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Final Report

Di-n-butyltin diacetate [DBTAc]
CAS number: 1067-33-0

In-vitro Metabolism Study

Author: Sven Hansen

Study Completion Date: November 20, 2019

Sponsor:

TIB Chemicals AG
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68219 Mannheim
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Test Facility:

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1. Summary

The dimeric distannoxane $[(DBTC)_2O]_2$ is the only in-vitro metabolite of DBTAc under the conditions of the study. Spectral data as well as melting point match very well with literature data (Davies 2004).

Dibutyltin dichloride (DBTC) – not even in traces - could not be detected in the reaction product.

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2. Study Statement

Title:

In-vitro Metabolism Study of Di-n-butyltin diacetate DBTAc]

Test Item:

Di-n-butyltin diacetate CAS-No.: 1067-33-0

Project staff

Study Director:	Sven Hansen
Laboratory:	Andreas Frais
Analytical (NMR):	Dr. Peter Graf, Universität Heidelberg
Analytical (DSC):	Andreas Frais
Analytical (ICP)	Paul Kolodziejczyk

Study phases:

Study Plan:	06.11.2019
Experimental phase:	07.11.-08.11.2019
Draft Report	20.11.2019
Final Report	20.11.2019

Good Laboratory Practice:

The study was not performed under the Good Laboratory Practice protocol.

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Archiving:

The following documents and materials will be archived for the periods specified in the operative national regulations of the Study Director:

- all raw data printouts
- the study plan
- any study plan amendments
- one certified copy of the final report
- one certified copy of any final report amendment or revised final report
- one sample of the test item and any reference item.

No raw data or material relating to the study will be discarded without the sponsor's prior written consent.

Signature:

This study, performed in the test facility of Galata Chemicals GmbH, was conducted in compliance with DIN, EN, ISO, OECD and EEC regulations. There were no circumstances that may have affected the quality or integrity of the study.

Sven Hansen
Study Director

Date: 2019 11 21

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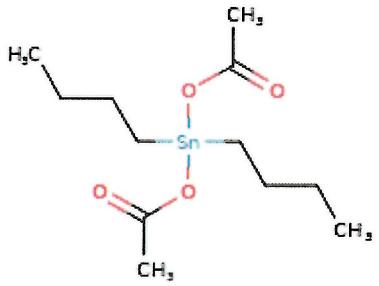
3. Test Item

The test item and the information concerning the test item were provided by the sponsor:

Name:	Di-n-butyltin diacetate
CAS-Nr.:	1067-33-0
IUPAC:	[acetyloxy(dibutyl)stannyl] acetate
Trivial Name:	Dibutytin diacetate, DBTAc, DBTA

Chemical Structure:



Batch No.:	Tk 233
Molecular Weight:	351.02
Aggregate State at RT/Colour:	colorless liquid
Purity:	> 99 % (by 119Sn-NMR Spectroscopy)
Storage:	In original container, at 15 °C to 25°C

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4. Test Method

Purpose of the study:

The purpose of the study was to verify the chemical pathways of the in-vitro metabolism of DBTAc with the aim to answer the following questions: is DBTAc metabolized via a hydrolytic mechanism at mammalian gastric pH? If so, what is the product of the metabolism?

Test Systems:

DBTAc was exposed at 40 °C for 4 hours to 0.1 M HCl (pH 1.2) solution. The reaction products were extracted with hexane and analyzed using 119Sn-NMR Spectroscopy and DSC. The aqueous phase remaining after the hexane extraction was analysed for it's tin content by ICP

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5. Analytical Method

119Sn-NMR Spectroscopy

The 119Sn-NMR was used to analyze the test item as well as the breakdown products of the test item, since it combines several unique aspects of analyzing organotin substances:

- 119Sn-NMR detects all tin-containing substances qualitatively and quantitatively at the same time.
- 119Sn-NMR is a direct and non-destructive method. It does not require any sample digestion or derivatization. Thus, it avoids errors associated with a) the sample derivatization and b) misinterpretation of the results associated with analyzing and quantifying the derivatives.
- The 119Sn-NMR spectra signals (peaks) are highly selective. They uniquely represent the corresponding tin compounds. Chemical shifts of differently substituted tin atoms are highly characteristic of the specific atom coordination.
- The 119Sn-NMR spectroscopy is very sensitive and reliable.
- The 119Sn-NMR method has been used for decades by the industry as a standard analytical method on tin compounds for the purpose of quality control, process development and research.

