CYTOTOXICITY ASSESSMENT OF HIGH-NITROGEN COMPOUNDS USING A NOVEL *IN VITRO* MULTI-CELLULAR APPROACH

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Disclaimer

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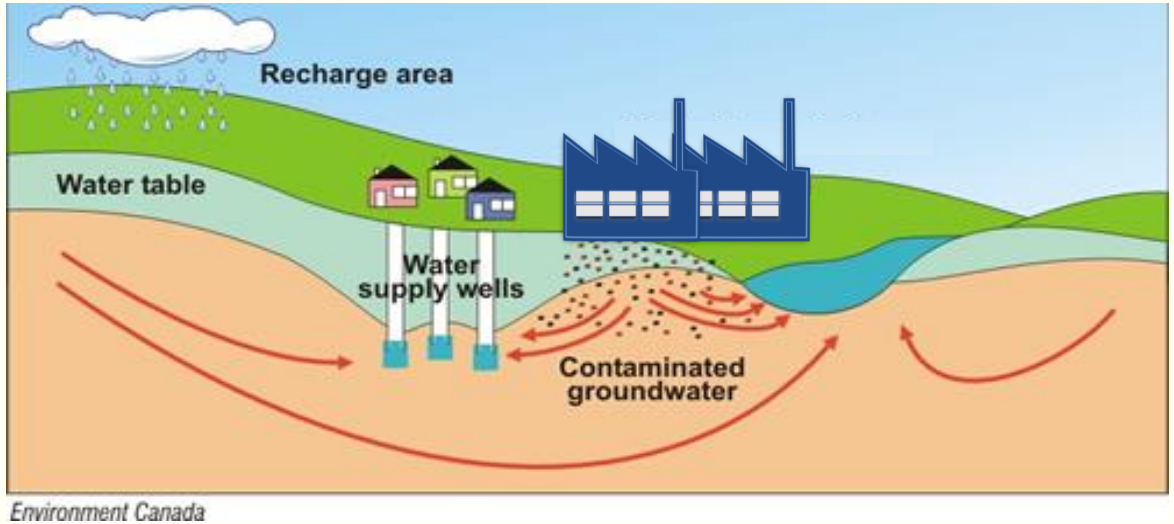
Background: Soil and Water Contamination by Explosives

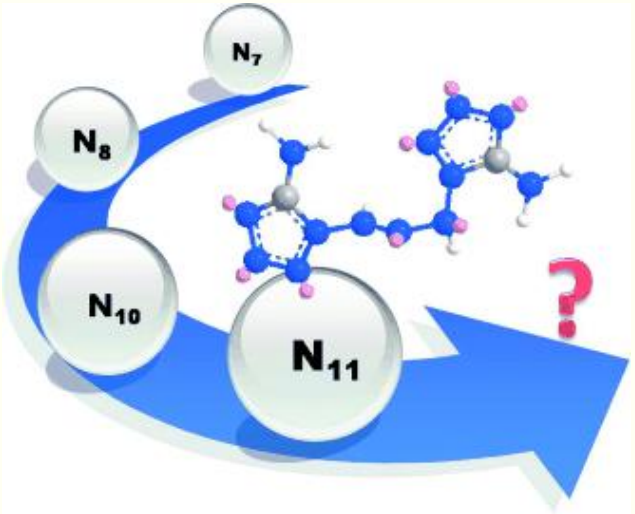
- 3.1 million metric tons a year (US, 2013)
- 21,000 contaminated military sites worldwide

- USA:
  - 1.2 million tons of contaminated soil
  - 583 sites with contaminated groundwater
  - TNT, RDX, HMX, perchlorate

