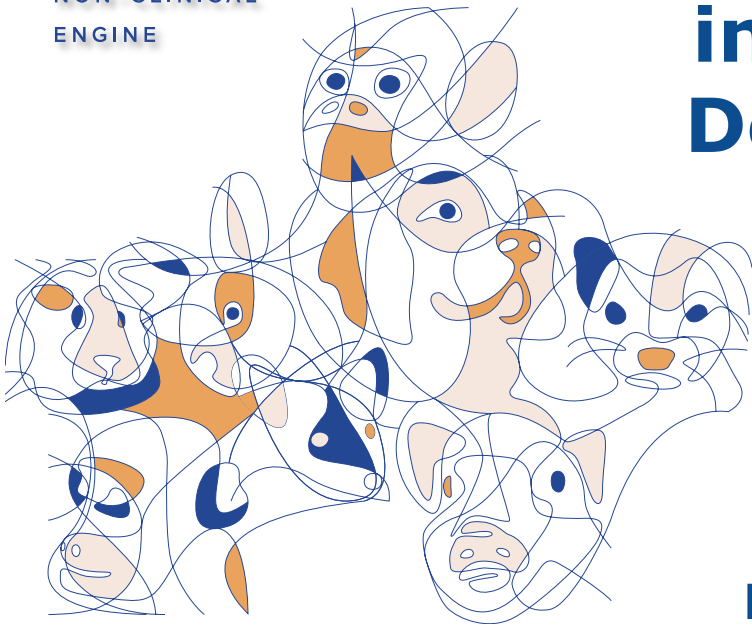




THE  
NON-CLINICAL  
ENGINE

# Hormone Monitoring in Preclinical Development



**Federico Pastori**  
(LabWare on behalf of ERBC)

**EBF - November 2023**



**Main objective** of the Preclinical development is to:

**Determine and address the primary toxicity endpoints** and all effects correlated with the New Chemical Entities (NCE) administration.

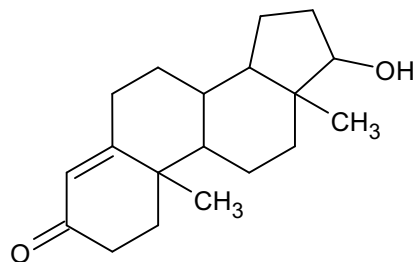
For chemical products, parallel evaluation of the **secondary toxicity effects on the endocrine system** is equally important.

The **panel** of the non-clinical studies to be designed **could be strongly affected** in the case in which a relevant secondary toxic effects occur.



## 52 weeks Chronic Toxicity Study – Hormone Panel

### TESTOSTERONE (TTE)



Molecular Formula:  $C_{19}H_{28}O_2$

Formula Weight: 288.431

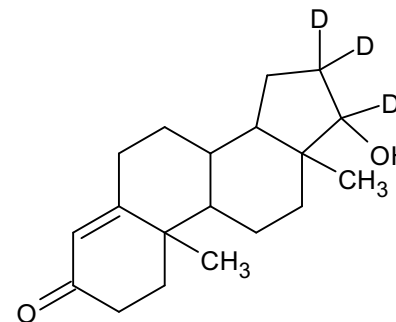
RD BE: 6

Monoisotopic Mass: 288.20893 Da

Nominal Mass: 288 Da

[M+H]<sup>+</sup>: 289.216207 Da

### TESTOSTERONE-D3 (TTE-D3,IS)



Molecular Formula:  $C_{19}H_{25}D_3O_2$

Formula Weight: 291.449306

RD BE: 6

Monoisotopic Mass: 291.22776 Da

Nominal Mass: 291 Da

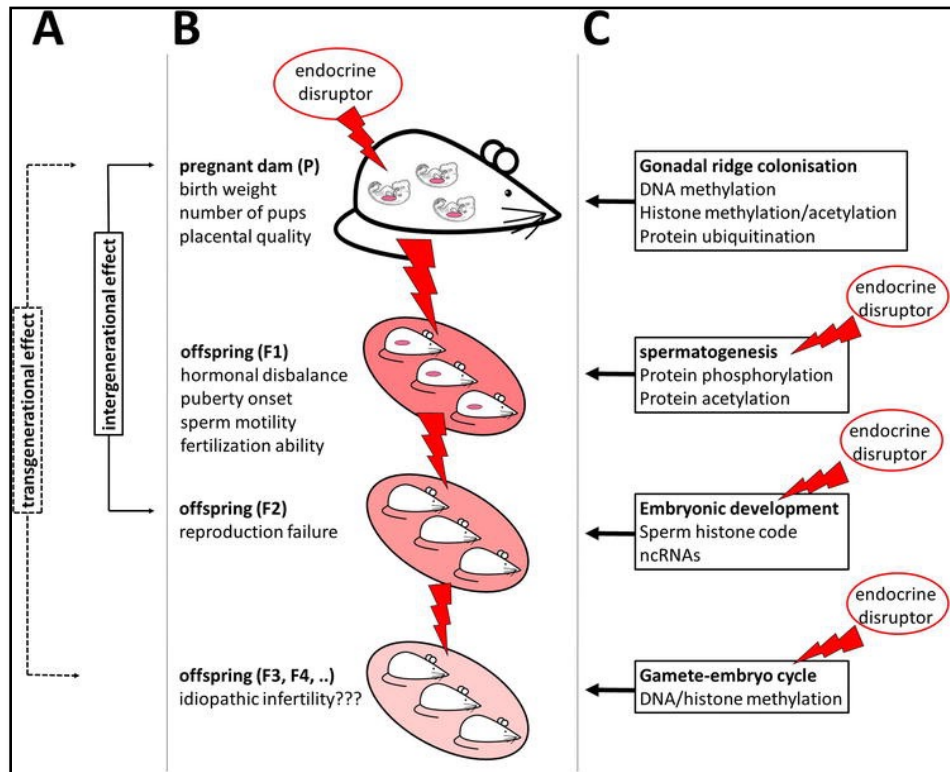
[M+H]<sup>+</sup>: 292.235037 Da

In this case

“**Endocrine disrupting chemicals** (EDCs) can interfere with normal hormonal balance and may exert adverse consequences on humans.

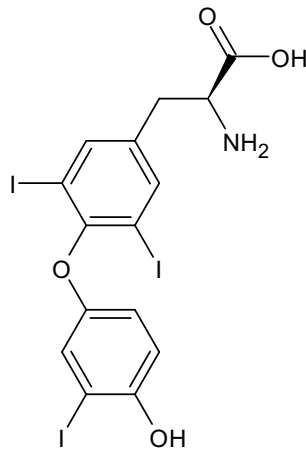
**The male reproductive system may be susceptible** to the effects of such environmental toxicants.”

In this case to properly assess the risk for human could be necessary to complete the **Chronic Toxicity** evaluation with a **Reproduction Toxicity Study** (OECD 421 or 423) to evaluate the impact of the disruptor on the reproductive system and the eventual teratogenic effects.



