Quantitative Analysis of Dried Blood Spots by DART/MS/MS without Sample Preparation

Justin Gordon[1]; Elizabeth Crawford[2]; Jing-Tao Wu[1]; Brian Musselman[2]; Ming-xiang Liao[1]; Bei-Ching Chuang[1]; Cindy Xia[1]; David Ho[3]; Lily Li[3]; Shaoxia Yu[1]

[1] Millennium Pharmaceuticals, Inc. 35 Landsdowne St., Cambridge, MA 02139
[2] IonSense, Inc., 999 Broadway, Suite 404, Saugus, MA 01906
[3] Tandem Labs, 35R Cabot Road, Woburn, MA 01801

27 May, 2010

Takeda MILLENNIUM
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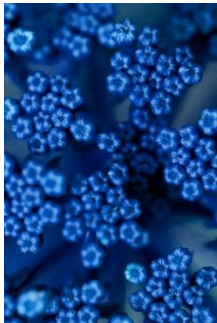
Overview

- Purpose
- Review DART Technology
- Millennium Research
  - DBS for *in-vivo* PK studies
  - *In-vitro* plasma stability studies
- Conclusion and Future Considerations
- Questions

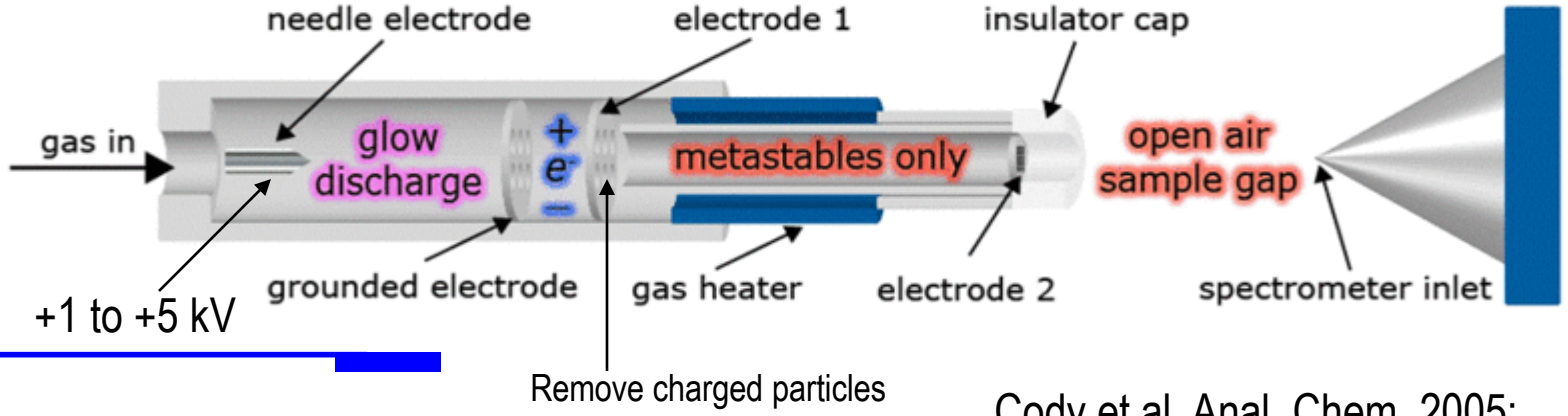
Purpose

- Apply DART/MS/MS technology directly to dried blood spots (DBS) to reduce sample prep
- Apply DART/MS/MS to other samples for faster turnaround time
- Utilize low input sample volume (5 μL) to allow **multiple** PK time points from a **single mouse**
  - Reduce animal-animal variability
  - Reduce in-life study costs, animal death



Review: DART Technology

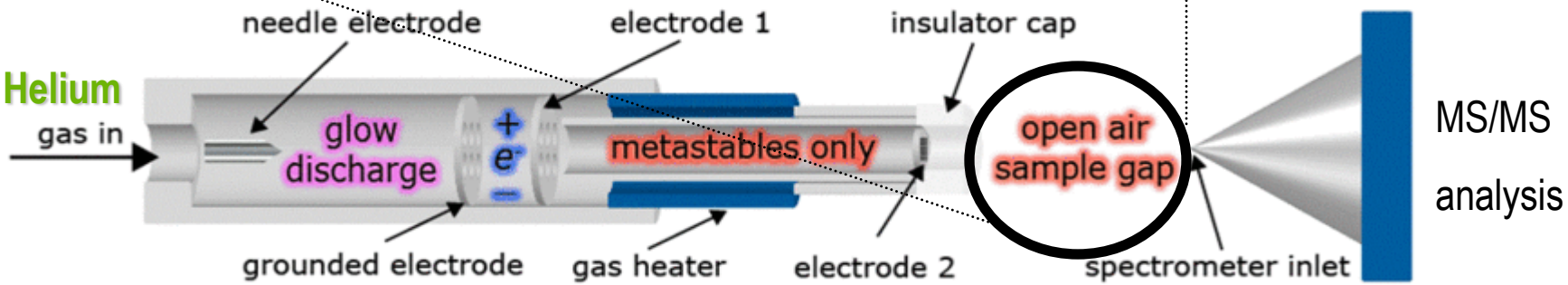
- *Direct* Analysis in Real Time
  - Direct sample ionization at ambient pressure
  - Ionization process (Penning ionization): interaction/ energy transfer between
    - sample ('**S**'- analytical molecule)
    - and excited gas molecule ('**M***' - metastable species)
    - $M^* + S \rightarrow S^{+\bullet} + M + e^-$



Cody et al. Anal. Chem. 2005;
Image: JOEL USA, Inc.

Review: DART Technology

- *Direct* Analysis in Real Time
  - $M^* + S \rightarrow S^{+\bullet} + M + e^-$
  - Where *M* is ~~*excited*~~ Helium ('positive' mode):
    - $\mathbf{He}(2^3s) + H_2O \rightarrow H_2O^{+\bullet} + \mathbf{He}(1^1s) + e^-$
    - $H_2O^{+\bullet} + H_2O \rightarrow H_3O^+ + OH^\bullet$
    - $H_3O^+ + nH_2O \rightarrow [(H_2O)_nH]^+$
    - $[(H_2O)_nH]^+ + \mathbf{S} \rightarrow \mathbf{S}H^+ + {}_nH_2O$



Cody et al. Anal. Chem. 2005;
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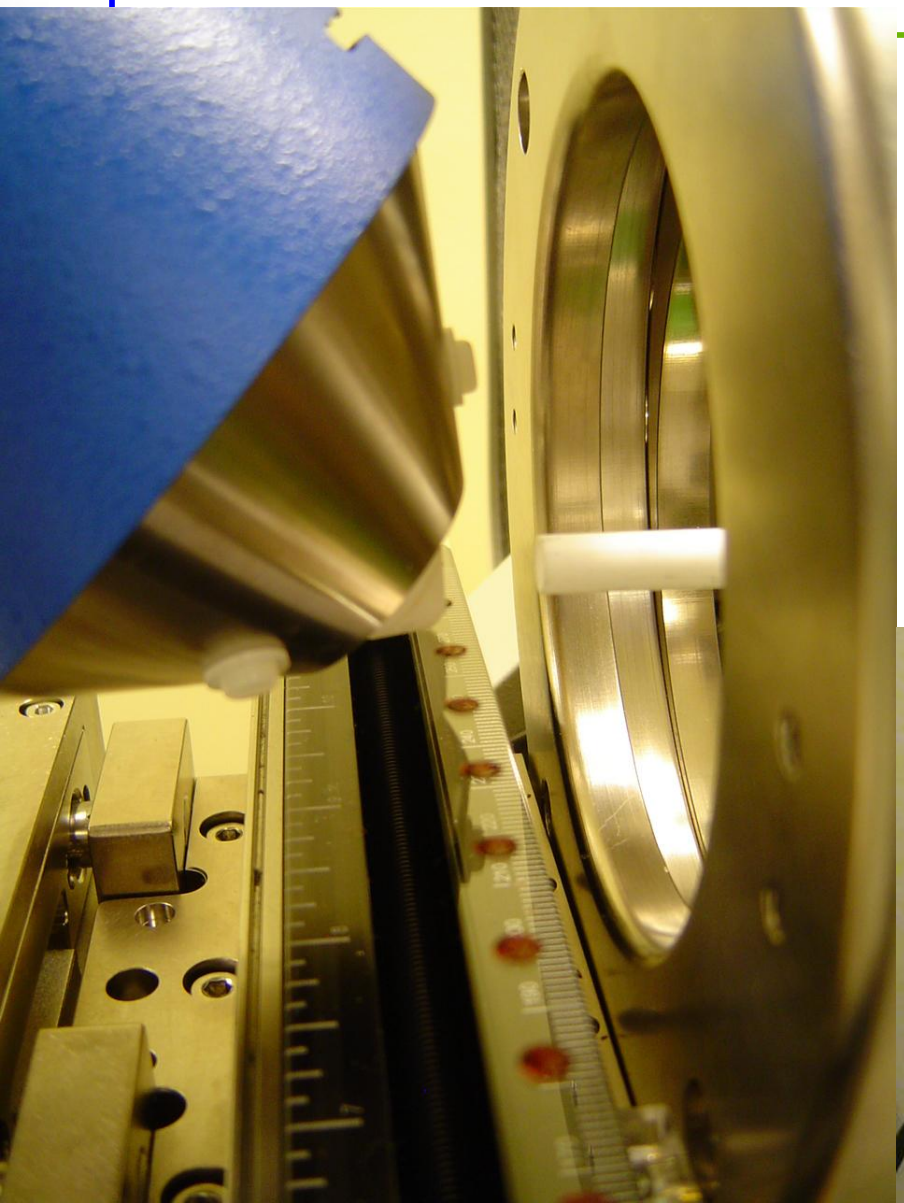
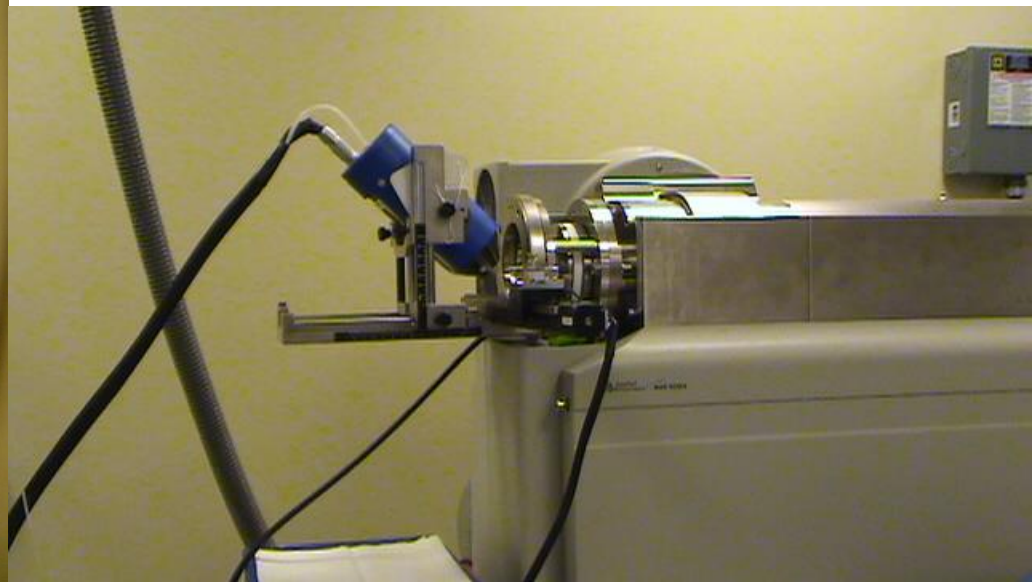
DART Technology



