantification of the related structure impurity content of the sample, confirming the conclusions of CCQM-K55.a that measurement of this general class of impurities is performed satisfactorily by most NMIs. Where relatively low values for total organic impurity were reported by an individual participant these were consistent with underestimation of either the isodrin (2) content or a failure to detect the long retention time impurity dechlorane (4).

There was also a good level of agreement on the quantification of the water, residual solvent and inorganics content of the material. Unlike the previous CCQM-P20.f (digoxin) and CCQM-K55.a (estradiol) comparisons, the majority of participants sought to specifically quantify each of these impurity components.

As discussed in detail in the main report, the disparity in the final results reported for the aldrin content, as evidenced from the DoE plot which shows a set of results which overestimate the aldrin content by approximately 10 mg/g, arose primarily from a failure of most participants using a mass balance approach to detect the presence of a significant level of non-volatile organic impurity in the sample. This class of impurity had not been present to any significant extent in any previous comparisons.

Inspection of the DoE plots by impurity sub-group obtained by participants using the mass balance approach provides a clear indication of the laboratories that are applying this approach satisfactorily, apart from missing the non-volatile impurity in this particular instance, and those which have more general issues to address.

Nevertheless the follow-up discussions revealed that several participants did have an indication from qNMR data that their mass balance procedure had overestimated the aldrin content, but decided to accept the mass balance result and assume a hidden bias in the NMR data. By contrast the laboratories that placed greater value on the qNMR result, notably the NMIJ who invested significant effort into resolving the discrepancy, were able to find evidence for the presence of non-volatile organic impurity at the requisite level to bring the mass balance and qNMR results into agreement.

A secondary issue was that some laboratories need to review their NMR data processing procedures and software as they obtained a high qNMR value for aldrin, which resulted in a false degree of confidence in their mass balance results.

In summary, the major conclusions coming out of the comparison were:

- experienced laboratories obtained good levels of agreement in the mass fraction assignments for related impurities, water and VOC content in CCQM-K55.b. This could be demonstrated clearly through the equivalence plots relative to the individual KCRVs for individual participants for these classes of impurities;

- a weakness in direct methods for detecting the presence of significant non-volatile organic impurities. The inferential methods that had been relied on in previous comparisons (elemental analysis, NMR, TGA) were not suitable for CCQM-K55.b;
- the varying perceptions prior to the comparison between laboratories of the reliability (or otherwise) of qNMR for direct assignment of the purity of the main component should be reviewed in the light of the actual results;
- a need in some cases for improved QC procedures and/or understanding of software processing issues such that laboratories can have confidence that qNMR data is not biased as a result of their processing parameters