## 52 weeks Chronic Toxicity Study – Hormone Panel

### 3,3',5-Triiodo-L-thyronine (T3)



Molecular Formula:  $C_{15}H_{12}I_3NO_4$

Formula Weight: 650.97741

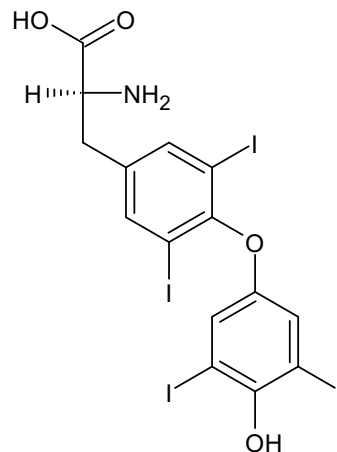
RDBE: 9

Monoisotopic Mass: 650.790013 Da

Nominal Mass: 651 Da

[M+H]<sup>+</sup>: 651.797289 Da

### L-Thyroxine (T4)



Molecular Formula:  $C_{15}H_{11}I_4NO_4$

Formula Weight: 776.87388

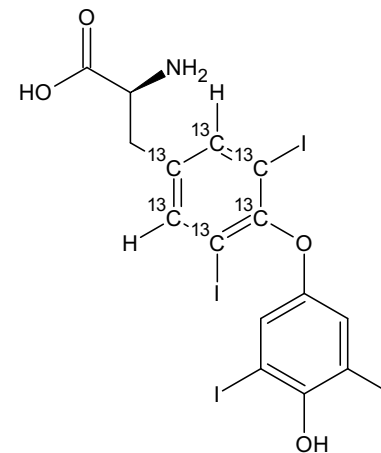
RDBE: 9

Monoisotopic Mass: 776.686648 Da

Nominal Mass: 777 Da

[M+H]<sup>+</sup>: 777.693924 Da

### L-Thyroxine-13C6 (T3-13C6, IS)



Molecular Formula:  $C_{15}H_{11}I_4NO_4$

Formula Weight: 782.82809

RDBE: 9

Monoisotopic Mass: 782.706777 Da

Nominal Mass: 783 Da

[M+H]<sup>+</sup>: 783.714053 Da

The **profile of TSH, T4, and T3** varies with the mechanism of toxicity affecting the thyroid.

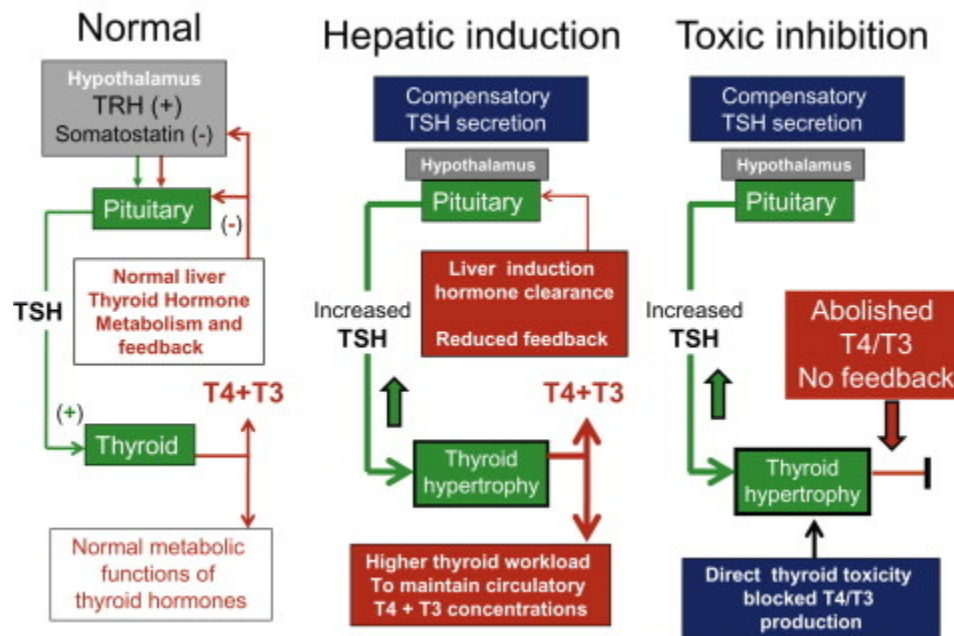
Functional levels of **T3** and **T4** correctly **regulates** the concentration of **TSH**

**Depletion of T3 and T4** lead to elevation of TSH could lead to **cancerogenic effect** on the thyroid.

T3 and T4 monitoring helps to properly assess the risk for human.

**Chronic Toxicity** studies could be completed with **Toxicity Study** to evaluate **carcinogenic effects**.

Thyroid hypertrophy: Differential mechanisms



Bioanalytical Method was set to determine the unconjugated compounds in serum samples:

### Application Ranges:

**TTE: 0.1 – 25 ng/mL**, ISTD: TTE-D3

**T3: 0.15 – 15 nmol/L**, ISTD: T3-13C6

**T4: 5 – 125 nmol/L**, ISTD: T3-13C6

**Matrix: SERUM**      **Sample Volume: 50uL**

### Sample Preparation:

- 50ul Serum sample
- 100ul of Acetonitrile
- Vortex
- Centrifugation 13000 rpm
- Injection

Analytes	Calib. Sample Criteria	QC Sample Criteria	Levels	Level Conc.	Certified Users	Analytical System	Preparation Reagents
1	BLANK	BLANK	P_ASTD_00117	0.00000 ng/mL	1.00000 50	µL	10.00000 UL
2	ISTD_ILLOQ_1		P_ASTD_00117	0.00000 ng/mL	1.00000 50	µL	10.00000 UL
3	SST		P_ASTD_00117	0.00000 ng/mL	1.00000 50	µL	10.00000 UL
4	C01		P_ASTD_00117	0.10000 ng/mL	1.00000 50	µL	10.00000 UL
5	C02		P_ASTD_00117	0.22000 ng/mL	1.00000 50	µL	10.00000 UL
6	QLOW		P_ASTD_00117	0.25000 ng/mL	1.00000 50	µL	10.00000 UL
7	C03		P_ASTD_00117	0.50000 ng/mL	1.00000 50	µL	10.00000 UL
8	C04		P_ASTD_00117	1.10000 ng/mL	1.00000 50	µL	10.00000 UL
9	C05		P_ASTD_00117	2.20000 ng/mL	1.00000 50	µL	10.00000 UL
10	C06		P_ASTD_00117	5.00000 ng/mL	1.00000 50	µL	10.00000 UL
11	Q MID		P_ASTD_00117	10.00000 ng/mL	1.00000 50	µL	10.00000 UL
12	C07		P_ASTD_00117	11.00000 ng/mL	1.00000 50	µL	10.00000 UL
13	QHIGH		P_ASTD_00117	20.00000 ng/mL	1.00000 50	µL	10.00000 UL
14	C08		P_ASTD_00117	25.00000 ng/mL	1.00000 50	µL	10.00000 UL

