DEVELOPMENT OF IMPROVED DTPA FOR RADIONUCLIDE CHELATION:

TRANSPORT ENHANCERS

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Medical Countermeasures against Nuclear Threats: Decorporation Agents, MD, September 17-18, 2007

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Need: Oral Treatment for Radionuclide Exposure

Am

Cm

Pu

Post 9/11 concerns

- increased risk of intentional exposure to trans-uranium radio-nuclides, e.g., “dirty bombs”
- Existing risk of accidental exposure from nuclear power plant mishap
- Need readily available treatment

One Existing Treatment

- Ca- & Zn-DTPA: Only approved product to treat exposure
  - NDA held by Hameln Pharmaceutical, Germany
- Drug treatment can span months or even years
  - Injectable formulation only
  - Impractical for widespread exposure or self-administration
  - Low oral bioavailability with present formulation (<5%)

Need an oral formulation of Ca- & Zn-DTPA

- Can be easily stockpiled for emergency use
- Easy to disseminate to exposed population
- Easy self-administration

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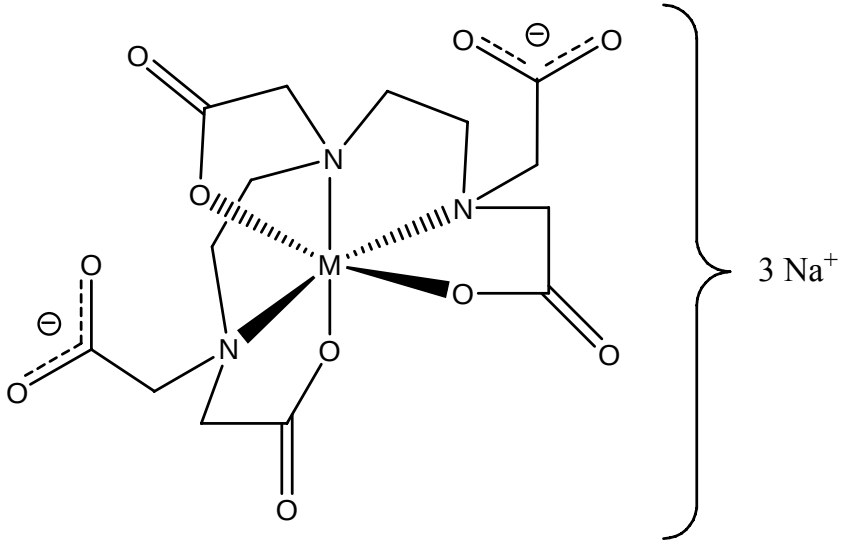
Problem: DTPA has VERY POOR ORAL BIOAVAILABILITY

Due To Poor Absorption

DTPA is a classic Class III Biopharmaceutics Classification System (BCS) Drug

- Low octanol/aqueous partitioning
- Presence of strongly charged functional groups
- High molecular weight
- High polar surface area
- Substantial number of hydrogen bonding functional groups



M = Zn, Ca

Trisodium zinc (or calcium) diethylenetriaminepentacetate

Poorly **A**bsorbed
Distributes rapidly after IV
Is not **M**etabolized
Eliminated rapidly in urine

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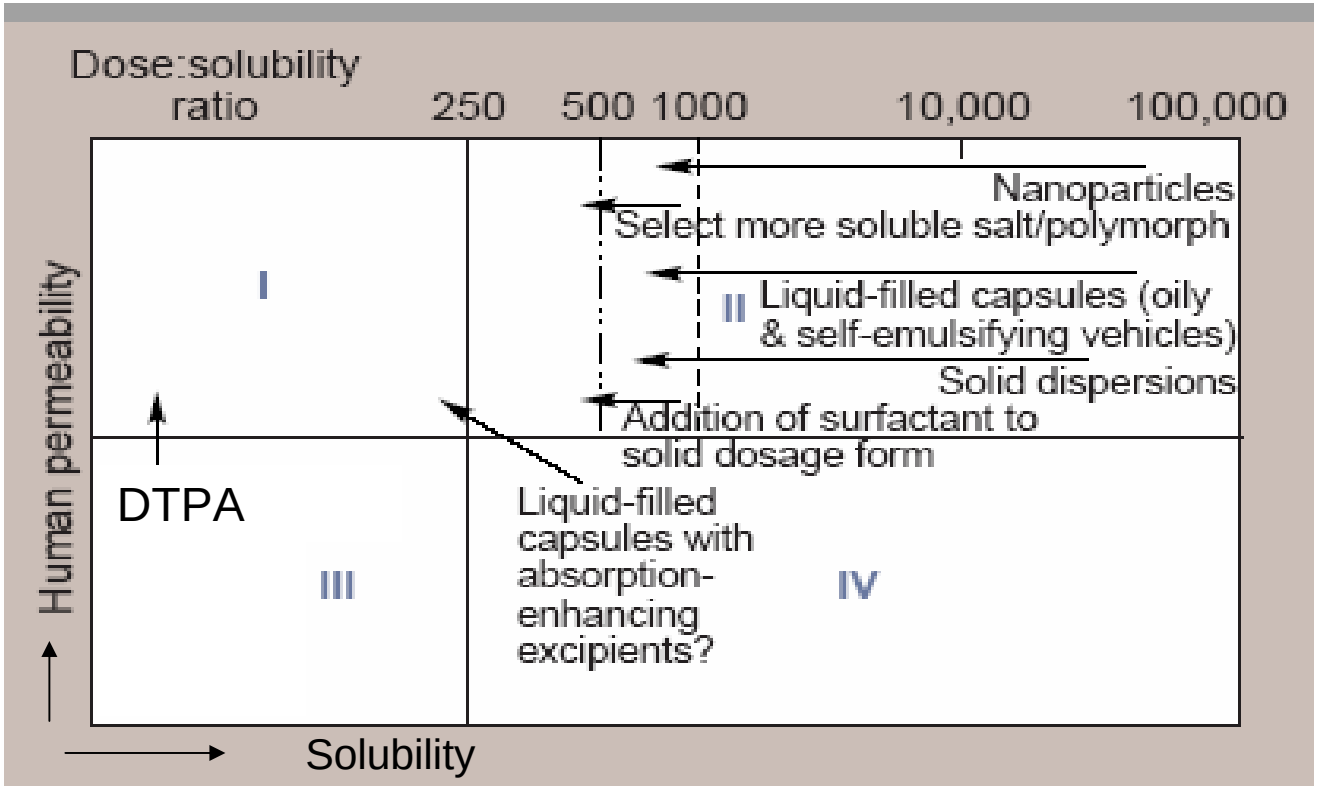
To improve druggability via formulation

Biopharmaceutics Classification System are:

- **Class I: High solubility, high permeability**
- **Class II: Low solubility, high permeability**
- **** Class III: High solubility, low permeability***
- **Class IV: Low solubility, low permeability**

DTPA will be formulated to move it from Class III to Class I



Reference: Jennifer Dressman et al., Pharmaceutical Technology, 2001, pp 68-76

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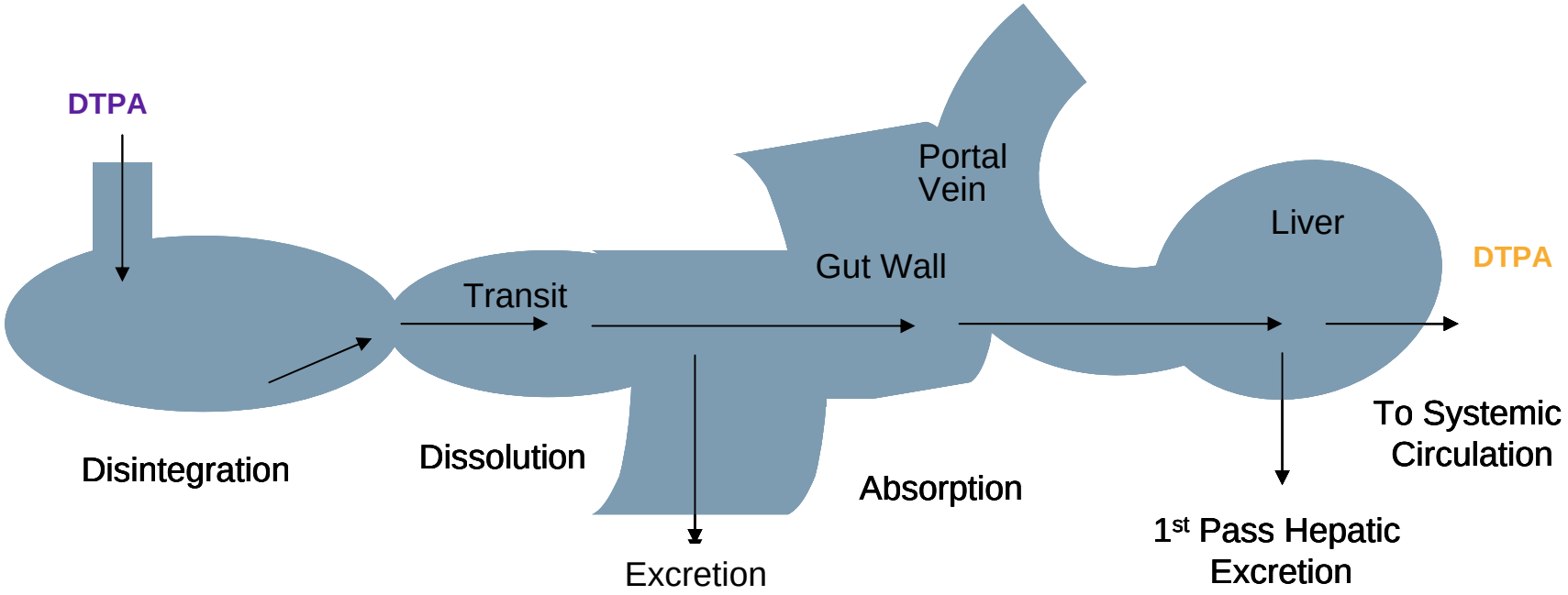
Oral Absorption Process

The amount into systemic circulation is the fraction of drug intact after going through these processes.