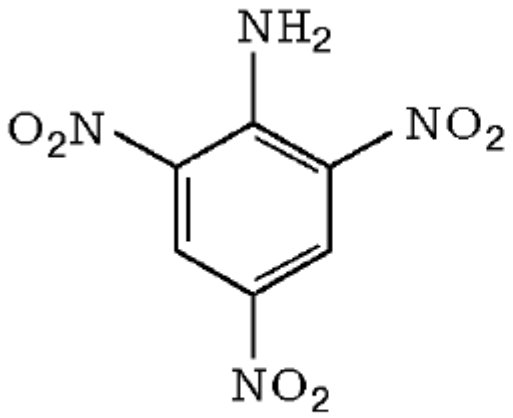


Background: High-Nitrogen Compounds

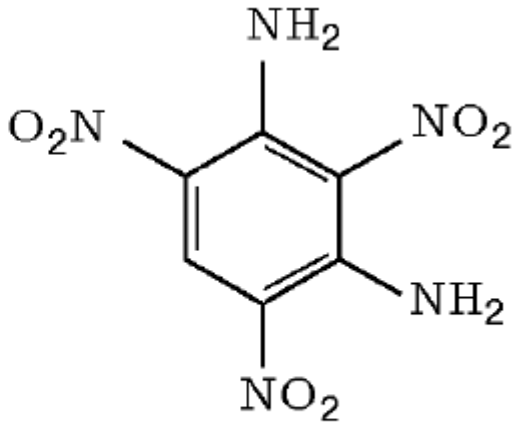
- Unique class of novel energetic materials
- Low sensitivity to heat and shock
- High nitrogen quantity = high density = generation of large quantity of gas per gram
- End product: N_2
- Little is known about their toxicity
- Need for a rapid high-nitrogen chemical hazard assessment tool



