

DEFENCE



DÉFENSE



HI-6: Oxime Research in Canada

Development of an Intravenous Formulation of HI-6

Defence R&D Canada

Nov 4, 2009



Defence Research and
Development Canada

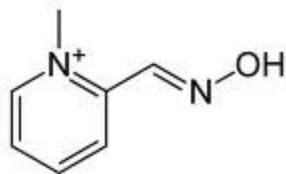
Recherche et développement
pour la défense Canada

Canada



Oxime Development Programs

- Before 1950 the only treatment for nerve agent or pesticide poisoning was atropine
- During the 1950's a group of chemicals known as "oximes" were developed that could reactivate cholinesterase inhibited by nerve agents
- The first clinically tested oxime was pralidoxime chloride (2-PAM)

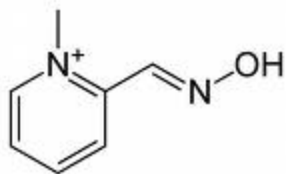


Pralidoxime

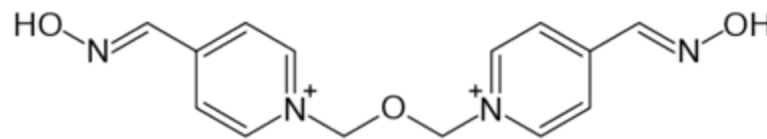


Oxime Development Programs

- During the 1960's obidoxime which was effective against GA was also licensed in several countries
- Both 2-PAM and obidoxime lacked efficacy against all nerve agents;
 - Soman (GD), cyclosarin (GF), Russian VX (RVX)
- This led most nations to initiate programs to develop improved oximes



Pralidoxime

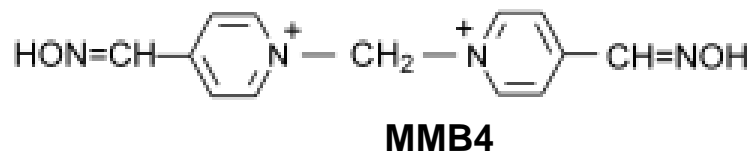
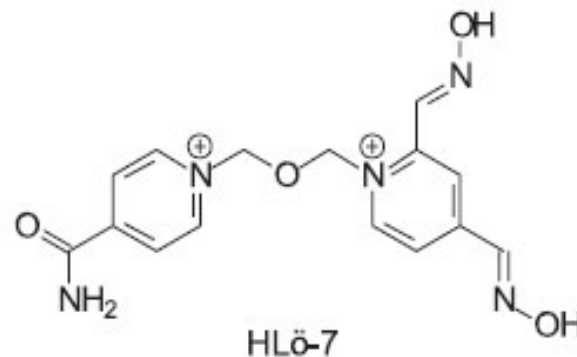
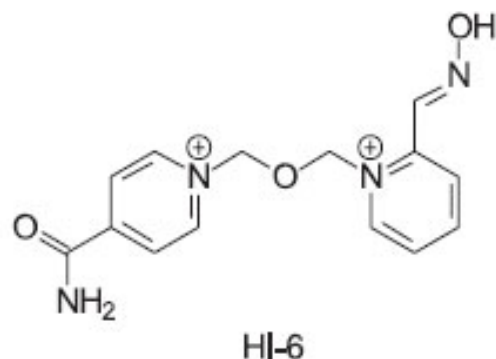


Obidoxime



Oxime Development Programs

- Effort to develop new oximes led to the synthesis of several oximes including;
 - HI-6, HLö7 and MMB4





HI-6

- HI-6
 - dichloride salt (2-Cl)
 - synthesized (1966) by Ilse Hagedorn and Irmo Stark (GER)
- HI-6 identified by several NATO nations as the primary future oxime based on;
 - improved efficacy against all nerve agents except GA
 - least toxic of all next generation oximes
(HI-6, HLö7 and MMB4)
 - has been fielded by Canada, Sweden and Czech Republic



