Electrochemical Generation of Toxicants and their Application as Chemotherapeutics

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MSc. Defense

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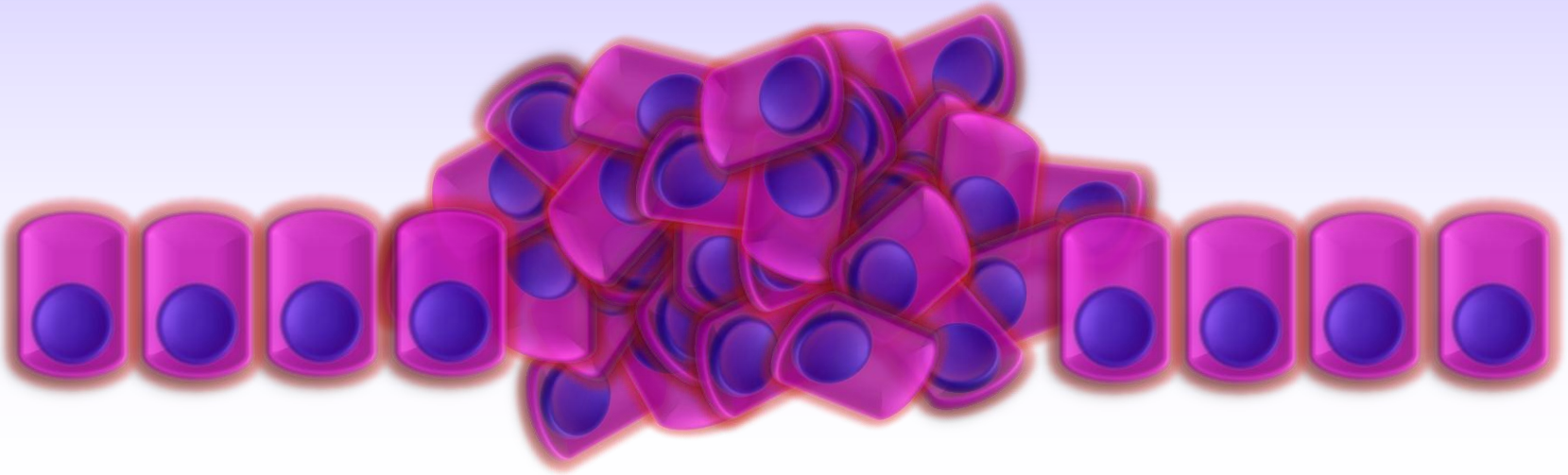


The Problem

- Significant collateral toxicity encountered in patients undergoing cancer treatment
- Systemic treatments target actively replicating cells (e.g., the cancer, hair, blood, G.I.)
- Treatments must differentiate cancerous and healthy cells via rate of cell division (i.e., cancer: fast, normal: slow)

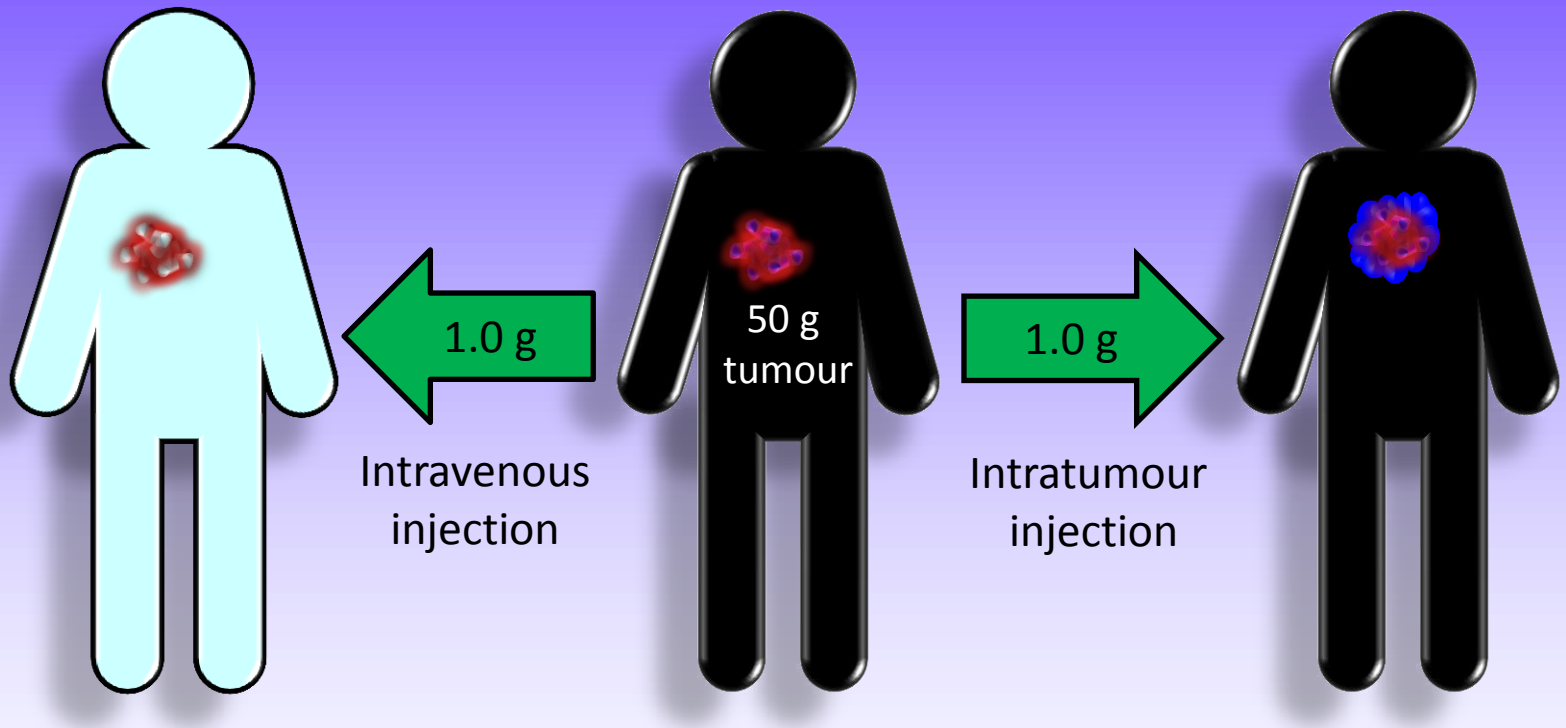
Treatment of Solid Tumours

- Surgical excision is preferred, but not always possible
- Inoperable or locally invasive tumours often require surgery, radiation, **and** chemotherapy
- Can you use a local treatment to treat local disease?

Localized Chemotherapy

- Localized chemotherapy bypasses systemic circulation
- Use higher local concentrations with smaller amounts of drug vs. conventional therapy
- Improves response and quality of life

Localized Chemotherapy



Dosage: 14 mg/kg
Tumour exposure: 0.75 mg
Exposure efficiency: 0.07%

70 kg patient

Dosage: 20 mg/g tumour
(~ 20,000 mg/kg)
Tumour exposure: 1.0 g

Localized Chemotherapy

- Mitotic toxicants that interfere with DNA replication are often carcinogenic (*de novo* cancers)
- Intratumour injection allows for use of non-mitotic toxicants
- Systemic use of non-mitotic toxicants would be lethal, as metabolic pathways are ubiquitous in all cells

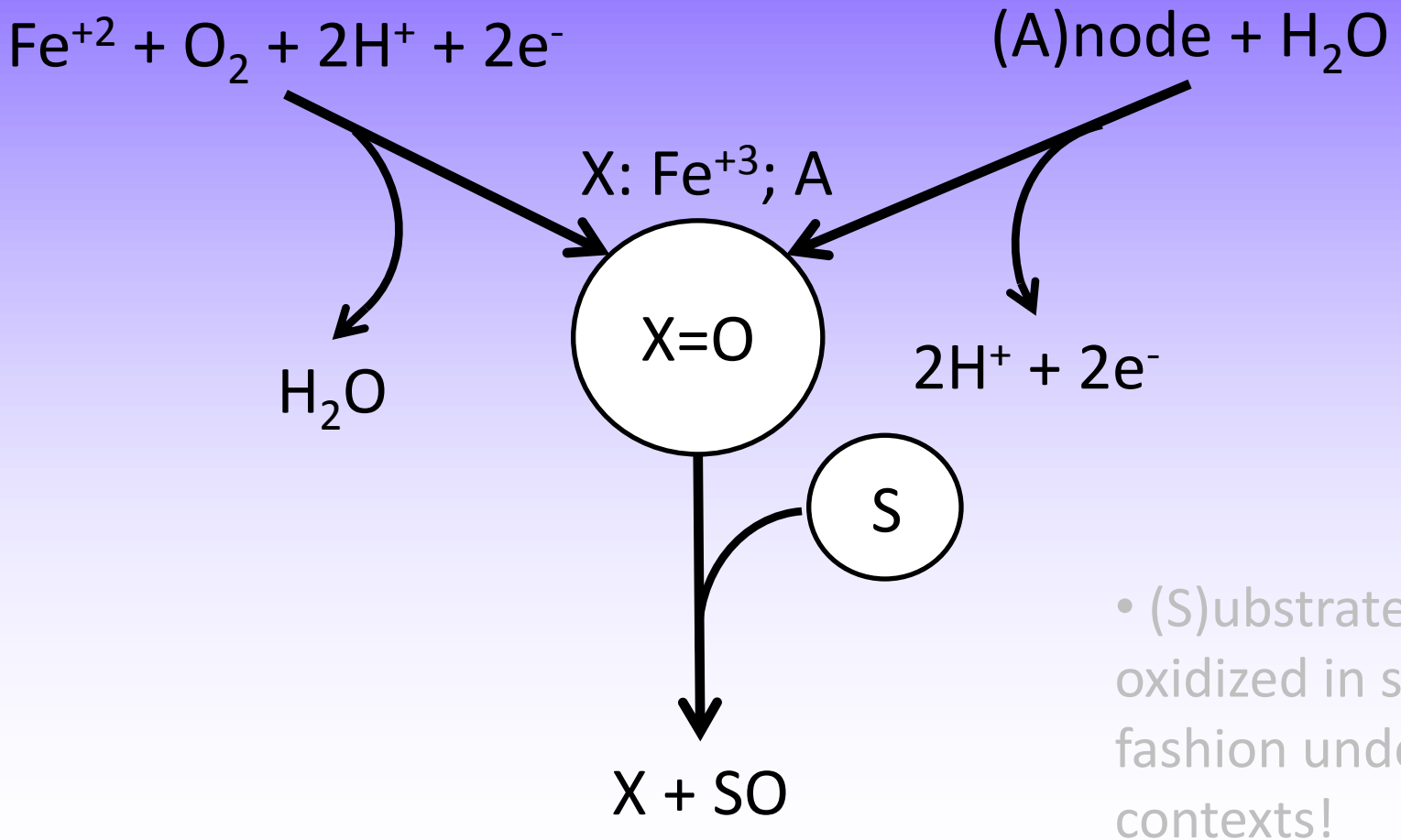
Drug Activation

- Allows for some control over distribution of reactive toxicants
- Many anti-cancer drugs are activated through oxidative metabolism (bioactivation; P450s)
- Concept of biomimicry via electrochemistry
- Some anode materials, such as graphite and Ti/RuO_2, mimic oxidative bioactivation

Drug Activation

P450:

Electrochemistry:



- (S)ubstrate is oxidized in similar fashion under both contexts!

