

HESI DART – ETS Thyroid Working Group Survey Data

Results & Path Forward

Pragati S. Coder, PhD, DABT



Background

- **April 2017** – The HESI DART Committee approved a new project to evaluate thyroid hormone assessments in laboratory animal species.
- **October 2017** – The HESI DART Committee approved combining project efforts with the existing ETS Thyroid Taskforce.
- **June 2018** – Targeted Thyroid Hormone Assessments Survey released (to members of HESI DART Committee, SOT/RDTSS, TS & ETS) with the goal of collecting data from laboratories that conducted regulated safety assessment studies.*
- Responses received from 12 laboratories from across US & EU.



Contributing Laboratories

- **BASF**
- **Bayer AG – Pharma**
- **Bayer SAS – Crop Sciences**
- **Charles River Ashland**
- **Charles River Den Bosch**
- **Charles River Horsham**
- **Charles River – Lyon**
- **Covance Laboratories**
- **Dow Agro Sciences**
- **DuPont Haskell**
- **Sequani Ltd.**
- **US EPA**

Data included in this presentation (and in the database) are blinded to contributor laboratory.



Survey Organization

- **Part 1: General Survey** – 25 Questions with sub-parts, for details.
- Inspired by the BfR Survey (2016)¹ and SOT Thyroid Round Table session (2017)²
- General Questions pertained to:
 - Frequency of Assessments
 - Assessment Types (Total & Free T3 & T4, & TSH)
 - Analytical Methods & Validation (Sample & Method type, LoD, LLoQ etc.)
 - Animal Species, Strain, Age/Life stage, Physiological Status, Sex etc.
 - Laboratory SOPs & Precautions (Time and route of blood collection, anesthesia, stress reduction?)
 - Information Sharing (i.e. willingness to share SOPs/Protocols, HC Data)

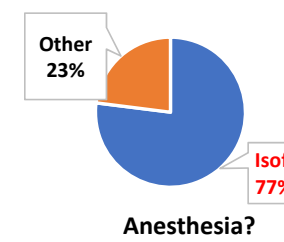
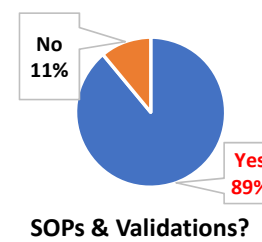
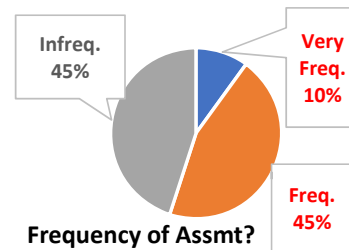
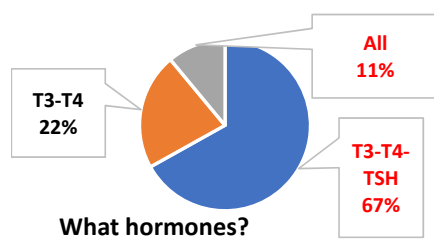


Survey Organization

- **Part 2: Data Submission Spreadsheets** (Total & Free T3 & T4, & TSH).
- Serve as the start of a Global HCD Repository.
 - Reg. Auth. have different requirements on TH assessments – but, analytical methodologies and quality criteria are not defined or recommended.
 - Samples are collected across at multiple life stages in a variety of studies.
 - Unlike humans, reference concentration ranges do not exist for lab animals.
 - Each lab has its own methods/HCD making comparisons across the industry difficult - especially for regulators who have to make decisions regarding these compounds.
 - Availability of a global HCD repository could aid in...
 - *Interpretation of equivocal datasets and allowing inter-laboratory comparisons**
 - *Understanding population ranges and variability (by assay and strain)*
 - *Inform decisions regarding methodologies appropriate for various life stages*



General Questions (9 of 12 labs responded)



- **Species** – 100% Rat; 10% mouse; 45% Dog; 22% NHP
- **Circadian rhythm** – 100% with AM sampling. Occasional datasets w PM.
- **Blood Sampling** – 66% single bleeds; 88% terminal bleeds
- **Blood Sampling** – 55% <30 animals at each interval, 45% <50/interval.
- **Blood Sampling** - 66% 30-60 sec/animal, 34% <30 sec/animal.



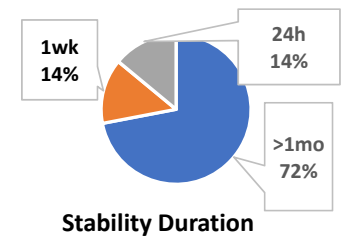
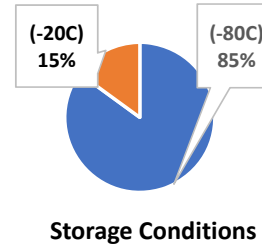
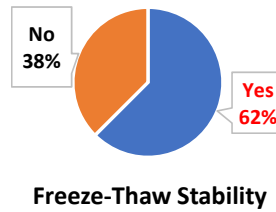
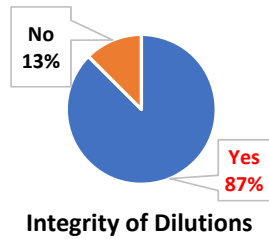
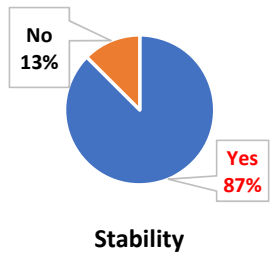
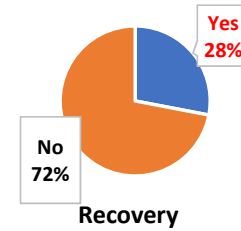
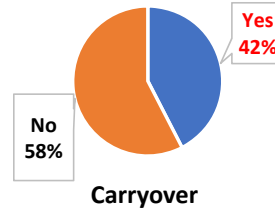
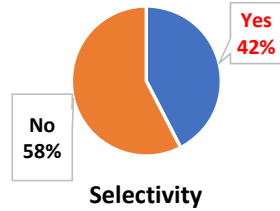
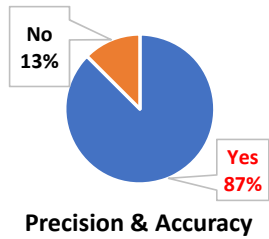
Assay Validation – Quality Criteria/Parameters