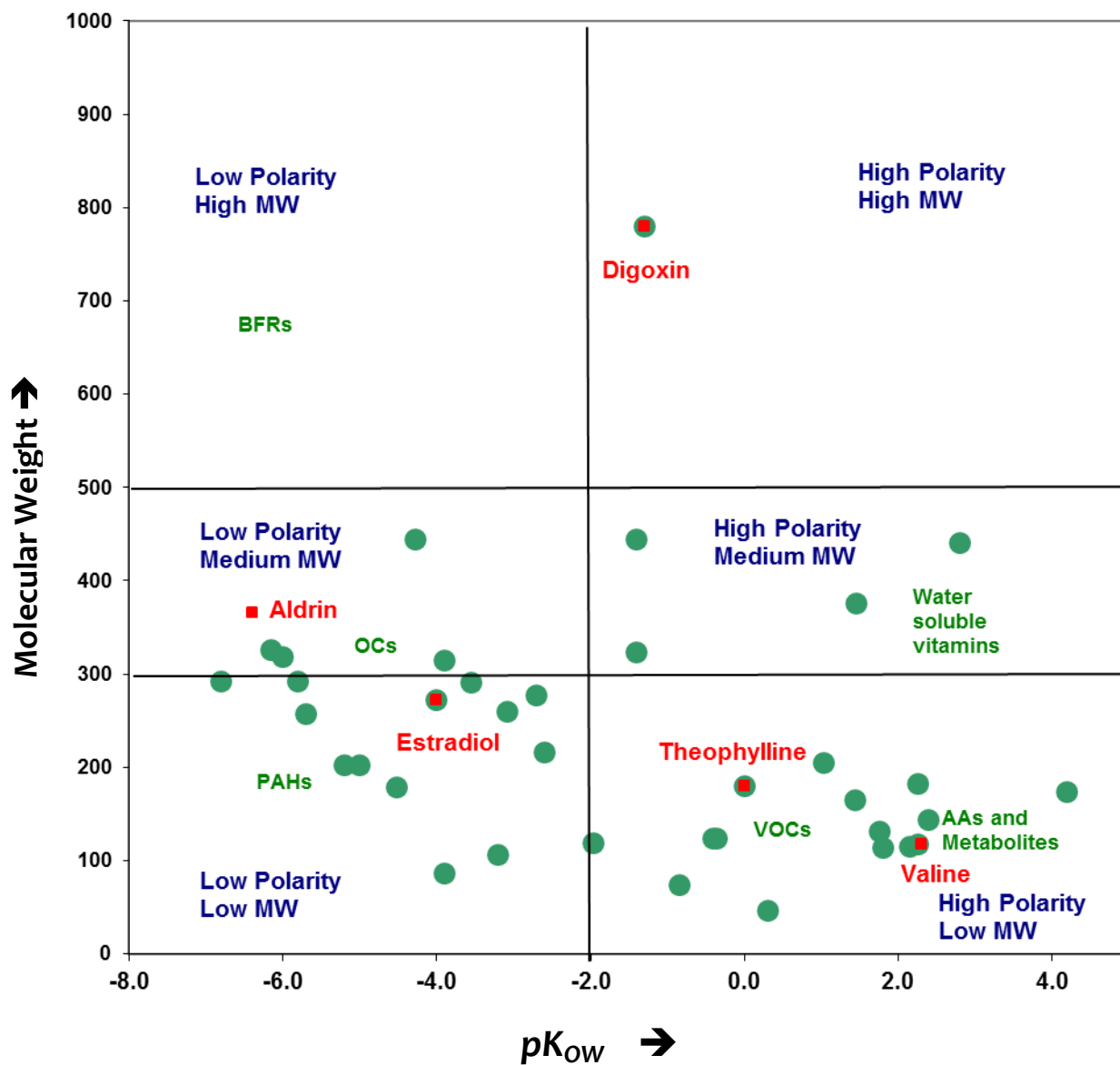
The results of the comparison provide a strong example of the need to undertake further investigations when notionally independent methods (mass balance v. qNMR in this instance) give significantly different main component purity results. A valid explanation for the cause of such differences should be established before one or other result is discarded. This is particularly so in a situation where the qNMR result is **lower** than the mass balance result, as it is not possible in this case to explain the discrepancy as arising simply from a contribution of NMR signals from unidentified impurities to the NMR signal being integrated to quantify the main component.

The study shows that in a case where a material is relatively impure both in the level and range of the impurity components, a capability to assign purity through either a mass balance approach or a direct qNMR approach with a moderate expanded uncertainty ($U_{95\%} < 0.6\%$ relative) can be achieved.

“How Far The Light Shines” Statement for CCQM-K55.b

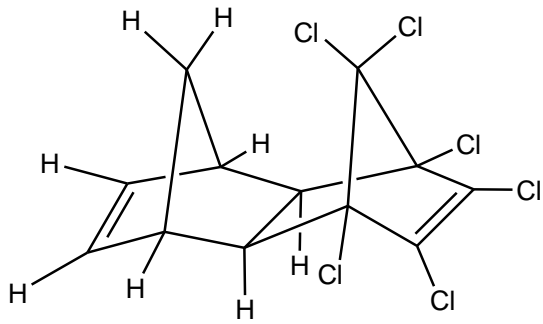
The comparison was intended to demonstrate a laboratory’s performance in determining the mass fraction of the main component in a high purity organic material. The measurement results should be indicative of the performance of a laboratory’s measurement capability for the purity assignment of organic compounds of medium structural complexity (molecular weight 300-500) and low polarity ($pK_{OW} < -2$) and for which related structure impurities are best quantified directly by capillary gas-phase chromatographic techniques.