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Making DTPA Orally BioAvailable

Original PO bioavailability in dogs ~5% in vivo
SRI formulation PO bioavailability in dogs and rats~30% in vivo
Successfully demonstrated decorporation of Am-241 from rats

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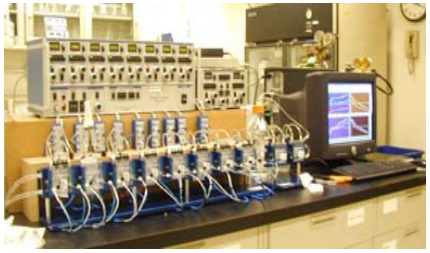
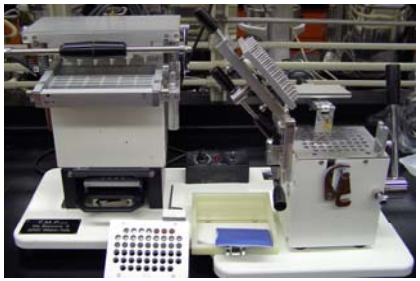
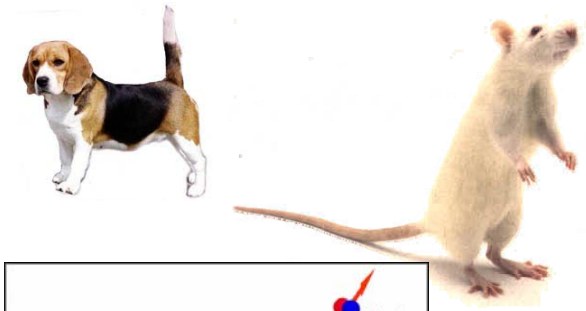
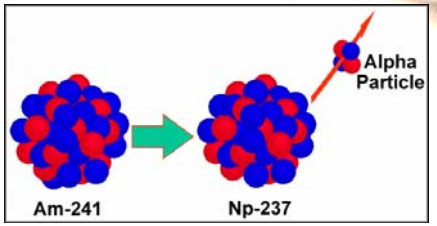
SRI's Approach









1. In Vitro Experiments

- Used in vitro method to identify the site of absorption of pure drug in the intestinal tract
- Screened many conditions and formulation components in vitro to identify best formulations/enhancers

2. Develop Dosage forms

- Capsule (Size 0) formulations including liquid drug-enhancer as well as enhancer containing drug beads were developed
- Enteric coating of capsules was optimized
- Developed equivalent mini-capsules (Size 9) to administer to rats
- Developing appropriate tablets for improved storage stability

3. In Vivo Studies

- Screened several formulations in rats. More replicates possible in rats and confirms in-vitro results.
- Beagles dogs were administered drug in capsules, like humans.
- Minicapsules to rats

4. Decorporation Experiments

- Demonstrate decorporation of ^{241}Am from male rats by administering enteric coated minicapsules containing CaDTPA and ZnDTPA.
- Estimate ^{241}Am in excreta, blood, liver, lungs, spleen and kidney

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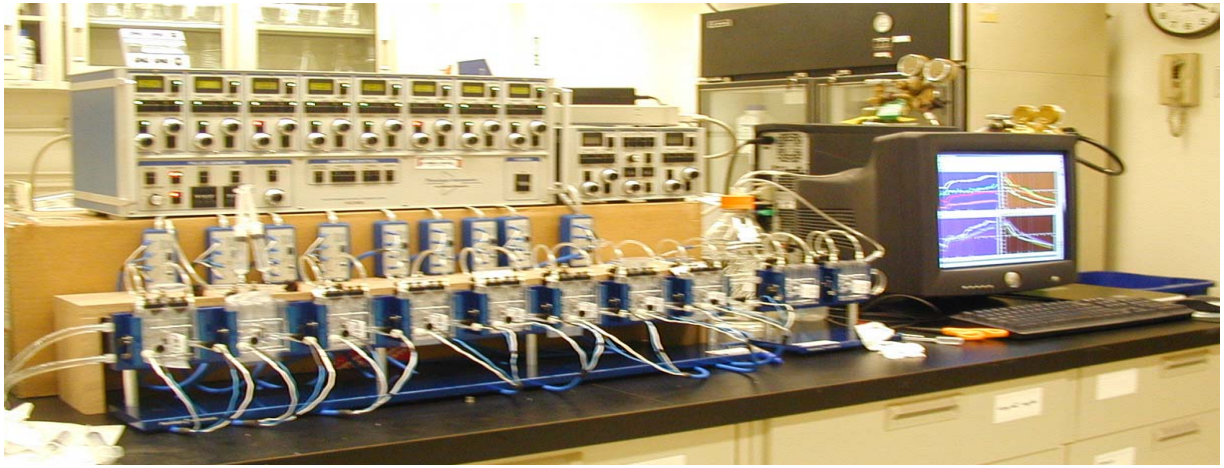
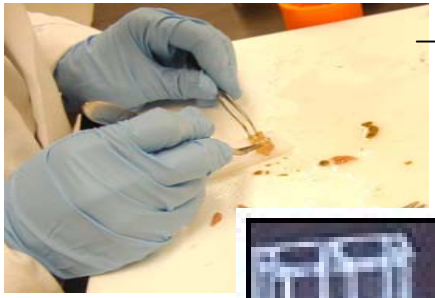
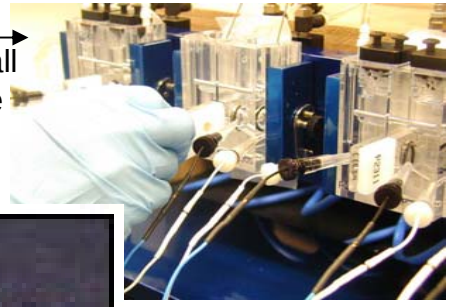
In Vitro Transport Studies for DTPA

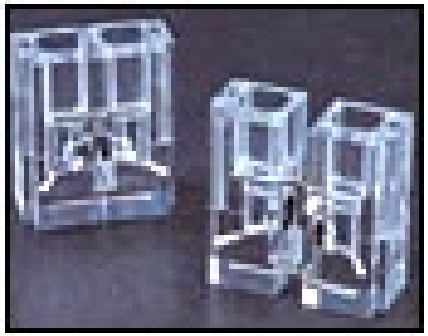


Ussing chambers hold many tissue sections from single rat



Rat small intestine





Measurable
Mucosal/Serosal transport
M to S/S to M

Living Tissue:
TEER values
Physiological conditions
Oxygen

Tissue type/size/replicates

Buffer compositions

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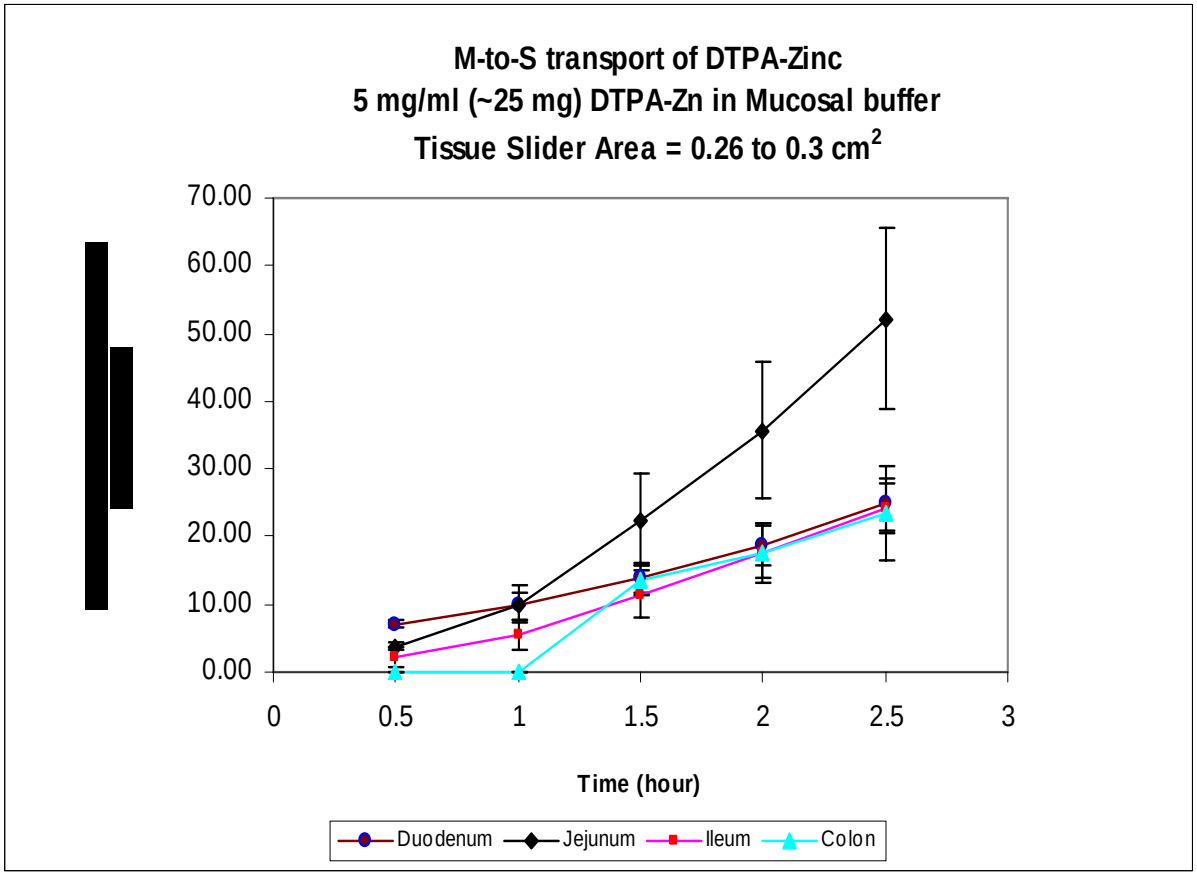
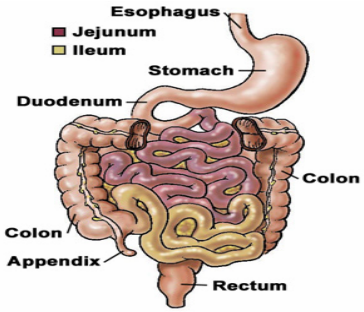
Jejunum is the site of maximum transport