Generally accepted, and published,^{1,2} criteria are considered independent of the assay methodology. Assays should be expected to meet pre-defined performance criteria (CV OECD 407/408- T3/T4 <25% and TSH <35%; EPA OPPTS Male Pubertal - T4 <27.5% and TSH <58%, Female pubertal – T4 <29%)

- Reproducibility and Sensitivity (LLOQ)
- Precision (%CV) & Accuracy (%RE) (inter- and intra-run)
- Selectivity and Cross-reactivity (for immunoassays)
- Measurement Range and Linearity.
- Matrix Effects, or lack thereof.
- Stability, including Freeze-Thaw Stability
- Quality Controls & Calibration Standards



Assay Validation Responses (100% Serum)



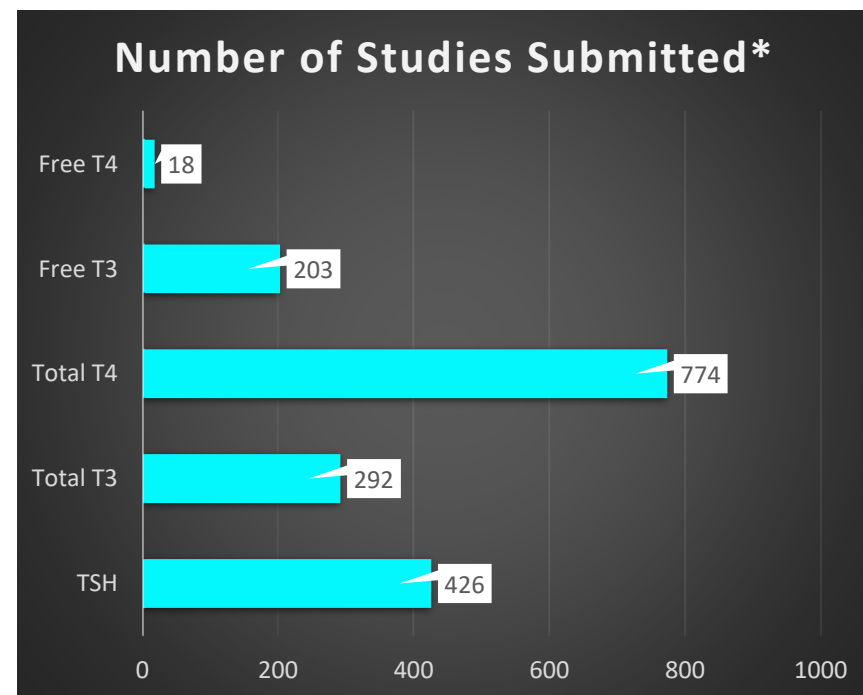
Stress & Thyroid Hormone Assessments

- Increases in TH in response to stress have been previously discussed and documented. Laboratories were asked to provide their stress reduction criteria and responses included ..
 - Animal handling/acclimation
 - Single housing of Gestating/Lactating animals
 - Group housing 5/grp vs 2-3/grp
 - Separation of aggressive animals
 - Environmental Conditions (temp, humidity, music?)
 - Minimal restraint for in-life sampling
 - Anesthesia – quick onset, if used
 - Use of holding room, or ante-room (55%)
 - Movement of animals pre-room to necr.
 - Timing of removal, restraint, sampling
 - Dam and pup maintained together for as long as feasible



Data Submission Section - Summary Statistics

- 12 of 12 Labs submitted data.
- ~1750 datasets received.
- Total T4 most robust
- TSH and T3 less robust
- Free T3 & T4 (insufficient)
- Required unit standardization
 - T3 & T4 (nmol/L)
 - TSH ($\mu\text{g/L}$)