Annex A : Analysis Space for Organic Primary Calibrators

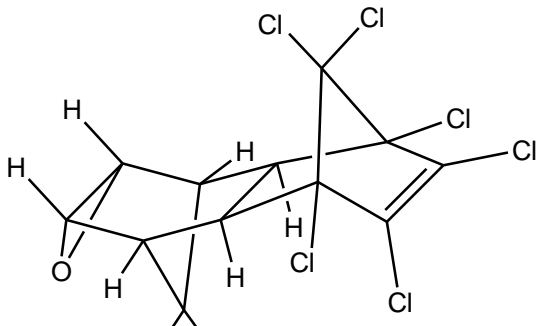


- CCQM-P20 & CCQM-K55 measurands
- CMC claims for pure substance calibrators or calibration solutions

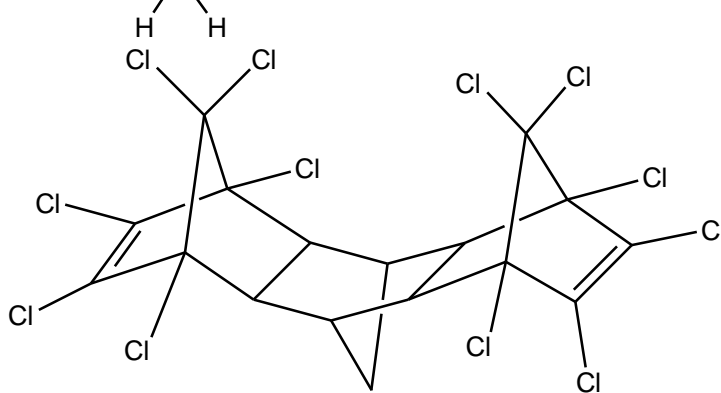
Annex B – Aldrin-related compounds identified in CCQM-K55.b



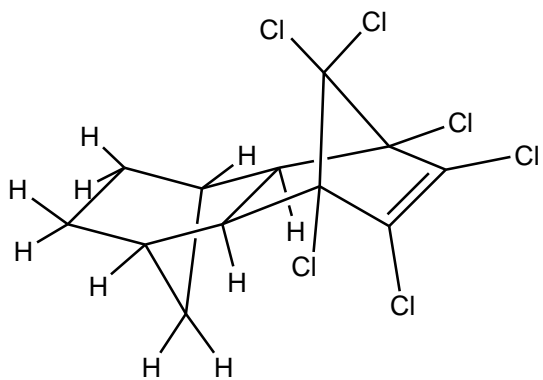
Isodrin (2)



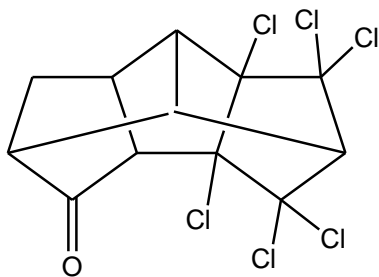
Dieldrin (3)



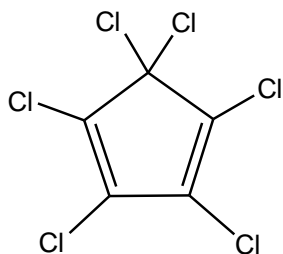
Dechlorane (4)



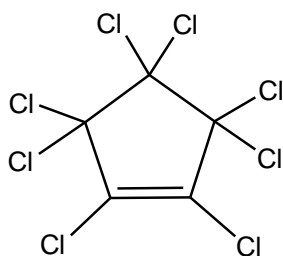
Dihydroaldrin (5)



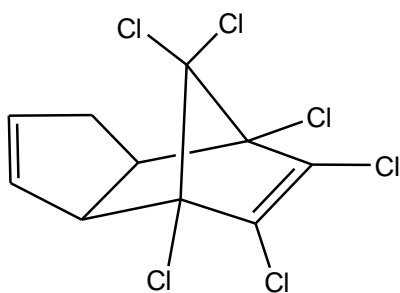
Endrin ketone (6)



Hexachlorocyclopentadiene (7)



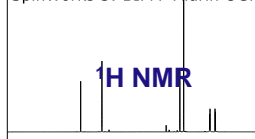
Octachlorocyclopentene (8)



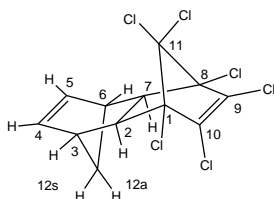
Chlordene (9)