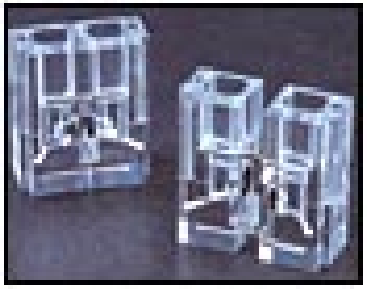
Duodenum **Jejunum** **Ileum** **Colon**



(Data in the absence of permeation enhancer)



$$Papp = \frac{dQ / dt}{C0 \times A}$$



PLAN: To study the effect of enhancers on M-to-S transport

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P_{app} Values for "Transport" of Drug using Different Permeation Enhancers Measured in Rat Jejunum Identify Best Formulations

FORMULATION	Papp (x10-6 cm/sec)	Std. Dev. (x10-6)
Enhancer A	4.63	0.41
Enhancer B	**6.29**	**0.77**
Enhancer C	5.43	1.08
Enhancer D	**11.76**	**1.76**
Enhancer E	11.61	2.05
Enhancer F	6.57	0.96
Enhancer G	7.27	0.97
Enhancer H	**14.04**	**4.45**
Enhancer I	7.85	0.67
Enhancer J	12.65	2.33
Enhancer K	2.83	0.35
Enhancer L	2.75	0.41
Enhancer M	3.61	0.29
Enhancer N	**13.51**	**0.69**

$$Papp = \frac{dQ / dt}{C_0 \times A}$$

where dQ/dt is the linear appearance of mass in receiver compartment, C_0 is the initial solute concentration in donor compartment and A is the surface area

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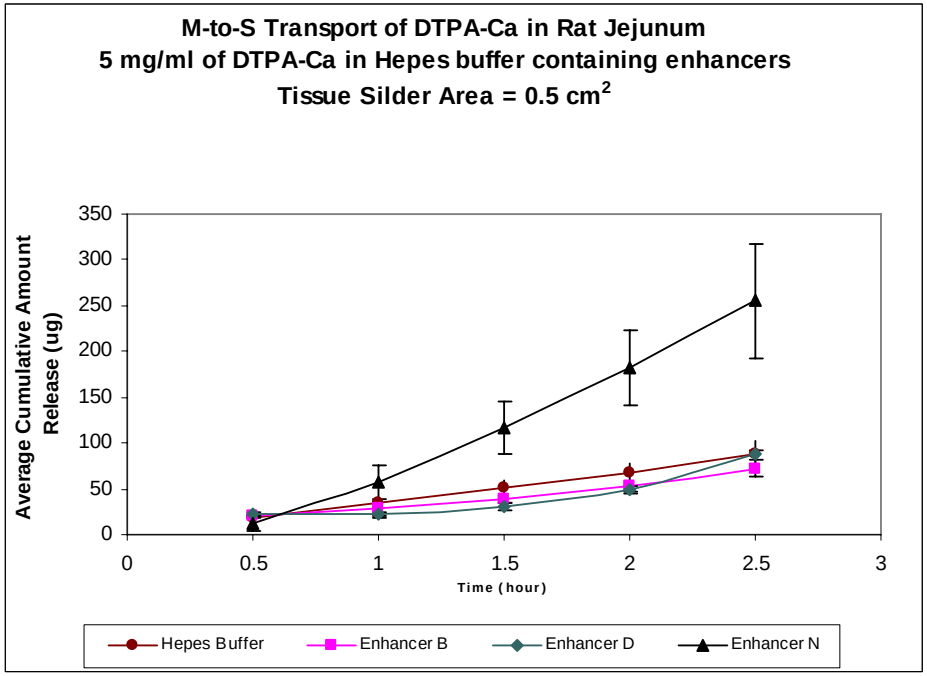
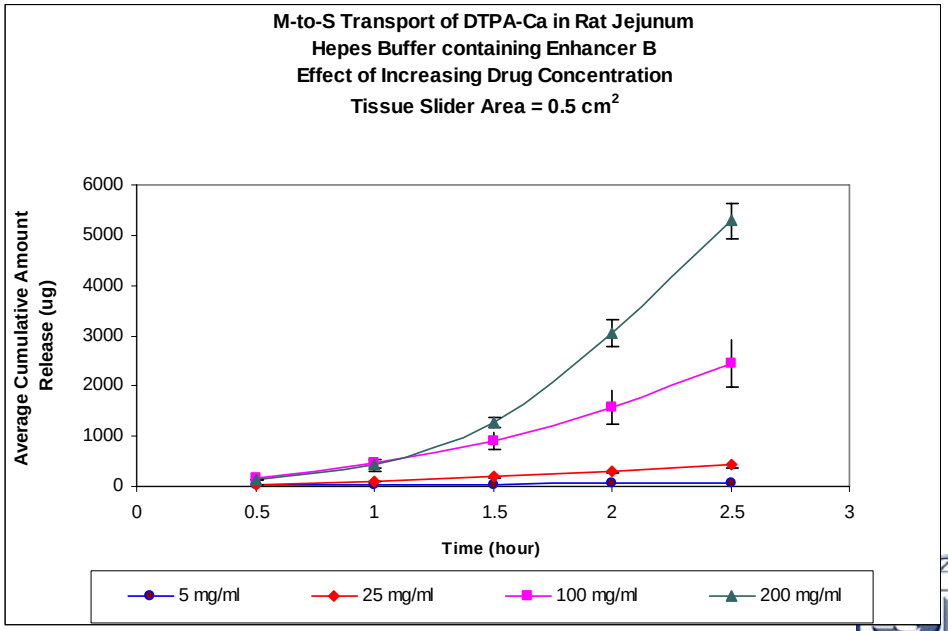


At low drug loadings, Increasing enhancer levels increases the rate of transport

At constant enhancer levels, increasing drug concentration results in increase in transport



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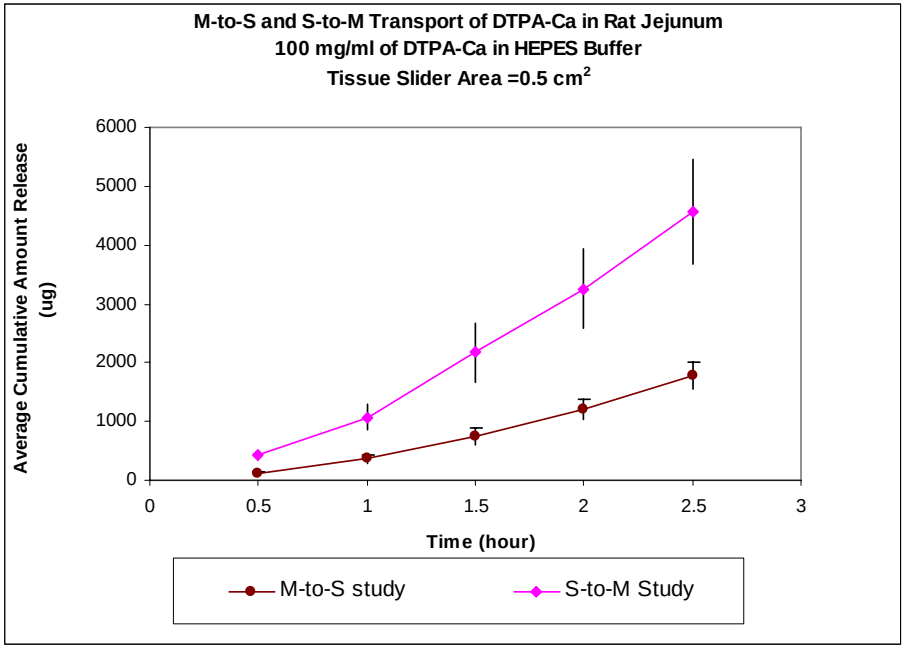
Is DTPA a P-gp Substrate?