Aim of the Thesis

- To provide proof of concept for a system which is:
  1. Capable of electrochemically activating a prodrug into a cytotoxic form;
  2. Subsequently using the activated compound against *in vitro* cancer cells

Project Overview

1. Choose cell line and cytotoxicity assay
2. Choose model toxicants: determine toxic potency
3. Electrochemical characterization of prodrug in batch cell
4. Toxicity of electrochemical product in batch cell
5. Flow cell design and toxicity of effluent

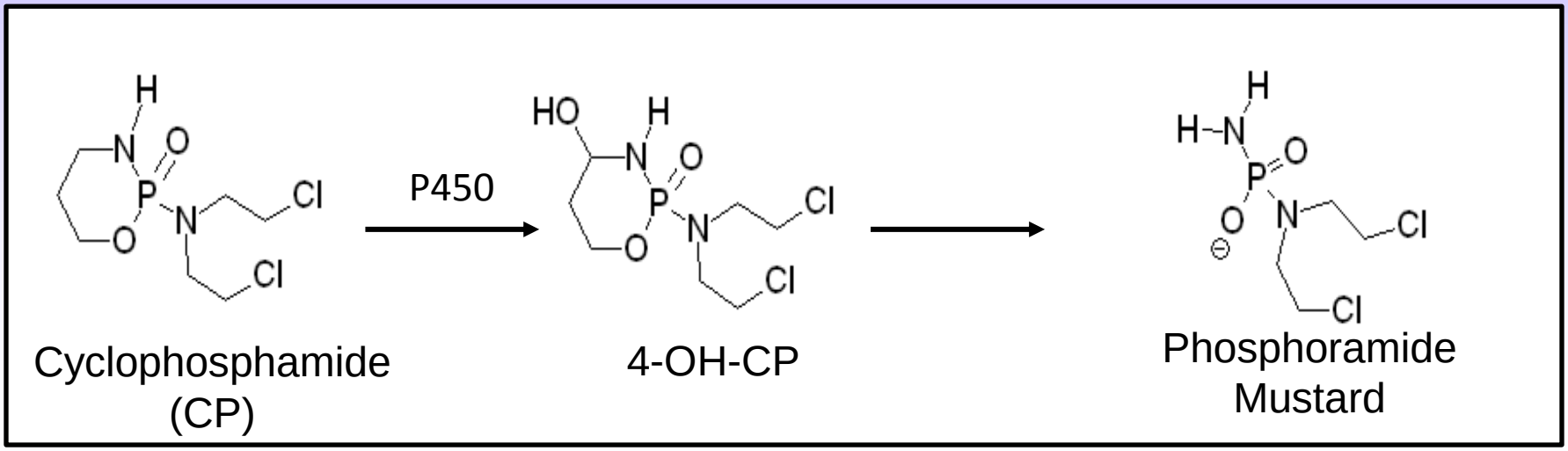
Cell Line and Cytotoxicity Assay

- Mouse mammary adenocarcinoma cell line: EMT-6
- Measure viability colourimetrically (MTS dye); visually confirm death with morphological changes in cells
- Living cells make NAD(P)H → reduces MTS dye → colour change (ox.: yellow; red.: blue) → measure colour change

Model Toxicant: Cyclophosphamide (CP)

- Bioactivated by P450s to give toxic product: the phosphoramide mustard (PM)

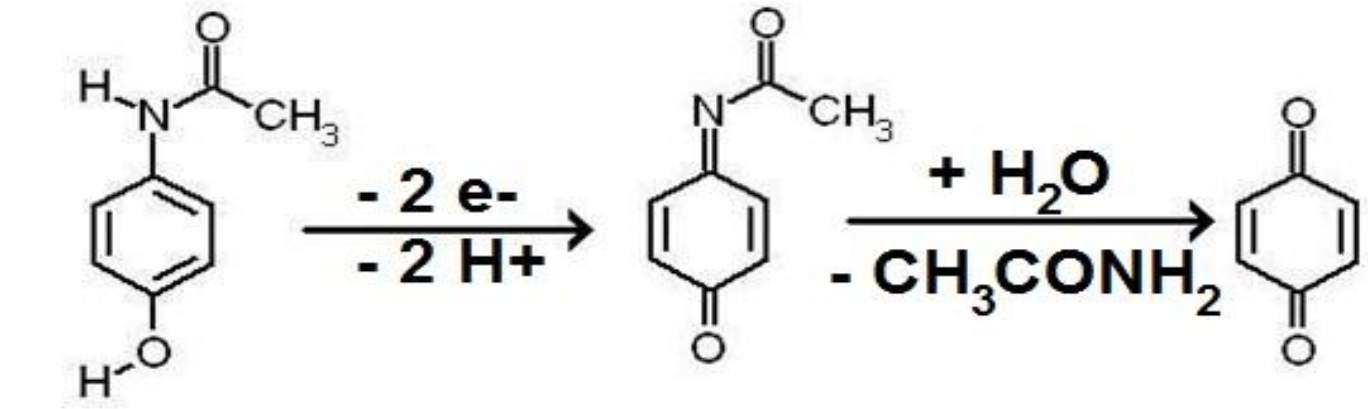
- Example of a mitotic toxicant (DNA alkylation)



Model Toxicant: Acetaminophen (AP)

- Bioactivated by P450s to give toxic products: N-acetyl-*p*-benzoquinonimine (NAPQI) and benzoquinone (BQ)

- Example of a non-mitotic toxicant (denaturation)



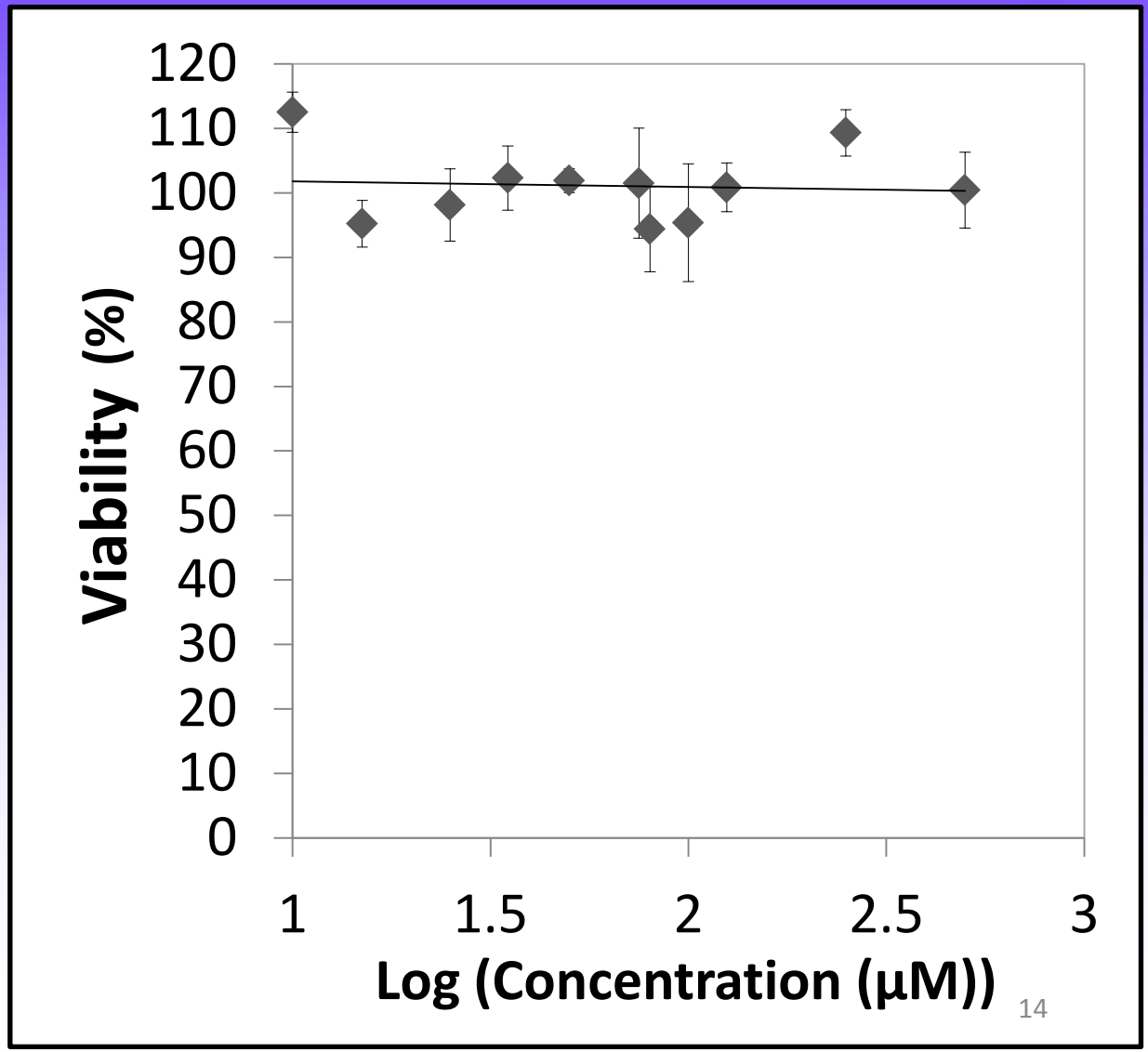
Acetaminophen (AP)

N-acetyl-*p*-quinonimine (NAPQI)

1,4-Benzoquinone (BQ)