- DART ion source commercially produced by IonSense, Inc.
- Latest generation
  - SVP (standard voltage and pressure)
  - Attached to API 4000 front end



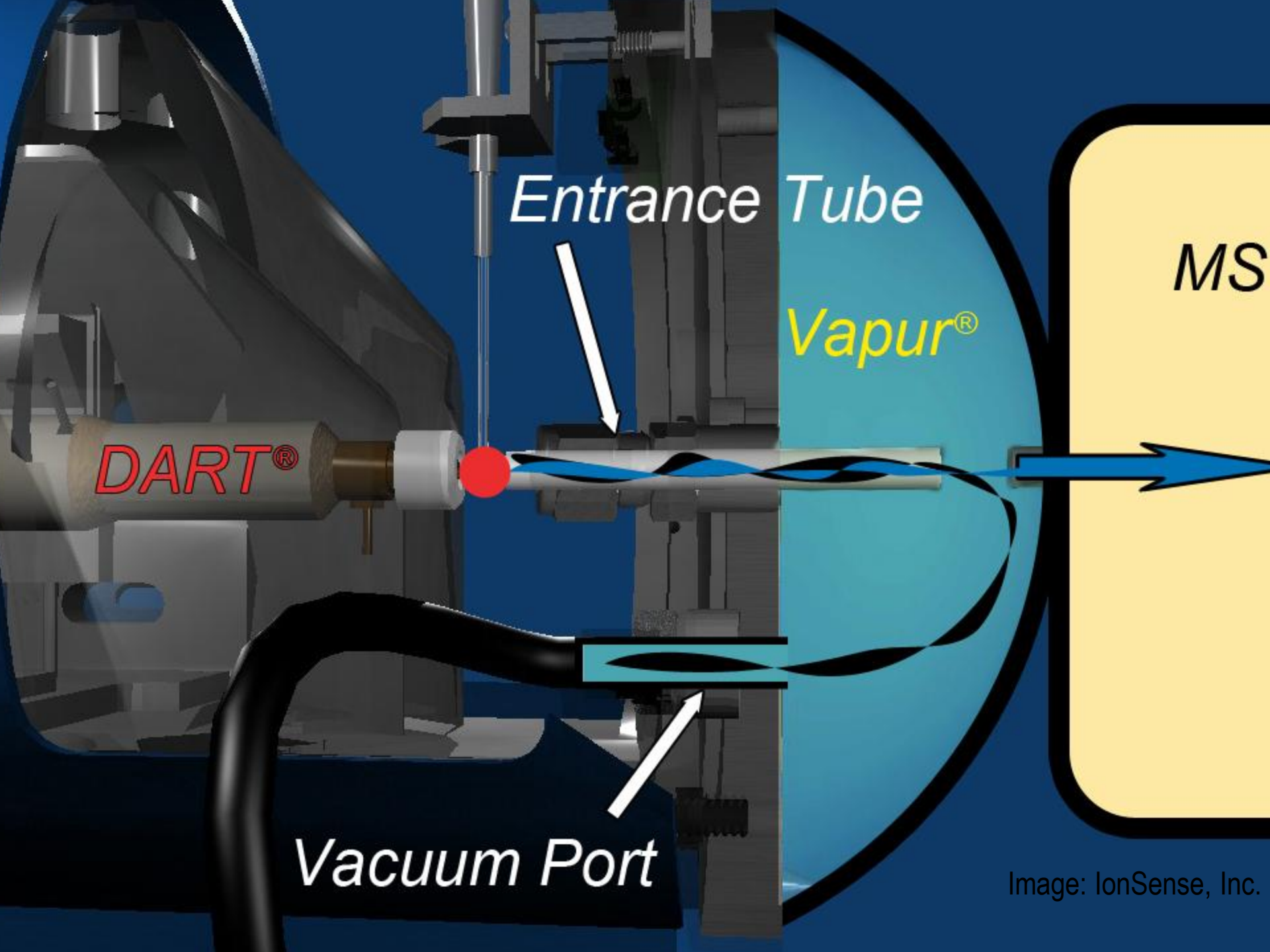
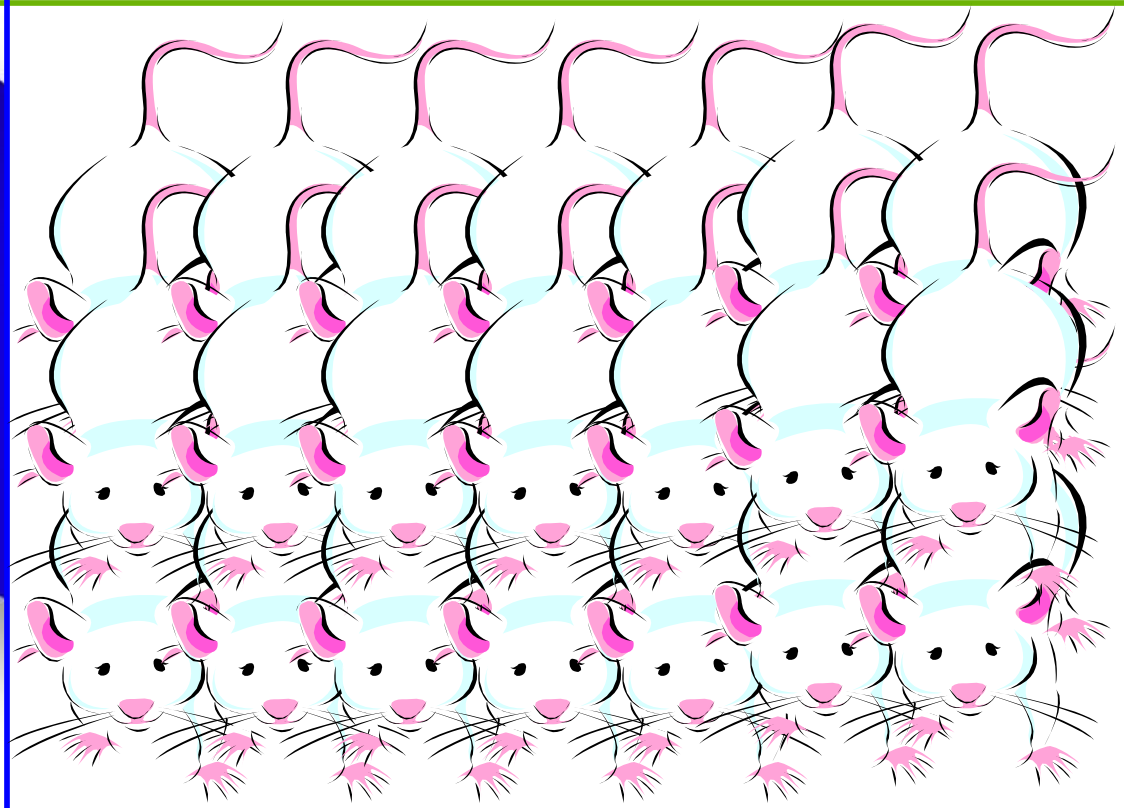


Image: IonSense, Inc.