Experimental:

NMR:

Apparatus:	Bruker Advance III 400
Temperature:	Ambient temperature
Sample preparation:	370 µl/330µl $CDCl_3$ (10 mg/ml CrAcAc)
Documentation:	The test conditions and spectra obtained were documented as raw data and the printouts

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DSC:

Apparatus:	Mettler Toledo DSC1 /W200
Documentation:	The test conditions and spectra obtained were documented as raw data and the printouts

ICP:

Apparatus:	Agilent 725-ES ICP-OES
Documentation:	The test conditions and spectra obtained were documented as raw data and the printouts

6. Experimental

Apparatus:

- 2 L Round bottom flask
- Ground-in stopper
- 0.1 M HCl aqueous solution (pH 1.2) available from and provided by VWR International GmbH
- Electrical stirrer
- Separatory funnel
- Sample container (headspace glass)

Analytical:

- 119 Sn-NMR / Bruker Advance III 400
- Bruker D2 Phaser
- Mettler Toledo DSC1 /W200

Test Temperature:

Ambient temperature (outside of the apparatus)

Documentation:

The test conditions and measurements obtained were documented as raw data.

Performing the test:

The test item was used without a co-solvent or a detergent.

3.51 g (10 mMol) Test Item was added to 1000 ml of 0.1 M (100 mMol) aqueous solution of hydrochloric acid that was preheated to 37 °C in a 2L round bottom flask flask.

The flask was pre-heated to 37°C. The test item was added to the flask and stirred with an electrical dispersion stirrer for 4 hours.

After the pre-determined exposure time, the solution was allowed to cool down to room temperature; extracted 3 times with 50 ml hexane; the phases were separated using a separatory funnel. The organic phase was transferred into a pre-weighted flask, and the solvent was removed in

a rotary evaporator (<40 °C, 10 mbar). The recovered mass was determined, and the sample analyzed by 119Sn-NMR Spectroscopy and DSC.

7. Results

The results of in-vitro metabolism of DBTAc are displayed in Table 1.

The test item (DBTAc) reacted under the study conditions almost quantitatively to form the dimeric dichloro-distannoxane $[(DBTCl)_2O]_2$. Two 119Sn-NMR signals at -91 ppm and -138 ppm are characteristic for the non-equivalent exo- and endocylic tin atoms in the structure given below (figure 1). The dimeric structure of distannoxanes is well described in literature (Chandarsekhar, 2002; Beckmann, 2002; Davies, 2004). The structures which are minimum dimeric are unequivocally proven by 119Sn-NMR spectral data and X-Ray diffraction. Monomeric distannoxanes exist only in in solid phase with very bulky ligands (Davies 2004).

Equilibria between dimeric and monomeric structures are not described in literature, they would be visible in the 119Sn-NMR spectra. The chemicals shift of a literature described monomeric distannoxane $\{[(Me_3Si)_2CH]_2Sn(OH)]_2\}O$ is reported to be in the region of 17 ppm (Edelmann, 1990)

Figure 1: structure of $[(DBTCl)_2O]_2$

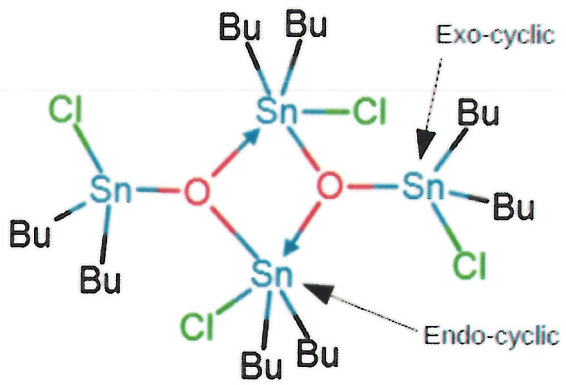

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Table 1: 119Sn-NMR chemical shifts