Chromatography Settings:

ACQUITY UPLC BEH C18 **1.7  $\mu\text{m}$  1x50mm** (Waters)

Eluent A: 0.1% Formic acid in water

Eluent B: 0.1% Formic acid in acetonitrile

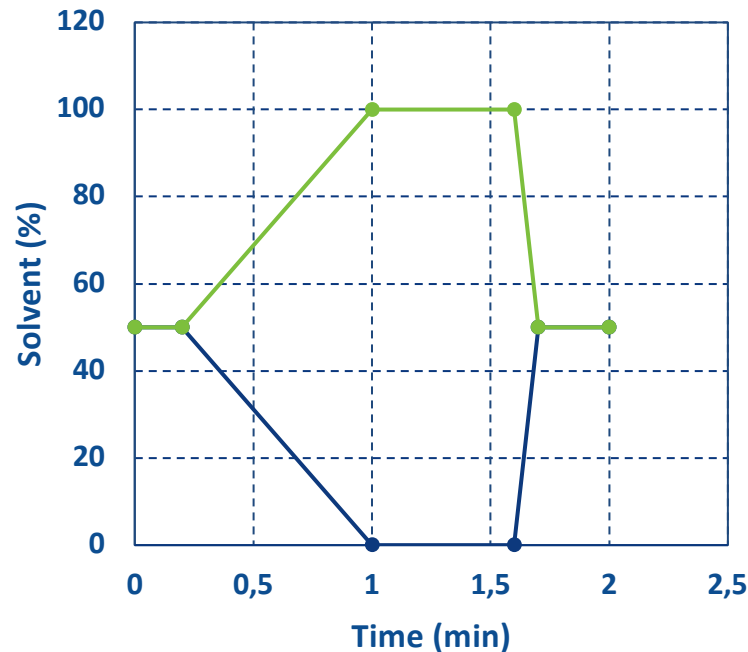
Injection Volume: **1.5uL**

Gradient for analysis of TTE and TTE D3 in rat serum

Time (min)	Flow ( $\mu\text{L}/\text{min}$ )	A (%)	B (%)
0	80	50	50
<b>0.2</b>		50	<b>50</b>
<b>1.0</b>		0	<b>100</b>
1.6		0	100
1.7		50	50
2		50	50

P = 62.5

### Gradient

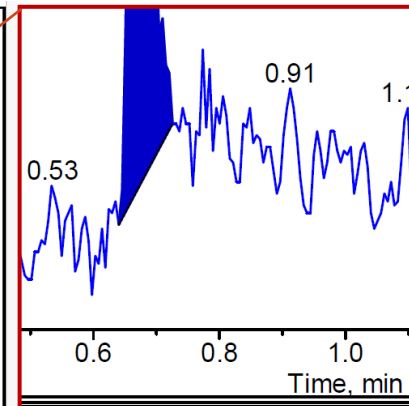
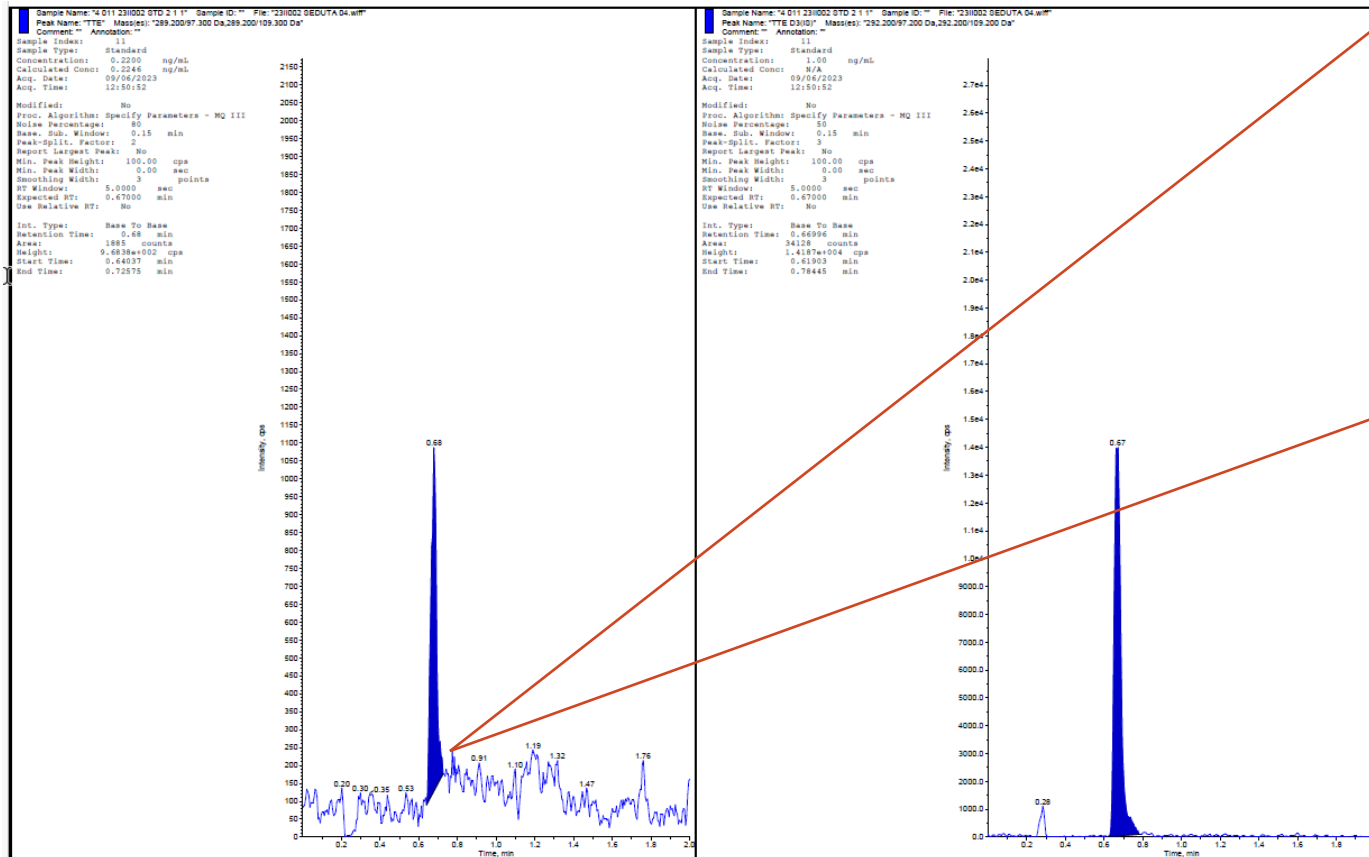


Great **gain in sensitivity**, due to:  
**Micro LC M5 (SCIEX), I.D.** and **Particle Size** of the column.