DRDC Oxime Program (2003)

- HI-6 Development
 - autoinjector dose limited by solubility of 2-Cl
 - dimethanesulphonate salt (DMS) developed to increase solubility
 - stability in solution
 - wet/dry autoinjector
 - use of suspensions or emulsions in single chamber
 - BCME synthesis route for HI-6 2-Cl
 - novel synthesis route for HI-6 DMS



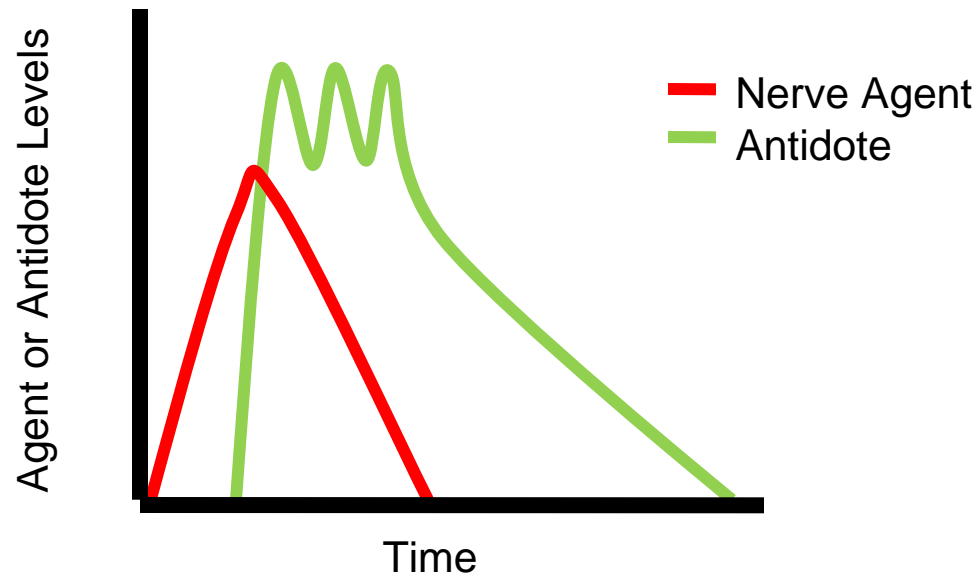
CA-NL-UK Development (2005)

- Trilateral agreement to license autoinjector
- Based on 3-in-1 autoinjector containing HI-6, atropine and avizafone (*water soluble diazepam*)
- Pre-clinical and clinical studies to support regulatory approval
 - Non-BCME method
 - Small-scale (3kg) GMP batches (late-2009)
 - Scale-up (15kg) GMP batches (2010-2011)
 - Clinical toxicology studies (2010-11)
 - Ph1 clinical study (2011-12)