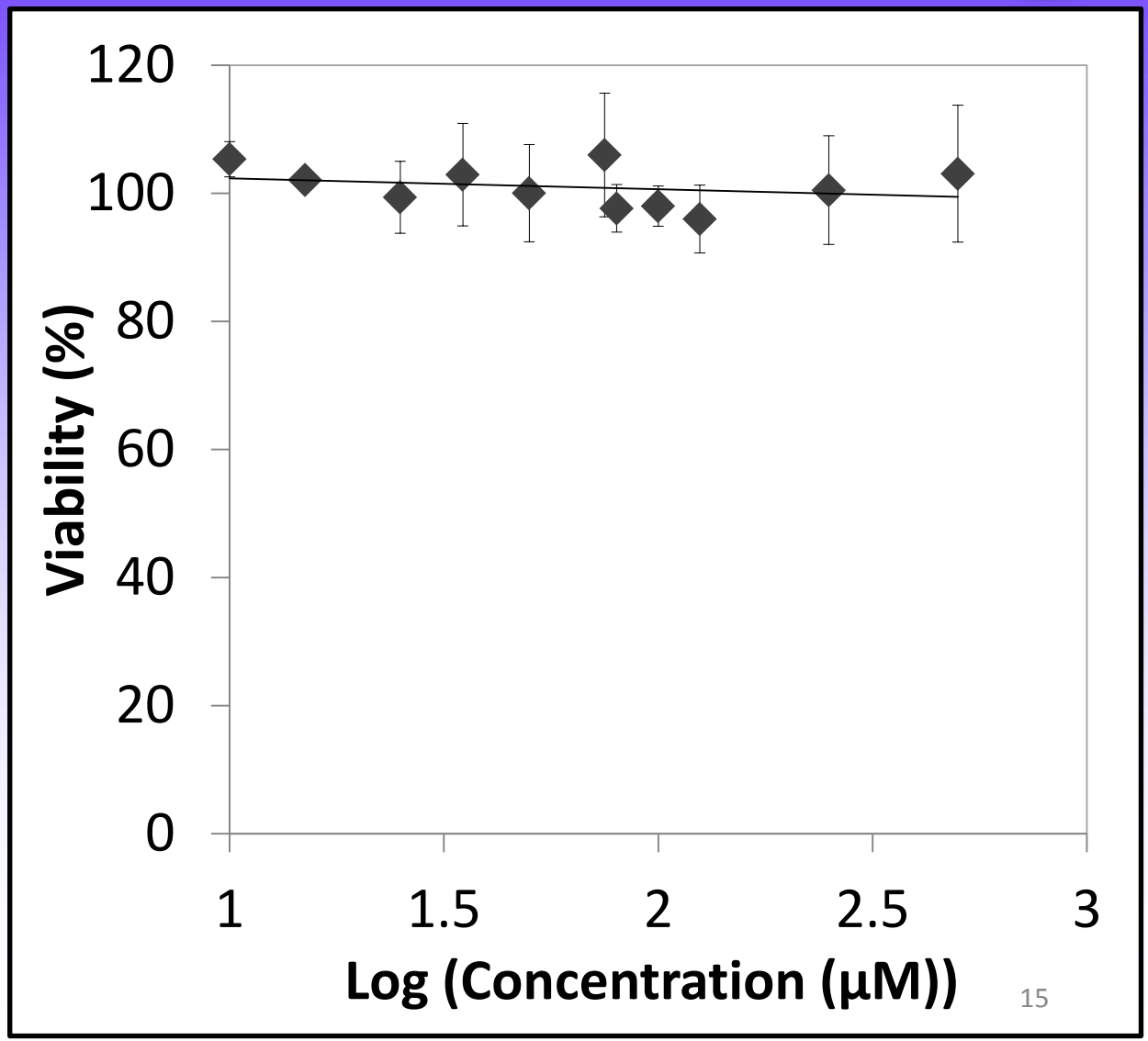
Toxicity: Cyclophosphamide

- CP is **non-toxic** across the concentrations examined
- Mean +/- SD; n=3
- Response relative to untreated controls



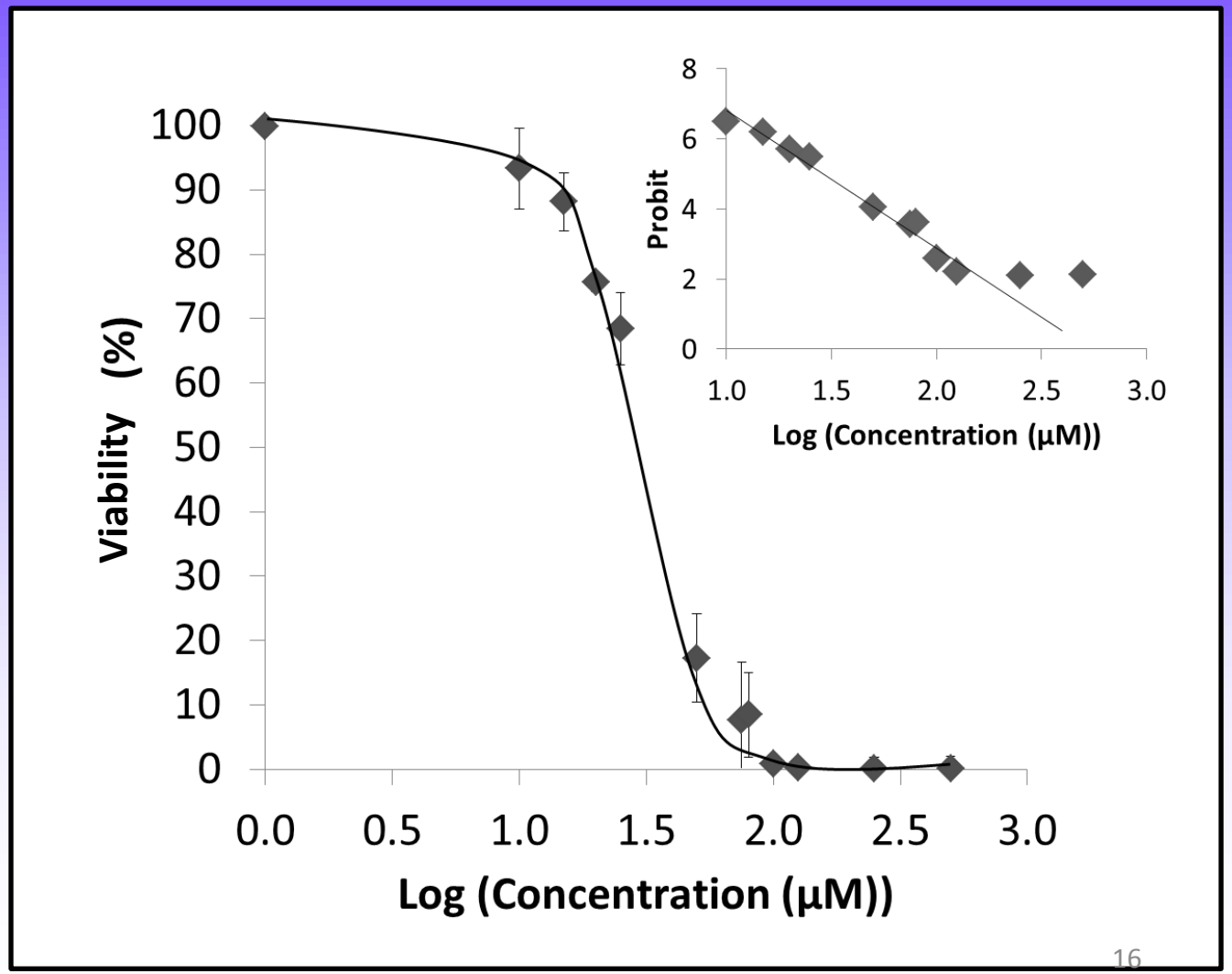
Toxicity: Acetaminophen

- AP is **non-toxic** across the concentrations examined.
- Mean +/- SD; n=3
- Response relative to untreated controls



Toxicity: Benzoquinone (BQ)

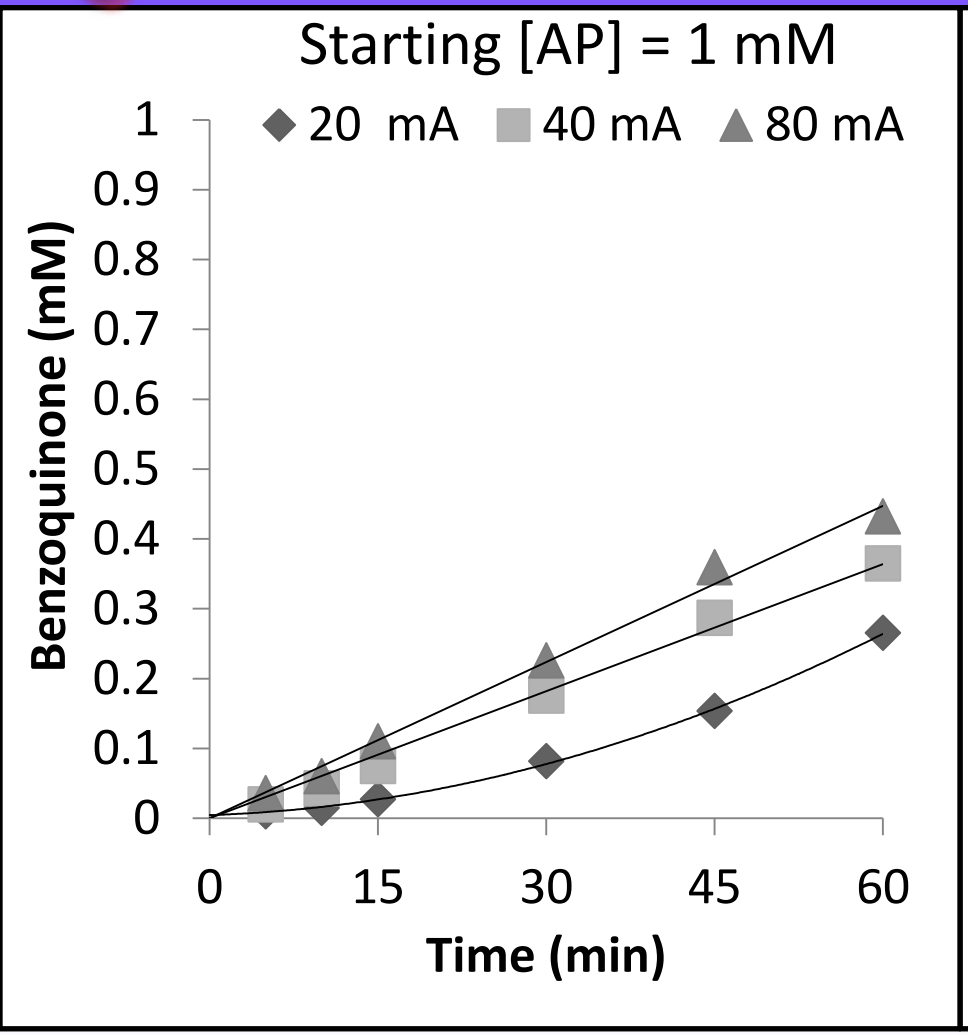
- BQ is **toxic** across the concentrations examined.
- Mean +/- SD; n=3
- Response relative to untreated controls