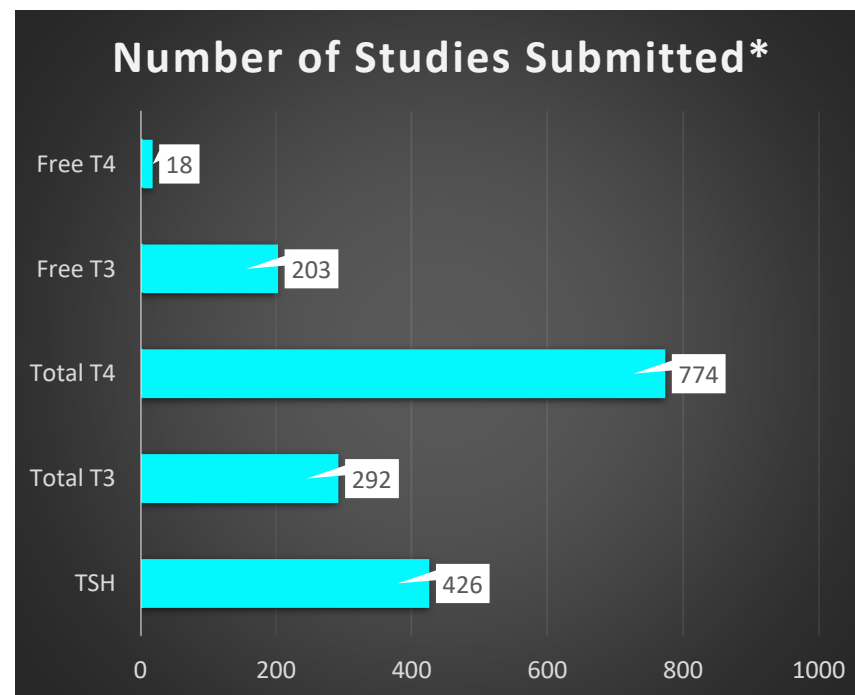
* Included data from General Toxicity Studies



Data Submission Section - Summary Statistics

Key Parameters Available:

- Species & Sex
- Age of Collection
- Method & Route of collection
- Analytical method
- Time of collection
- Anesthesia Status
- Fasting Status



* Included data from General Toxicity Studies

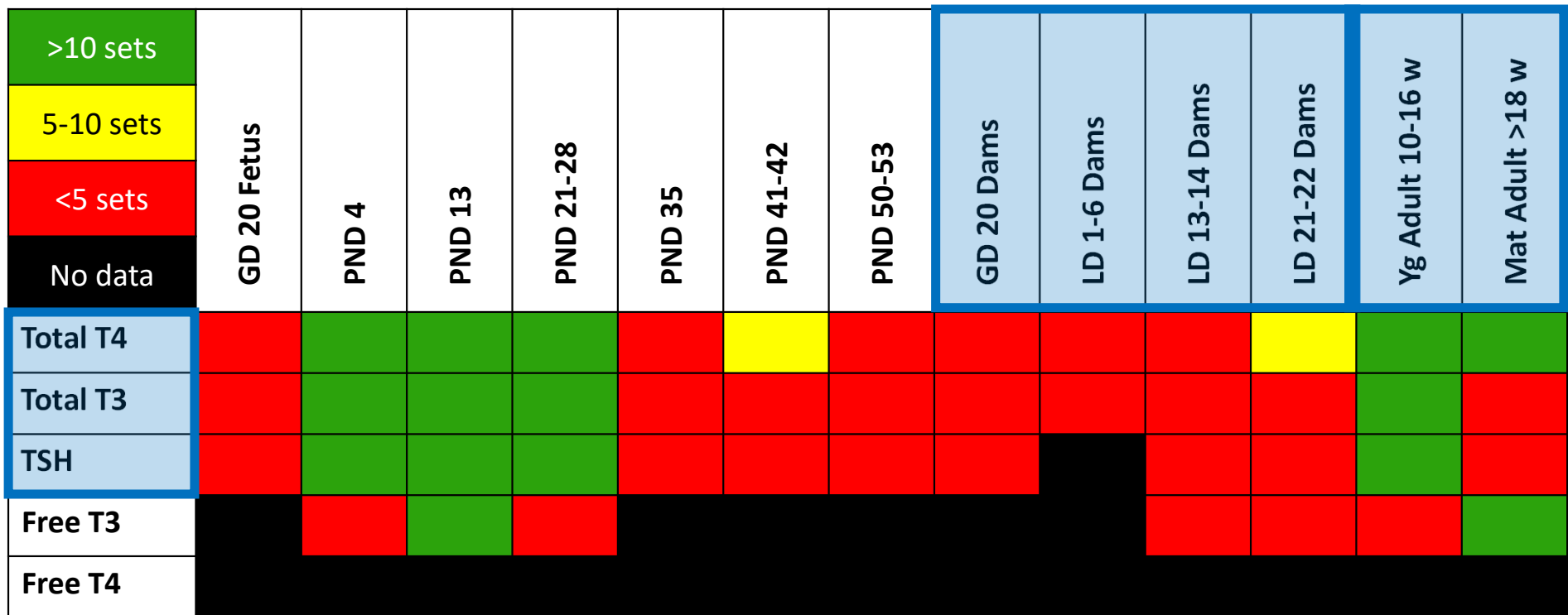


Selected Parameters - Analysis Constraints

- Species– **Rat**, Dog (Beagle) and NHP (Cyno).
- Rat Strains– **Sprague Dawley, Han Wistar** and Long Evans
- Study Types– OECD 407, 408, **421/422, 443, EPA CTA, Pubertal Assays**, Mechanistic/Investigative Studies etc.
- Age Categories– GD 20 Fetuses, **PND 4, PND 13, PND 21-28 (weanlings)**, PND 41-42 (pubertal), PND 50-53 (pubertal), **Adults** (10-16w and >18w), and **Maternal females** at GD 20, LD 1-6, LD -14 and LD 21-22.
- Data Categories- **Sex, Analytical Method**, Route of Collection, Fasting and Anesthesia status.