In-vivo Mouse PK studies

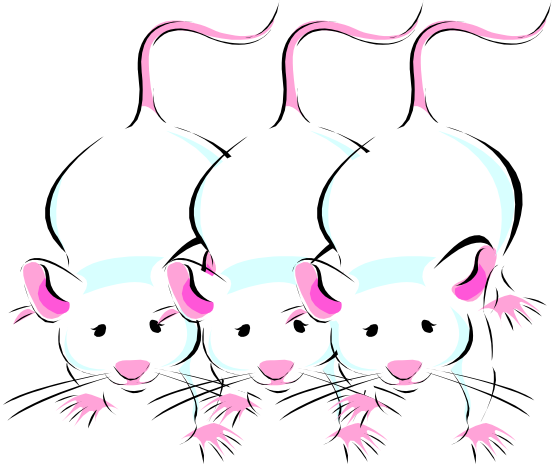
- Dried blood spots (DBS)
  - 3 *in-vivo* studies: Verapamil and Millennium Compounds A and B (MLN-A, MLN-B)
    - Seven time point, terminal PK protocol
      - Each animal sacrificed at time point (n=3, 21 mice total)
    - Seven time point, single mouse, serial bleed protocol
      - Take 25uL six time points plus terminal (n=3, 3 mice total)

Mice Required for In-vivo PK Study



LC/MS/MS Method

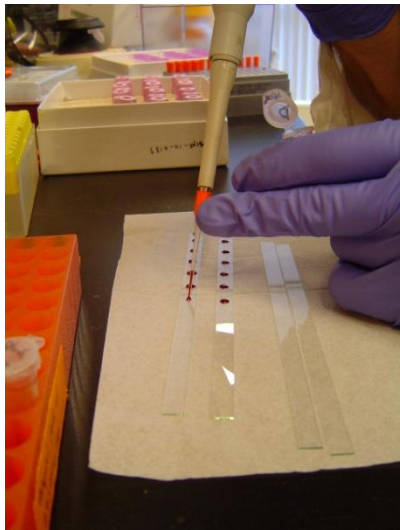
DART/MS/MS Method



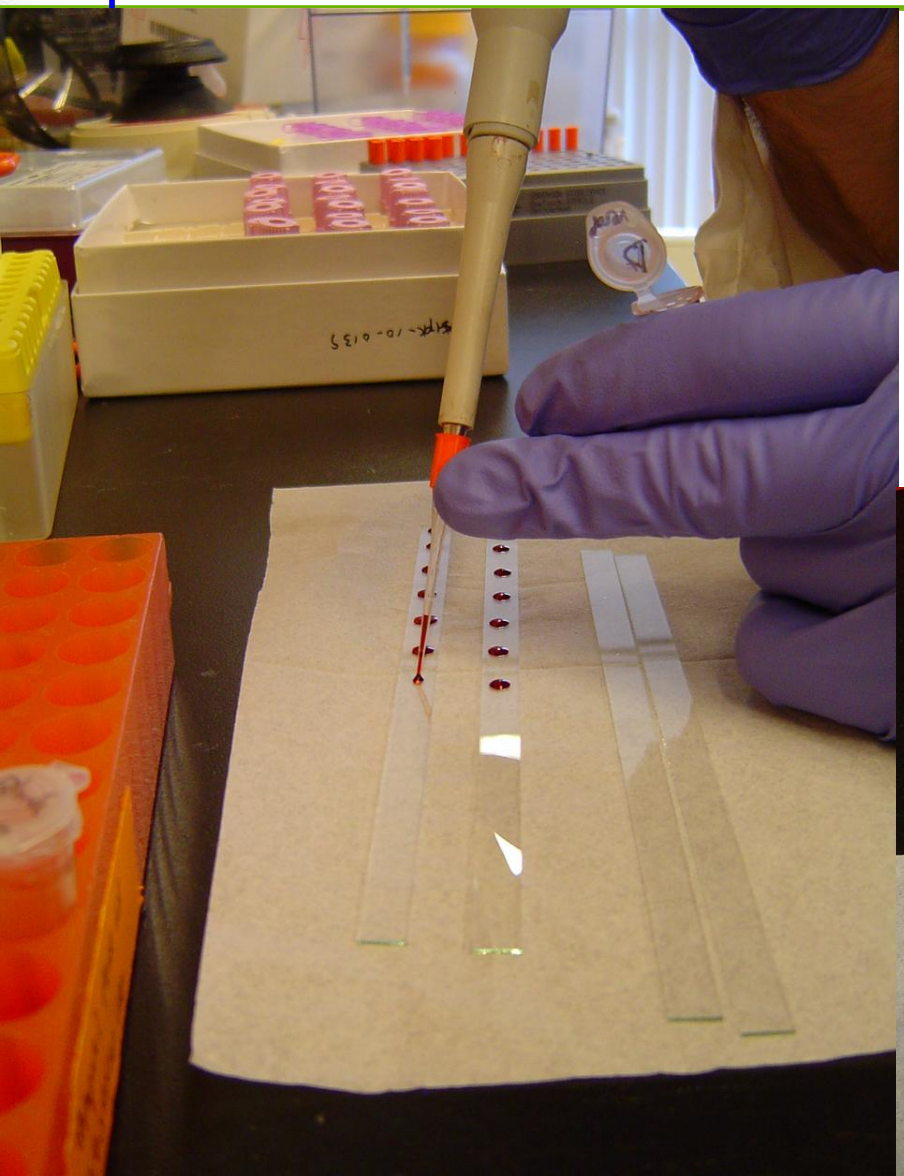
LC/MS and DART Process Comparison

- LCMS Sample Prep:
  - Sample extraction
    - various methods
  - Overnight run

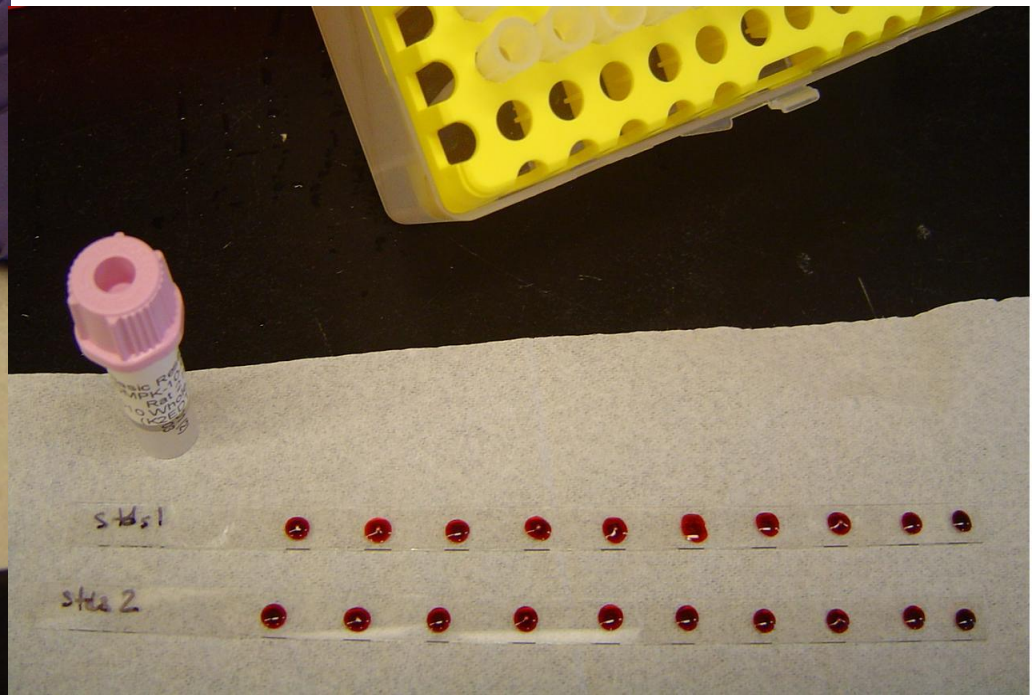
- DART Sample Prep:
  - Sample spotting
  - Run samples on DART
    - Glass slides: (0.2mm/s, 120mm of sample each slide = ~10min/slide; less than 1min/sample)
  - “Real-time”