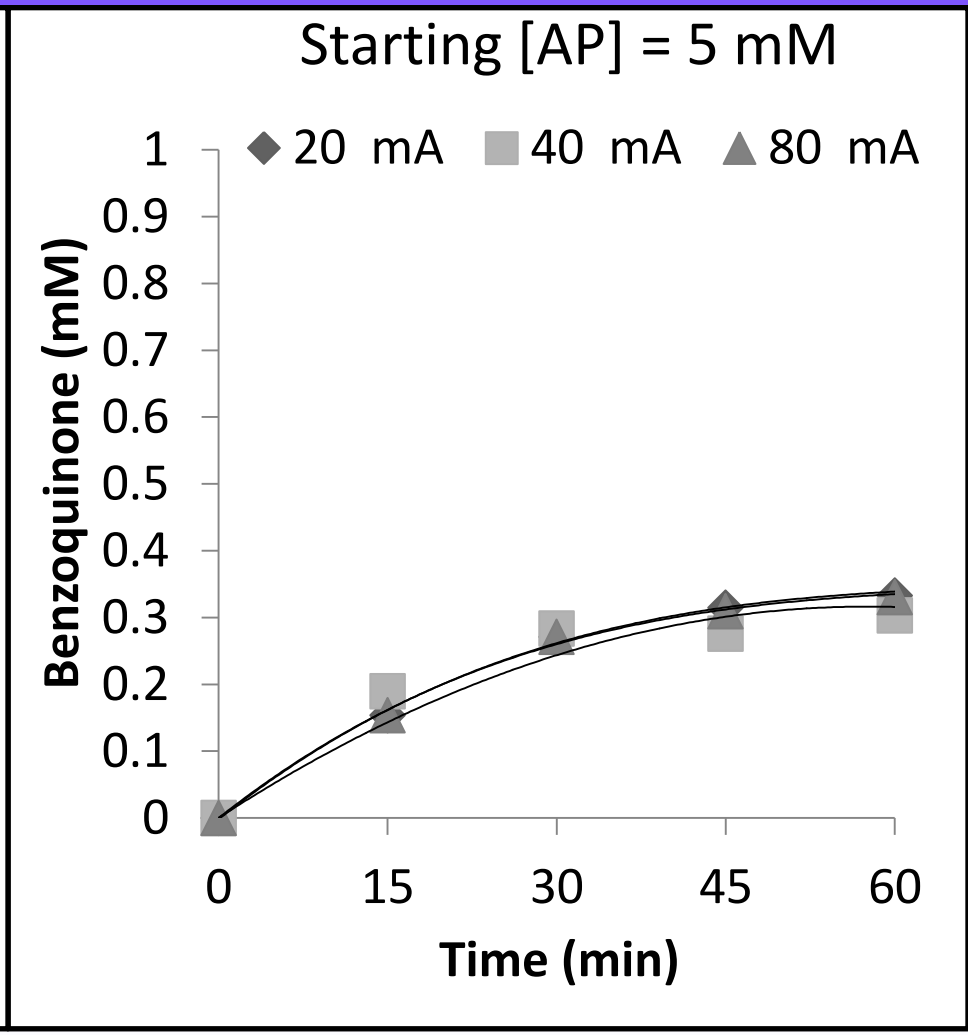


Toxicity: Benzoquinone

- LC_{50}: 30 µM; LC_{99}: 138 µM
- Cytotoxicity assay causes a 3-fold sample dilution to occur; reflective of tumour dilution
- Therefore, electrolyses are required to produce three times the LC_{99}: 414 µM

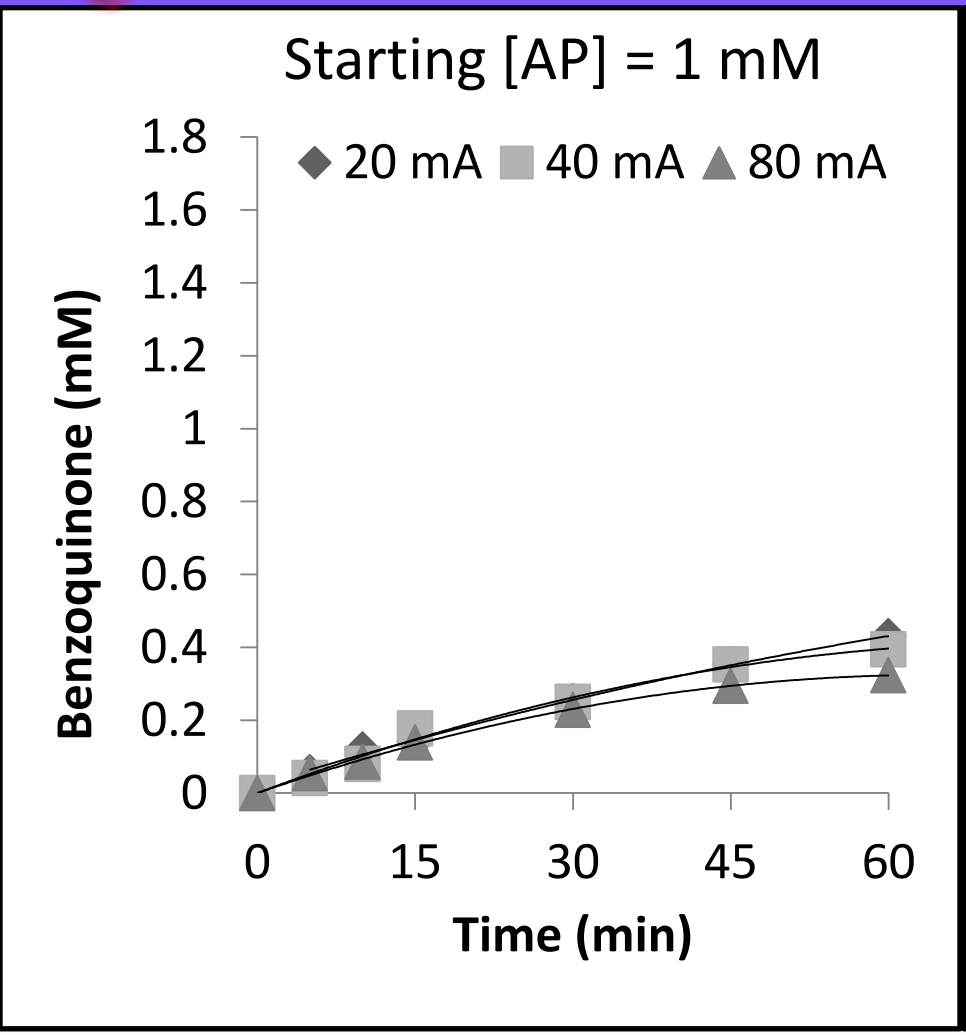
Acetaminophen Electrolysis: Ti/RuO_2

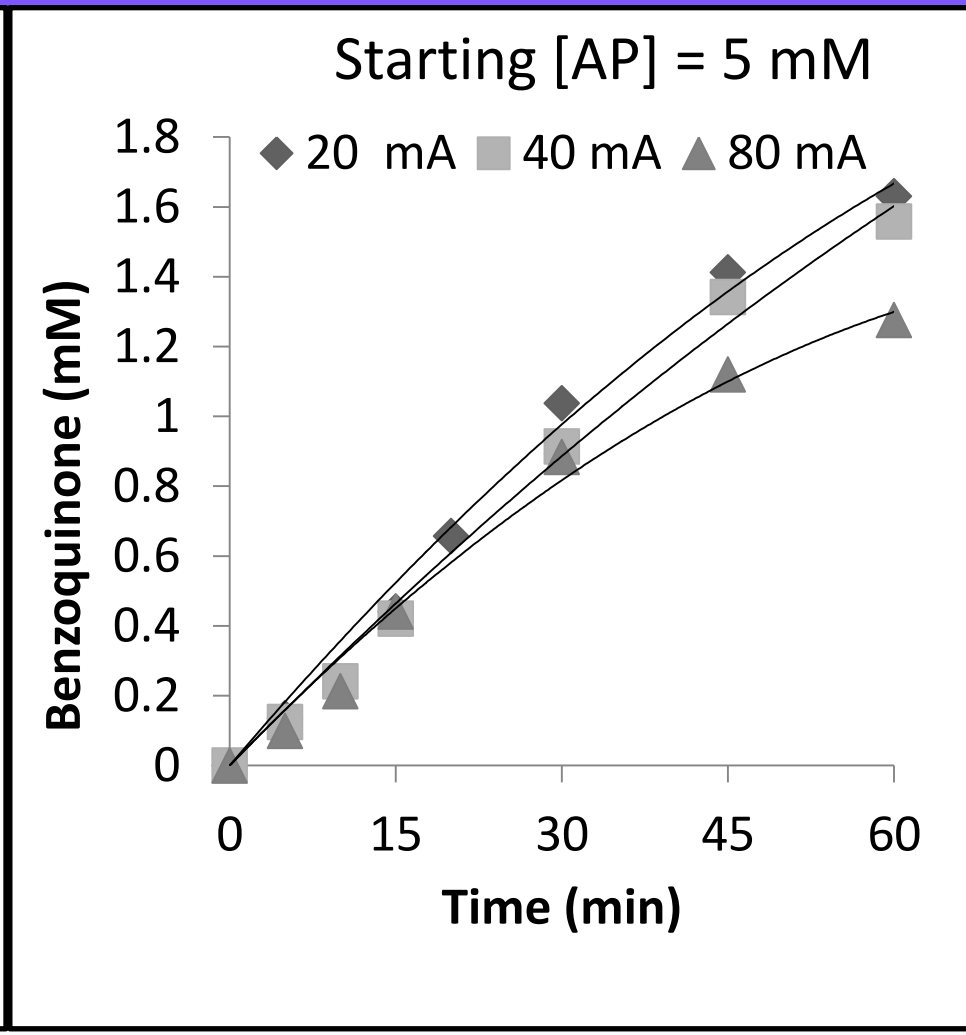




25 mM SO_4^{-2} ; 50 ml Volume; divided batch cell;