Parenteral Formulation Program

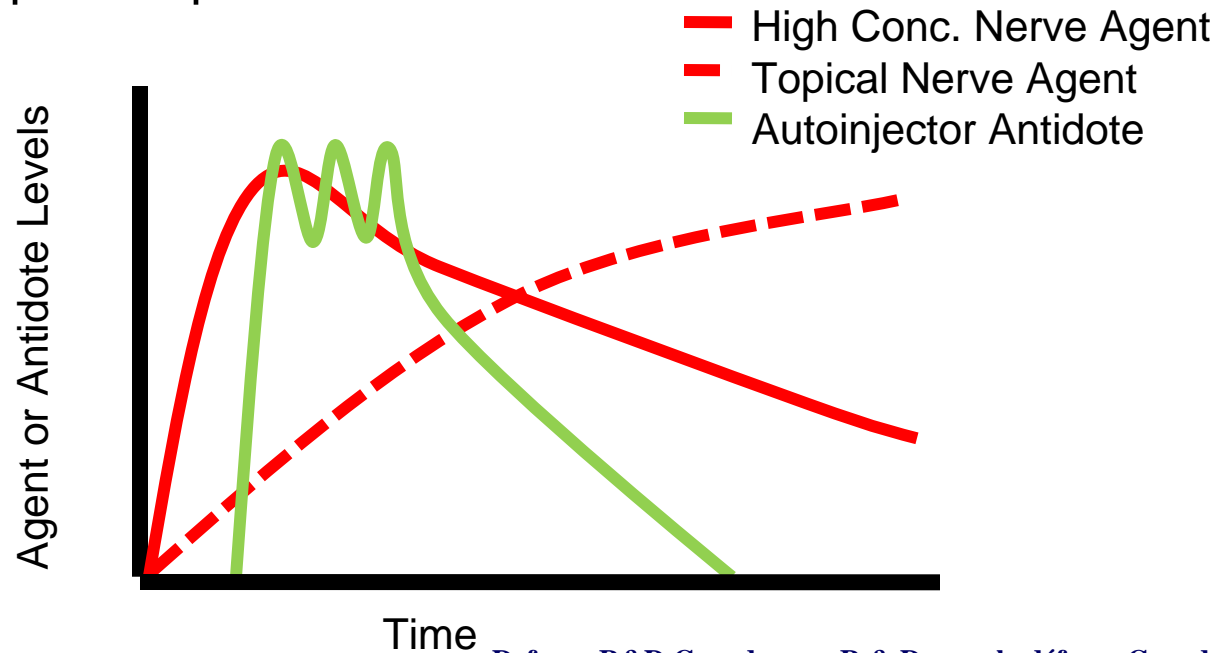
- Current nerve agent antidote treatment regimens are designed for field treatment of nerve agent exposure.
 - 3 autoinjectors, one at 1, 15 and 30 min