# Hormone Monitoring in Preclinical Development

## ERBC - BMV - Case Study



Peak Time

$$t_1 - t_0 = 0.74 - 0.63 \text{ min} \\ = 0.11 \text{ min} = 6.6 \text{ sec}$$

To grant 10 points for a good peak shape acquisition instrument duty cycle time should be less than **660 msec**



### High-Resolution MS Systems:

- No. 1: M5 - API 5600
- No. 2: M5 - API 6600+

The Pilot GLP Study for hormone monitoring was performed on the entry level SCIEX API 4000.

### Low-Resolution MS System:

- No. 2: M3 - API 4000
- No. 1: M5 - API 5500
- No. 2: M5 - API 7500

A comparable sensitivity with RIA obtained with LC-MS will be then used to scale-up the method more sensible MS System, scaling down the Sample Volume





### Bioanalytical process Flow

Validation Protocol Definition

Sample Planning and Login

Worklist Preparation

Bi-directional interfacing between LabWare and SCIEX Analyst

Regression in LabWare

Reporting

METH\_VAL: METH\_VAL-23-00004 - [PASTORIF] Federico Pastori

Recent File SOP Audit Plot Document History Options Validation Evaluation Report Filter Study Evaluation Variable Function Weighting Study

Summary Method Validation Protocol Samples

Accuracy and Precision		
QC Batch	Set Batch	QC_2023-FPA-00048
Storage	Set Storage	RT
Condition	Set Condition	NORMAL_LIGHT
Tube Type	Set Tube Type	EP
Number of Periods/Days	Set Number of Periods/Days	3
Nominal Levels	Set Nominal Levels	QLLOQ, QHIGH, QLOW, QMID
Number Aliquots/Replicates	Set Aliquots	6

Validation Scope	Period/Day Number	QC Batch	Nominal Level	Aliquot	Sample Name
Full Validation	1	QC_2023-FPA-00048	QLLOQ	1	V_AP1_QC_2023-FPA-00048_QLLOQ_1
Full Validation	1	QC_2023-FPA-00048	QLLOQ	2	V_AP1_QC_2023-FPA-00048_QLLOQ_2
Full Validation	1	QC_2023-FPA-00048	QLLOQ	3	V_AP1_QC_2023-FPA-00048_QLLOQ_3
Full Validation	1	QC_2023-FPA-00048	QLLOQ	4	V_AP1_QC_2023-FPA-00048_QLLOQ_4
Full Validation	1	QC_2023-FPA-00048	QLLOQ	5	V_AP1_QC_2023-FPA-00048_QLLOQ_5
Full Validation	1	QC_2023-FPA-00048	QLLOQ	6	V_AP1_QC_2023-FPA-00048_QLLOQ_6
Full Validation	1	QC_2023-FPA-00048	QHIGH	1	V_AP1_QC_2023-FPA-00048_QHIGH_1
Full Validation	1	QC_2023-FPA-00048	QHIGH	2	V_AP1_QC_2023-FPA-00048_QHIGH_2
Full Validation	1	QC_2023-FPA-00048	QHIGH	3	V_AP1_QC_2023-FPA-00048_QHIGH_3
Full Validation	1	QC_2023-FPA-00048	QHIGH	4	V_AP1_QC_2023-FPA-00048_QHIGH_4
Full Validation	1	QC_2023-FPA-00048	QHIGH	5	V_AP1_QC_2023-FPA-00048_QHIGH_5
Full Validation	1	QC_2023-FPA-00048	QHIGH	6	V_AP1_QC_2023-FPA-00048_QHIGH_6
Full Validation	1	QC_2023-FPA-00048	QLOW	1	V_AP1_QC_2023-FPA-00048_QLOW_1
Full Validation	1	QC_2023-FPA-00048	QLOW	2	V_AP1_QC_2023-FPA-00048_QLOW_2
Full Validation	1	QC_2023-FPA-00048	QLOW	3	V_AP1_QC_2023-FPA-00048_QLOW_3
Full Validation	1	QC_2023-FPA-00048	QLOW	4	V_AP1_QC_2023-FPA-00048_QLOW_4
Full Validation	1	QC_2023-FPA-00048	QLOW	5	V_AP1_QC_2023-FPA-00048_QLOW_5
Full Validation	1	QC_2023-FPA-00048	QLOW	6	V_AP1_QC_2023-FPA-00048_QLOW_6
Full Validation	1	QC_2023-FPA-00048	QMID	1	V_AP1_QC_2023-FPA-00048_QMID_1
Full Validation	1	QC_2023-FPA-00048	QMID	2	V_AP1_QC_2023-FPA-00048_QMID_2
Full Validation	1	QC_2023-FPA-00048	QMID	3	V_AP1_QC_2023-FPA-00048_QMID_3
Full Validation	1	QC_2023-FPA-00048	QMID	4	V_AP1_QC_2023-FPA-00048_QMID_4
Full Validation	1	QC_2023-FPA-00048	QMID	5	V_AP1_QC_2023-FPA-00048_QMID_5
Full Validation	1	QC_2023-FPA-00048	QMID	6	V_AP1_QC_2023-FPA-00048_QMID_6
Full Validation	2	QC_2023-FPA-00048	QLLOQ	1	V_AP2_QC_2023-FPA-00048_QLLOQ_1

### Bioanalytical process Flow

Validation Protocol  
Definition

Sample Planning and  
Login

Worklist Preparation

Bi-directional interfacing  
between LabWare  
and  
SCIEX Analyst

Regression in LabWare

Reporting

Worklist: 2023-FPA-00049\_ACC 24 BEN 12 CA 8 QC 6 TES 11  
Created By: PASTORIF  
Updated On: 04/11/2023 10:08:35