Acetaminophen Electrolysis: Graphite





25 mM SO_4^{-2}; 50 ml volume; divided batch cell;

Batch Cell Efficiency (20 mA)

Anode	AP (mM)	LC_{99} Time (min)*	BQ_{max} (µM)§	BQ_{max} Yield (%)¤	BQ Current Efficiency (%)
Graphite	1	57	430	43.0	10.4
	5	11.5	1630	32.6	23.1
Ti/RuO_2	1	> 60	265	26.5	1.4
	5	> 60	330	6.6	2.1

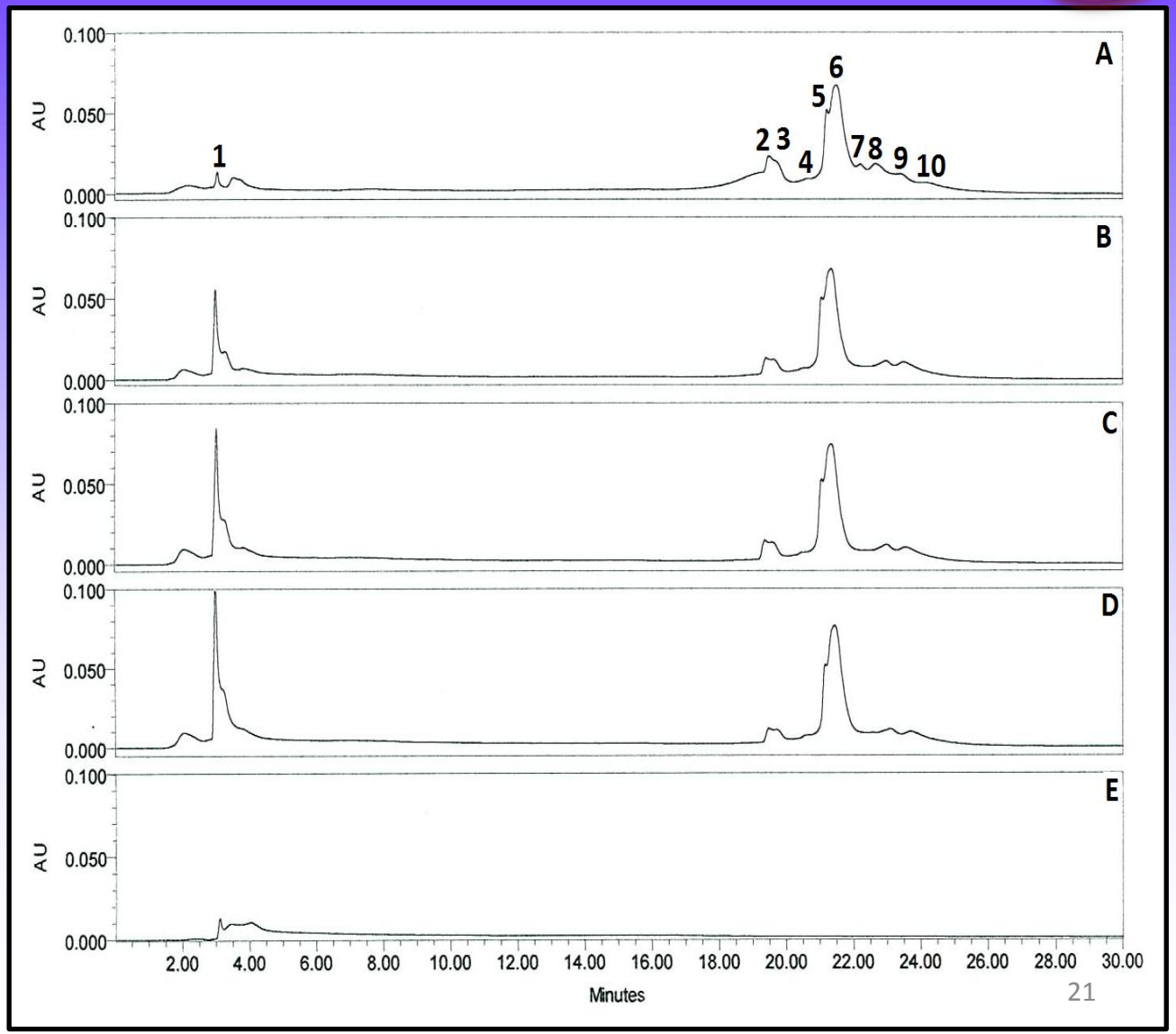
*: electrolysis time required to reach diluted LC_{99} (414 µM)

§: during 60 minutes electrolysis time

¤: BQ concentration as a function of AP lost after 60 min

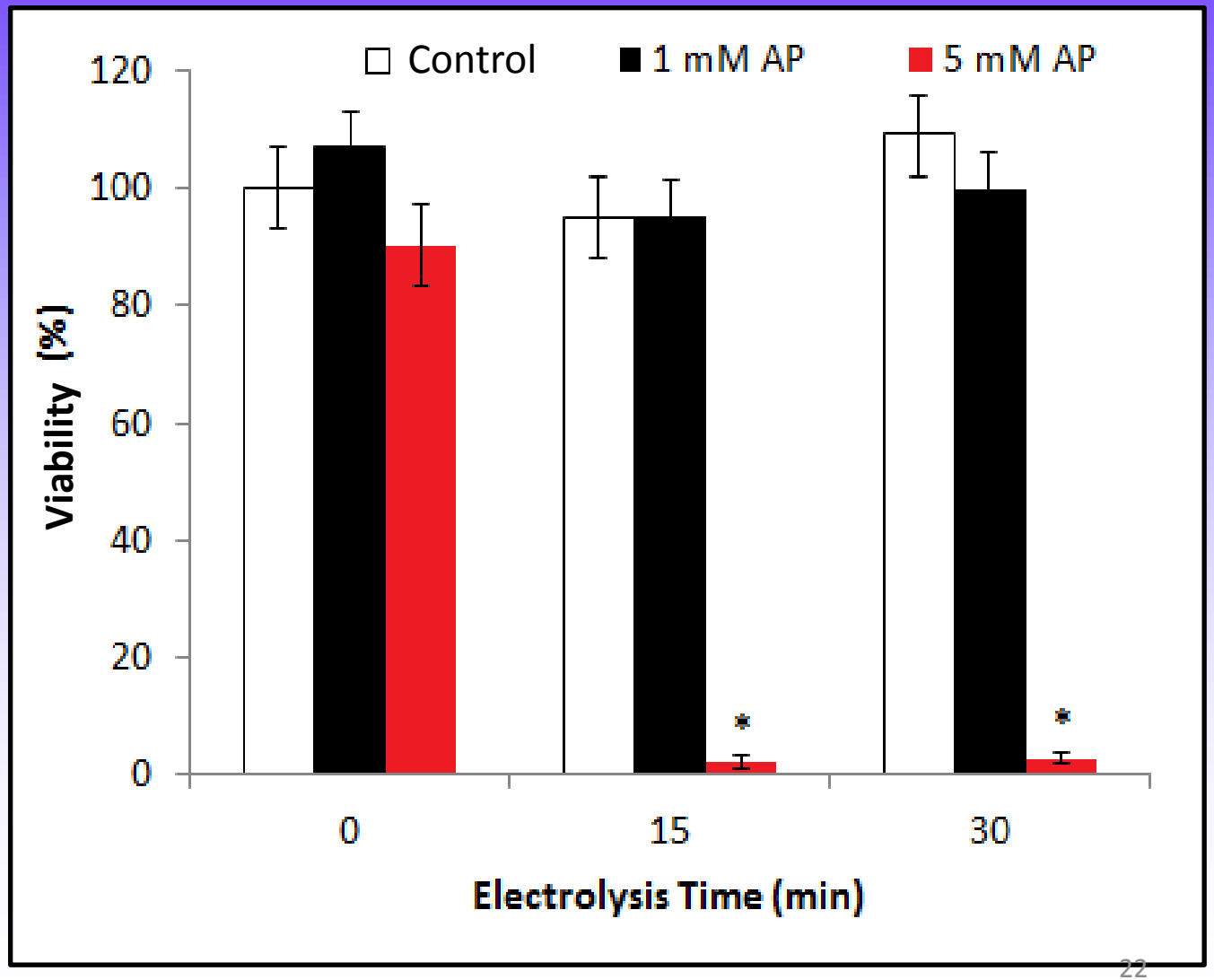
Cyclophosphamide Electrolysis: Graphite

- 500 µM CP, 5 mA
- 30 min. derivatization
- A: t=30;
  B: t=60;
  C: t=90;
  D: t=120;
  E: t=0
- Same results at Ti/RuO_2 (not shown)