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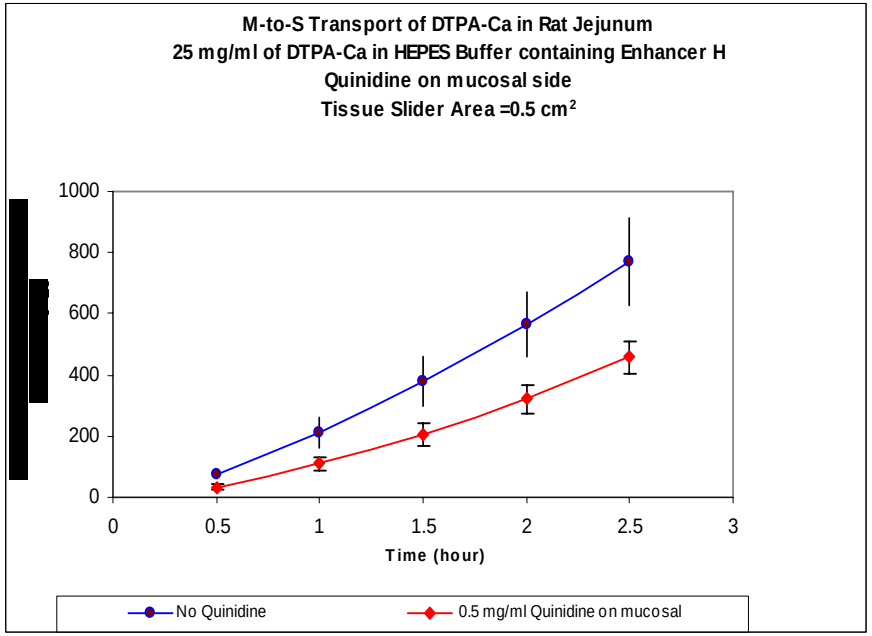
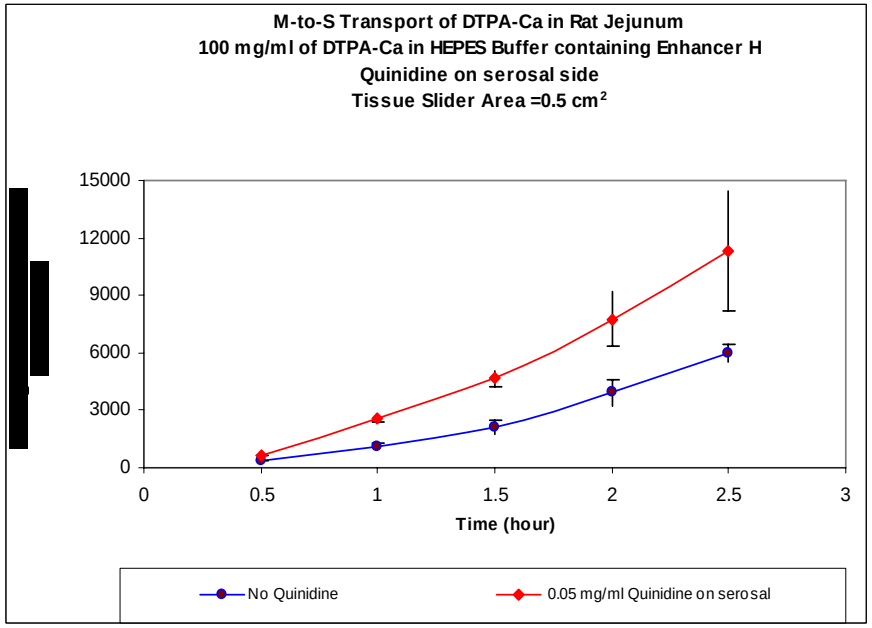
Effect of P-gp Inhibitor (Quinidine) on transport





Inhibitor on Mucosal side

Inhibitor on Serosal side

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In-vivo Effect of Enhancers on DTPA transport: Rat Study

- Radioactive (C-14) DTPA included in doses administered
- Male Sprague Dawley rats, administered DTPA-Ca doses, 20 mg/kg (~200 µCi/kg)
- One iv and six oral gavage treatment groups, n = 3 rats/group
- Blood was collected and processed to plasma at several time points
  - IV dose groups: 5, 10, 20, 30 min, and 1, 2, 3, 9, and 24 hours post-dose.
  - Oral dose groups: 15, and 30 minutes, 1, 2, 4, 6, 8, 10 and 24 hours post-dose.
- Total radioactivity was determined by liquid scintillation counting

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Effect of Enhancer on Oral Bioavailability of DTPA in Rats (20mg/kg)

DTPA	$AUC_{0\rightarrow last}$ (hr*ng/ml)[a]	Mean +/-SD	Bioavailability	Mean +/-SD
Group 1 IV CaDTPA	17.3	17.73 +/- 0.63	100	100
	18.5			
	17.4			
Group 2 Oral CaDTPA	1..24	1.22 +/- 0.05	7.00	6.87 +/- 0.27
	1.16		6.56	
	1.25		7.06	
Group 3 Oral Free acid DTPA	1.47	1.21 +/- 0.29	8.29	6.80 +/- 1.66
	0.89		5.02	
	1.26		7.10	
Group 4 Oral Enhancer D CaDTPA	1.69	2.15 +/- 0.41	9.53	12.14 +/- 2.29
	2.31		13.05	
	2.45		13.82	
Group 5 Oral Enhancer B CaDTPA	1.47	1.26 +/- 0.24	8.30	7.12 +/- 1.37
	1.00		5.62	
	1.32		7.43	
Group 6 Oral Enhancer H CaDTPA	1.35	1.40 +/- 0.15	7.62	7.90 +/- 0.84
	1.28		7.24	
	1.57		8.85	
Group 7 Oral 0.15mM Qunidine *then* Enhancer D CaDTPA	3.39	1.81 +/- 1.39	19.10	10.23 +/- 7.82
	1.30		7.28	
	0.77		4.32	

Optimum enhancer →

Pgp Inhibition ▸

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Roadmap for Solid Dosage (Capsule) Development

Developed Size 0 (Adult) Coated Capsules

- Confirmed resistance to disintegration in acid media
- Assay, dissolution
- Tested bioavailability in Beagle Dogs
- Performed accelerated stability studies
- Minicapsules: same proportions and coated
  - Assay, dissolution, integrity of coating
  - Bioavailability in Rats
  - Decorporation Efficiency in Rats

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Capsule Formulations

Stability?

I. Liquid Filled Capsule Formulation

Each Capsule (size “0”) contains:
DTPA Ca/Zn
Enhancer D

Efficacy

II. Bead Filled Capsule Formulation

Each Capsule (size “0”) contains:

DTPA Ca(Zn)
Enhancer D

ALL CAPSULES WERE ENTERIC COATED

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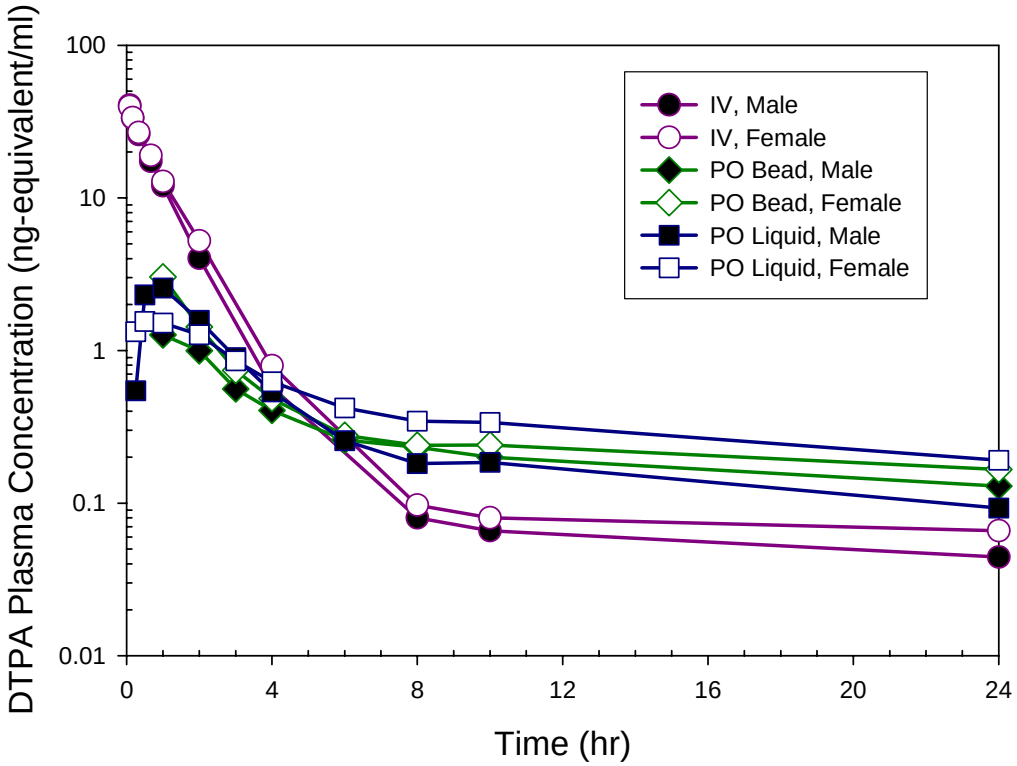
Plasma Levels of ^{14}C-DTPA in Beagle Dogs (10mg/kg) – Bead and Liquid Capsule Formulations



Formulation	Dog		Route	C_{max} (ng/ml)	AUC (hr*ng/ml)	$t_{1/2}$ (hr)	F (%)[a]
Beads	1	M	PO	1.32	11.337	21.70	28.6
	2	F	PO	3.18	16.400	27.32	38.2
Liquid	3	M	PO	2.69	12.006	16.41	30.3
	4	F	PO	1.62	16.426	18.27	38.3