Parenteral Formulation Program

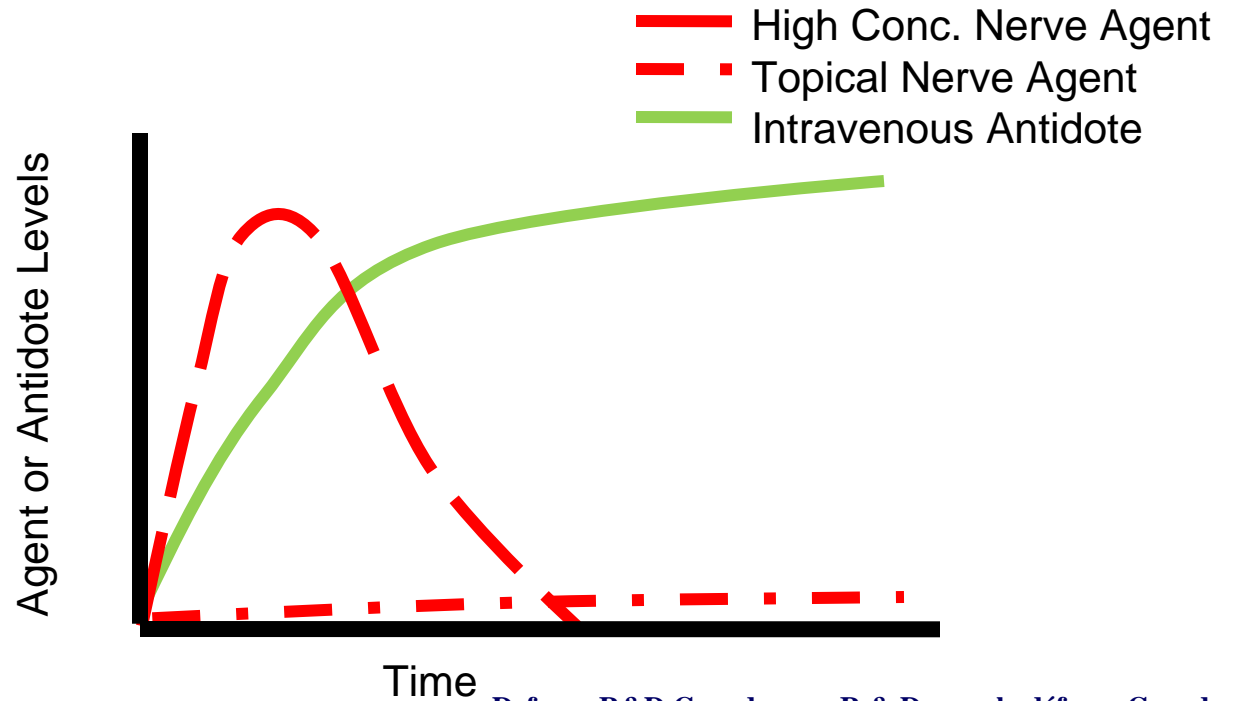
- How do we continue required treatment following extraction from the area or in a medical facility?
 - Exposure to high concentrations
 - Topical exposures