Heat Map of Submitted Data



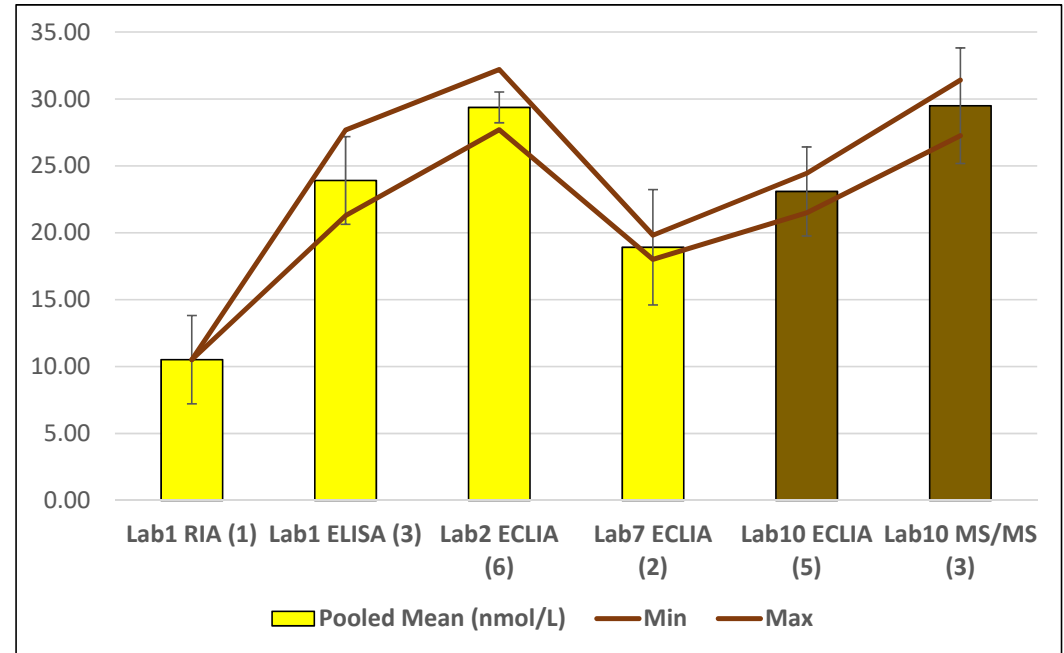
Data included in this presentation (and in the database) is blinded to contributor laboratory.



Total T4 – Neonate (PND 4) Pups

➤ 20 studies. 4 Labs. 4 Methods. 2 Rat strains. Samples pooled, regardless of sex.

Method	LoD	LLOQ	Mean %CV
	(nmol/L)		
RIA (Lab 1, n=1) 2008	-	10.6	31.4
ELISA (Lab 1, n=3)	5.4	6.8	13.53
ECLIA (Lab 2, n=6)	NL	26.0	NL
ECLIA (Lab 7, n=2)	3.7	12.9	NL
ECLIA (Lab 10, n=5)	5.4	7.0	14.4
HPLC/MS (Lab 10, n=3)	0.01	0.16	14.4



NL = Not Listed Acceptability criteria OECD 407/408- T3/T4 CV <25%



 SD Rat

 Han Wistar Rat



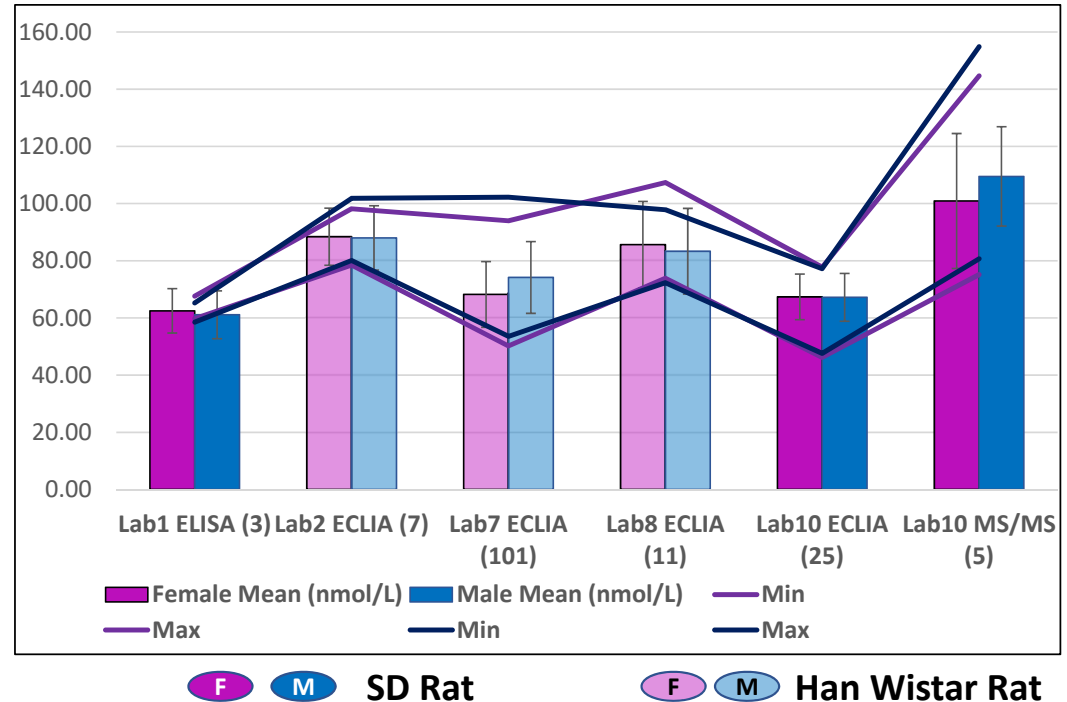
Total T4 – Pre-weanlings (PND 13) Pups

➤ 152 studies, 5 Labs. 3 Methods; 2 Rat strains; Samples separated by sex.