Test-No.	comment	δ [ppm]	Tin component
TK233	Untreated Test item	-155	DBTAc
TK233 111119 Hydrolysis	Metabolized test item extracted with hexane	-91, -138	$[(DBTCl)_2O]^2$

Melting point:

The melting point of the hydrolysis product of Dibutyltin diacetate was determined by DSC to be 110.29 °C (Literature: 111°C, Davis, 2004)

Aqueous phase:

The aqueous phase remaining after extraction with hexane has been analysed for water soluble tin compounds by ICP. The tin content of the aqueous phase remained below 0.5 mg/L. I must be concluded that no water soluble tin species was formed under the conditions of the study.

Dibutyltin dichloride – even in traces - could not be detected.

The 119Sn-NMR spectra in Annex 1-3 show that the chemical shift of DBTC (127 ppm) appears at significantly lower field as the test Item (-148 ppm) and it's metabolite $[(DBTC)_2O]_2$ (-91 / -138 ppm)

8. Conclusion and Discussion

The dimeric distannoxane $[(DBTC)_2O]_2$ is the only in-vitro metabolite of DBTAc under the conditions of the study. Spectral data as well as melting point match very well with literature data (Davies 2004).

DBTC – not even in traces - could not be detected in the reaction product.

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9. Abbreviations

DSC	Differential Scanning Calorimetry
DBTAc	Di-n-butyltin diactetate
DBTC	Dibutyltin dichloride
$[(DBTCl)_2O]_2$	bis[chloro-dibutyltin stannoxane]
ICP	Inductively coupled plasma mass spectrometry
NMR	Nuclear Magnetic Resonance

10. Literature

1) Davies A. G. (2004). Difunctional distannoxanes, XR2SnOSnR2X. J. Chem. Res. (5) 309-314. http://discovery.ucl.ac.uk/35579/

2) J. Beckmann, D. Dakternieks, F.S. Kuan and E.R.T. Tiekink, J. Organomet. Chem., 2002, 659, 73.

3) V. Chandrasekhar, S. Nagendran, and V. Baskar, Coord. Chem.Rev., 2002, 235, 1.

4) M.A. Edelman, P.B. Hitchcock and M.F. Lappert, J. Chem. Soc.,Chem. Commun., 1990, 1116.