Summary	Samples	Results	Report
>	8 T_BLANK/2	(SAMPLE)	
>	9 T_BLANK/3	(SAMPLE)	
>	10 C_0,1_CA_2023-FPA-00047/1	(SAMPLE)	
>	11 C_0,22_CA_2023-FPA-00047/1	(SAMPLE)	
>	12 C_0,5_CA_2023-FPA-00047/1	(SAMPLE)	
>	13 C_1,1_CA_2023-FPA-00047/1	(SAMPLE)	
>	14 C_2,2_CA_2023-FPA-00047/1	(SAMPLE)	
>	15 C_5_CA_2023-FPA-00047/1	(SAMPLE)	
>	16 C_11_CA_2023-FPA-00047/1	(SAMPLE)	
>	17 C_25_CA_2023-FPA-00047/1	(SAMPLE)	
>	18 T_BLANK/1	(SAMPLE)	
>	19 T_BLANK/2	(SAMPLE)	
>	20 66988 V_API_OC_2023-FPA-00048_QLLOQ_1/1	(SAMPLE)	
>	21 66989 V_API_OC_2023-FPA-00048_QLLOQ_2/1	(SAMPLE)	
>	22 66990 V_API_OC_2023-FPA-00048_QLLOQ_3/1	(SAMPLE)	
>	23 66991 V_API_OC_2023-FPA-00048_QLLOQ_4/1	(SAMPLE)	
>	24 66992 V_API_OC_2023-FPA-00048_QLLOQ_5/1	(SAMPLE)	
>	25 66993 V_API_OC_2023-FPA-00048_QLLOQ_6/1	(SAMPLE)	
>	26 67006 V_API_OC_2023-FPA-00048_QMID_1/1	(SAMPLE)	
>	27 67007 V_API_OC_2023-FPA-00048_QMID_2/1	(SAMPLE)	
>	28 67008 V_API_OC_2023-FPA-00048_QMID_3/1	(SAMPLE)	
>	29 67009 V_API_OC_2023-FPA-00048_QMID_4/1	(SAMPLE)	
>	30 67010 V_API_OC_2023-FPA-00048_QMID_5/1	(SAMPLE)	
>	31 67011 V_API_OC_2023-FPA-00048_QMID_6/1	(SAMPLE)	
>	32 Q_10_QC_2023-FPA-00048/1	(SAMPLE)	
>	33 67000 V_API_OC_2023-FPA-00048_QLOW_1/1	(SAMPLE)	
>	34 67001 V_API_OC_2023-FPA-00048_QLOW_2/1	(SAMPLE)	
>	35 67002 V_API_OC_2023-FPA-00048_QLOW_3/1	(SAMPLE)	
>	36 67003 V_API_OC_2023-FPA-00048_QLOW_4/1	(SAMPLE)	
>	37 67004 V_API_OC_2023-FPA-00048_QLOW_5/1	(SAMPLE)	
>	38 67005 V_API_OC_2023-FPA-00048_QLOW_6/1	(SAMPLE)	
>	39 Q_10_QC_2023-FPA-00048/2	(SAMPLE)	
>	40 66994 V_API_OC_2023-FPA-00048_QHIGH_1/1	(SAMPLE)	
>	41 66995 V_API_OC_2023-FPA-00048_QHIGH_2/1	(SAMPLE)	
>	42 66996 V_API_OC_2023-FPA-00048_QHIGH_3/1	(SAMPLE)	
>	43 66997 V_API_OC_2023-FPA-00048_QHIGH_4/1	(SAMPLE)	
>	44 66998 V_API_OC_2023-FPA-00048_QHIGH_5/1	(SAMPLE)	
>	45 66999 V_API_OC_2023-FPA-00048_QHIGH_6/1	(SAMPLE)	
>	46 Q_0,25_QC_2023-FPA-00048/2	(SAMPLE)	
>	47 67019 V_BENCHTOP1_OC_2023-FPA-00048_RT_NORMAL_LIGHT_QLOW_D0_4H_1/1	(SAMPLE)	
>	48 67020 V_BENCHTOP1_OC_2023-FPA-00048_RT_NORMAL_LIGHT_QLOW_D0_4H_2/1	(SAMPLE)	
>	49 67021 V_BENCHTOP1_OC_2023-FPA-00048_RT_NORMAL_LIGHT_QLOW_D0_4H_3/1	(SAMPLE)	
>	50 67022 V_BENCHTOP1_OC_2023-FPA-00048_RT_NORMAL_LIGHT_QLOW_D0_4H_4/1	(SAMPLE)	

Analyst - [C:\ANALYST DATA\PROJECTS\F\_E001-23002-TR...]  
File Edit View Tools Window Script Help  
Quantitate  
Configure  
Security Configuration  
Hardware Configuration  
Report Template Editor  
Tune and Calibrate  
Compound Optimization  
Instrument Optimization  
Manual Tuning  
Acquire  
IDA Method Wizard  
Build Acquisition Method  
Build Acquisition Batch  
Explore (1)  
Open Data File  
Open Compound Database  
Quantitate (1)  
Build Quantitation Method  
Quantitation Wizard  
Review Results Table  
Companion Software  
Reporter 3.2  
Formulas  
1 T\_BLANK  
2 T\_BLANK  
3 T\_BLANK  
4 T\_BLANK  
5 T\_BLANK  
6 T\_BLANK  
7 T\_BLANK  
8 T\_BLANK  
9 T\_BLANK  
10 C\_0,1\_CA  
11 C\_0,22\_CA  
12 C\_0,5\_CA  
13 C\_1,1\_CA  
14 C\_2,2\_CA  
15 C\_5\_CA  
16 C\_11\_CA  
17 C\_25\_CA  
18 T\_BLANK  
19 T\_BLANK  
20 V\_API\_OC  
21 V\_API\_OC  
22 V\_API\_OC  
23 V\_API\_OC  
24 V\_API\_OC  
25 V\_API\_OC  
26 V\_API\_OC  
27 V\_API\_OC  
28 V\_API\_OC  
29 V\_API\_OC  
30 V\_API\_OC  
31 V\_API\_OC  
32 Q\_10\_QC  
33 V\_API\_OC  
34 V\_API\_OC  
35 V\_API\_OC  
36 V\_API\_OC  
37 V\_API\_OC  
38 V\_API\_OC  
39 Q\_10\_QC  
Start, press F1

# Hormone Monitoring in Preclinical Development

## Full Validation: Sample and Data Processing

### Bioanalytical process Flow

Validation Protocol Definition

Sample Planning and Login

Worklist Preparation

Bi-directional interfacing between LabWare and SCIEX Analyst

Regression in LabWare

Reporting

Analyst - [C:\ANALYST DATA\PROJECTS\P\_E001-2311002-TTE-SER-RAT\RESULTS\2023-FPA-00049.RDB] ANALYST\_INTERFACES-10.100.240.66