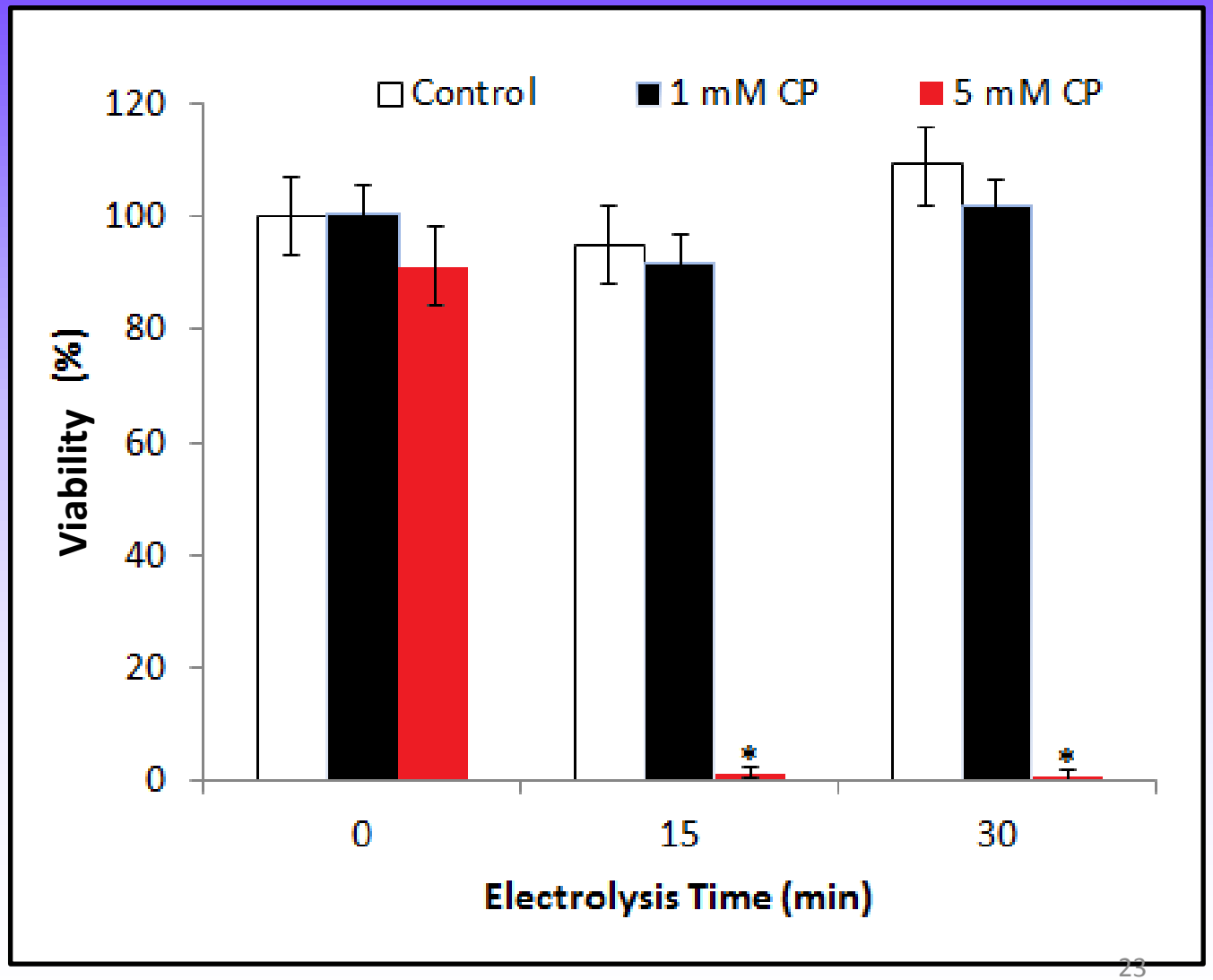
Electrolysis Toxicity: Acetaminophen

- 20 mA; divided batch cell; 25 mM Na_2SO_4
- EMT-6 cell exposure: 24 hours
- Control: 25 mM Na_2SO_4

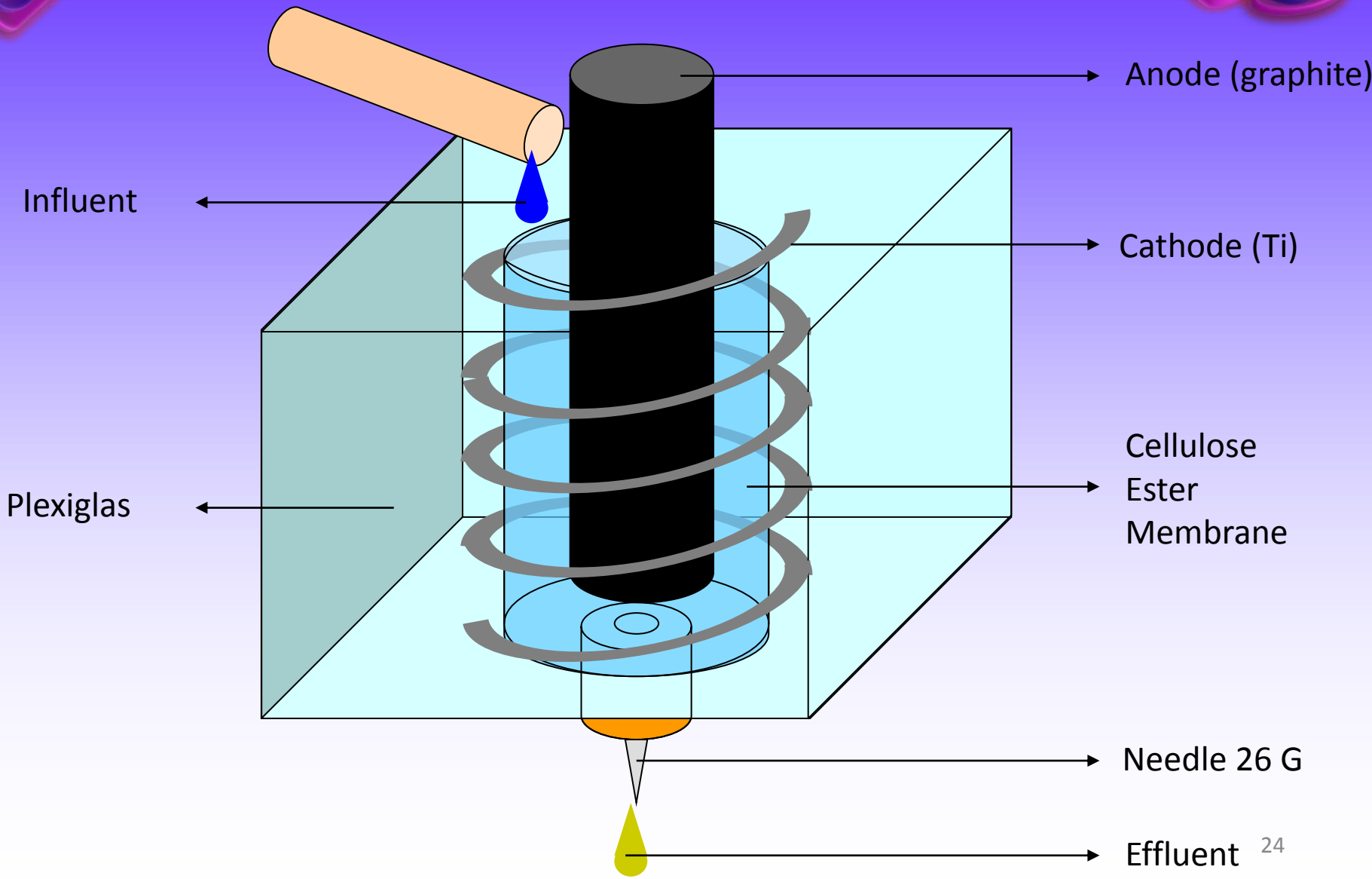


Electrolysis Toxicity: Cyclophosphamide

- 20 mA; divided batch cell; 25 mM Na_2SO_4
- EMT-6 cell exposure: 24 hours
- Control: 25 mM Na_2SO_4

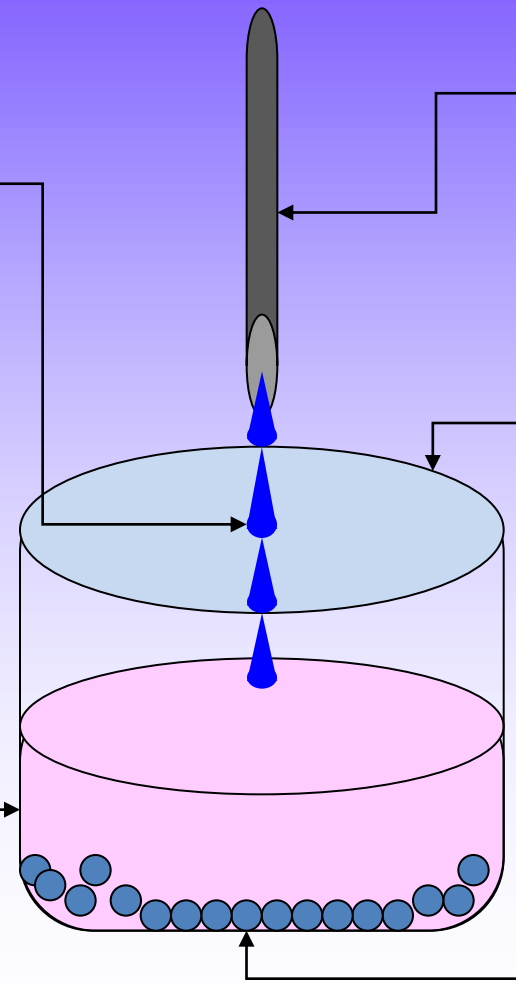


Flow Cell


Anode (graphite)
Influent
Cathode (Ti)
Cellulose
Ester
Membrane
Plexiglas
Needle 26 G
Effluent

Flow Cell: Experiment

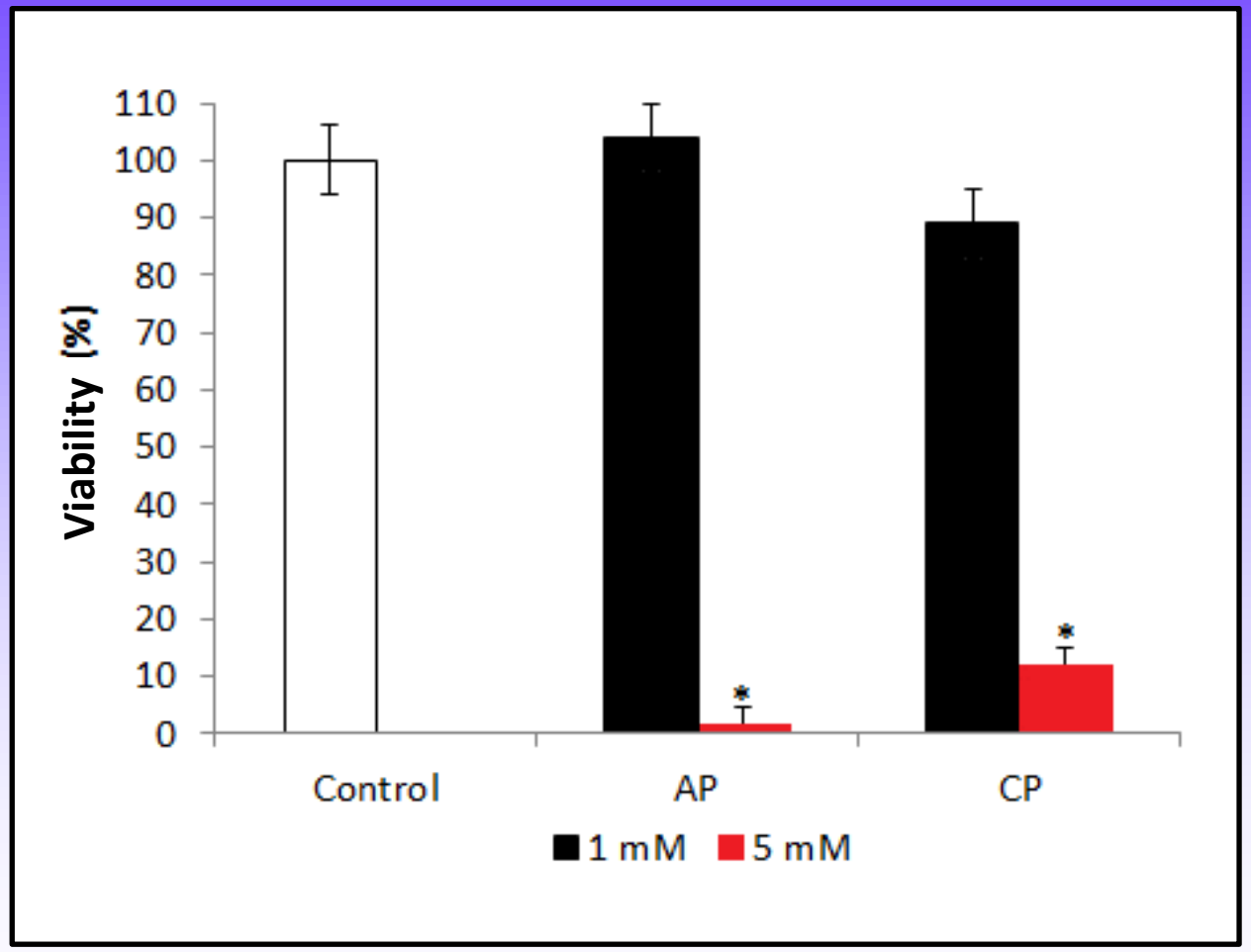
- Prodrug solution +/- electrolysis
- Growth medium