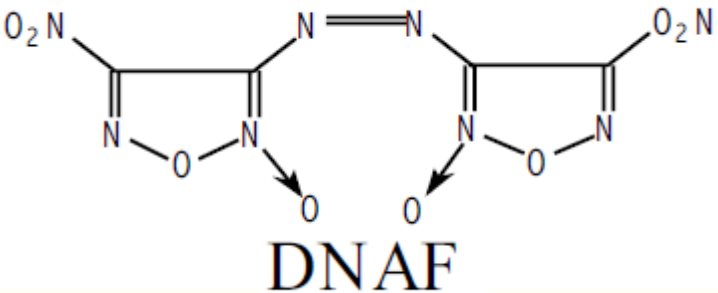
Background: High-Nitrogen Compounds



MATB

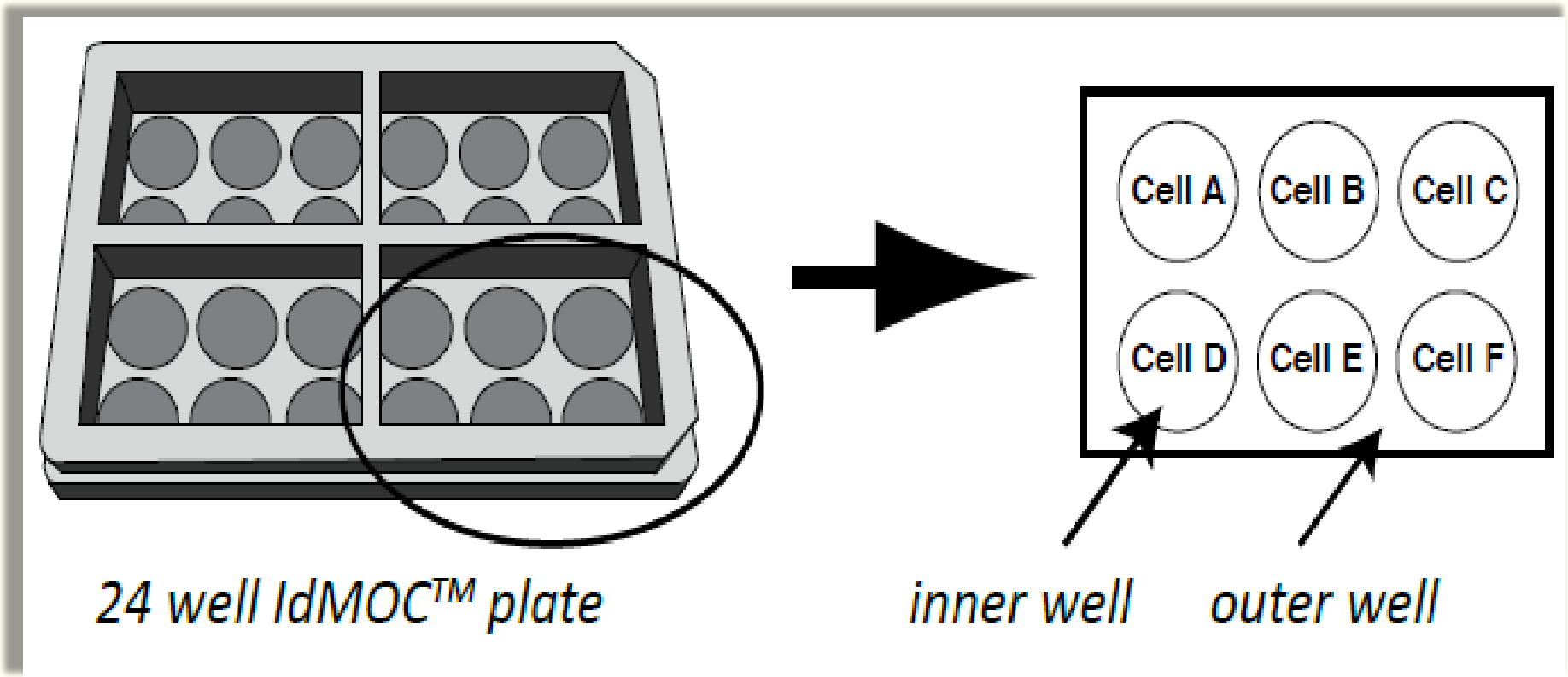


DATB



DNAF

Background: Integrated Discrete Multi-Organ Co-culture (IdMOC) system



Statement of purpose and hypothesis

To assess the cytotoxicity of 2,4-dinitroanisol (DNAN) via the IdMOC system validated with TNT.

Hypothesis

Human cell types representative of multiple organs exposed in co-culture to DNAN versus TNT will have unique gene expression profiles indicative of systemic toxicity and reflecting the lower relative toxicity of DNAN.

Public Health Relevance

- Greater emphasis on understanding chemical perturbations of pathways
- Rapid assessment of new high-nitrogen compounds
- Potential of improving efficiencies in hazard assessments
- Ultimately: prevent exposure to hazardous chemicals

Conceptual Model

In vivo studies =  × Hundreds of new high-N compounds

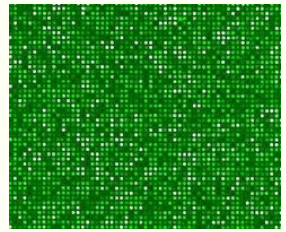
In vitro studies = 

IdMOC = (screening process) 
Hundreds of new high-N compounds

Conceptual representation of experimental procedure:

Functional analysis and metabolic flux balance analysis

Microarray analysis



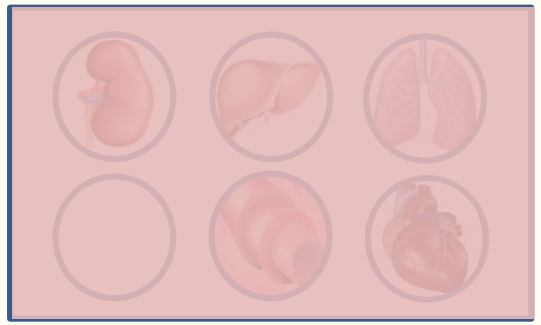
RT-qPCR and RNA quantification

IdMOC cells only

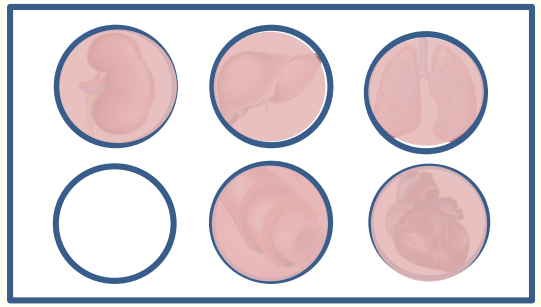
Total RNA Extraction

Cells

IdMOC



Monoculture



Media

Functional Assays

NGAL

Albumin

Surfactant protein B

Endothelin-I

Troponin I

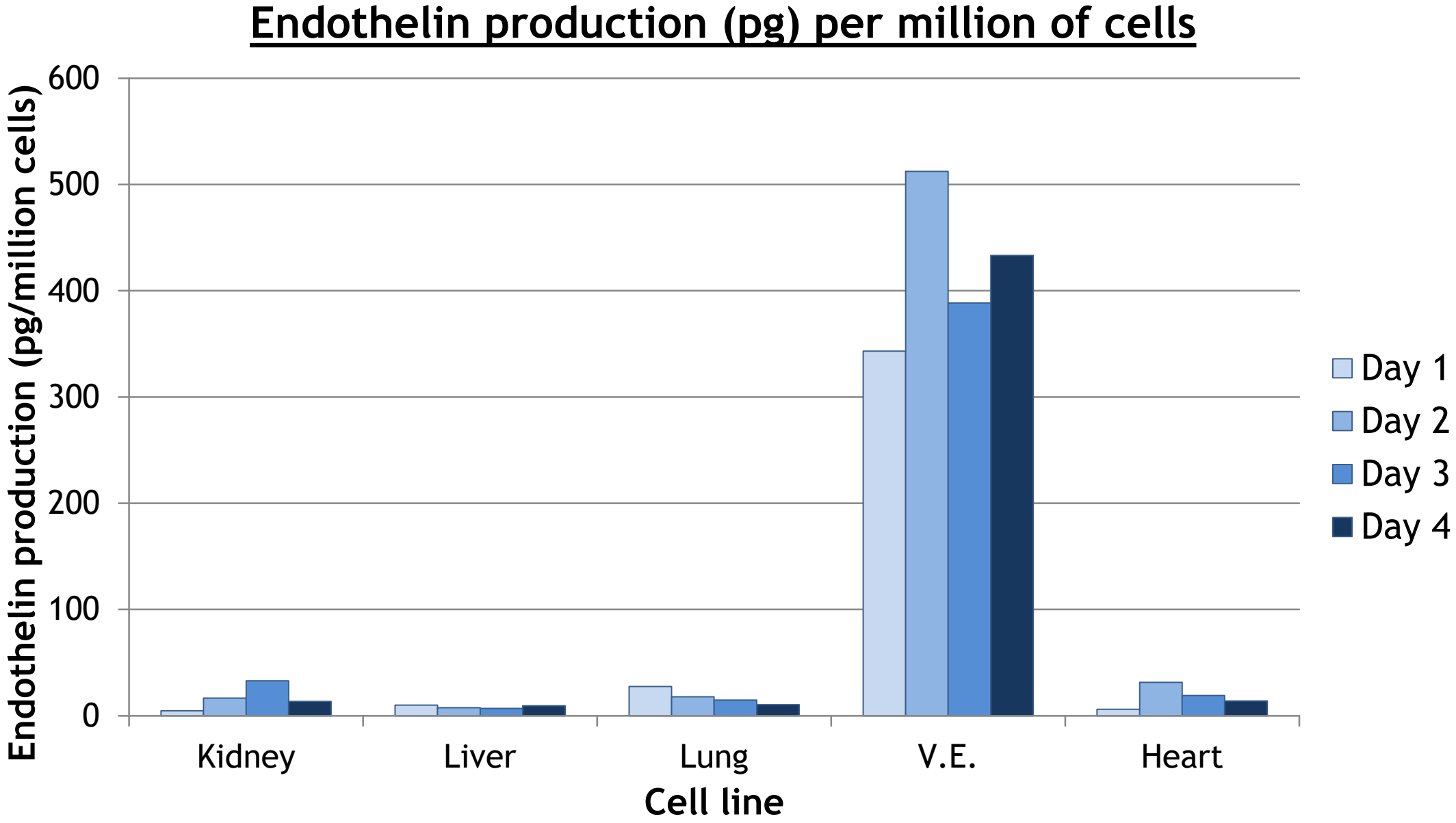
X 4 concentrations + 2 controls (DMSO+; DMSO-)
X 4 replicates
X 2 chemicals (TNT and DNAN)