[a] $(AUC_{PO}/AUC_{IV}) \times 100$

100% DTPA is excreted (released) in urine within 8 hours after intravenously administration

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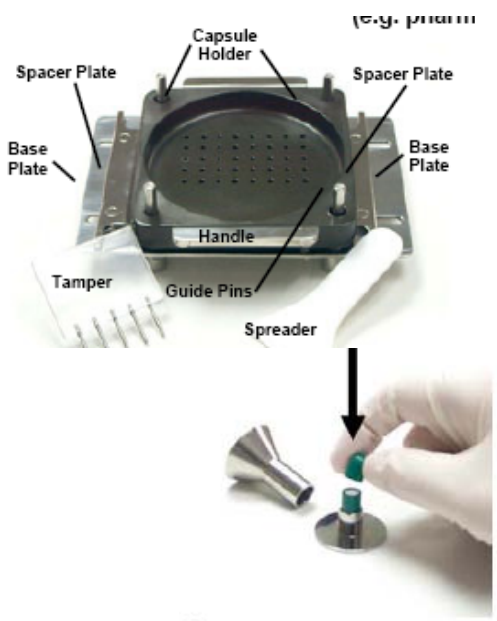
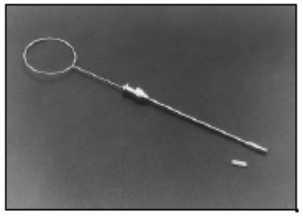
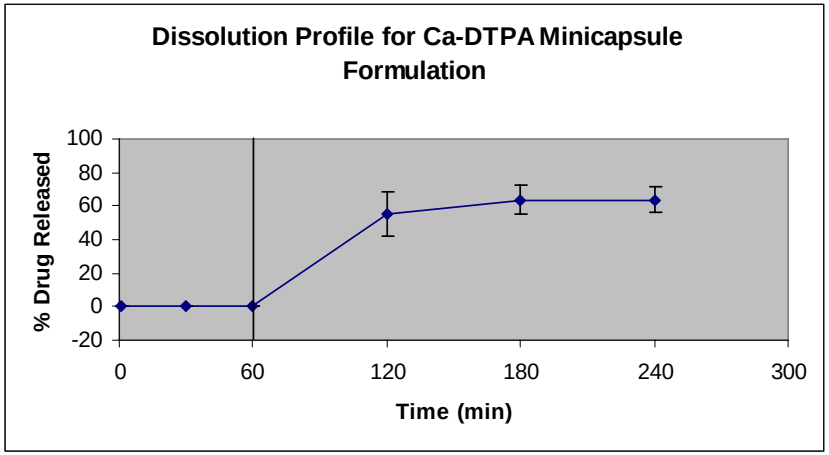
Development of Mini-Capsule Formulation for Rats

- Mini-capsules suitable for rats >200 g.
- Capsule is ejected into the animal's esophagus.
- Capsules were filled with proportional amounts of DTPA/enhancers as in SRI's prior disclosed pilot batches of bead-filled capsules
- All capsules were enteric coated.







Mini-gelatin capsule and apparatus for filling and dose administration to rats

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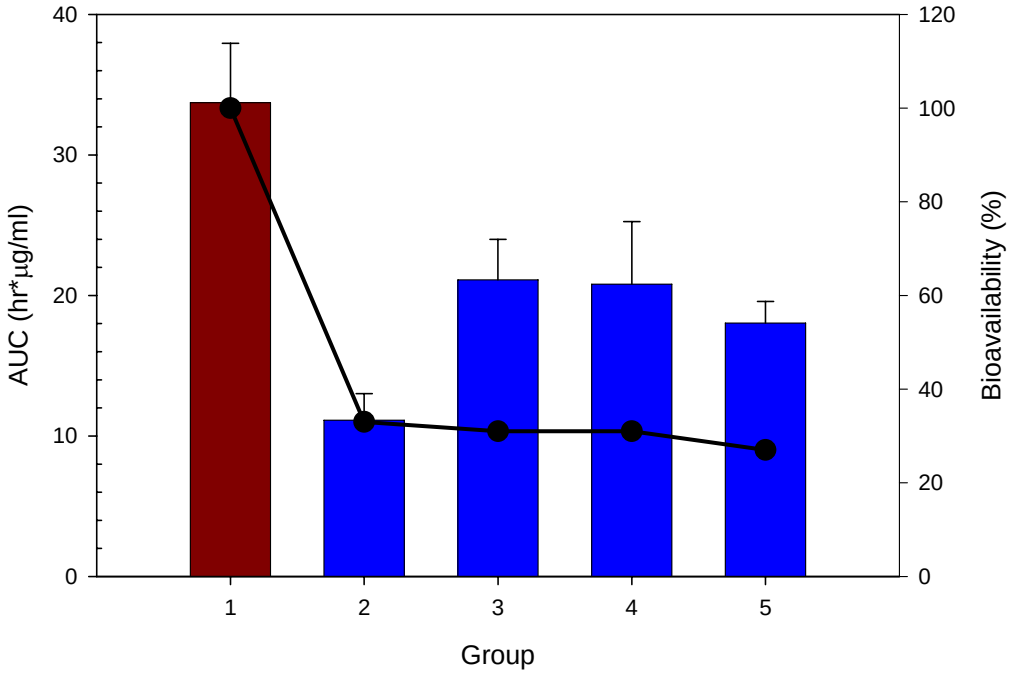
Rat Capsule Bioavailability/Transporter Study



- Exposure to DTPA is proportional to dose
- All oral formulation groups exhibited **~30% oral bioavailability**

Group 1: 20 mg/kg, iv, in saline
Group 2: 20 mg/kg, po, Formulation 1 (minicapsule)
Group 3: 40 mg/kg, po, Formulation 2 (minicapsule)
Group 4: 40 mg/kg, po, Formulation 2**,** (minicapsule) + sodium decanoate
Group 5: 40 mg/kg, po, Formulation 2**,** (minicapsule) + mannitol

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DECORPORATION

Decorporation Efficacy ~ Chelation Ability

What we already know:

- DTPA Ca/Zn effectively chelates Am, Cm, Pu
- DTPA-Ca has superior chelation ability than Zn
- DTPA-Ca repeat doses causes toxicity
- DTPA-Zn safe for pediatric and pregnant population
- Current regimen Ca-DTPA on day 1 followed by Zn-DTPA on subsequent days

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Decorporation Study Design

- Objective: To demonstrate the decorporation of ^{241}Am from male rats by a single dose of orally administered CaDTPA or ZnDTPA
- Fischer 344 rats, males only, n = 2 or 3 per group
- Fast all rats overnight prior to start of study; feed 8 hr after ^{241}Am administration.
- Determine radioactivity in tissues and excreta

Group #	Rats (n)	^{241}Am (µCi)[c]	DTPA Route	CaDTPA[a] Administered after Am (hr)	ZnDTPA Administered after Am (hr)	Sample Collection[b]
1	3	0	none	none	none	96 hr
2	2	0.8	none	none	none	96 hr
3	2	0.8	IV	1	24, 48, 72	96 hr
4	2	0.8	Oral	1	24, 48, 72	96 hr

[a] Food was withdrawn from all rats the night before study start and returned to rats 8 hr after Am administration on study Day 1

[b] Feces and urine was collected every 24 hr for the duration of the study. Blood, lung, liver, kidney and spleen was collected from each rat at necropsy at the indicated time point.

[C] A 100 µl volume containing 0.8 µCi was injected per rat

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Procedure for estimating of Am-241 in biological samples

Samples were ashed at 550°C for 24 hours

(feces 48 hours) and then digested in nitric acid

↓

analyzed by liquid scintillation counting

(Detection limit is 23pCi/g)

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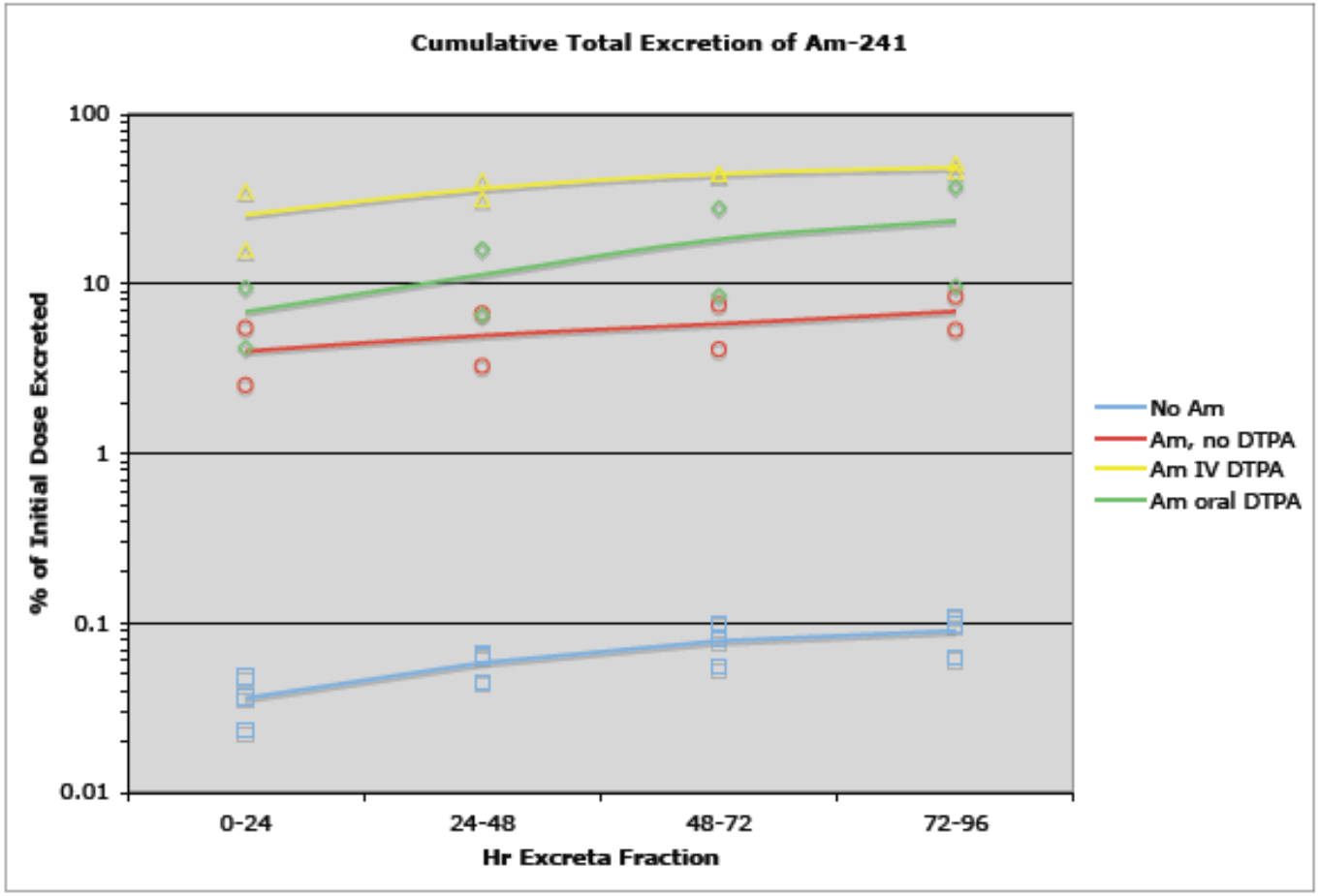
Cumulative Total Excretion of Am-241