- 26 G needle
- Well
- EMT-6 cell monolayer

Flow Cell: Toxicity

- 5 mA with graphite
- 1 hr electrolysis time – steady state
- 230 µl/min flow rate
- Control: 25 mM Na_2SO_4



Flow Cell: Design Considerations

- Minimum pressure of 18 mg/mm^2 required for flow
- Tight spaces prone to gas bubble accumulation → flow stoppage
- High current densities decompose graphite anode → flow stoppage

Summary

- Unelectrolyzed AP and CP are non-toxic to EMT-6 cells
- Both graphite and RuO_2 anodes are capable of oxidizing CP in aqueous solutions
- Graphite oxidizes AP more effectively than RuO_2
- Constructed flow cell is capable despite limitations

Summary

Electrolyzed solutions produce significant toxicity compared to prodrugs!

Future Works

1. Assess conceptual feasibility *in vivo*
   - How does it compare to standard treatments?
2. Reconsider flow cell design
   - Address ease of use; integration in to existing medical technology (I.V.)
3. Investigate suitable toxicants
   - Are CP and AP the best compounds?

Acknowledgements

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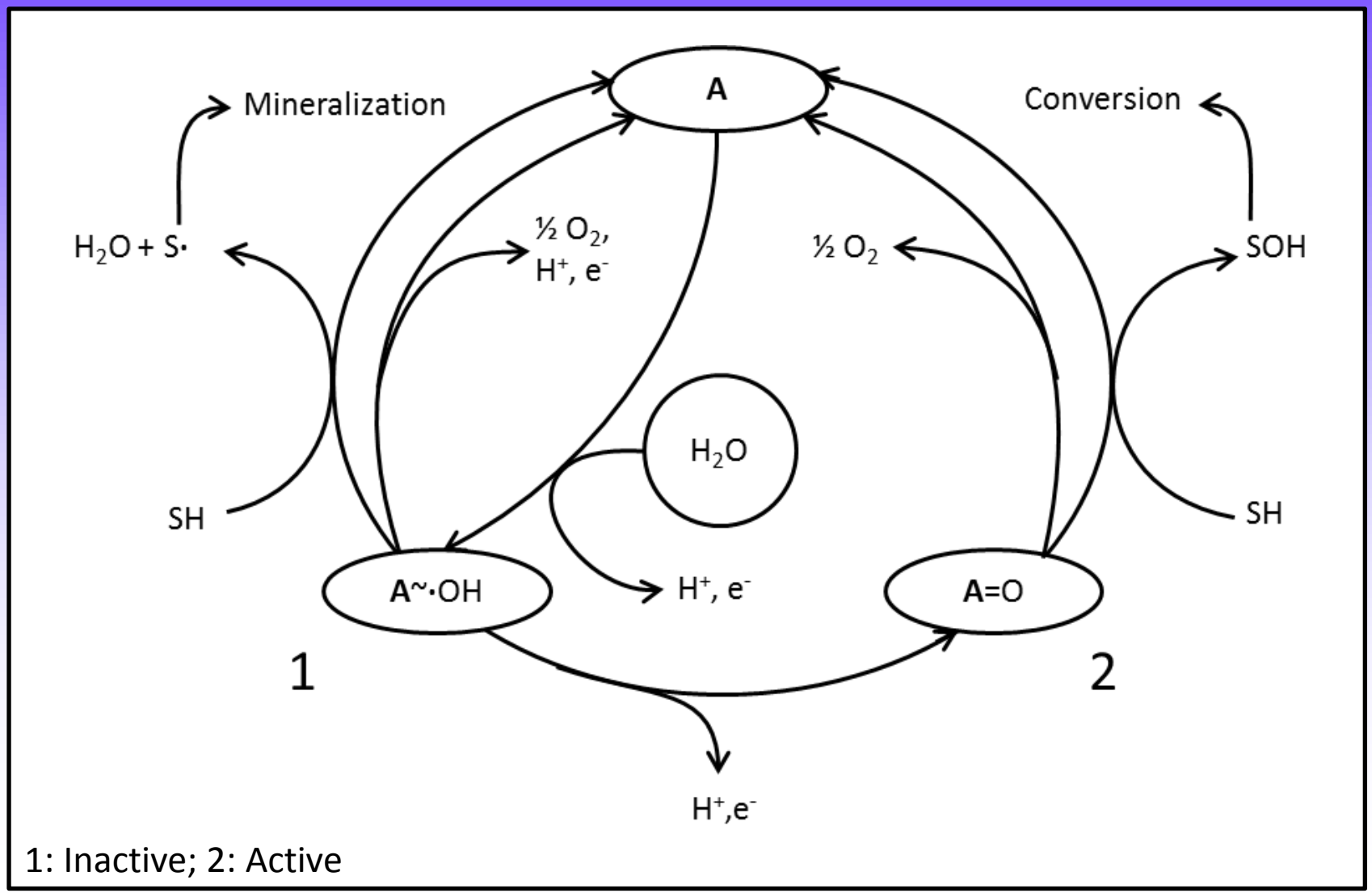
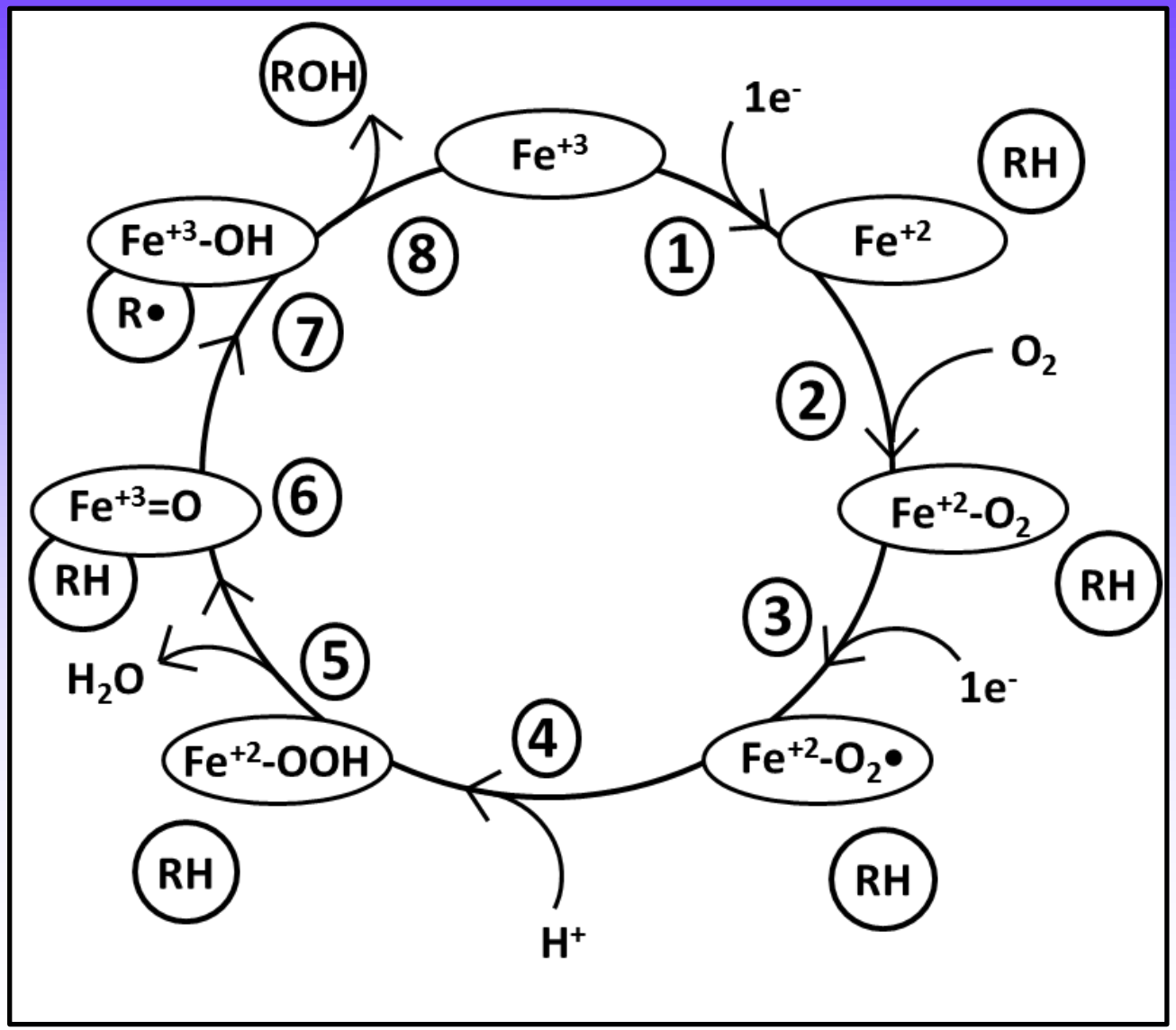
- Examination committee
  - Dr. France-Isabelle Auzanneau
  - Dr. Mark Baker
- NSERC