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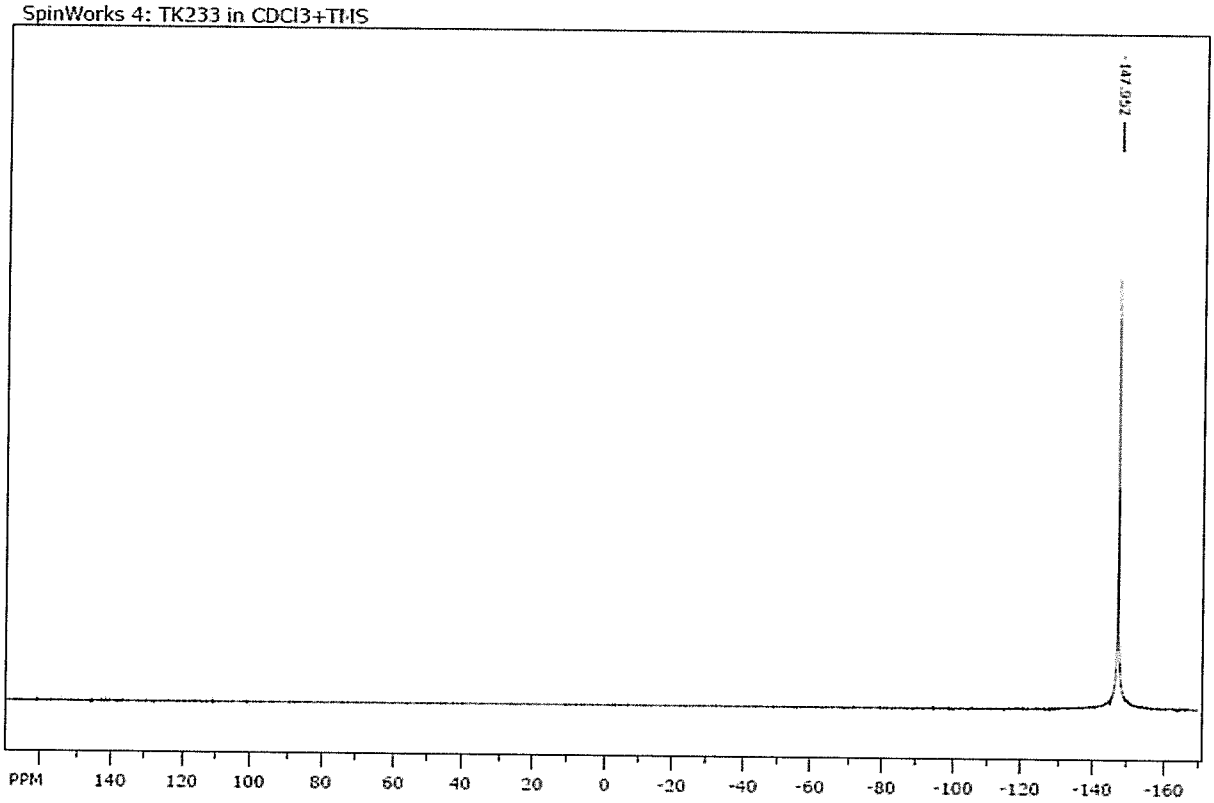
11.Annexes

Annex 1: 119Sn-NMR spectrum of untreated test item (DBTAc)



file: ... diacatate\c191112baf.TK233\3\fid expt: <zgig30>
transmitter freq.: 149.285587 MHz
time domain size: 131072 points
width: 51020.41 Hz = 341.7638 ppm = 0.389255 Hz/pt
number of scans: 1024

freq. of 0 ppm: 149.285587 MHz
processed size: 65536 complex points
LB: 2.000 GF: 0.0000
Hz/cm: 2040.816 ppm/cm: 13.67055