Annex C – ¹H NMR spectra of CCQM-K55.b

SpinWorks 3: BIPM Aldrin OGP-014 110 (10mg) in 0.6ml CDCl₃ (over K₂CO₃) QNP600



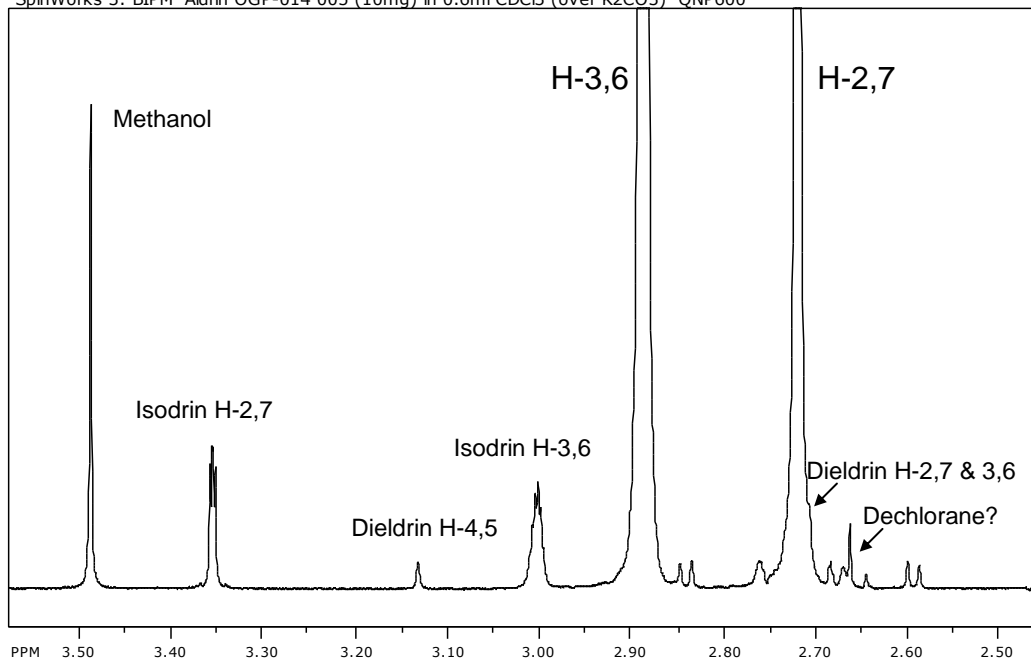
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 Hz: 600.133001 599.999999 599.866997 599.733995 599.600993 599.467991 599.334989 599.201987 599.068985 598.935983 598.802981 598.669979 598.536977 598.403975 598.270973 598.137971 598.004969 597.871967 597.738965 597.605963 597.472961 597.339959 597.206957 597.073955 596.940953 596.807951 596.674949 596.541947 596.408945 596.275943 596.142941 596.009939 595.876937 595.743935 595.610933 595.477931 595.344929 595.211927 595.078925 594.945923 594.812921 594.679919 594.546917 594.413915 594.280913 594.147911 594.014909 593.881907 593.748905 593.615903 593.482901 593.349899 593.216897 593.083895 592.950893 592.817891 592.684889 592.551887 592.418885 592.285883 592.152881 592.019879 591.886877 591.753875 591.620873 591.487871 591.354869 591.221867 591.088865 590.955863 590.822861 590.689859 590.556857 590.423855 590.290853 590.157851 590.024849 589.891847 589.758845 589.625843 589.492841 589.359839 589.226837 589.093835 588.960833 588.827831 588.694829 588.561827 588.428825 588.295823 588.162821 588.029819 587.896817 587.763815 587.630813 587.497811 587.364809 587.231807 587.098805 586.965803 586.832801 586.699799 586.566797 586.433795 586.300793 586.167791 586.034789 585.901787 585.768785 585.635783 585.502781 585.369779 585.236777 585.103775 584.970773 584.837771 584.704769 584.571767 584.438765 584.305763 584.172761 584.039759 583.906757 583.773755 583.640753 583.507751 583.374749 583.241747 583.108745 582.975743 582.842741 582.709739 582.576737 582.443735 582.310733 582.177731 582.044729 581.911727 581.778725 581.645723 581.512721 581.379719 581.246717 581.113715 580.980713 580.847711 580.714709 580.581707 580.448705 580.315703 580.182701 580.049699 579.916697 579.783695 579.650693 579.517691 579.384689 579.251687 579.118685 578.985683 578.852681 578.719679 578.586677 578.453675 578.320673 578.187671 578.054669 577.921667 577.788665 577.655663 577.522661 577.389659 577.256657 577.123655 576.990653 576.857651 576.724649 576.591647 576.458645 576.325643 576.192641 576.059639 575.926637 575.793635 575.660633 575.527631 575.394629 575.261627 575.128625 574.995623 574.862621 574.729619 574.596617 574.463615 574.330613 574.197611 574.064609 573.931607 573.798605 573.665603 573.532601 573.399599 573.266597 573.133595 572.999593 572.866591 572.733589 572.600587 572.467585 572.334583 572.201581 572.068579 571.935577 571.802575 571.669573 571.536571 571.403569 571.270567 571.137565 571.004563 570.871561 570.738559 570.605557 570.472555 570.339553 570.206551 570.073549 569.940547 569.807545 569.674543 569.541541 569.408539 569.275537 569.142535 569.009533 568.876531 568.743529 568.610527 568.477525 568.344523 568.211521 568.078519 567.945517 567.812515 567.679513 567.546511 567.413509 567.280507 567.147505 567.014503 566.881501 566.748499 566.615497 566.482495 566.349493 566.216491 566.083489 565.950487 565.817485 565.684483 565.551481 565.418479 565.285477 565.152475 565.019473 564.886471 564.753469 564.620467 564.487465 564.354463 564.221461 564.088459 563.955457 563.822455 563.689453 563.556451 563.423449 563.290447 563.157445 563.024443 562.891441 562.758439 562.625437 562.492435 562.359433 562.226431 562.093429 561.960427 561.827425 561.694423 561.561421 561.428419 561.295417 561.162415 561.029413 560.896411 560.763409 560.630407 560.497405 560.364403 560.231401 560.098400 559.965398 559.832396 559.699394 559.566392 559.433390 559.300388 559.167386 559.034384 558.901382 558.768380 558.635378 558.502376 558.369374 558.236372 558.103370 557.970368 557.837366 557.704364 557.571362 557.438360 557.305358 557.172356 557.039354 556.906352 556.773350 556.640348 556.507346 556.374344 556.241342 556.108340 555.975338 555.842336 555.709334 555.576332 555.443330 555.310328 555.177326 555.044324 554.911322 554.778320 554.645318 554.512316 554.379314 554.246312 554.113310 553.980308 553.847306 553.714304 553.581302 553.448300 553.315298 553.182296 553.049294 552.916292 552.783290 552.650288 552.517286 552.384284 552.251282 552.118280 551.985278 551.852276 551.719274 551.586272 551.453270 551.320268 551.187266 551.054264 550.921262 550.788260 550.655258 550.522256 550.389254 550.256252 550.123250 550.000000