Method	LoD	LLOQ	Mean %CV
	(nmol/L)		
ELISA (Lab 1, n=3)	5.4	6.8	12.5-13.7
ECLIA (Lab 2, n=7)	NL	26.0	NL
ECLIA (Lab 7, n=100)	3.7	12.9	NL
ECLIA (Lab 8, n=11)	3.7	12.9	17.6-18.0
ECLIA (Lab 10, n=25)	5.4	7.0	11.9-12.6
HPLC/MS (Lab 10, n=5)	0.01	0.16	15.9-22.1

NL = Not Listed

Acceptability criteria OECD 407/408- T3/T4 CV <25%



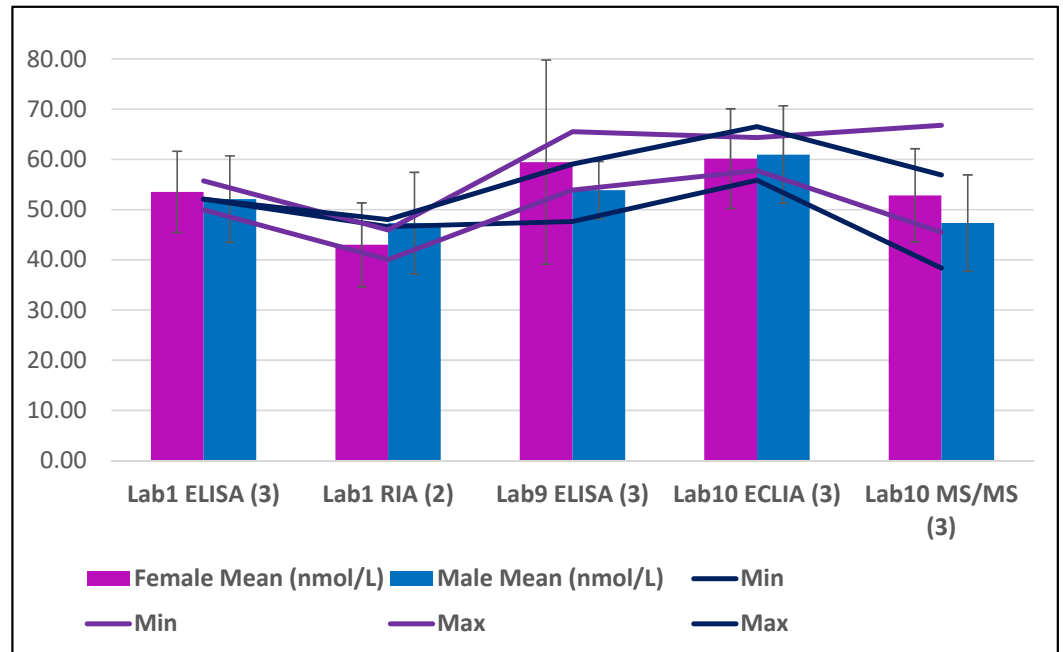
Total T4 – Weaning (PND 21-28) Pups

➤ 14 studies. 3 Labs. 4 Methods. 1 Rat strain. Samples separated by sex.

Method	LoD	LLOQ	Mean %CV
	(nmol/L)		
RIA (Lab 1, n=2)	NL	10.6	19.3-21.4
ELISA (Lab 1, n=3)	5.4	6.8	15-16.5
ELISA (Lab 9, n=3)	12.9	12.9	10.6-34.6
ECLIA (Lab 10, n=3)	5.4	7.0	16-16.6
HPLC/MS (Lab 10, n=3)	0.01	0.16	19.9-20.4