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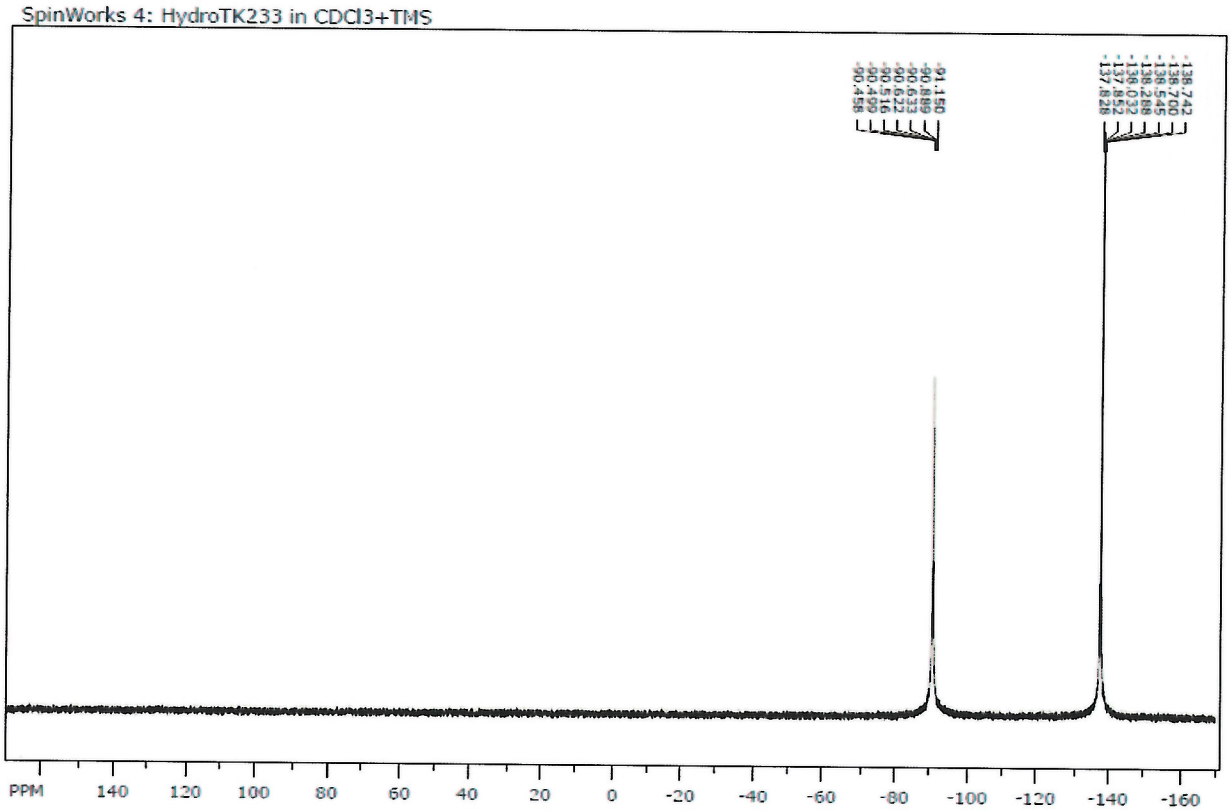
Annex 2: 119Sn-NMR spectrum of the metabolized test item at 40 °C/pH 1.2/4h



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time domain size: 131072 points
width: 51020.41 Hz = 341.7638 ppm = 0.389255 Hz/pt