Parenteral Formulation Program

- Intravenous injection or infusion
 - optimize therapeutic concentrations





Parenteral Study Outline

- GLP Implementation at DRDC Suffield 2007
- Determine pharmacokinetics (PK) of HI-6 2-Cl and DMS salts in guinea pigs (GLP) and domestic swine
- Develop an infusion protocol to maintain a target plasma concentration of 100 $\mu\text{Mol/L}$
 - 8 hour infusion period
 - with and without atropine sulfate
- Efficacy against percutaneous nerve agent exposure (3Q 2010)



GLP-Validated HPLC Method for Quantification of HI-6 in Plasma

- Mobile Phase
 - Component A
 - PIC-B7 (acetic acid, methanol, alkane sulfonate salts), water
 - triethylamine
 - Water
 - Component B
 - 1 part Component A
 - 1 part Methanol
- Linear Gradient Elution (A:B - 60:40 to 0:100)
- UV detection (302 nm)



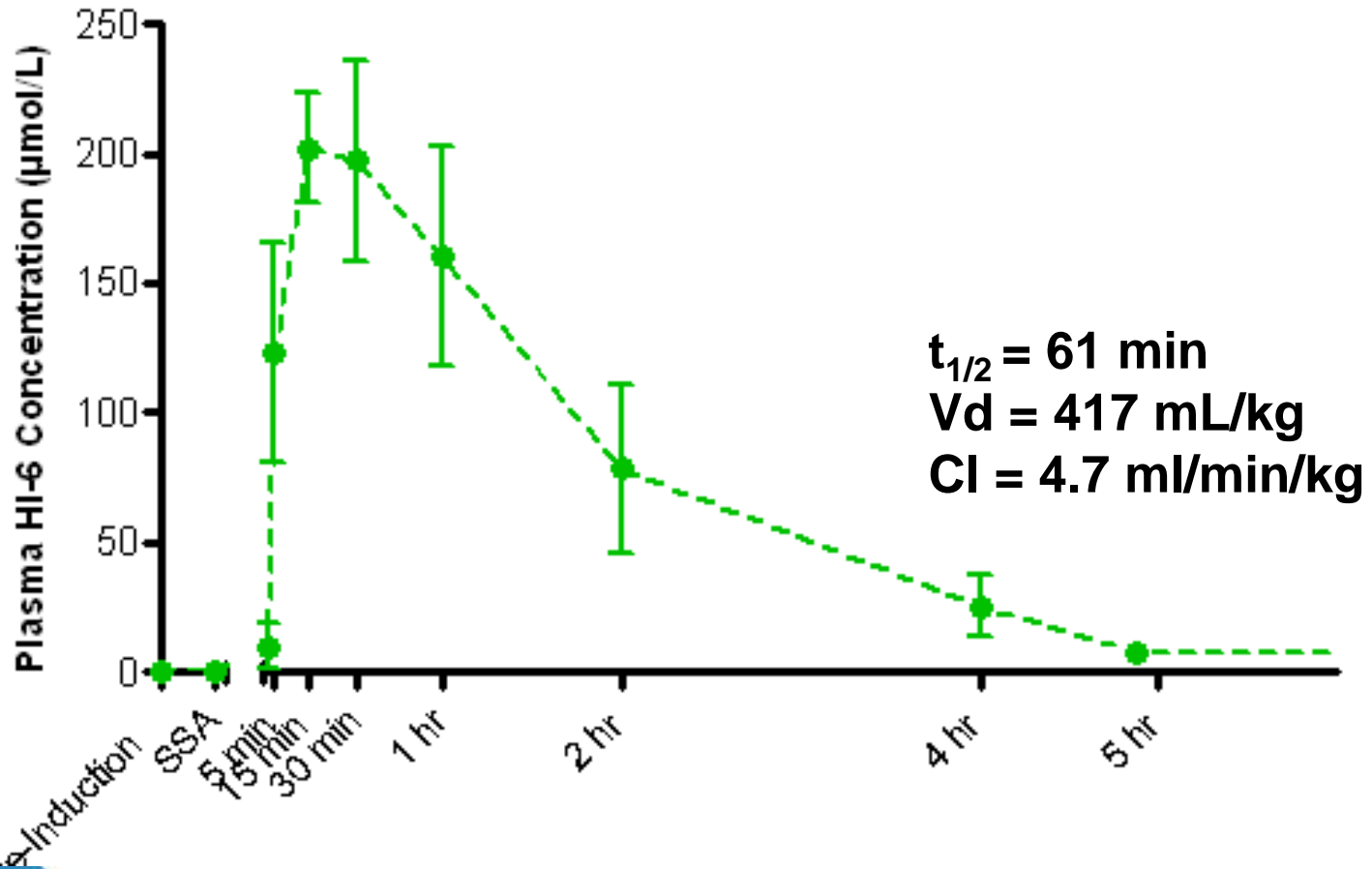
Plasma Sample Preparation

- ~ 100 μL of whole blood collected via an indwelling catheter located in the jugular vein (guinea pig)
- Plasma is collected and then stored at -80°C
- 35 μL of plasma mixed with 35 μL 0.10 mg/mL 2-PAM (internal standard)
- Precipitate proteins with 10% TCA, centrifuge, remove supernatant
- Neutralize with 0.2 M NaOH and filter
- Aliquot filtrate into glass insert
- 2-PAM used as an internal standard