NL = Not Listed

Acceptability criteria OECD 407/408- T3/T4 CV <25%



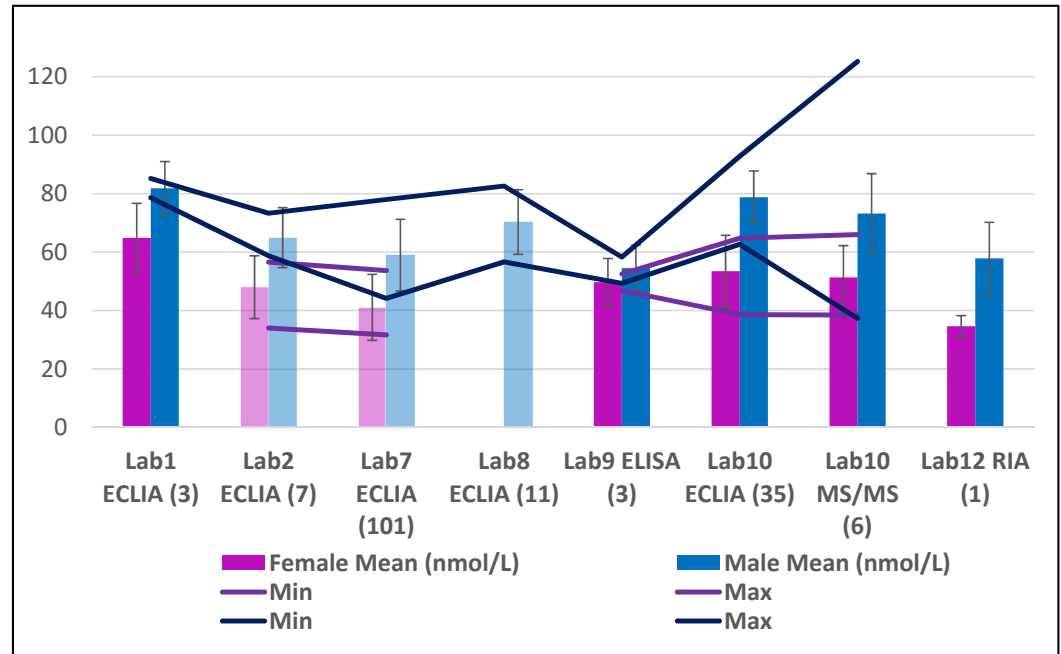
F M SD Rat



Total T4 – Adult Animals (Males & Females)

➤ 167 studies, 7 Labs. 4 Methods. 2 Rat strains. Samples separated by sex.

Method	LoD	LLOQ	Mean %CV
	(nmol/L)		
ECLIA (Lab 1, n=3)	5.4	6.8	11.1-18.2
ECLIA (Lab 2, n=7)	NL	26	NL
ECLIA (Lab 7, n=101)	3.7	12.9	NL
ECLIA (Lab 8, n=11)	3.7	12.9	16.0
ELISA (Lab 9, n=3)	12.9	12.9	14.3-16.2
ECLIA (Lab 10, n=35)	5.4	7.0	11.4-23.6
UHPLC/MS (Lab 10, n=6)	0.01	0.16	20.2-21.9
RIA (Lab 12, n=1)	NL	12.9	10.4-12.3



NL = Not Listed Acceptability criteria OECD 407/408- T3/T4 CV <25%

F M SD Rat

F M Han Wistar Rat

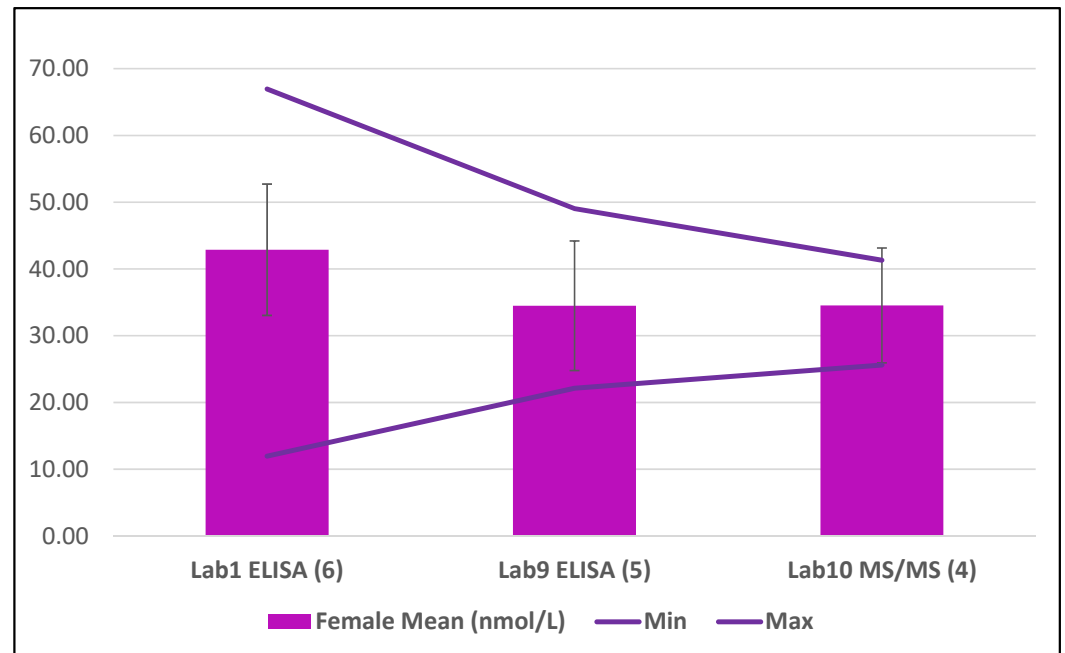


Total T4 – Gestating/Lactating Dams

➤ 15 studies. 3 Labs; 2 AC methods; 1 rat strain

Method	LoD	LLOQ	%CV
	(nmol/L)		
ELISA (Lab 1, n=6)	5.4	6.8	20.8
ELISA (Lab 9, n=5)	12.9	12.9	31.1
UHPLC/MS (Lab 10, n=4)	0.01	0.16	24.8