number of scans: 1024

freq. of 0 ppm: 149.285587 MHz
processed size: 65536 complex points
LB: 2.000 GF: 0.0000
Hz/cm: 2040.816 ppm/cm: 13.67055

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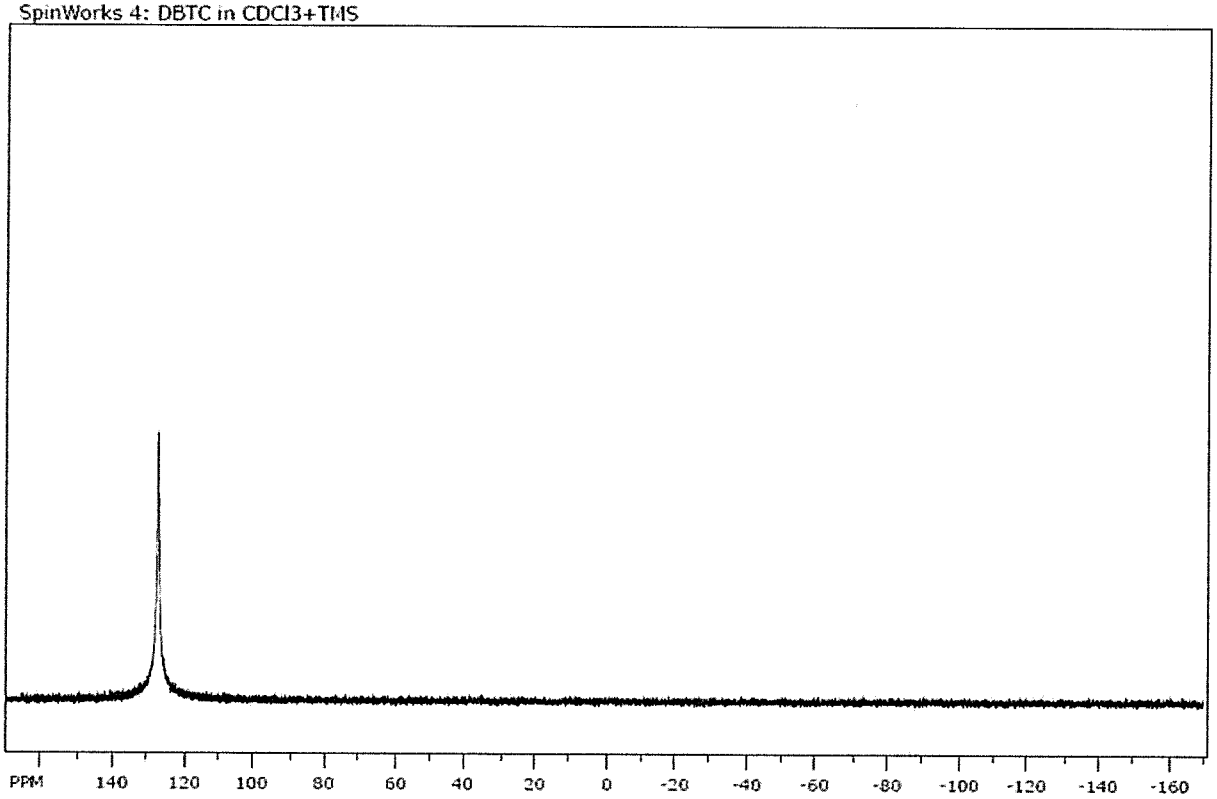
Annex 3: 119Sn-NMR spectrum of DBTC (reference)