Quantitate Mode P\_E001-2311002-TTE-SER-RAT

Analyte Layout: TTE  
Query: None  
Idle  
Sort: Unsorted

	Sample Name	Sample Type	Use Record	Analyte Concentration (ng/mL)	Calculated Concentration (ng/mL)	Deviation %	Accuracy (%)	Analyte Peak Name	Analyte Retention Area (min)	Analyte Peak Area (a.u.)	IS Peak Area (a.u.)	Area Ratio
1	T_BLANK / 1	Unknown		N/A	#DIV/0!	N/A	N/A	TTE	0.00	0	0	#DIV/0!
2	T_BLANK / 1	Unknown		N/A	#DIV/0!	N/A	N/A	TTE	0.00	0	0	#DIV/0!
3	T_BLANK / 1	Unknown		N/A	#DIV/0!	N/A	N/A	TTE	0.67	60624	0	#DIV/0!
4	T_BLANK / 1	Unknown		N/A	#DIV/0!	N/A	N/A	TTE	0.00	0	0	#DIV/0!
5	T_BLANK / 1	Unknown		N/A	0.02975	N/A	N/A	TTE	0.68	204	20304	1.0066e-002
6	T_BLANK / 1	Unknown		N/A	#DIV/0!	N/A	N/A	TTE	0.00	0	0	#DIV/0!
7	T_BLANK / 1	Unknown		N/A	#DIV/0!	N/A	N/A	TTE	0.00	0	0	#DIV/0!
8	T_BLANK / 2	Unknown		N/A	0.5733	N/A	N/A	TTE	0.68	7	49	1.4738e-001
9	T_BLANK / 3	Unknown		N/A	< 0	N/A	N/A	TTE	0.69	16	30400	5.3147e-002
10	C_0_1_CA_2023-FPA-00047 / 1	Standard	<input checked="" type="checkbox"/>	0.1000	0.1023	2.27	102.27	TTE	0.68	804	28311	2.8396e-002
11	C_0_22_CA_2023-FPA-00047 / 1	Standard	<input checked="" type="checkbox"/>	0.2200	0.2127	-3.33	96.669	TTE	0.68	1732	30777	5.6277e-002
12	C_0_5_CA_2023-FPA-00047 / 1	Standard	<input checked="" type="checkbox"/>	0.5000	0.4799	-4.01	95.985	TTE	0.68	3852	31115	1.2379e-001
13	C_1_1_CA_2023-FPA-00047 / 1	Standard	<input checked="" type="checkbox"/>	1.100	1.114	1.32	101.32	TTE	0.68	8096	28496	2.8410e-001
14	C_2_2_CA_2023-FPA-00047 / 1	Standard	<input checked="" type="checkbox"/>	2.200	2.208	0.38	100.38	TTE	0.68	15123	26984	5.6044e-001
15	C_5_CA_2023-FPA-00047 / 1	Standard	<input checked="" type="checkbox"/>	5.000	4.743	-5.14	94.856	TTE	0.68	34490	28725	1.2007e+000
16	C_11_CA_2023-FPA-00047 / 1	Standard	<input checked="" type="checkbox"/>	11.00	10.30	-6.38	93.617	TTE	0.68	77535	29774	2.6041e+000
17	C_25_CA_2023-FPA-00047 / 1	Standard	<input checked="" type="checkbox"/>	20.00	22.98	14.91	114.91	TTE	0.68	173030	29789	5.8086e+000
18	T_BLANK / 1	Unknown		N/A	31.58	N/A	N/A	TTE	0.67	39	5	7.9815e-002
19	T_BLANK / 2	Unknown		N/A	13.82	N/A	N/A	TTE	0.68	31	9	3.4929e-002
20	V_AP1_QC_2023-FPA-00048_QLLOQ_1 / 1	Quality Control	<input checked="" type="checkbox"/>	0.1000	0.08138	-18.62	81.378	TTE	0.68	642	27762	2.3109e-002
21	V_AP1_QC_2023-FPA-00048_QLLOQ_2 / 1	Quality Control	<input checked="" type="checkbox"/>	0.1000	0.08346	-16.54	83.458	TTE	0.68	654	27659	2.3034e-002
22	V_AP1_QC_2023-FPA-00048_QLLOQ_3 / 1	Quality Control	<input checked="" type="checkbox"/>	0.1000	0.26325	-16.75	83.252	TTE	0.67	655	27765	2.3522e-002
23	V_AP1_QC_2023-FPA-00048_QLLOQ_4 / 1	Quality Control	<input checked="" type="checkbox"/>	0.1000	0.1945	4.48	104.48	TTE	0.67	856	25673	2.8945e-002
24	V_AP1_QC_2023-FPA-00048_QLLOQ_5 / 1	Quality Control	<input checked="" type="checkbox"/>	0.1000	0.09874	-1.26	98.739	TTE	0.67	829	30147	2.7494e-002
25	V_AP1_QC_2023-FPA-00048_QLLOQ_6 / 1	Quality Control	<input checked="" type="checkbox"/>	0.1000	0.08048	-19.52	80.483	TTE	0.67	667	29155	2.2893e-002
26	V_AP1_QC_2023-FPA-00048_QMID_1 / 1	Quality Control	<input checked="" type="checkbox"/>	10.00	10.06	0.61	100.61	TTE	0.68	72262	28403	2.5442e+000
27	V_AP1_QC_2023-FPA-00048_QMID_2 / 1	Quality Control	<input checked="" type="checkbox"/>	10.00	11.30	13.03	113.03	TTE	0.68	77340	27060	2.8581e+000
28	V_AP1_QC_2023-FPA-00048_QMID_3 / 1	Quality Control	<input checked="" type="checkbox"/>	10.00	9.242	-7.58	92.425	TTE	0.68	65679	28098	2.3375e+000
29	V_AP1_QC_2023-FPA-00048_QMID_4 / 1	Quality Control	<input checked="" type="checkbox"/>	10.00	11.48	14.77	114.77	TTE	0.68	80543	27754	2.9020e+000
30	V_AP1_QC_2023-FPA-00048_QMID_5 / 1	Quality Control	<input checked="" type="checkbox"/>	10.00	10.42	4.19	104.19	TTE	0.67	80716	30635	2.6348e+000
31	V_AP1_QC_2023-FPA-00048_QMID_6 / 1	Quality Control	<input checked="" type="checkbox"/>	10.00	10.76	7.62	107.62	TTE	0.68	83473	30673	2.7214e+000
32	Q_10_QC_2023-FPA-00048 / 1	Quality Control	<input checked="" type="checkbox"/>	10.00	10.47	4.67	104.67	TTE	0.67	79105	29886	2.6469e+000
33	V_AP1_QC_2023-FPA-00048_QLOW_1 / 1	Quality Control	<input checked="" type="checkbox"/>	0.2500	0.2205	-11.79	88.205	TTE	0.68	1688	28972	5.8258e-002
34	V_AP1_QC_2023-FPA-00048_QLOW_2 / 1	Quality Control	<input checked="" type="checkbox"/>	0.2500	0.2516	0.64	100.64	TTE	0.67	1864	28199	6.6113e-002
35	V_AP1_QC_2023-FPA-00048_QLOW_3 / 1	Quality Control	<input checked="" type="checkbox"/>	0.2500	0.2556	2.23	102.23	TTE	0.67	1983	29547	6.7114e-002
36	V_AP1_QC_2023-FPA-00048_QLOW_4 / 1	Quality Control	<input checked="" type="checkbox"/>	0.2500	0.2634	5.35	105.35	TTE	0.67	2202	31865	6.9089e-002
37	V_AP1_QC_2023-FPA-00048_QLOW_5 / 1	Quality Control	<input checked="" type="checkbox"/>	0.2500	0.1885	-32.58	87.417	TTE	0.67	1226	27168	4.5129e-002
38	V_AP1_QC_2023-FPA-00048_QLOW_6 / 1	Quality Control	<input checked="" type="checkbox"/>	0.2500	0.2751	10.03	110.03	TTE	0.67	2162	30010	7.2043e-002
39	Q_10_QC_2023-FPA-00048 / 2	Quality Control	<input checked="" type="checkbox"/>	10.00	9.791	-2.09	97.908	TTE	0.67	69717	28157	2.4760e+000