Acceptability criteria OECD 407/408- T3/T4 CV <25%



Total T4 – Preliminary Reference Ranges

Strain	Age	#Datasets	Mean Value (nmol/L)	Min Value (nmol/L)	Max Value (nmol/L)
Han Wistar	PND 4	8	24.14	18.02	32.20
	PND 13 Males	118	81.82	53.54	102.19
	PND 13 Females	119	80.75	50.19	107.34
	Adult Males	119	64.72	44.14	82.63
	Adult Females	17	44.53	31.66	56.60
Sprague Dawley	PND 4	12	21.75	10.52	31.40
	PND 13 Males	33	79.27	47.62	154.88
	PND 13 Females	33	76.91	46.20	144.66
	PND 21-28 Males	12	52.32	38.34	66.54
	PND 21-28 Females	14	53.79	40.04	66.80
	Adult Males	48	56.98	37.34	125.23
	Adult Females	15	50.80	34.63	66.00



Total T4 – Preliminary Reference Ranges

Strain	Age	#Datasets	Mean Value (nmol/L)	Min Value (nmol/L)	Max Value (nmol/L)
Han Wistar	GD 20 Fetuses	1	4.29	4.29	4.29
Sprague Dawley	GD 20 Fetuses	2	9.22	6.82	11.97
	Gest/Lact Dams	15	37.30	11.97	66.96
	GD 20 Dams	4	28.28	22.14	34.41
	LD 21-22 Dams	5	48.87	31.27	66.96

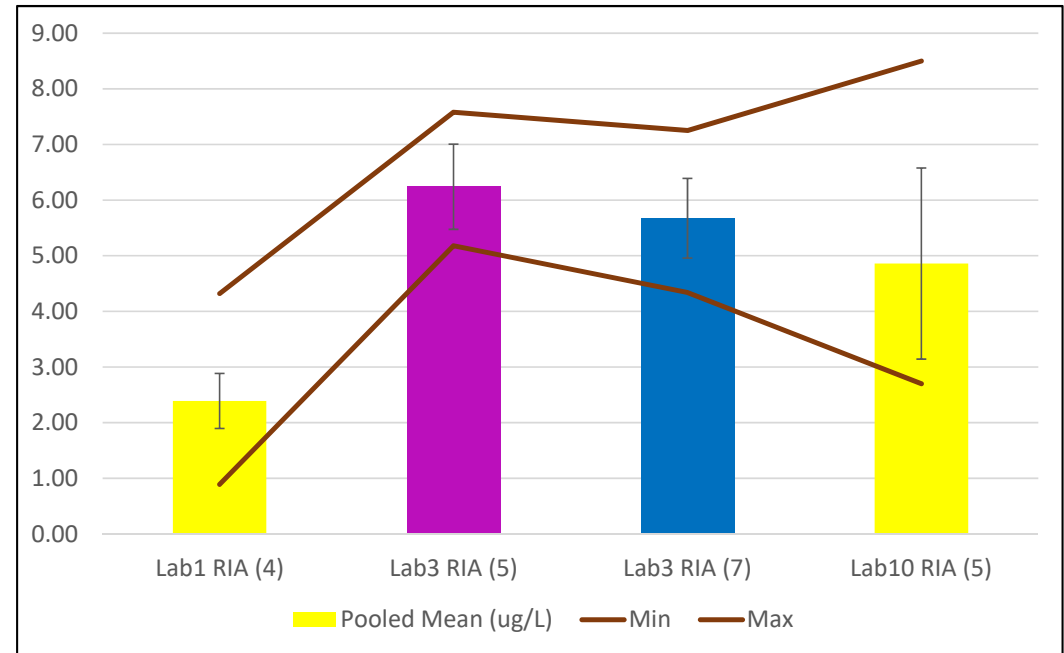


TSH – Neonate (PND 4) Pups

- 16 studies; 3 labs; 1 method; 2 rat strains

Method	LoD	LLOQ	%CV
	(µg/L)		
RIA (Lab 1, n=4)	1	2	23.82
RIA (Lab 3, n=5, Fem)	0.5	1	12.45
RIA Lab 3, n=7, Mal)	0.5	1	12.14
RIA Lab 10, n=5	1.4	2	38.64

Acceptability criteria OECD 407/408- TSH CV <35%



Pooled **SD Rat**
F
M **Han Wistar Rat**



Serum TSH – Preliminary Reference Ranges

Strain	Age	#Datasets	Mean Value (µg/L)	Min Value (µg/L)	Max Value (µg/L)
Sprague Dawley	PND 4	9	3.63	0.89	8.50
	PND 13 Males	4	4.65	3.85	5.46
	PND 13 Females	4	5.20	4.41	5.77
	PND 21-28 Males	10	3.60	0.95	8.6
	PND 21-28 Females	10	3.37	0.87	7.4
	Adult Males	14	7.3	4.00	15.60
	Adult Females	13	4.4	3.00	8.70
	GD 20 Fetuses	3	5.18	3.82	6.53
	Gest/Lact Dams	10	9.59	4.66	13.10
	GD 20 Dams	3	9.08	7.28	10.36
	LD 21-22 Dams	3	7.79	4.66	9.50



Serum TSH – Preliminary Reference Ranges

Strain	Age	#Datasets	Mean Value (µg/L)	Min Value (µg/L)	Max Value (µg/L)
Han Wistar	PND 4 Males	5	5.67	4.34	7.25
	PND 4 Females	7	6.24	5.18	7.58
	PND 13 Males	2	4.55	4.49	4.6
	PND 13 Females	2	5.02	4.99	5.04
	PND 21-28 Males	5	4.67	3.9	5.23
	PND 21-28 Females	5	4.39	4.26	4.54