RESULTS

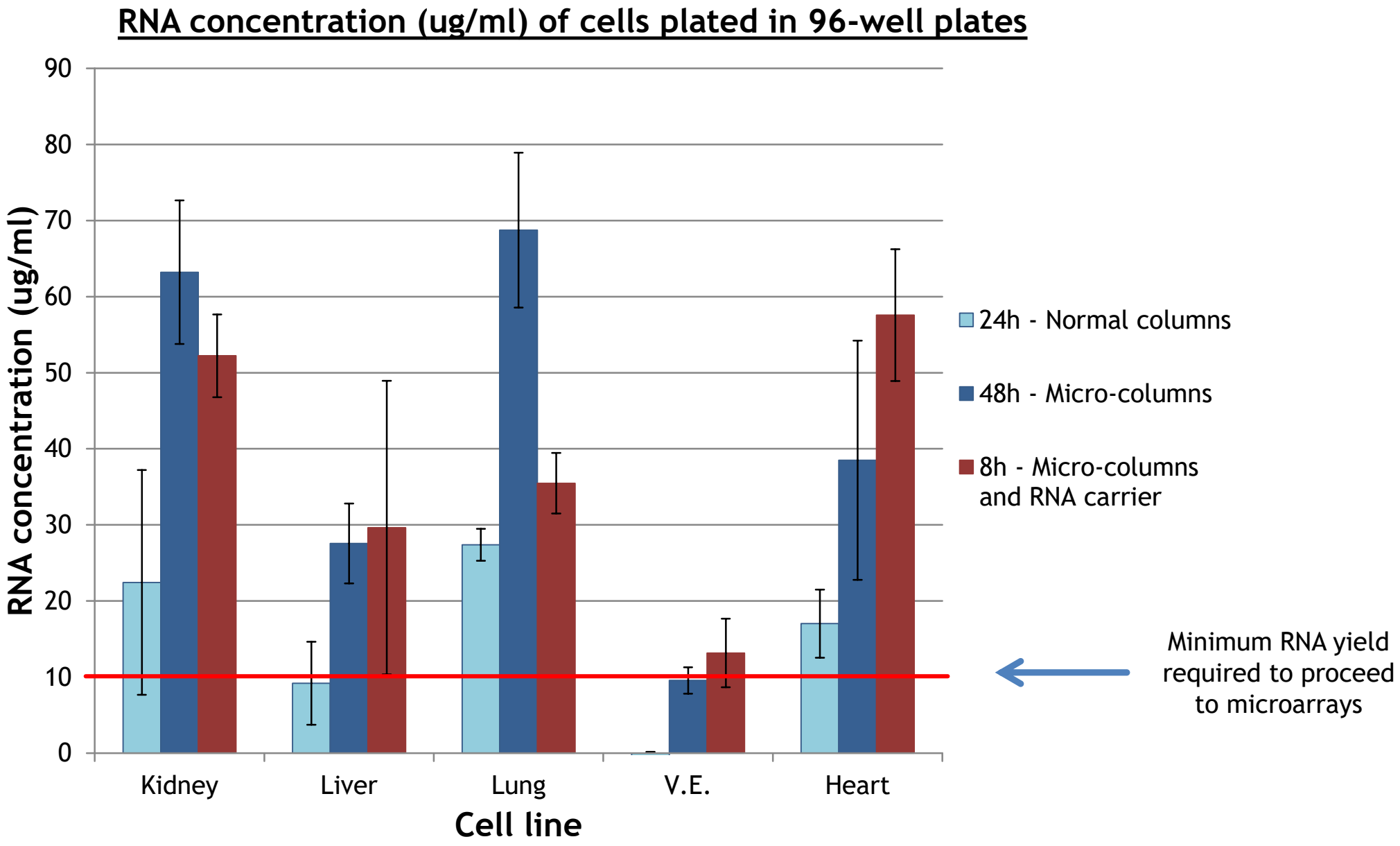
Assessment of the initial production of functional proteins

- All cell lines produced the expected functional proteins
- Non-specific for some
- Importance of mono-culture setting