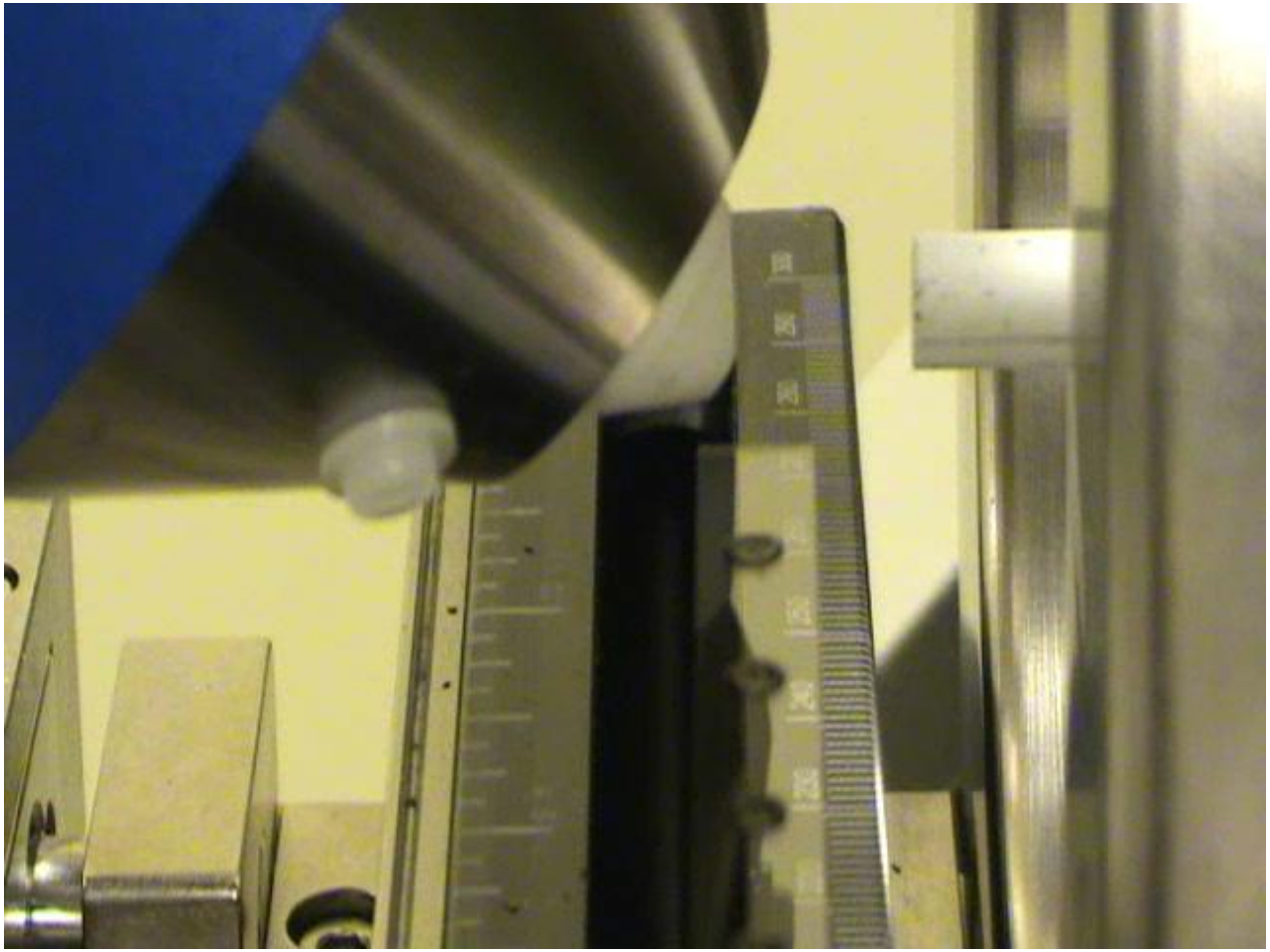
DBS Sampling: Bench



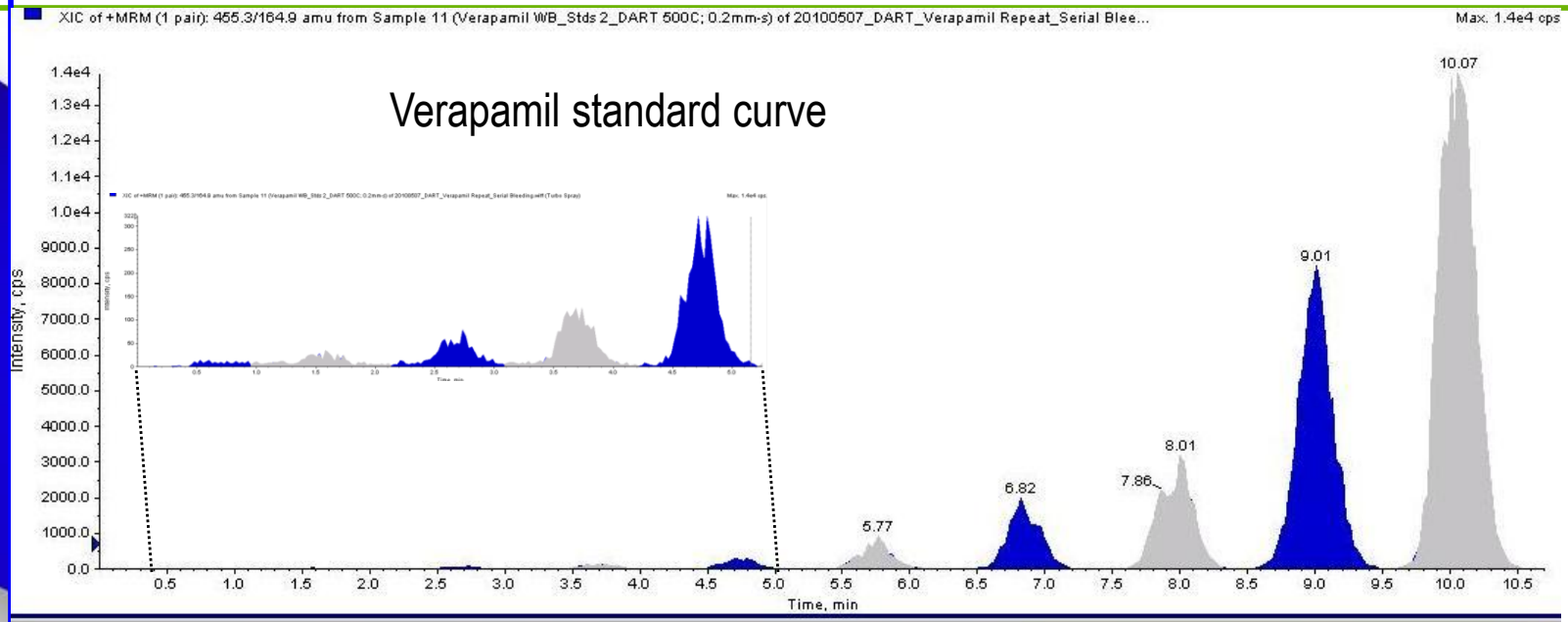
- paper substrate desorption not optimal, moved on to glass slides for evaluations



DBS Sampling: DART



DART Data Acquisition and Processing

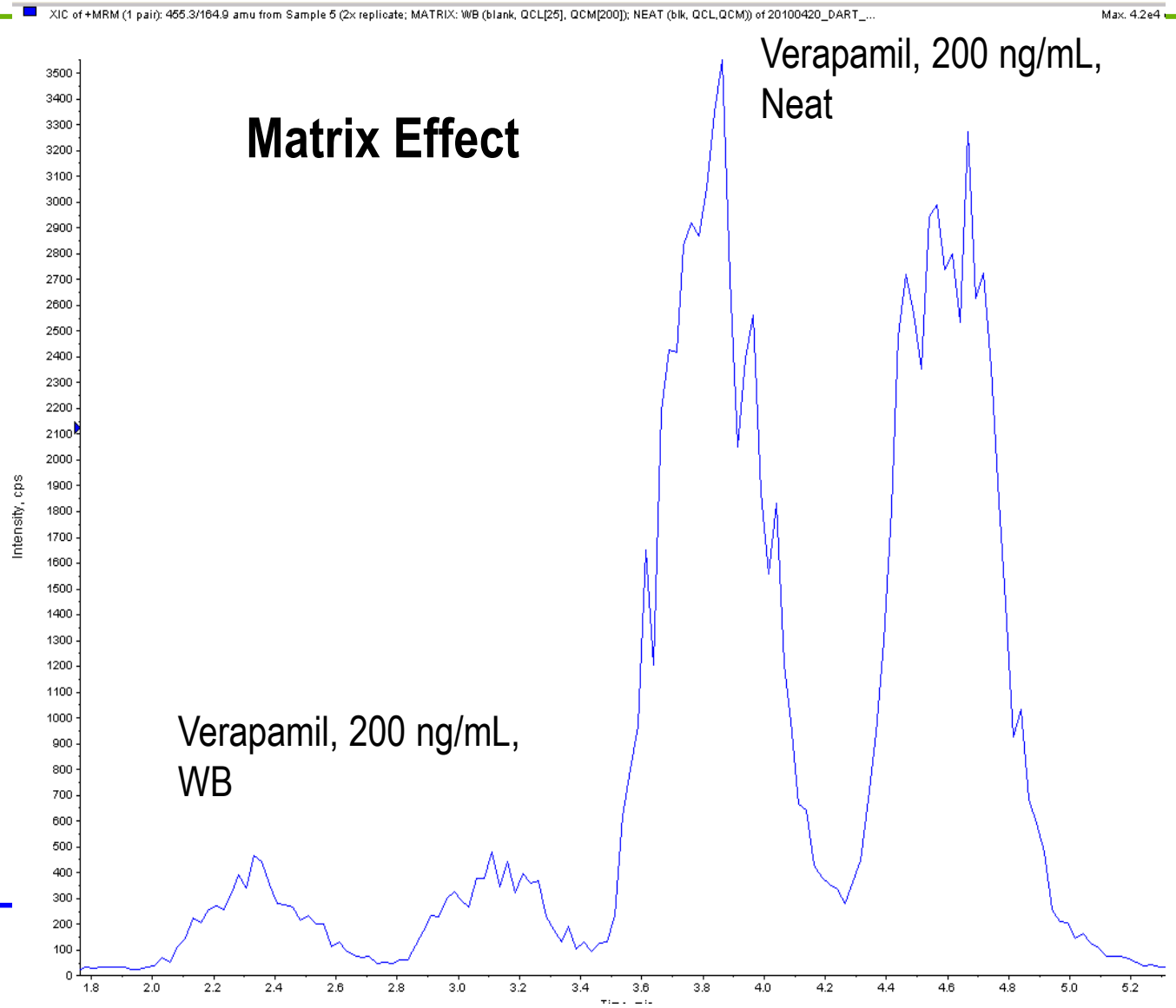


XIC of +MRM (1 pair): 455.3/164.9 amu from Sample 11 (Verapamil WB_Stds 2_DART 500C; 0.2mm-s) of 20100507_DART_Verapamil Repeat_Serial Bleeding.wiff (Tur...

Data List | Peak List

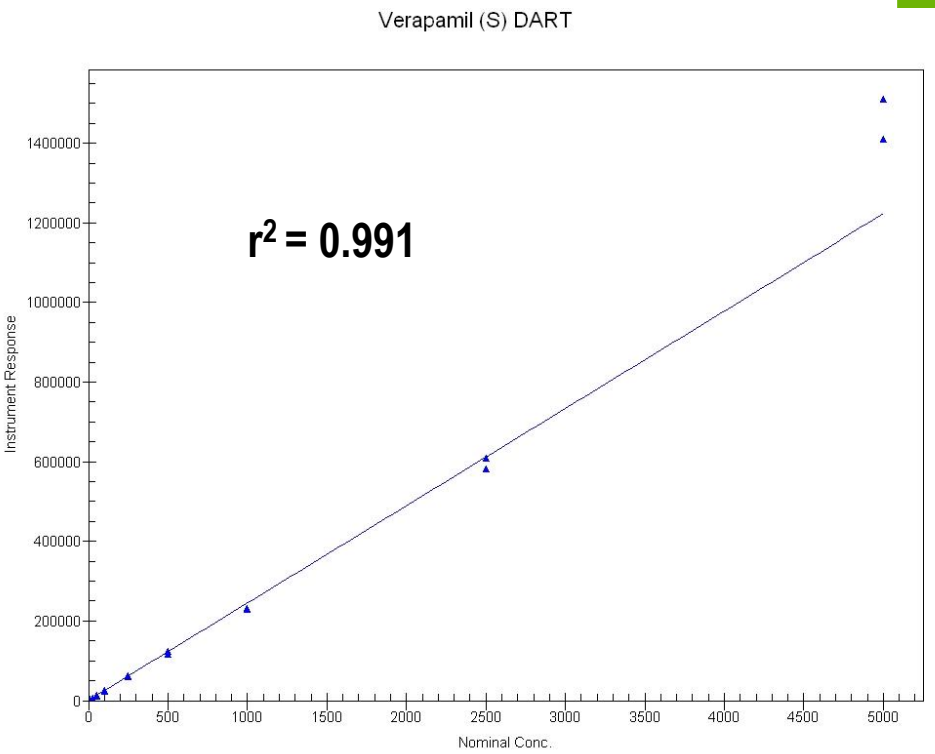
	Time (min)	Area (counts)	% Area	Height (cps)	% Height	Width (min)	Baseline Type
1	0.5984	286.5508	0.0541	14.7636	0.0504	0.5769	Valley
2	1.5875	880.6236	0.1663	36.1615	0.1235	1.1789	Valley
3	2.7385	1407.6588	0.2659	79.6969	0.2723	0.9532	Valley
4	3.6826	2586.1433	0.4885	126.3408	0.4316	1.1288	Valley
5	4.7160	5428.1849	1.0252	321.4005	1.0979	1.0034	Valley
6	5.7672	1.2999e4	2.4553	903.6064	3.0868	1.0285	Base to Base
7	6.8240	3.0875e4	5.8314	2005.8361	6.8521	1.1288	Base to Base
8	8.0105	5.3062e4	10.0219	3262.1611	11.1438	1.1288	Base to Base
9	9.0091	1.4735e5	27.8298	8515.9485	29.0911	1.0786	Base to Base
10	10.0668	2.7458e5	51.8616	1.4007e4	47.8505	1.0786	Base to Base