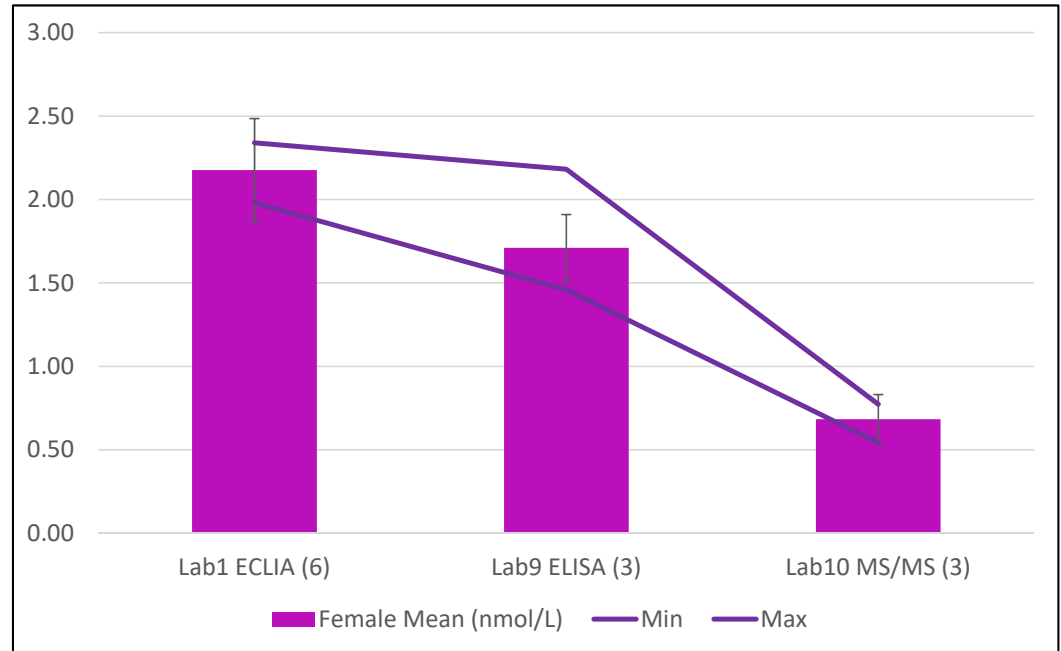


T3 – Gestating/Lactating Dams

- 12 studies; 3 labs; 3 methods; 1 rat strain

Method	LoD	LLOQ	%CV
	(nmol/L)		
ECLIA (Lab 1, n=6)	0.30	0.40	14.34
ELISA (Lab 9, n=3)	0.54	0.54	11.20
MS/MS (Lab 10, n=3)	0.0031	0.0077	20.71

Acceptability criteria OECD 407/408- T3/T4 CV <25%



F SD Rat



Total T3 – Preliminary Reference Ranges

Strain	Age	#Datasets	Mean Value (nmol/L)	Min Value (nmol/L)	Max Value (nmol/L)
Sprague Dawley	PND 4	6	5.74	0.32	30.62
	PND 13 Males	5	1.30	0.88	1.70
	PND 13 Females	5	1.26	0.83	1.72
	PND 21-28 Males	7	2.47	1.29	3.67
	PND 21-28 Females	7	2.47	1.22	3.36
	Adult Males	8	9.1	0.65	68.67
	Adult Females	6	9.7	0.77	53.64
	GD 20 Fetuses	3	1.00	0.02	2.02
	Gest/Lact Dams	12	1.52	0.54	2.34
	GD 20 Dams	4	1.38	0.77	2.34
	LD 21-22 Dams	4	1.71	0.73	2.26



What can we conclude from these data?

- The database is still weak, except for T4 and only at specific life stages.
 - **Regular data submission is necessary to strengthen database.**
- Every laboratory currently uses different units for presenting data. Reporting unit harmonization across the industry is important.
 - **Ease of incorporation of data into global repository**
 - **To allow use of reference ranges**
 - **To allow for inter-laboratory comparisons.**
- 4 methodologies are being used predominantly (RIA, ECLIA, ELISA and HPLC/MS)
 - **Establishment of universal quality criteria is necessary to support data irrespective of methodology used.**



What can we learn from these data?

- Which assay methodology is the best? What method should we validate in the lab? Which assay works best for X, Y or Z?

All or None! The answers depend on the question being asked..

- (a) Age of assessment (e.g. onset of TH production)
- (b) Sample Volume (and limitations thereof)
- (c) Sensitivity of the Assay (LLOQ)
- (d) Precision (%CV – closeness of repeated individual measures)
- (e) Accuracy (closeness of determined values to nominal (QC))
- (f) Reproducibility & Reliability (ability of the assay to repeatedly give the same result)



The Path Forward ..

- **Teratology Symposium**
 - Teratology Society Annual Meeting (San Diego, CA, June 2019)
- **Working Group Update**
 - European Teratology Society Meeting (Helsinki, Finland, Sep 2019)
- **Workshop Report**
 - A summary of the presentations and discussions held at this meeting, including conclusions and recommendations from the breakout groups to be published (2019-2020)
- **Global HCD Repository**
 - To be maintained as a living database (public location yet to be determined), preferably with annual updates; available for public use.



Questions?