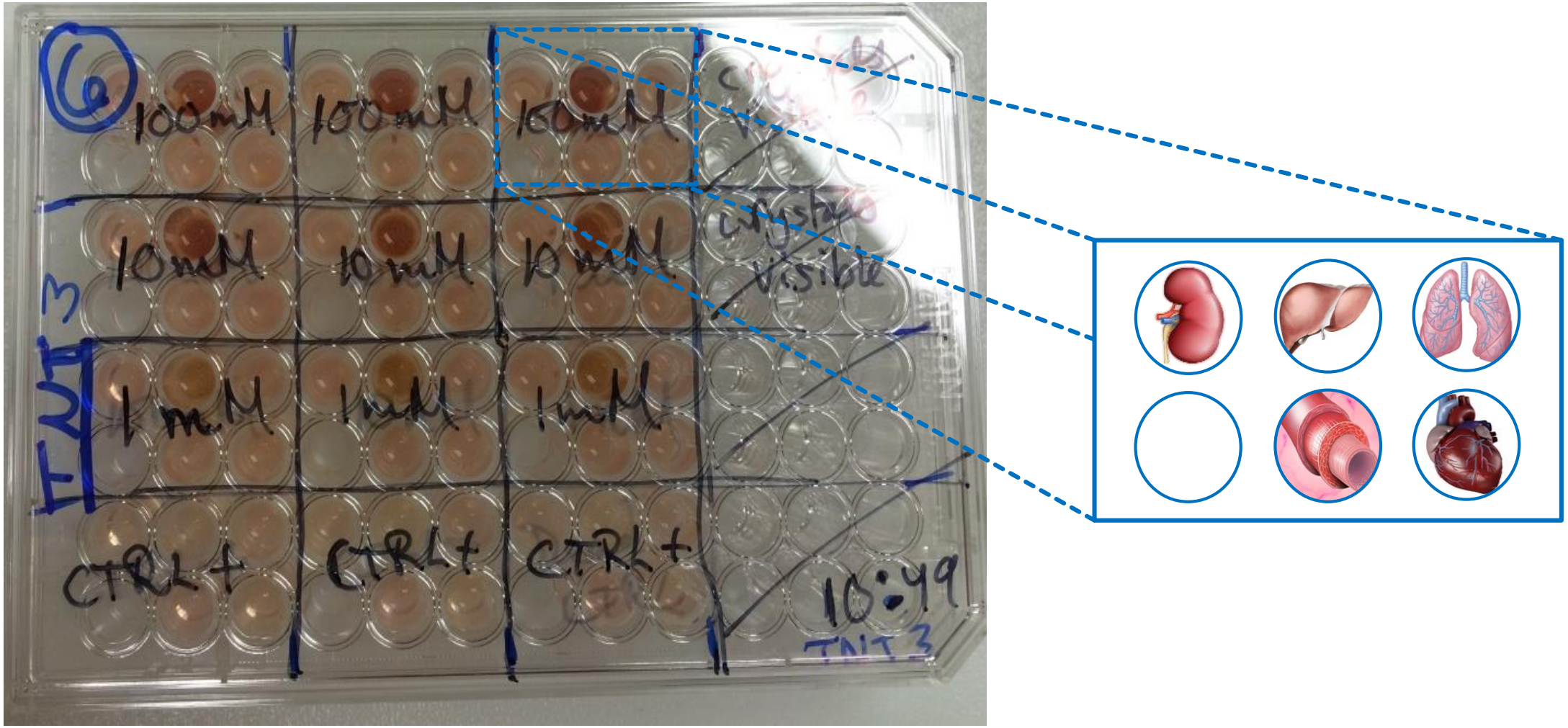
Initial Functional Protein Production

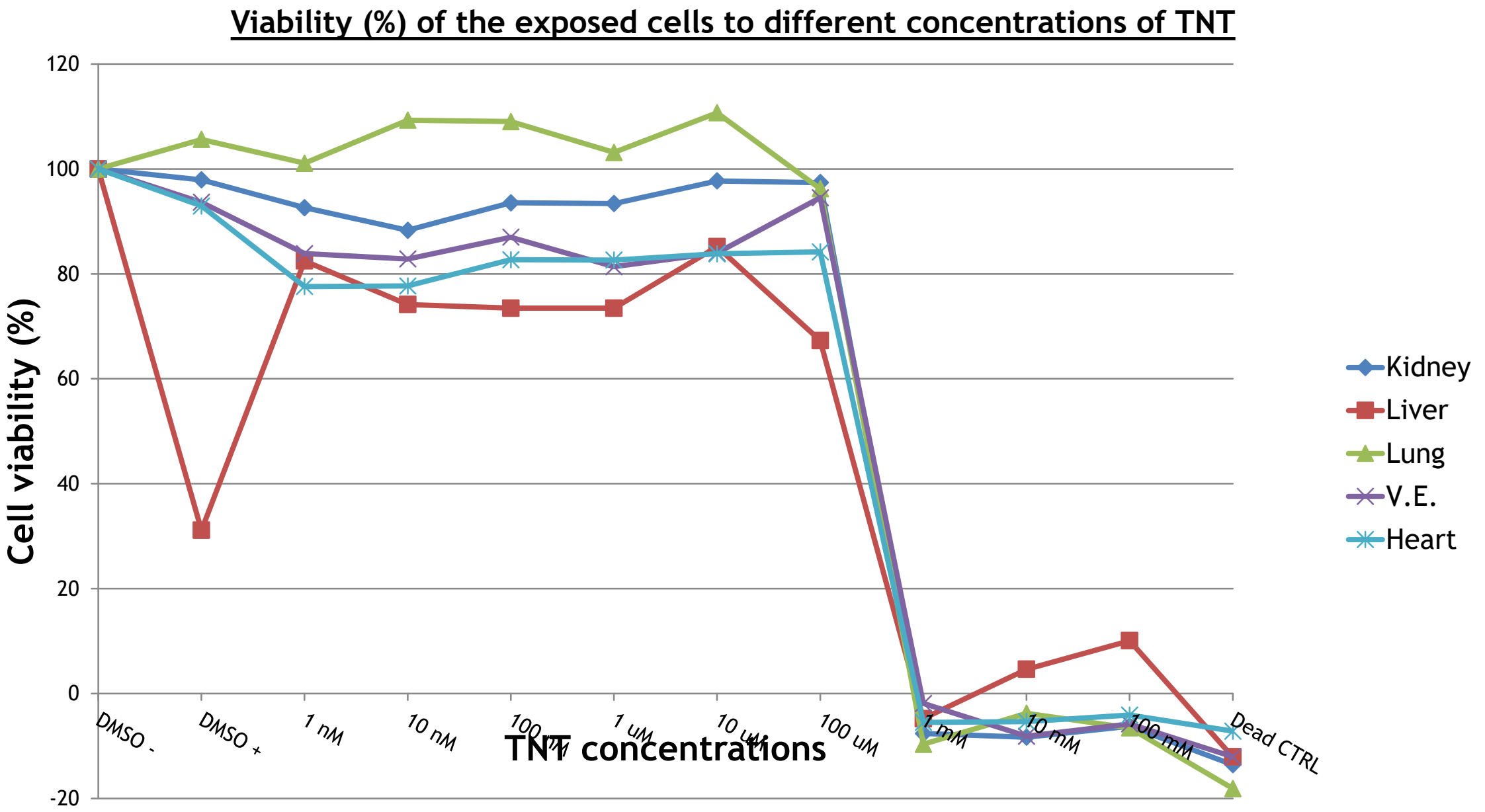


RNA Extraction

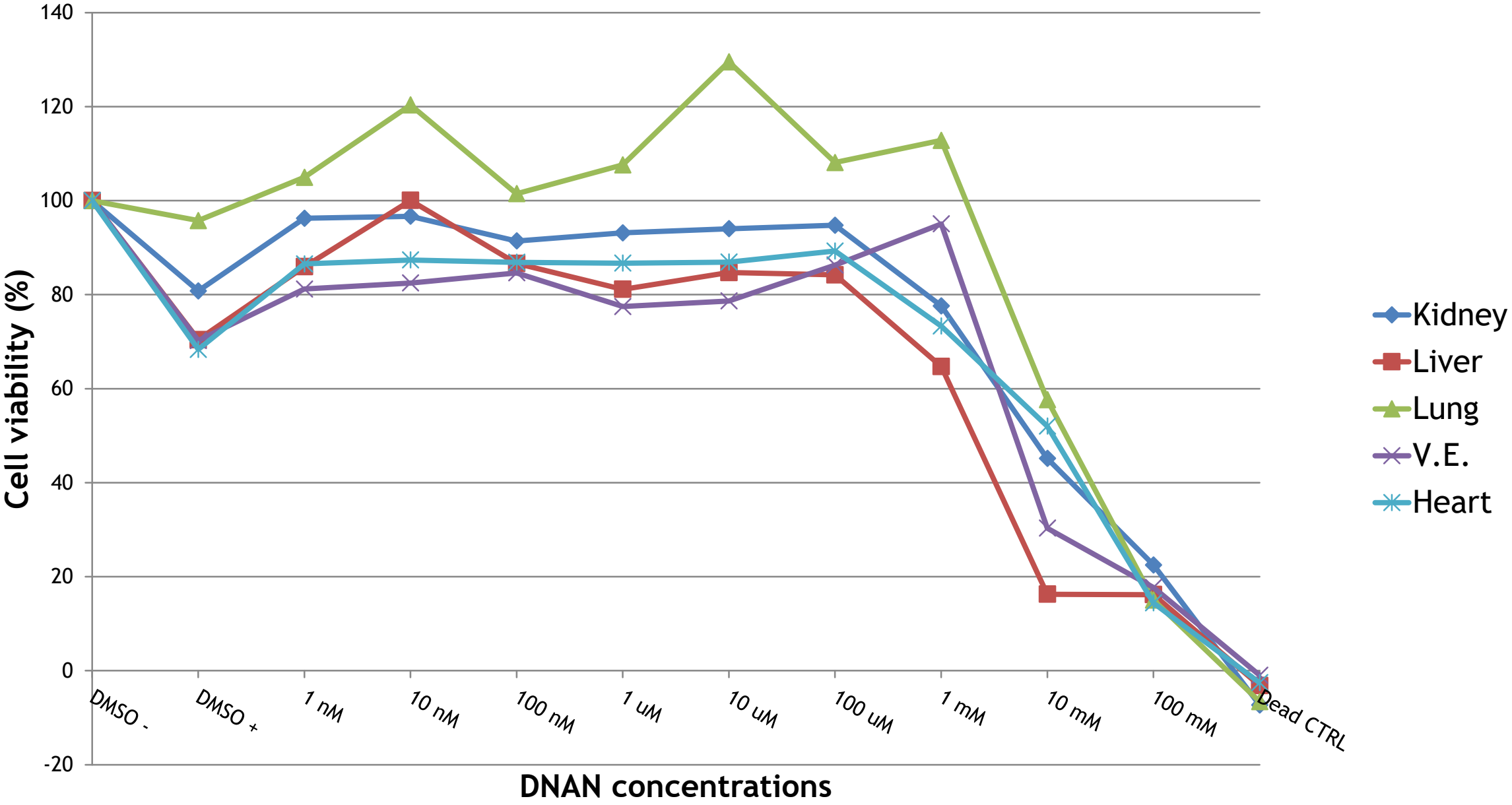


Range finding experiment




Viability (%) of the exposed cells to different concentrations of TNT
120
100
80
60
40
20
0
-20
Cell viability (%)
DMSO -
DMSO +
1 nM
10 nM
100 nM
1 uM
10 uM
100 uM
1 mM
10 mM
100 mM
Dead CTRL
TNT concentrations
Kidney
Liver
Lung
V.E.
Heart

Viability (%) of the exposed cells to different DNAN concentrations



Conclusion

- All cell lines produced the associated functional proteins
- RNA quantity and quality are sufficient to proceed with microarrays
- Range used:
  - TNT: 100 nM to 100 uM
  - DNAN: 1 uM to 1 mM
- Gene expression profile expected to reflect viability testing results

QUESTIONS?