Observed Matrix Effect for Verapamil in WB



Verapamil Calibration Curve and QCs

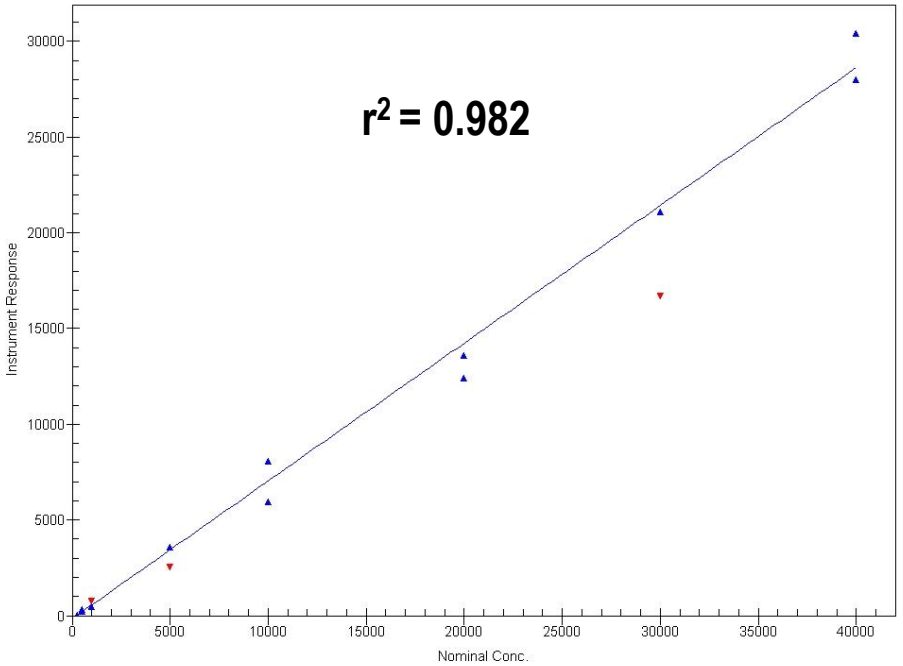
	QC.1	QC.2	QC.3
Theor. Conc.	25	200	4000
Found Conc.			
#1	21.5	184	3860
#2	21.5	179	3430
#3	20.6	180	2960
%CV	2.5	1.5	13.2
%Theoretical	**84.8**	**90.5**	**85.5**



	STD.1	STD.2	STD.3	STD.4	STD.5	STD.6	STD.7	STD.8	STD.9
Theor. Conc.	10	25	50	100	250	500	1000	2500	5000
Found Conc.									
#1	9.57	26.4	45	100	250	477	931	2370	5770
#2	10.6	25.1	49	89.5	243	506	947	2490	6170
Mean	10.1	25.8	47	94.8	247	492	939	2430	5970
%Theoretical	**101**	**103.2**	**94**	**94.8**	**98.8**	**98.4**	**93.9**	**97.2**	**119.4**

Data Processing MLN-A

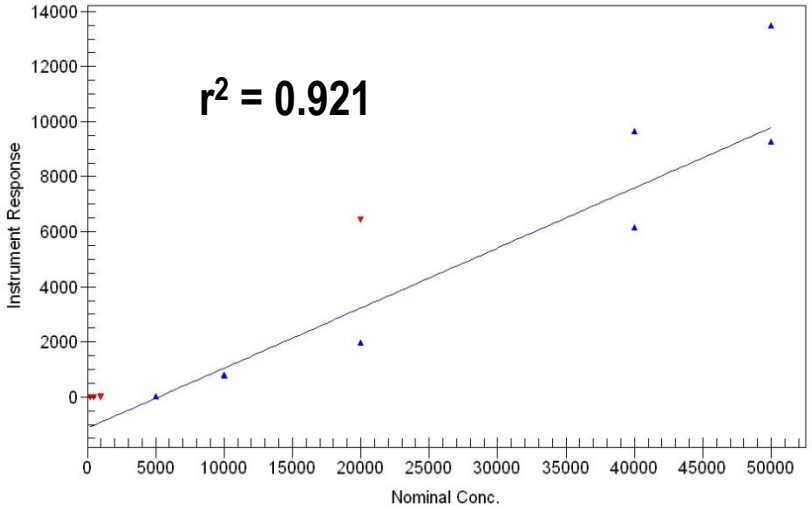
	QC.1	QC.2	QC.3
Theor. Conc.	1750	7000	35000
Found Conc.			
#1	1010	5290	37000
#2	1820	5680	38300
#3	1340	5640	32600
%CV	29.3	3.9	8.3
%Theoretical	**79.4**	**79.1**	**102.9**



	STD.1	STD.2	STD.3	STD.4	STD.5	STD.6	STD.7	STD.8
Theor. Conc.	250	500	1000	5000	10000	20000	30000	40000
Found Conc.								
#1	227	654	1300	5230	11400	19100	29500	39100
#2	227	563	855	3760	8480	17500	23400	42500
Mean	227	609	855	5230	9940	18300	29500	40800
%Theoretical	**90.8**	**121.8**	**85.5**	**104.6**	**99.4**	**91.5**	**98.3**	**102**

Data Processing MLN-B

- Reproducibility issue
  - more challenging compound

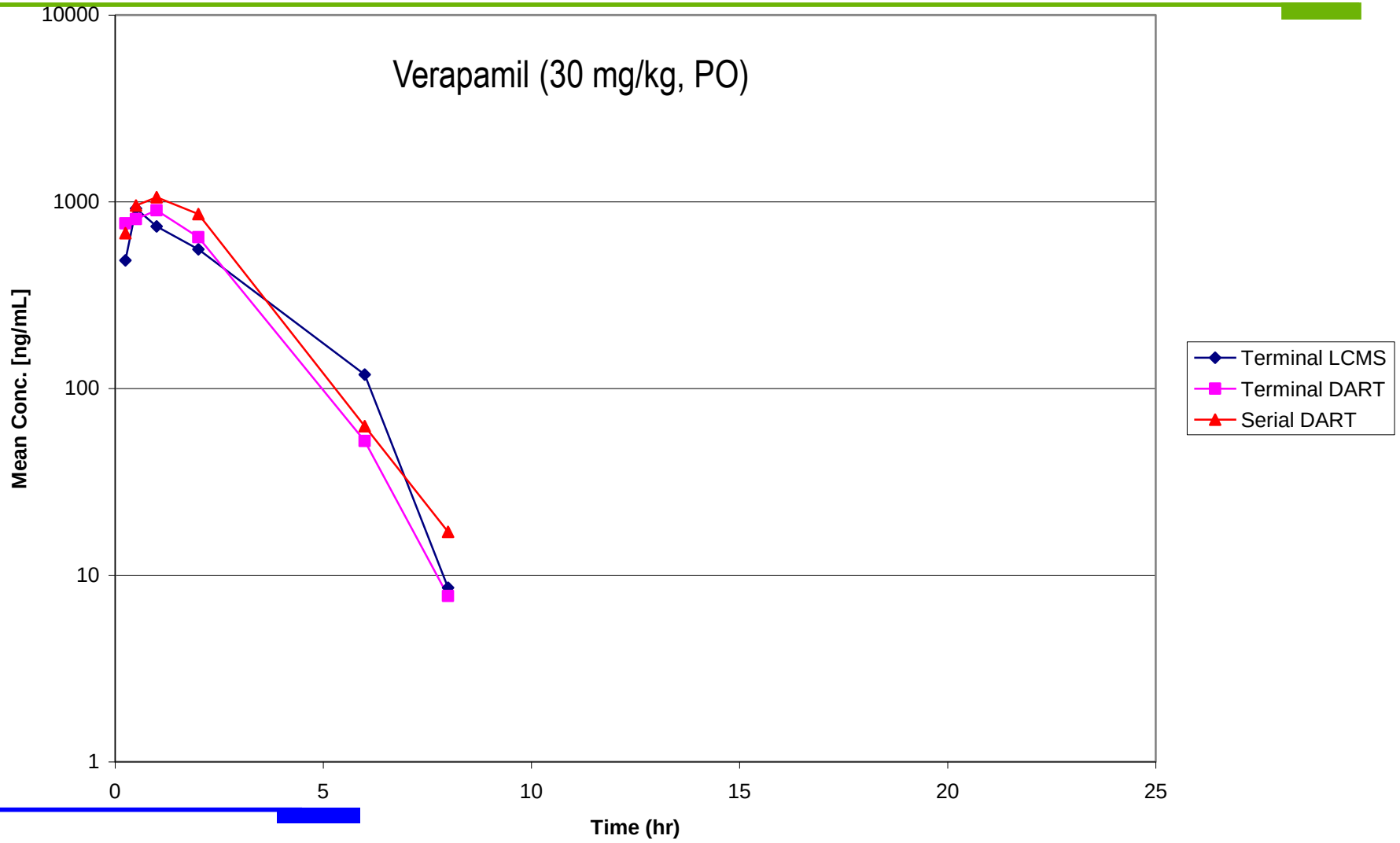


	QC.1	QC.2	QC.3
Theor. Conc.	1750	7000	35000
Found Conc.			
#1	1230	5880	30600
#2	1380	5680	29700
#3	1560	5720	24000
%CV	11.9	1.8	12.7
%Theoretical	**79.4**	**82.3**	**80.3**