Pharmacokinetics - Intramuscular

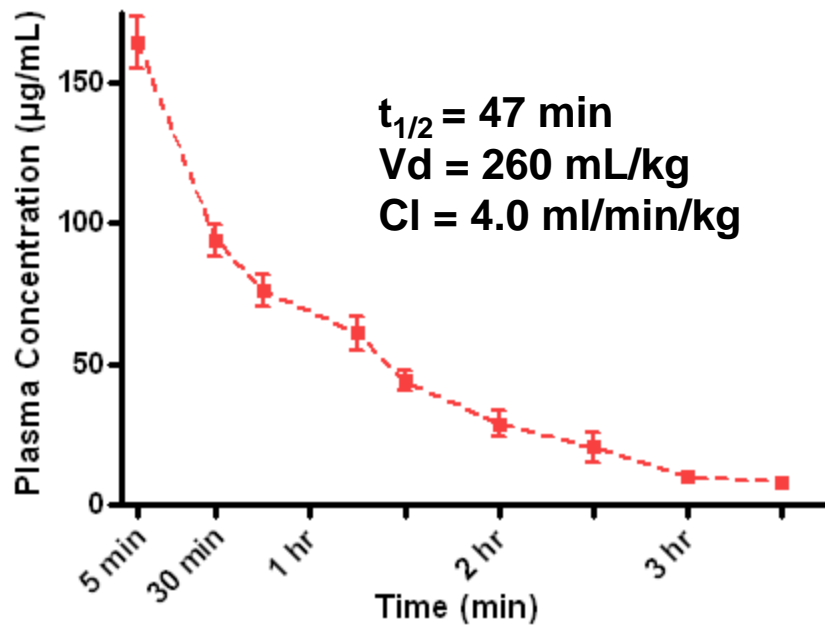
HI-6 2CI IM



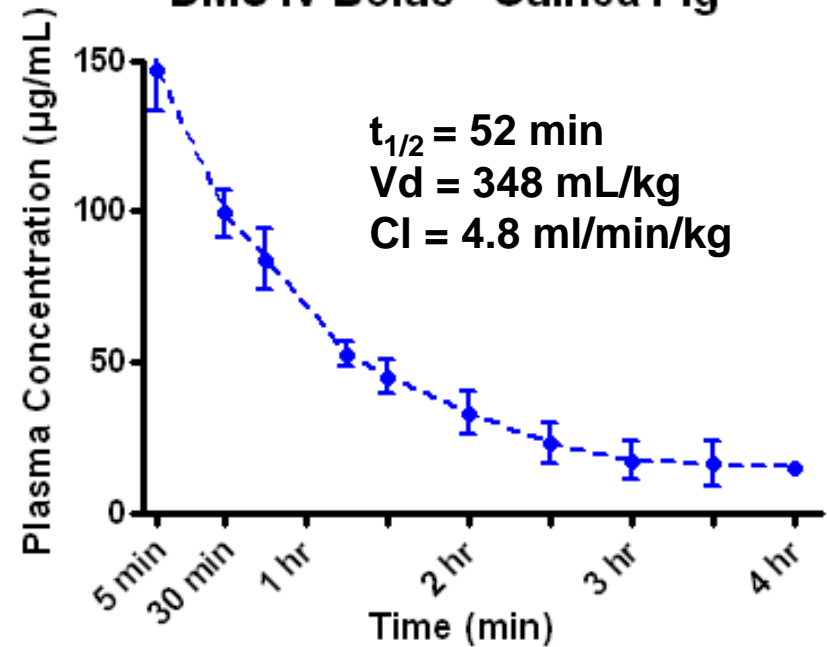


Intravenous Pharmacokinetics – Guinea Pig

2Cl IV Bolus - Guinea Pig



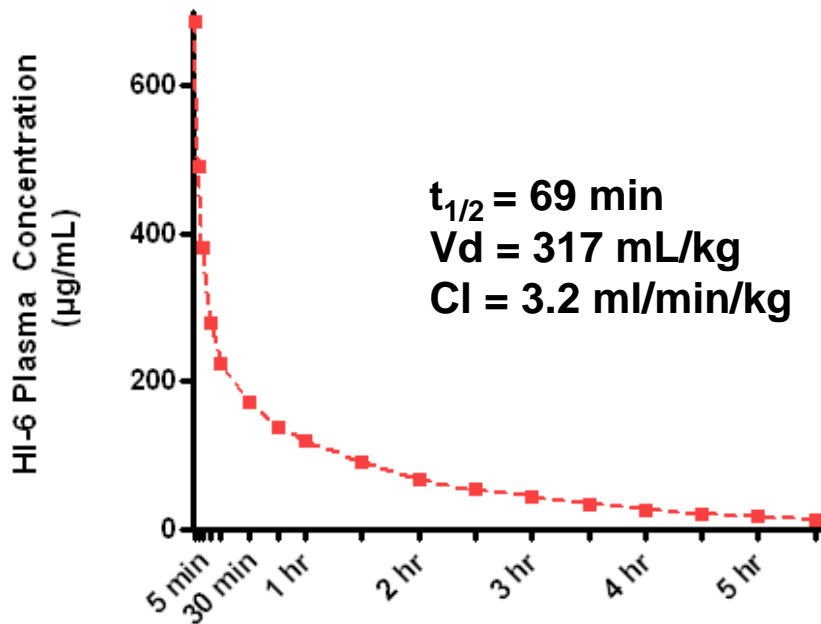
DMS IV Bolus - Guinea Pig