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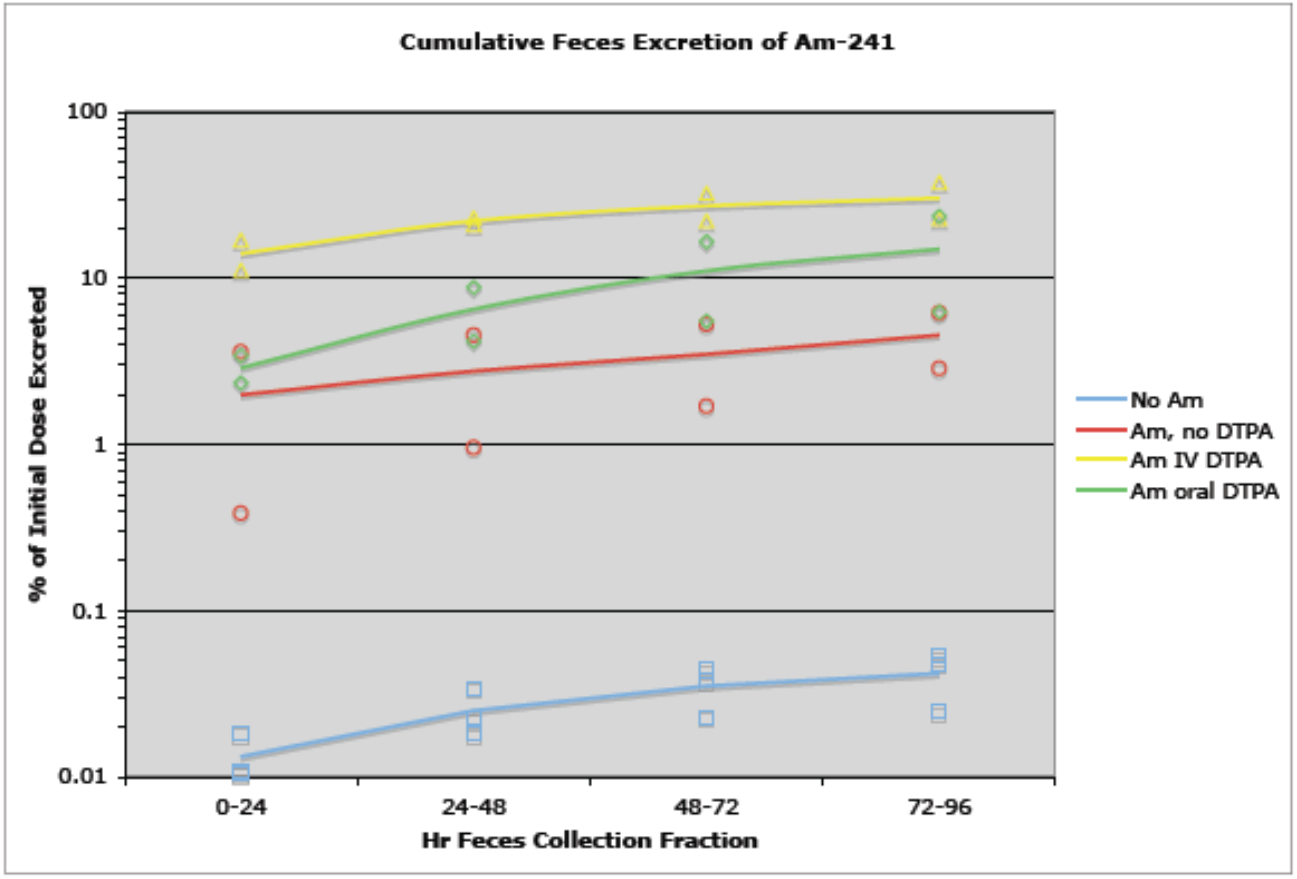
Cumulative Feces Excretion of Am-241



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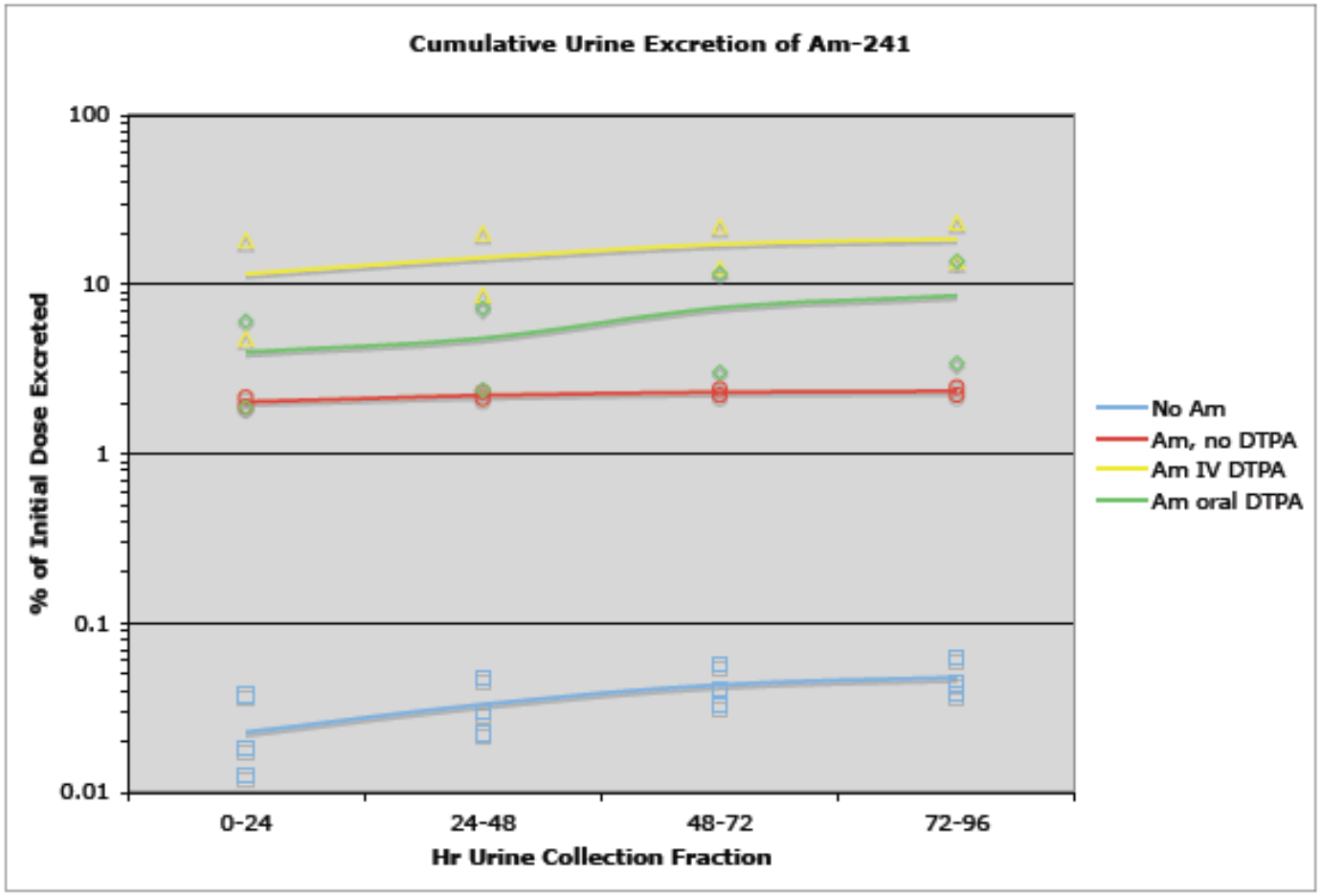
Cumulative Urine Excretion of Am-241