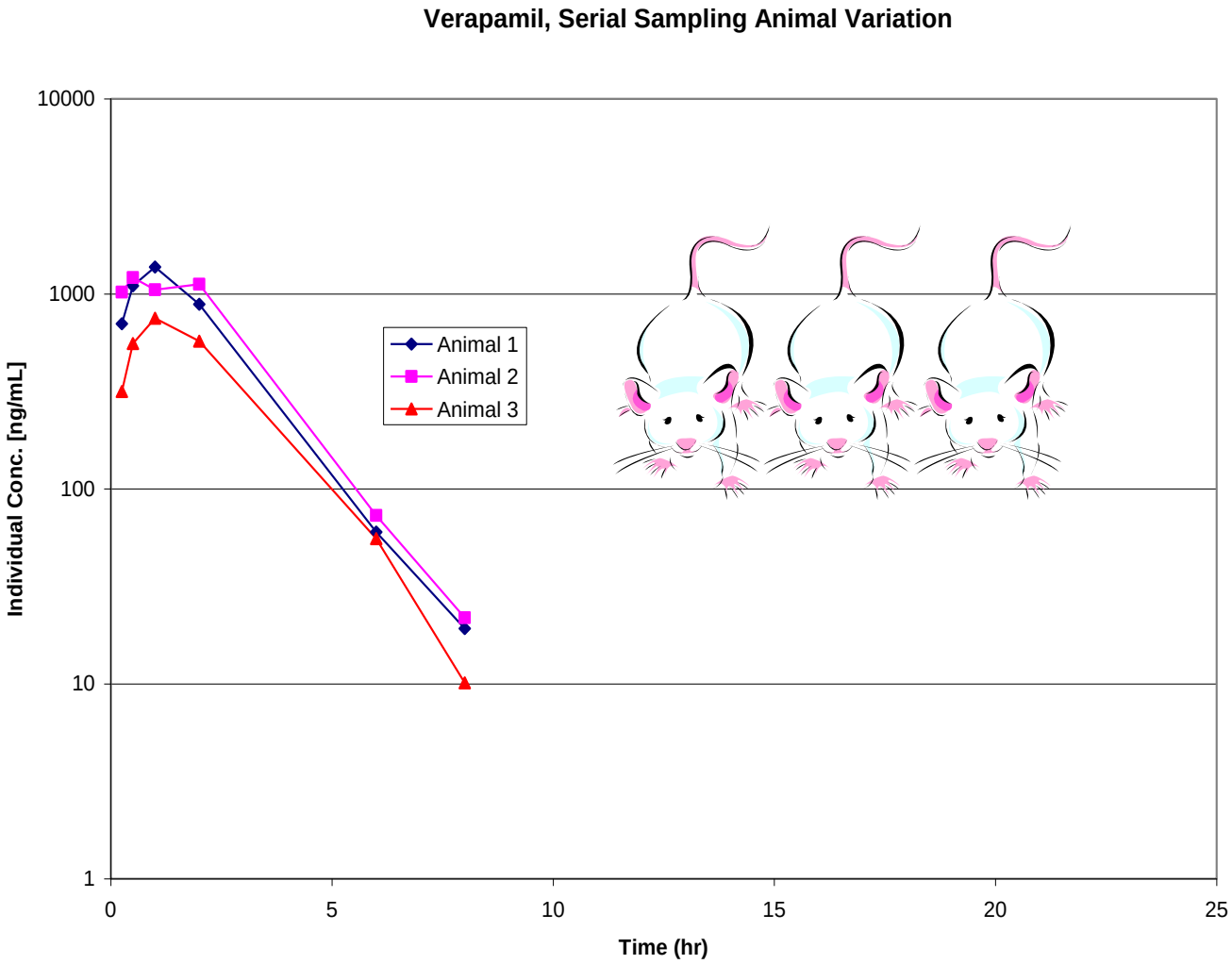
Sample Analysis

- Terminal Samples (n=21)
  - Run on LC/MS, extracted (sample prep)
  - Run on DART, DBS, same samples for comparison
- Serial Samples (n=3)
  - Run on DART, DBS, compared to terminal samples

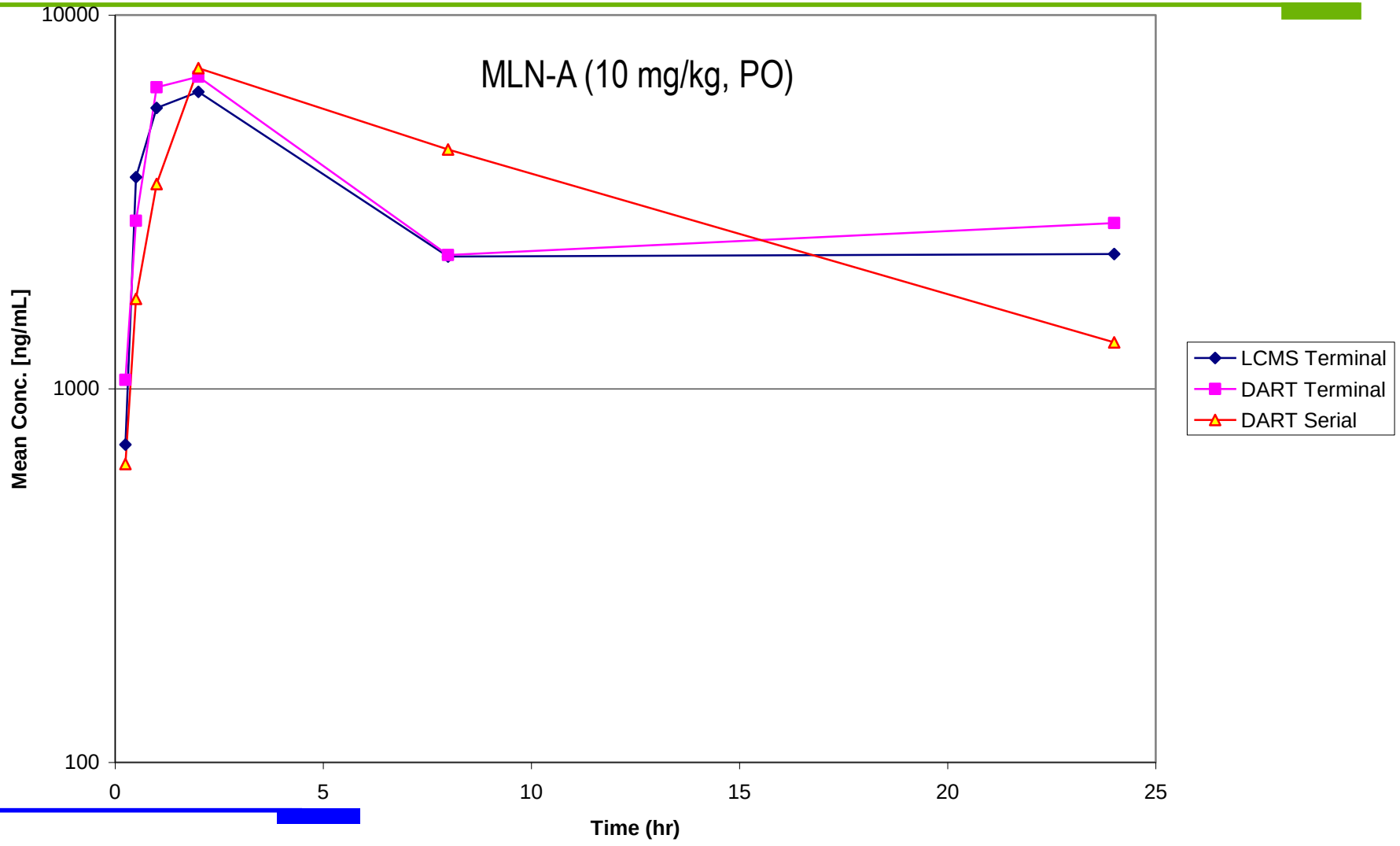
Results: Verapamil Time-Concentration Profile