Start | press F1

## Bioanalytical process Flow

Validation Protocol Definition

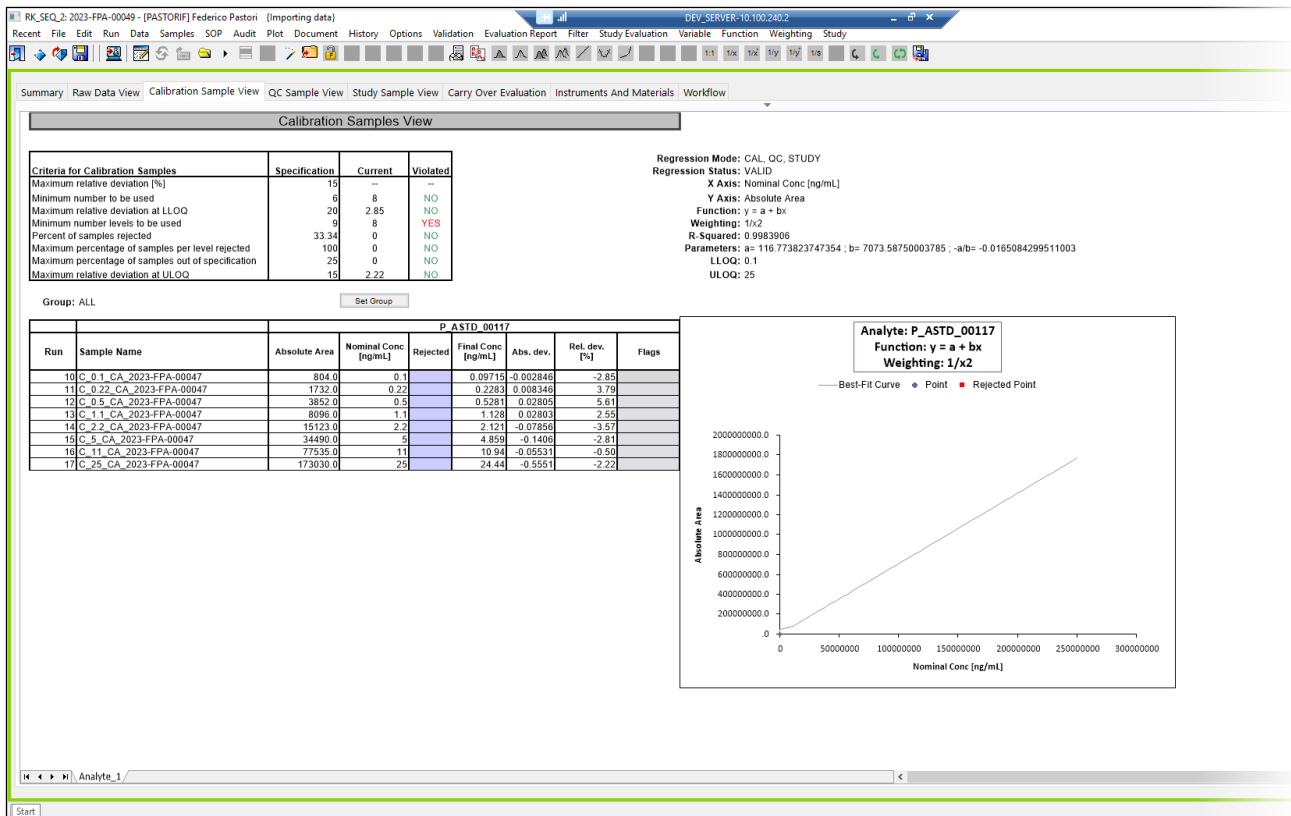
Sample Planning and Login

Worklist Preparation

Bi-directional interfacing between LabWare and SCIEX Analyst

Regression in LabWare

Reporting



### Bioanalytical process Flow

Validation Protocol Definition

Sample Planning and Login

Worklist Preparation

Bi-directional interfacing between LabWare and SCIEX Analyst

Regression in LabWare

Reporting

The screenshot displays the Genealogy Window interface. On the left, a file tree shows a project structure including Validation Plan (1), Sequences (1), and Evaluation Reports (4). The selected report is 'ACCANDPREC - QC Batch: QC\_2023-FPA-00048'. The main area shows an 'Evaluation Report' for 'Accuracy and Precision for P\_ASTD\_00117'. The report includes fields for Labware, Project Drug Substance, Matrix, Method, Species, Study Number, and QC Batch. It also lists Rejected Sequences (NA) and a table of results for 'Between-Run' and 'Within-Run' (2023-FPA-00049) with columns for n, mean [ng/mL], S.D., CV [%], and accuracy [%].

Generated On: 06 nov 2023  
Generated By: Federico Pastori

Light: Normal Light  
Temperature: Room Temperature  
Tube Type: Eppendorf

Sign / Date

	QLLOW (0.08 - 0.12)	QLLOW (0.21 - 0.29)	GMID (8.50 - 11.50)	QHGH (17.00 - 23.00)
Acceptance range	0.100	0.250	10.0	20.0
Nominal / Reference	1.00 [ng/mL]	1.000 [ng/mL]	1.00 [ng/mL]	1.00 [ng/mL]
Dil factor	1.00	1.000	1.00	1.00
Sequence				
2023-FPA-00049	0.0787	0.224	10.5	27.4
	0.0808	0.256	11.8	20.5
	0.0806	0.261	9.65	22.7
	0.103	0.269	12.0	21.6
	0.0967	0.170	10.9	21.1
	0.0776	0.281	11.2	22.1
Between-Run				
n	6	6	6	6
mean [ng/mL]	0.0860	0.243	11.0	22.6
S.D.	0.0110	0.0410	0.866	2.51
CV [%]	12.8	16.9	7.9	11.1
accuracy [%]	86.0	97.2	110.1	112.9
Acceptance Criteria	Passed	Passed	Passed	Passed
Within-Run (2023-FPA-00049)				
n	6	6	6	6
mean [ng/mL]	0.0860	0.243	11.0	22.6
S.D.	0.0110	0.0410	0.866	2.51
CV [%]	12.8	16.9	7.9	11.1
accuracy [%]	86.0	97.2	110.1	112.9
Acceptance Criteria	Passed	Passed	Passed	Passed