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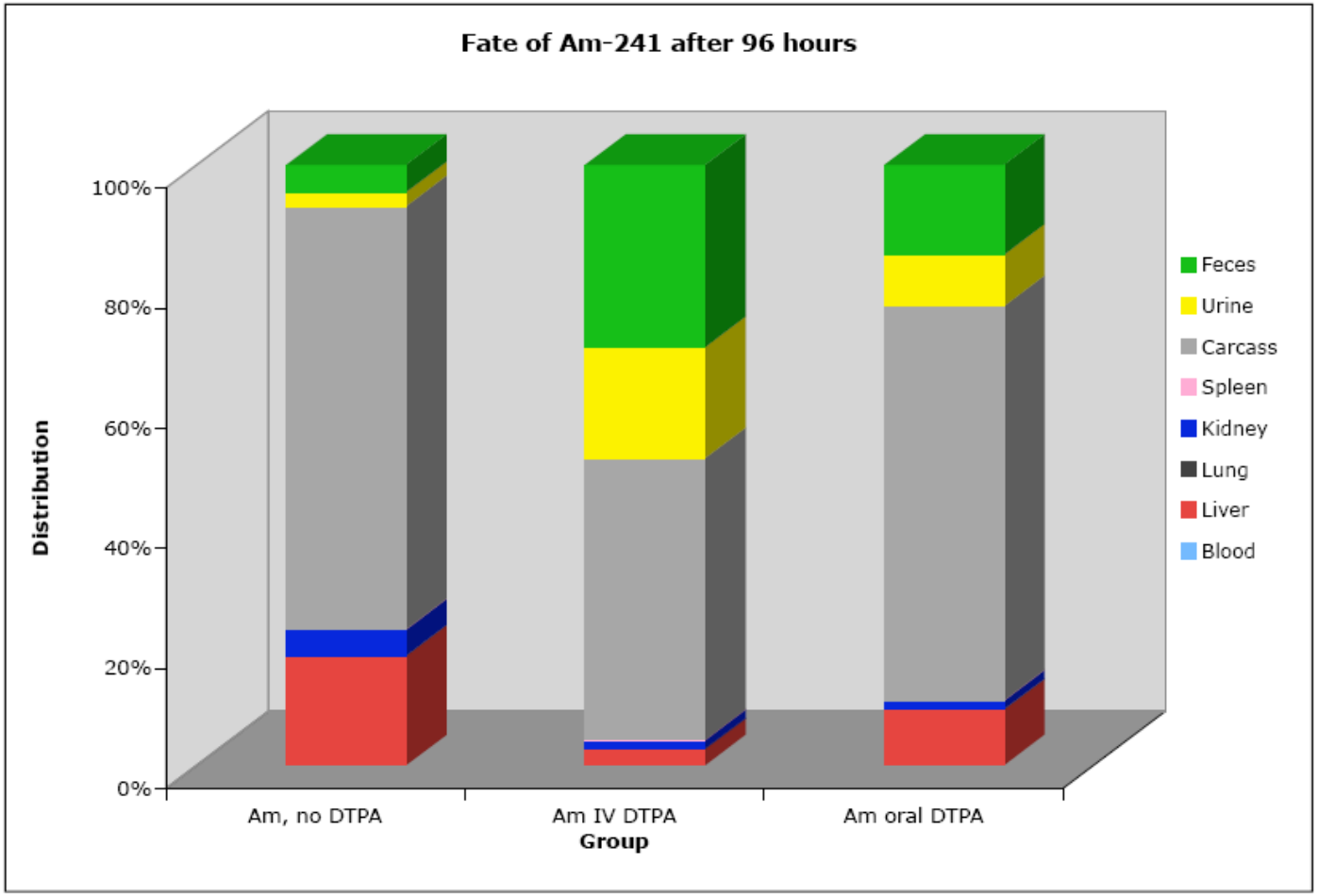
Am-241 Body burden after excretion at 4 days after injection



Body burden highest in carcass (which includes femur)

* Carcass calculated based on the dose given minus the excreted Am, minus the measured tissue burdens for blood, liver, lung, kidney and spleen

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Distribution of Am-241 in rats (published data)

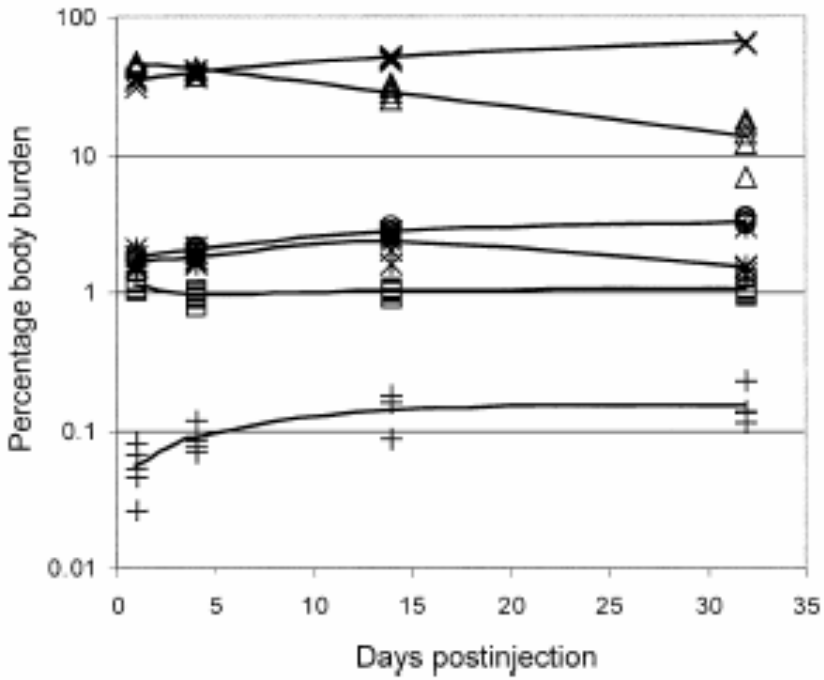

FIG. 4. Changes in distribution pattern of americium-241in the rat as a function of time postinjection. The results for individual animals and the means are presented. ×, carcass; △, liver; □, kidneys; ○, femur; +, spleen; *, gastrointestinal tract.

At 4 days after injection, Am-241 burden is 45% in liver and 35% in carcass (excluding femur)

Female rats, Am-241 2.8 kBq/rat injected via femoral vein in a citrate solution at pH 6.5

Reference: Priest, ND, Comparative biokinetics.., Rad Res 168, 327-331 (2007)

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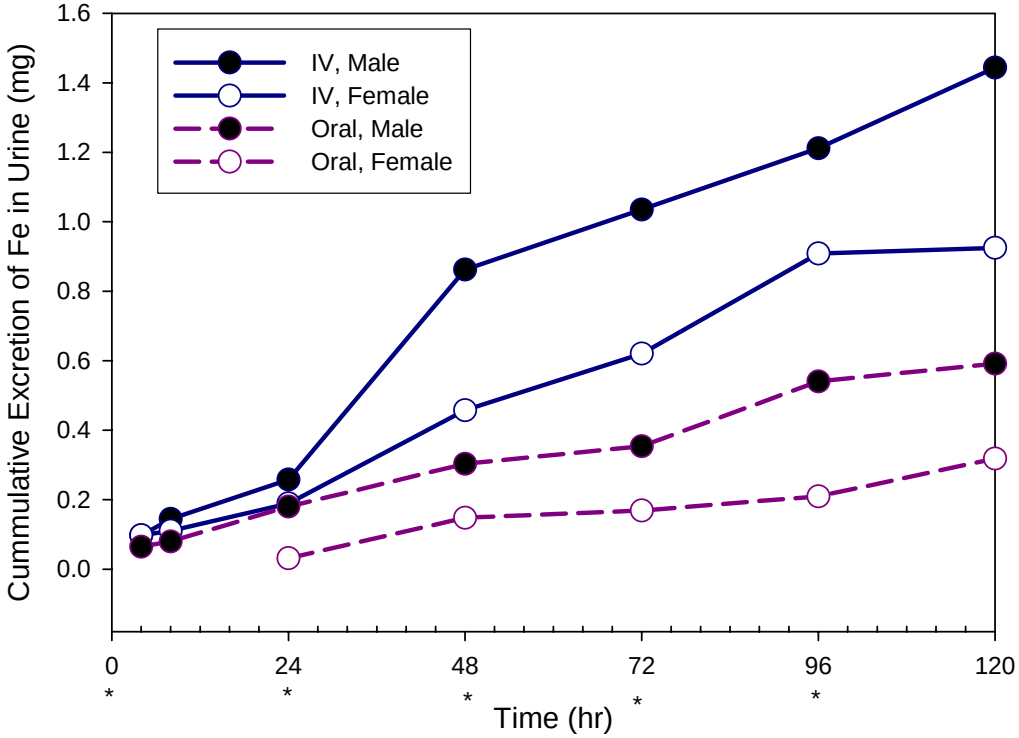
Can DTPA Chelate Iron? Used Fe-Loaded Dogs



	Fe (mg)				Ca (mg)				Zn (mg)			
	IV		Oral		IV		Oral		IV		Oral	
Time (hr)	1M	2F	3M	4F	1M	2F	3M	4F	1M	2F	3M	4F
0	0.1297	0.1158	0.0947	NS[a]	11.390	14.375	16.148	5.457	ND	0.988	1.192	NS
4	0.0970	0.0975	0.0645	NS	2.665	7.498	6.333	NS	0.756	0.405	4.014	NS
8	0.0471	0.0124	0.0153	NS	4.541	1.296	2.437	NS	1.103	2.651	0.009	NS
24	0.1136	0.0792	0.1000	0.0314	8.038	16.636	40.513	1.200	0.065	4.186	1.064	0.414
48	0.6044	0.2686	0.1232	0.1162	10.107	25.846	12.374	5.943	9.846	4.376	7.689	0.365
72	0.1732	0.1623	0.0506	0.0210	25.084	9.811	18.581	2.424	1.042	0.163	1.475	1.677
96	0.1762	0.2884	0.1869	0.0406	25.462	12.313	15.812	2.750	0.212	1.181	0.383	0.200
120	0.2324	0.0166	0.0506	0.1089	25.081	2.535	1.463	4.282	3.696	0.097	1.145	0.109
Total[b]	1.444	0.925	0.591	0.318	100.98	75.93	97.51	16.60	16.72	13.06	15.78	2.76

[a] NS = No sample

[b] Total calculated by summing the value for excretion 4 hr to 120 hr.