SpinWorks 4: DBTC in CDCl3+TMS



file: ...n diacatate\c191112ba\.DBTC\1\fid expt: <zgig30>
transmitter freq.: 149.285587 MHz
time domain size: 131072 points
width: 51020.41 Hz = 341.7638 ppm = 0.389255 Hz/pt
number of scans: 1024

freq. of 0 ppm: 149.285587 MHz
processed size: 65536 complex points
LB: 2.000 GF: 0.0000
Hz/cm: 2040.816 ppm/cm: 13.67055

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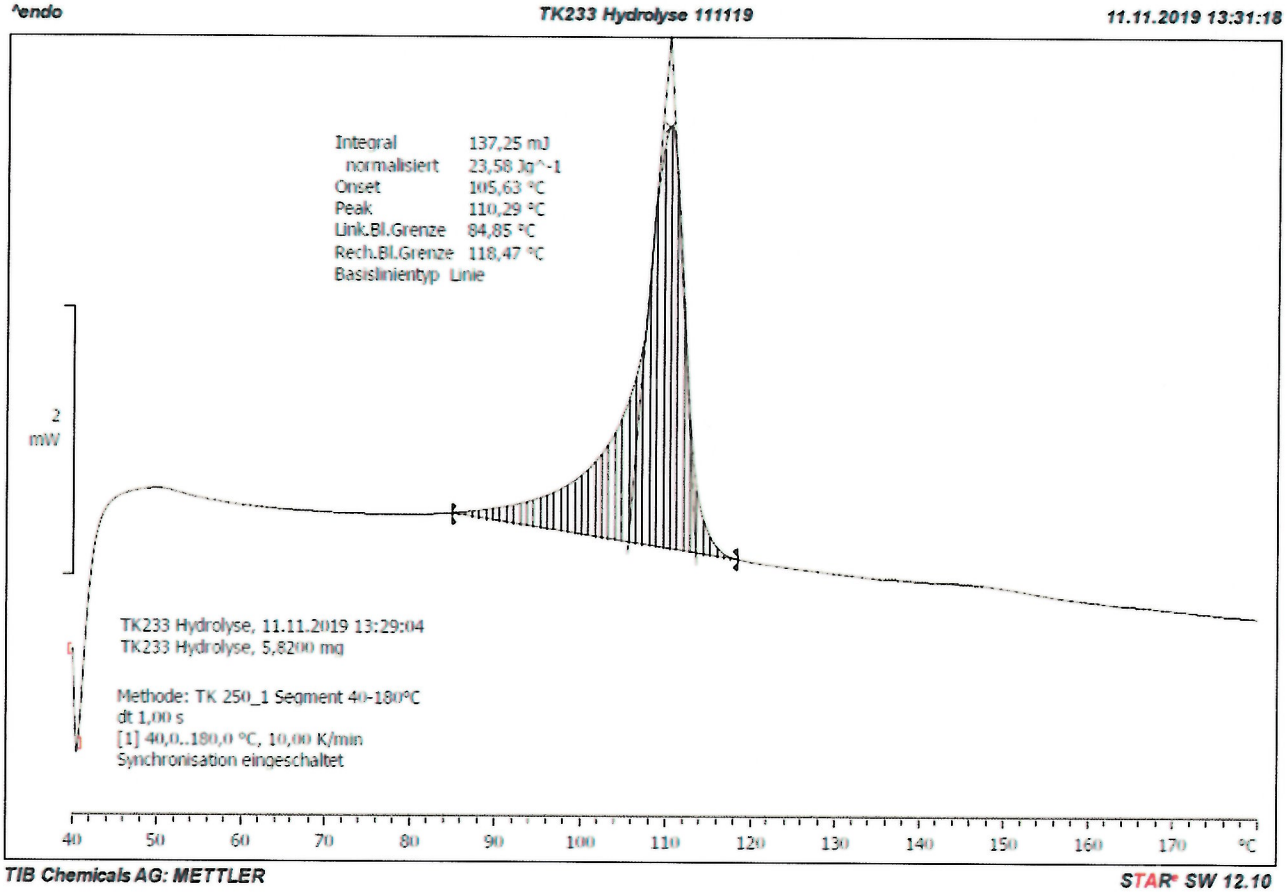
Annex 4 – DSC Measurement – Melting Point



Annex 6 – NMR and Melting Point reference data

Table 1 Dimeric distannoxanes, $(XR_2SnOSnR_2X)_2$

$[XR_2SnOSnR_2X]_2$	Structure	M.p./°C	$\delta^{119}Sn$	Ref.
$[ClMe_2SnOSnMe_2Cl]_2$	4–4–4	>280	−62.6, −114.4	36
$[(Me_3SiO)Me_2SnOSnMe_2(OSiMe_3)]_2$	4–4–4	167–168	−136.3, −155.8	12
$[BrEt_2SnOSnEt_2Br]_2$	4–4–4	176–178	−79.3, −128.9	36
$[(ClBu_2SnOSnBu_2Cl)]_2$	4–4–4	111–112	−91.4, −140.3	36
$[(AcO)Bu_2SnOSnBu_2(OAc)]_2$	4–4–6–6	206–210	−171.4, −184.6	36
$[(PhO)Bu_2SnOSnBu_2(OPh)]_2$	4–4–4	134	−176.8, −177.4	37
$[(TfO)Bu_2SnOSnBu_2(OH)]_2$	4–4–4	75–76	−137, −170	38
$[ClBu_2SnOSnBu^t_2Cl]_2$	4–4–4–4–4	178–181	−150.7, −157.6	6
$[FCy_2SnOSnCy_2F]_2$	4-4-4	>295dec	−212.0, −237.6	36
$[IOct_2SnOSnOct_2I]_2$		90–95	−169.9, −174.6	36
$[ClPh_2SnOSnPh_2Cl]_2$	4–4–4	194–196		39
$[ClR^f_2SnOSnR^fCl]_2$ [a]	4-4-4	90–91	−178.3, −202.5	40
10	4–4–4 [b]	304–305	−96.1, −132.9	8
$(HO)R_2SnOSnR_2(OH)$ [c]	Monomer		17.45	14

[a] $R^f = C_6F_{13}CH_2CH_2$; [b]Double ladder; [c]$R = (Me_3Si)_2CH$.