H-2,7
 H-4,5
 H-3,6
 CDCl₃
 H-12a
 H-12s

¹H NMR spectrum of OGP.014 in CDCl₃ : full scale

SpinWorks 3: BIPM Aldrin OGP-014 005 (10mg) in 0.6ml CDCl₃ (over K₂CO₃) QNP600



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 time domain size: 32768 points
 width: 6613.76 Hz = 11.0205 ppm = 0.201836 Hz/pt
 number of scans: 32

freq. of 0 ppm: 600.130013 MHz
 processed size: 32768 complex points
 LB: 0.200 GF: 0.0000
 Hz/cm: 26.962 ppm/cm: 0.04493

¹H NMR spectrum of OGP.014 in CDCl₃ : expansion of δ 2.5 – 3.5 ppm

References

- ¹ Westwood, S., Josephs, R. D., Daireaux, A., Wielgosz, R., et al.: An international comparison of mass fraction purity assignment of theophylline: CCQM Pilot Study CCQM-P20.e (Theophylline), *Metrologia*, **46** (2009) 1A, 08019
- ² Westwood, S., Josephs, R. D., Choteau, T., Mesquida, C., Daireaux, A., Wielgosz, R., et al: An international comparison of mass fraction purity assignment of digoxin: CCQM Pilot Study CCQM-P20.f (Digoxin), *Metrologia* **48** (2011) 1A, 08013.
- ³ Westwood, S., Josephs, R. D., Daireaux, A., Wielgosz, R., et al; Final report on key comparison CCQM-K55.a (Estradiol): An international comparison of mass fraction purity of estradiol, *Metrologia*, **49** (2012) 1A, 08009
- ⁴ J. Sangster, *Octanol-water Partition Coefficients: Fundamentals and Physical Chemistry*, John Wiley & Sons, Chichester (1997).