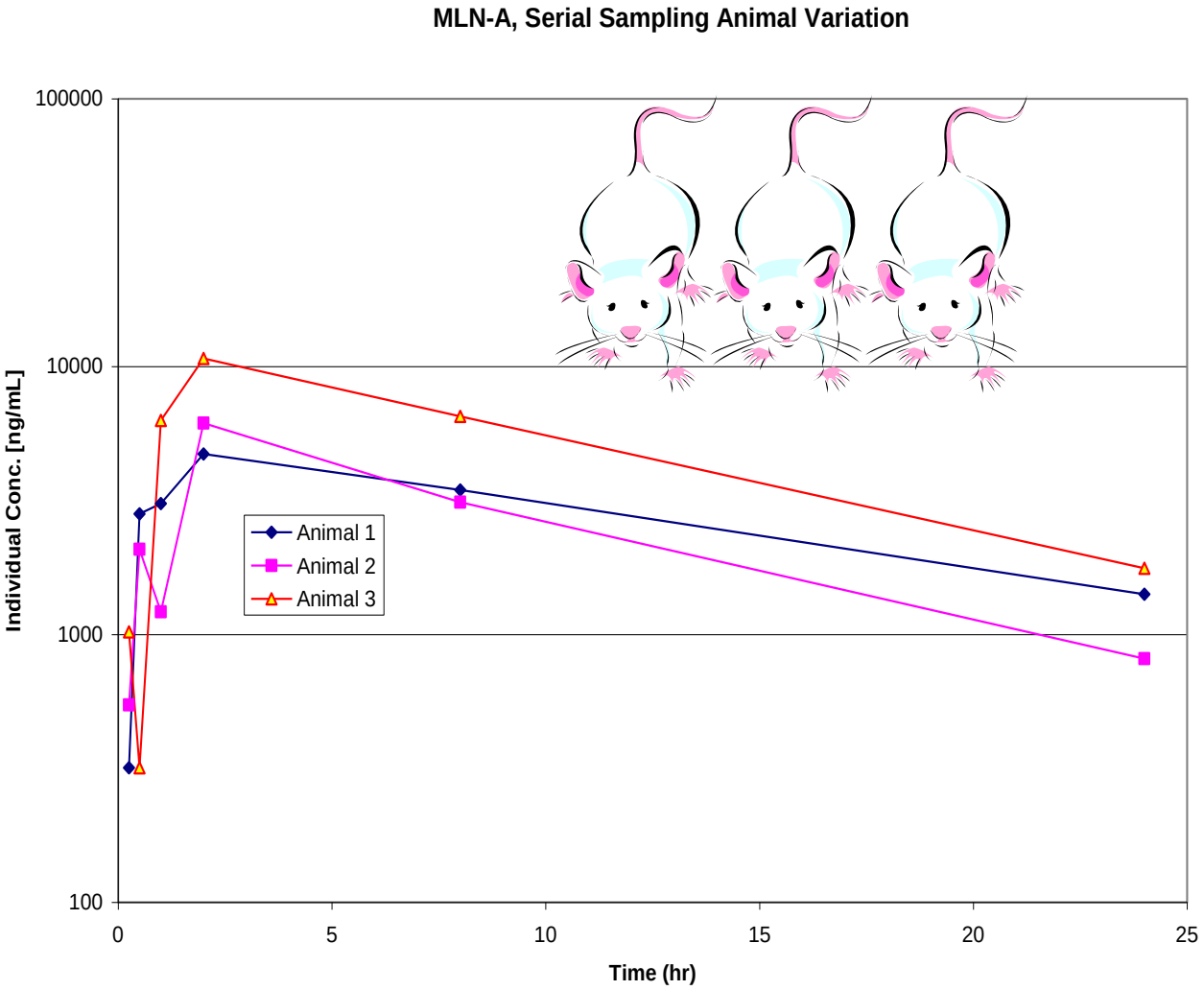
Time-Concentration Plot for Serial Bleeding: Individual Mice Results for Verapamil



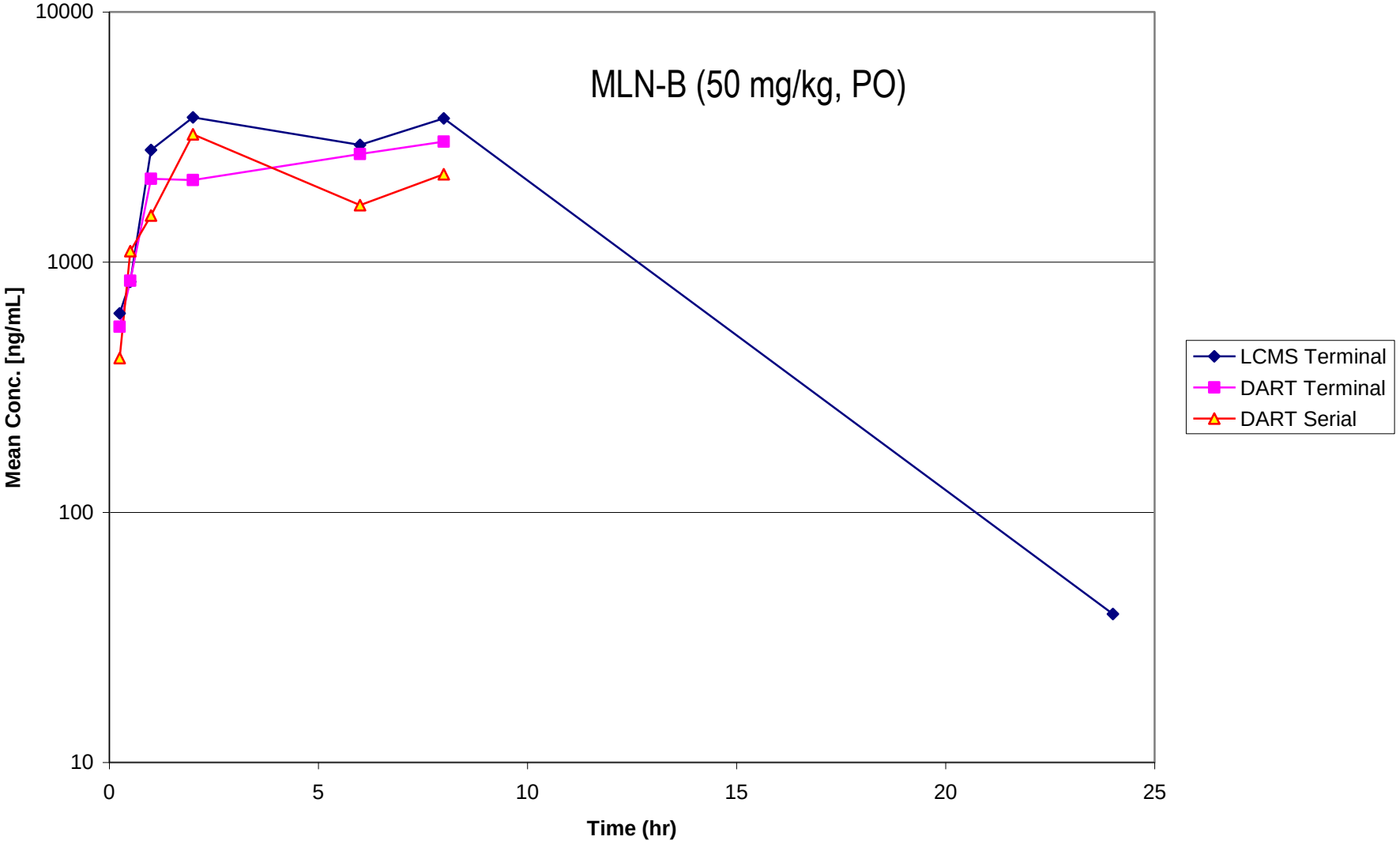
Results: MLN-A Time-Concentration Profile



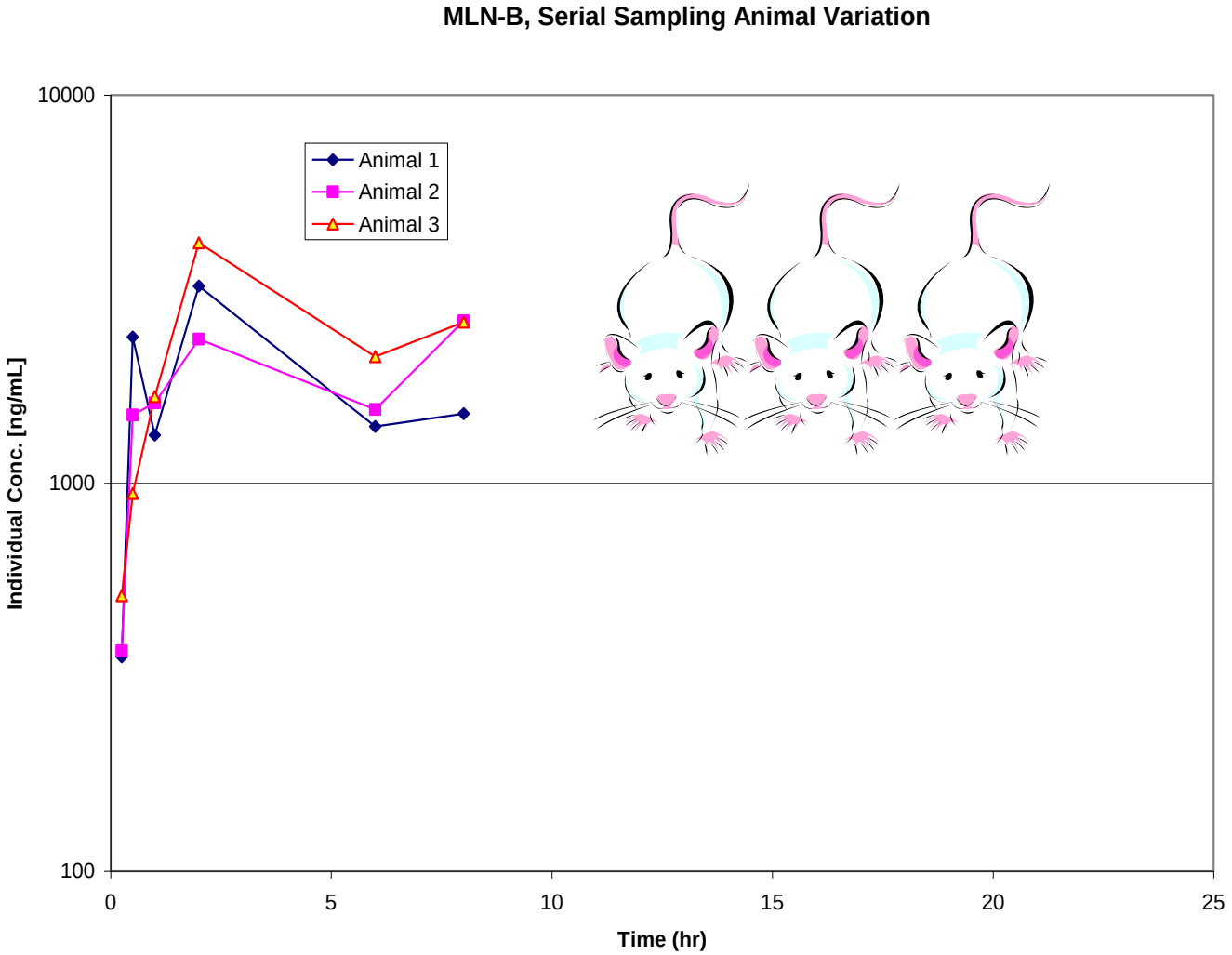
Time-Concentration Plot for Serial Bleeding: Individual Mice Results for MLN-A