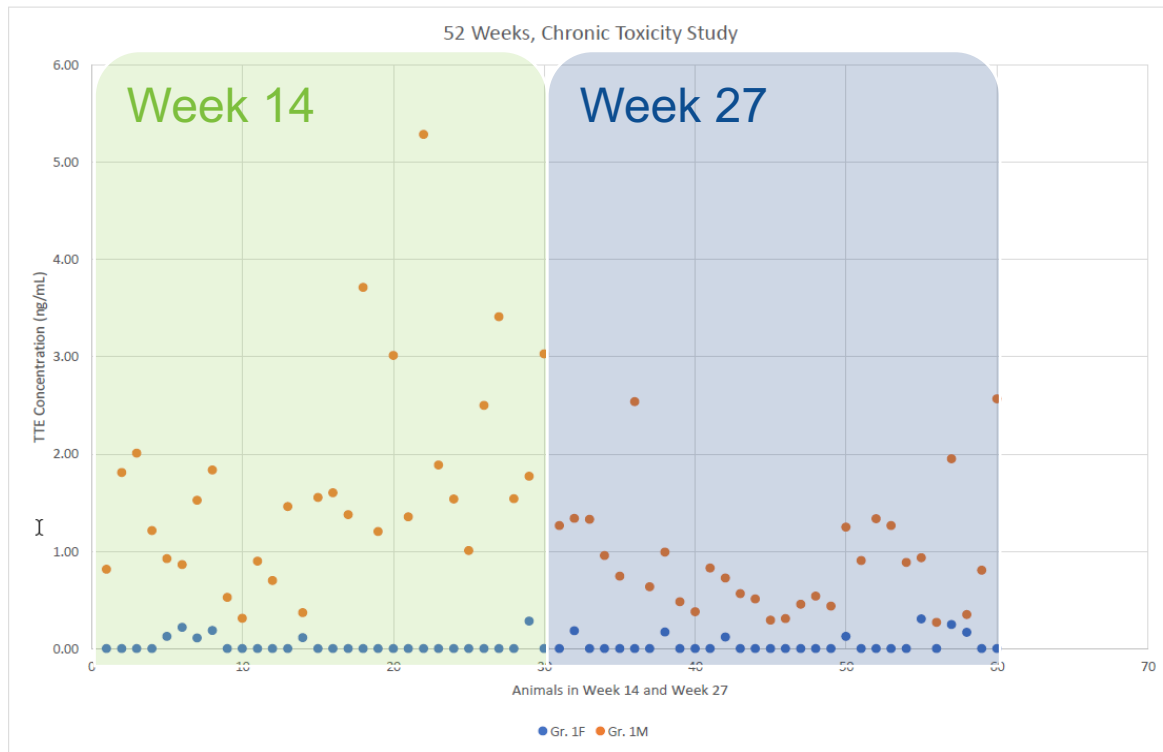


## Test Item - Treatment Schema:

Gr. No.	Dose Level (mg/kg/day)	Level	No. of Animals
1	0	Control	30
2	1000	Low	20
3	2000	Medium	20
4	4000	High	30

Results of the Control Group are used as baseline to detect eventual increase of the TTE.

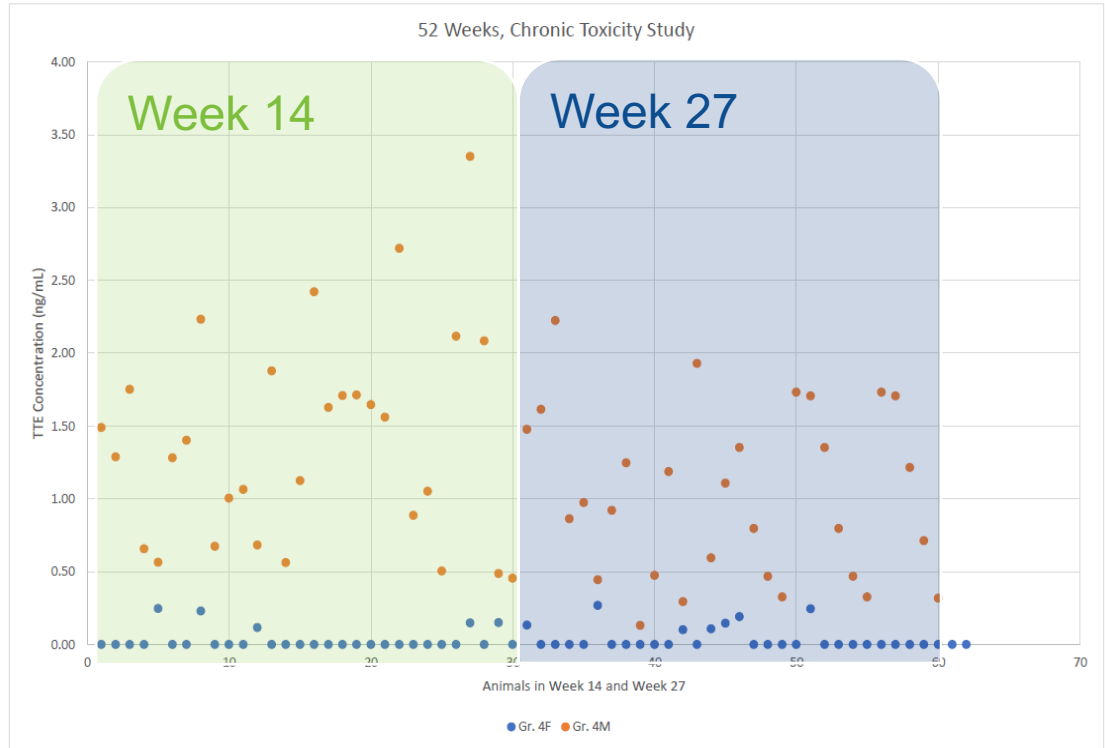
**50ul of Serum** were sampled in the **Main Groups** and not from a satellite ones.



Results of the Control Group  
Does not show any alteration  
of the physiological level of  
TTE.

The study is on-going and  
samples of 52 Weeks are not  
still sampled.

## Group 4 Samples



- Pilot Validation Study shown very good achievements in sensitivity using: Micro HPLC and M5 – ACQUITY BEH 1.7um 1x50mm – API4000.
- **Physiological Levels of TTE in Serum** have been properly described.
- **2 min chromatographic run** is a key to **reduce the analysis time impact** on the lab workload.
- Data management under **GxP compliance** from the Validation to the Sample analysis was realized with **LabWare LIMS**.

- TTE, T3 and T4 monitoring with LC-MS will be used in **all the Chronic Toxicity Studies in ERBC** substituting RIA Analytical Method.
- **Hormone panel will be extended** with potential ones that could be target of Test Item acting as endocrine disruptor.
- **Sensitivity will be increased** using more sensible **machine therefore the Volume of the Serum could be decreased**.
- Volume decrease is fundamental to reduce the animal stress. (3R-Reduction)
- **Volume Reduction** will be fundamental to sample blood from the litter in the reproductive Studies using **capillary sampling**.
- **LabWare LIMS** will always be there to help this process.

**ERBC:**

**Francesca R. Calfapietra**

**Davide Massari**

**Chiara Olmi**

**Marianna Gentili**

**Flavia Bonoli**

**Rosaria Cicalese**

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**LabWare:**

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**Aaike Oosterkamp**

“Differences should be much more appreciated and could only help us in doing Better”