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Dose Profile?

- What should be the daily dose?
- What is the toxicity level of such multiple doses?
- Should it be multiple doses per day at select intervals?
- Should it be one capsule per day at higher strength?
- Is Ca-DTPA better given IV, followed by oral ZnDTPA?
- Or should the regimens include Ca-DTPA at regular time intervals?
- How long should the treatment last?
- How do we titer such doses for pediatric population?

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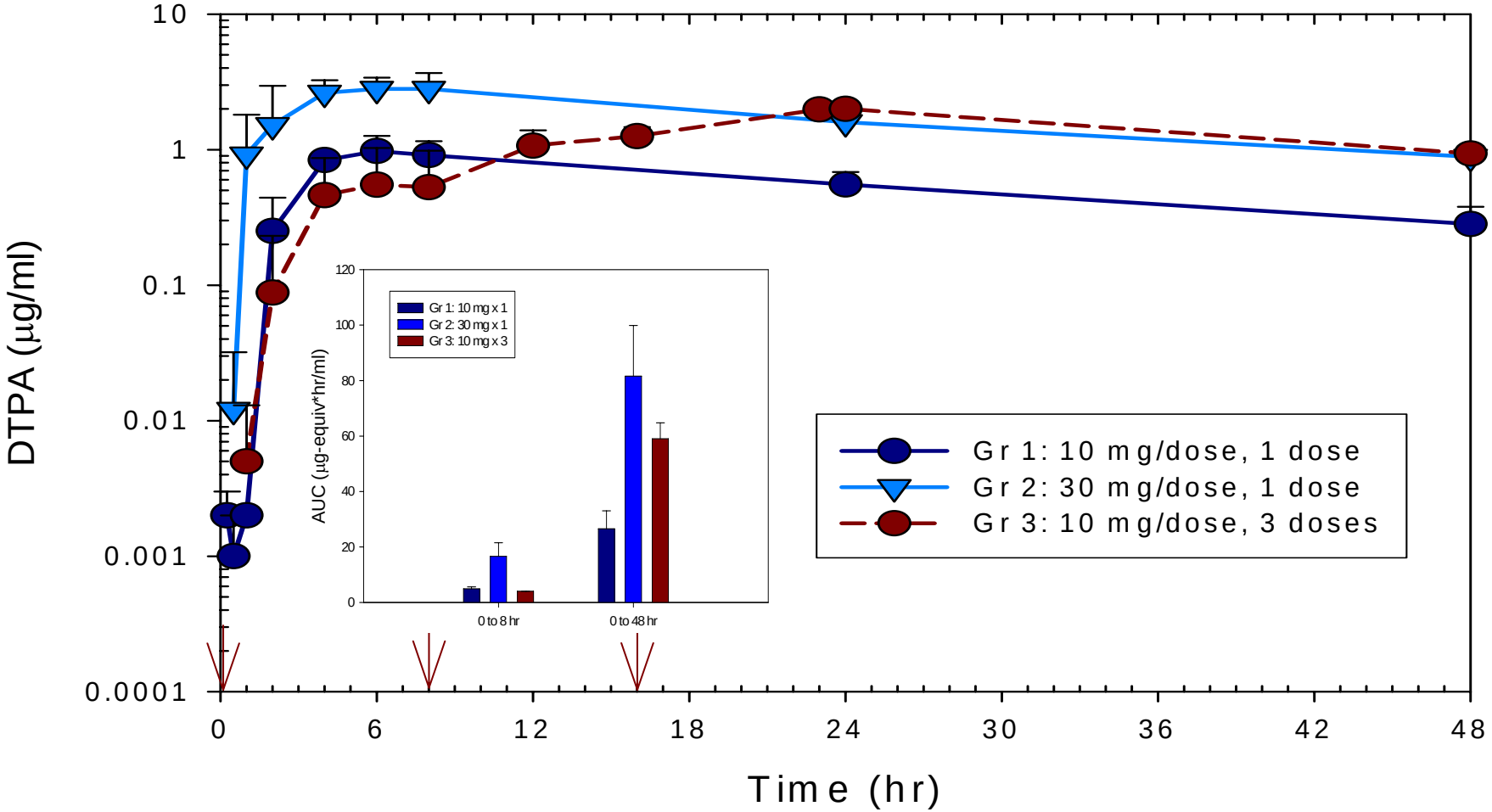
DTPA Time Course in Plasma - Rats



Tmax:	**Gr 1 = 6.0 hr**	
	Gr 2 = 6.7 hr	
	Gr 3 = 23.7 hr	

Cmax:	**Gr 1 = 0.98 µg/ml**	
	Gr 2 = 2.96 µg/ml	
	Gr 3 = 2.00 µg/ml	

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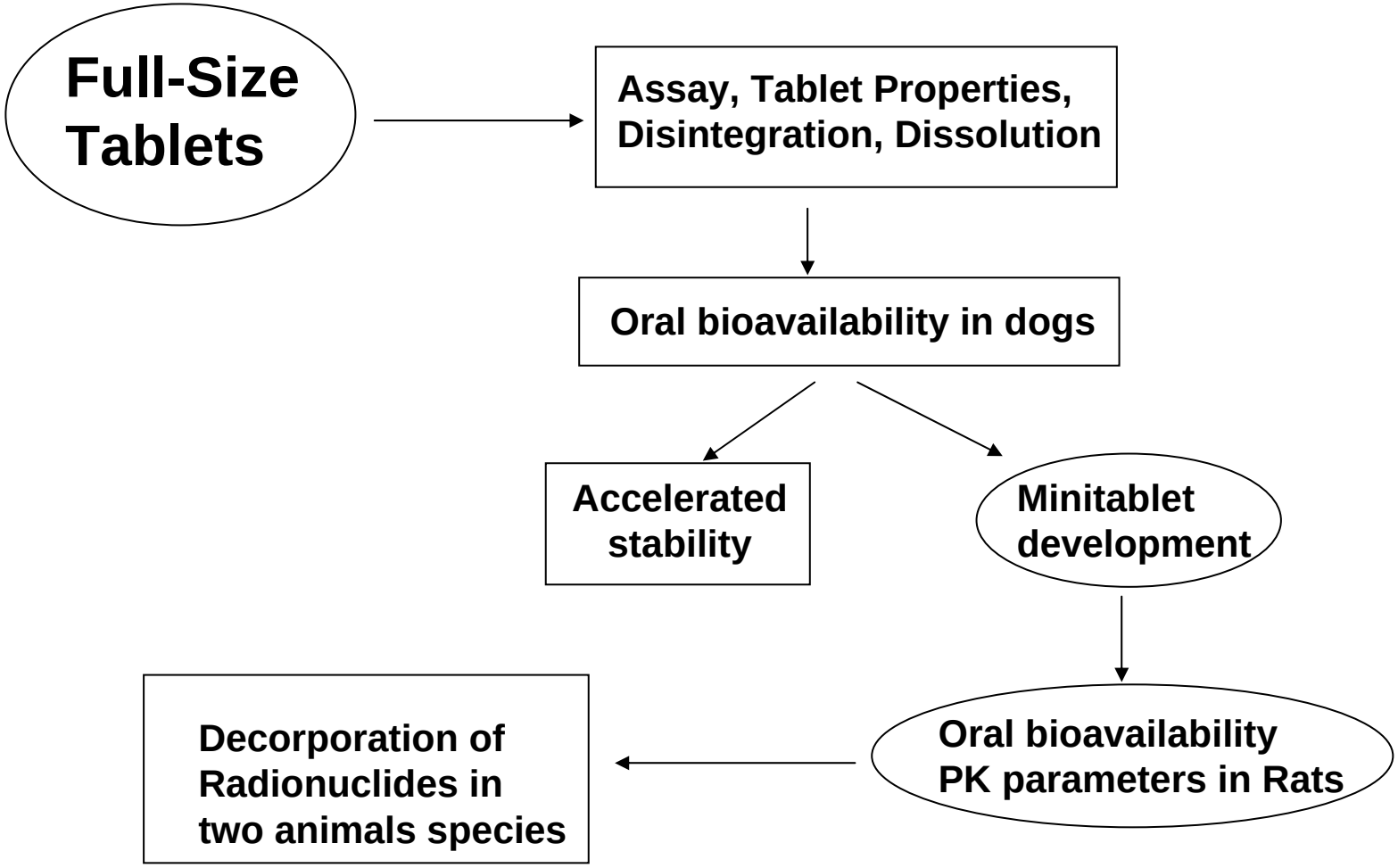
Roadmap for Tablet Development



Advantages: storage stability, controlled release, extended release

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