Results: MLN-B Time Concentration Profile



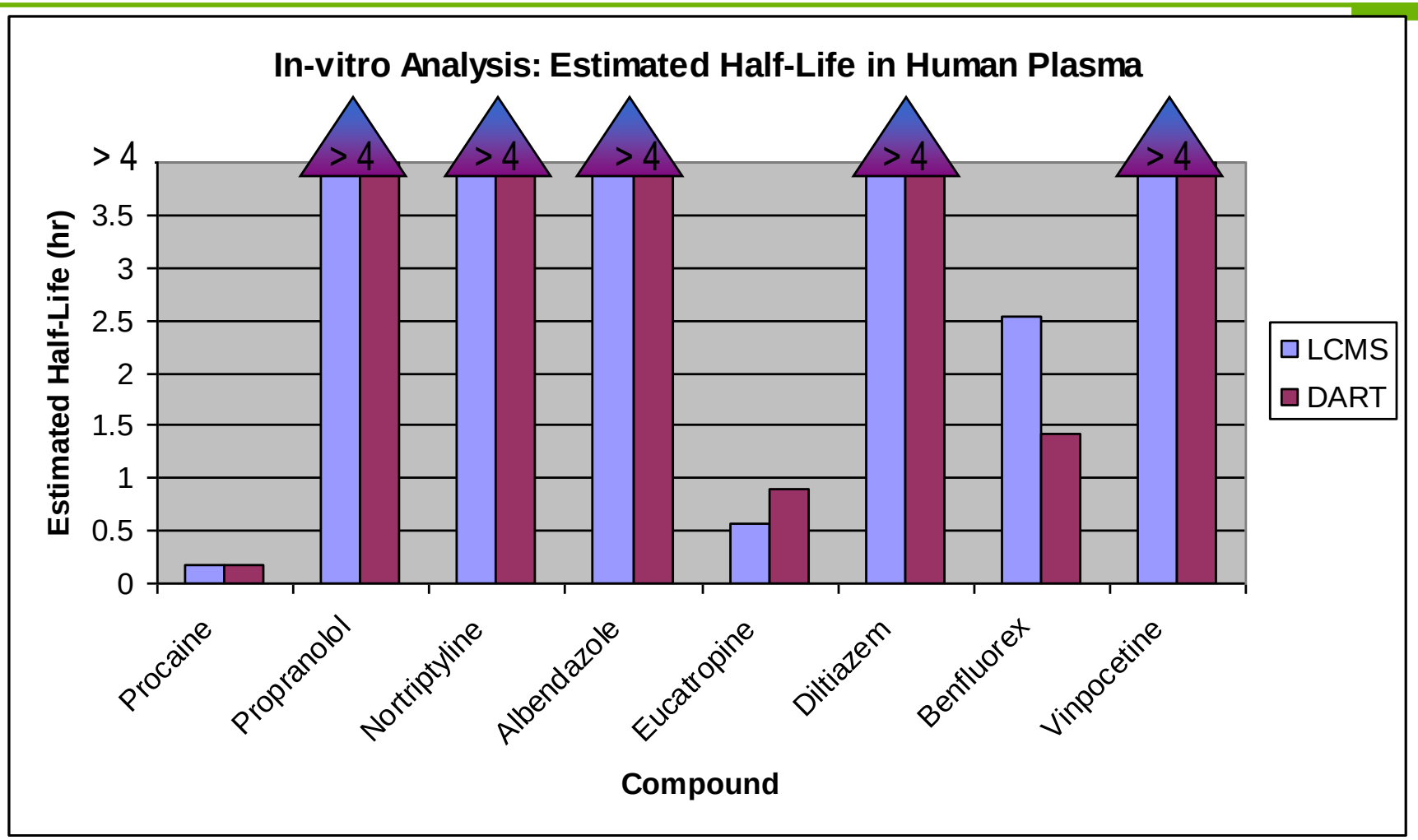
Time-Concentration Plot for Serial Bleeding: Individual Mice Results for MLN-B



In-vitro Sample Analysis

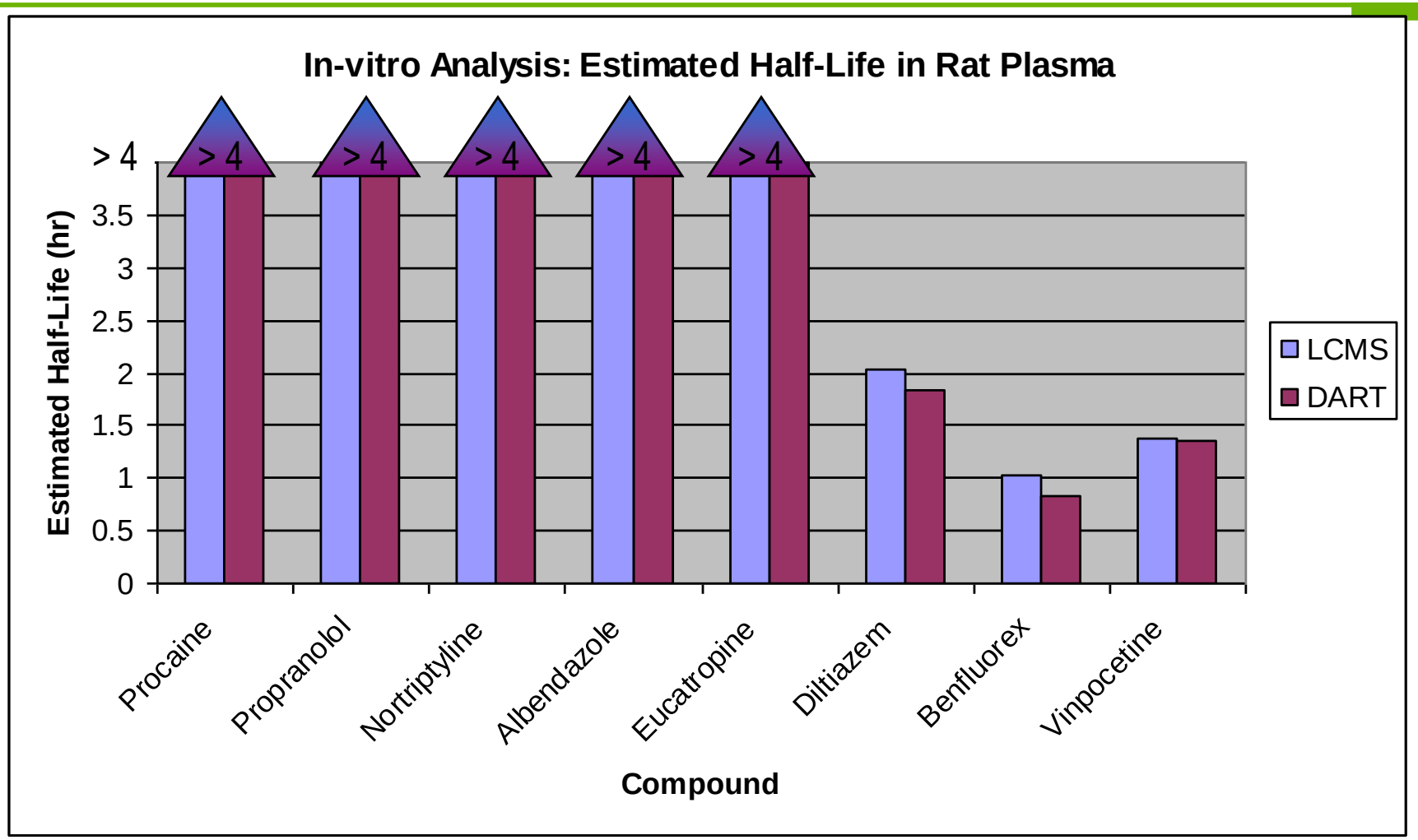
- Applying DART to in-vitro samples
- Plasma stability (ACN supernatant)
  - Various compound stabilities (low, med, high)
  - Samples run via LC/MS/MS at Tandem Labs
  - Samples run via DART/MS/MS
- Estimated Half-Life(s) compared

In-vitro Study: Stability in Human Plasma



LCMS Data: David Ho and Lily Li (Tandem Labs)

In-vitro Study: Stability in Rat Plasma



LCMS Data: David Ho and Lily Li (Tandem Labs)

Conclusion

- DART Strengths
  - DBS-DART/MS/MS promising for quantitation of single-mouse PK and some in-vitro ADME samples
  - Good for fast, “real-time” analysis in biological matrices
  - No sample prep (at all)
- DART Limitations
  - Sensitivity less optimal compared to LCMS with current instrumentation, which also limits sampling speed
  - Compounds with low sensitivity are challenging to consistently generate reproducible data
  - No chromatographic separation

The Future Application of DART at Millennium

- For WB, best used in high concentrated samples
  - Implement for AQL preventing
  - Possible use in quantitation for Tox studies
- Quick formulation dose comparison
- In-vitro sample analysis: microsome/plasma stability, etc.
  - Less matrix effect
- Further investigation of paper substrate for DBS-DART/MS/MS

Acknowledgements

- Kym Cardoza, Emily Guan, Shylah Wyllie, Mi-Sook Kim, Sandy Gould, Larry Cohen, Susan Chen, Anastasia Leyden, Joe Tice, Mike Johnson

Questions?