Anode H_2O Mechansim

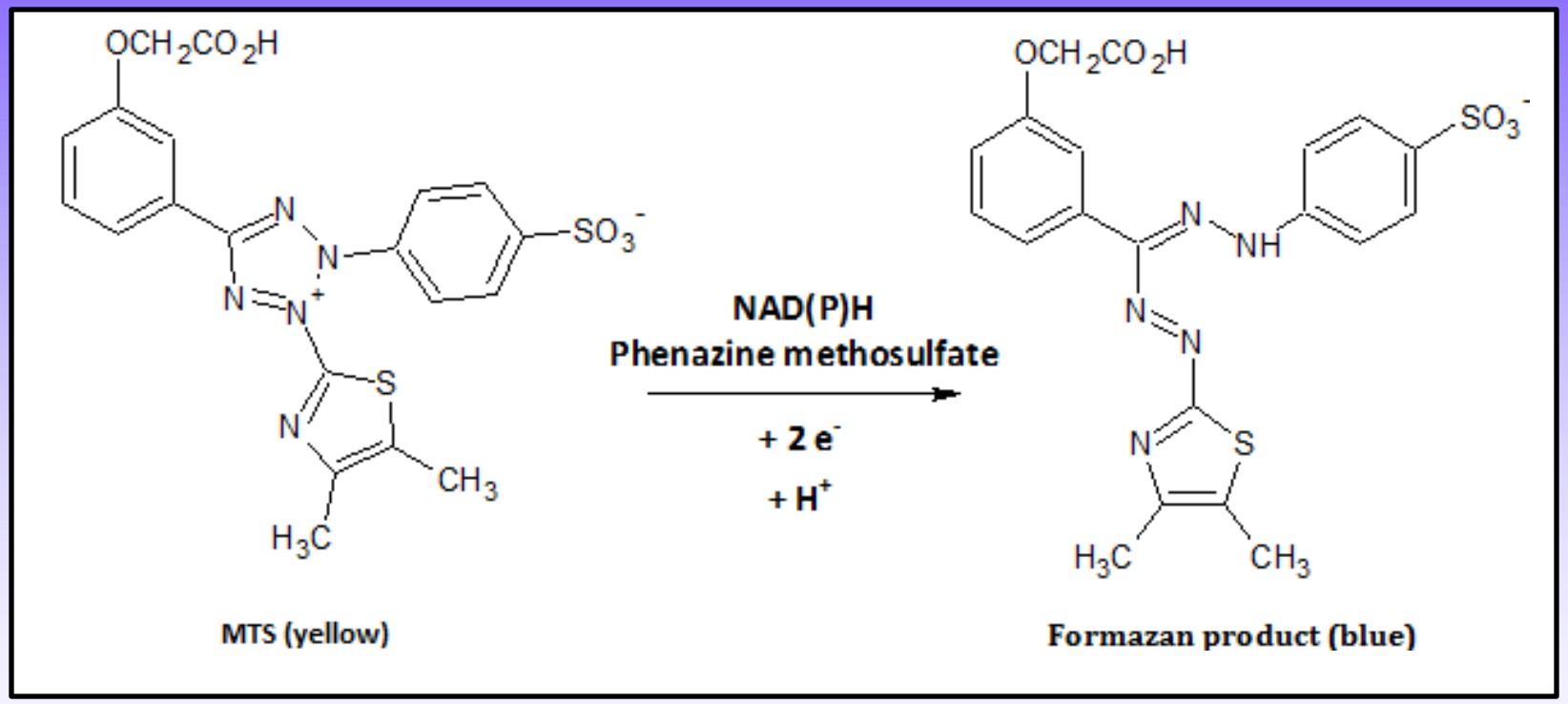


1: Inactive; 2: Active

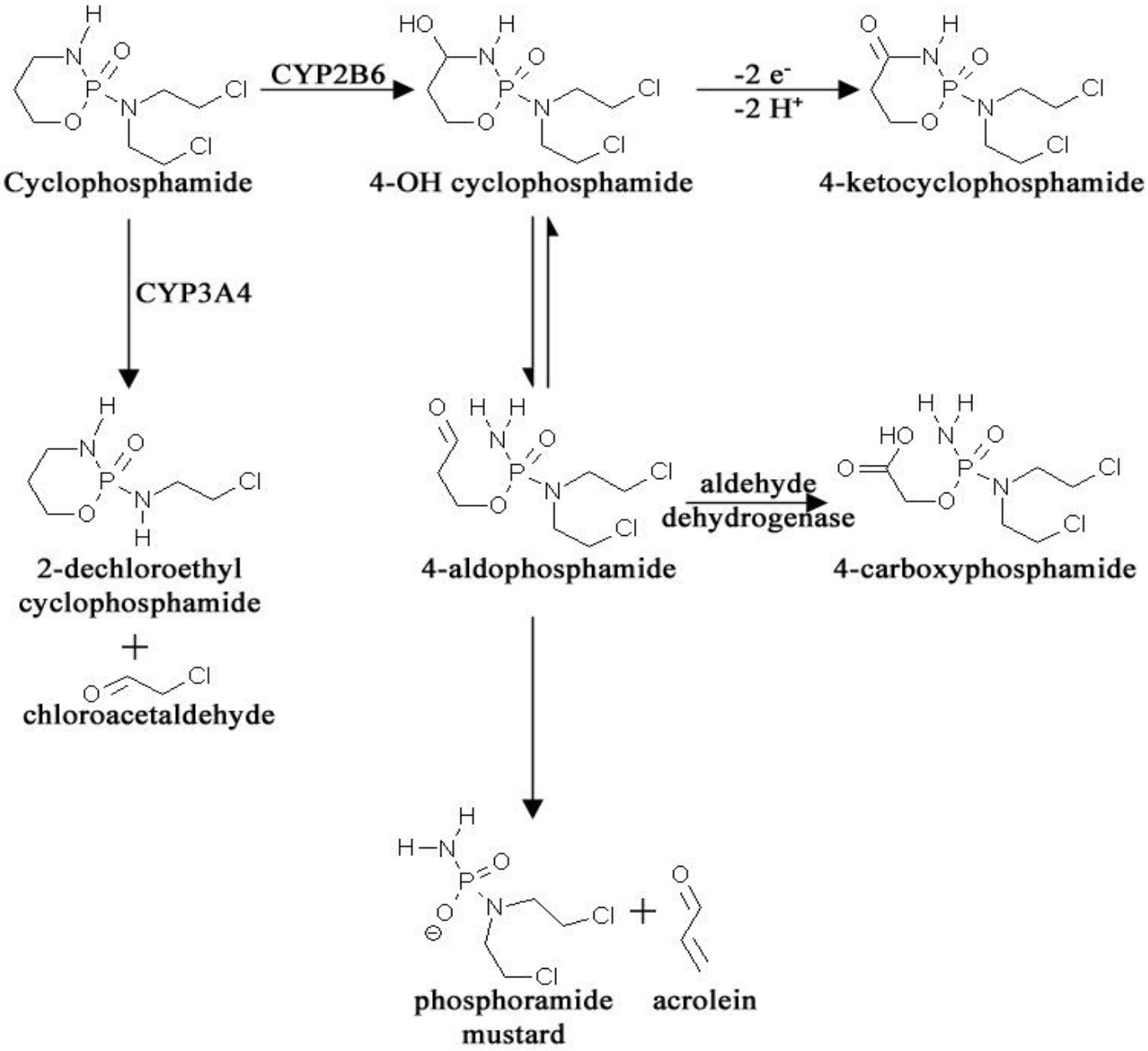
P450 Catalysis



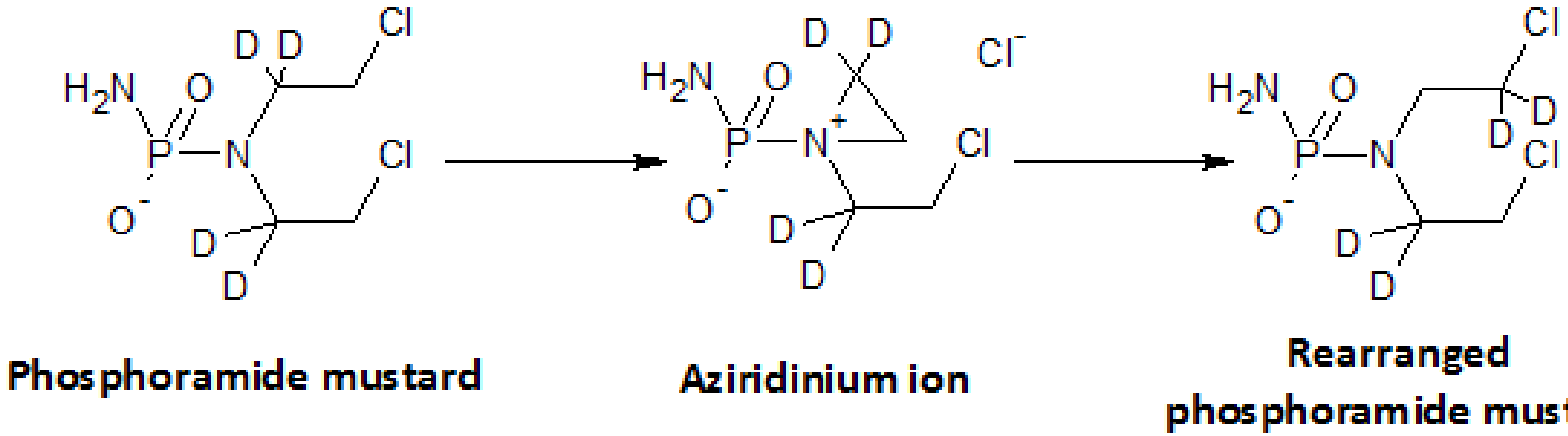
MTS Dye



3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium, inner salt,


Cyclophosphamide
CYP2B6
4-OH cyclophosphamide
-2 e⁻
-2 H⁺
4-ketocyclophosphamide
CYP3A4
2-dechloroethyl cyclophosphamide
+
chloroacetaldehyde
4-aldophosphamide
aldehyde dehydrogenase
4-carboxyphosphamide
phosphoramide mustard
+
acrolein

CP Mechanism



- Here Cl^- = nucleophile