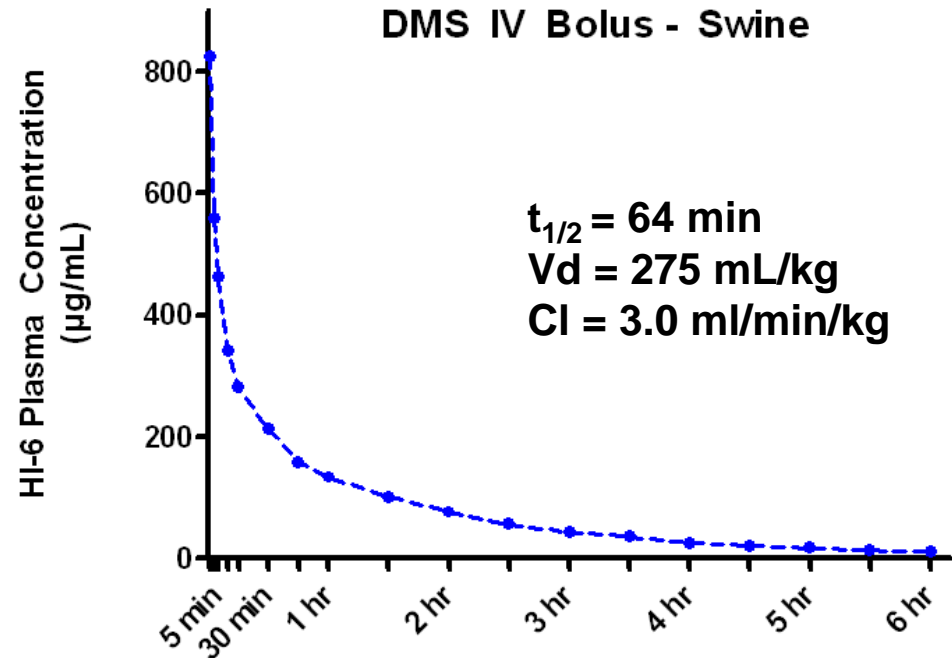


Intravenous Pharmacokinetics - Swine

2CI IV Bolus - Swine



DMS IV Bolus - Swine





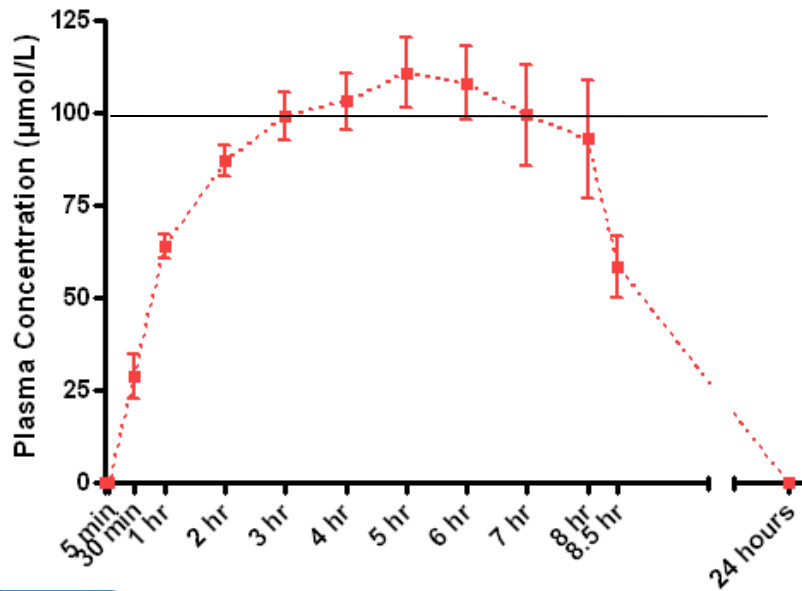
Comparison of HI-6 PK

	Guinea Pig			Swine		Human
	IM (2-Cl)	IV (2-Cl)	IV (DMS)	IV (2-Cl)	IV (DMS)	IM (2-Cl)
$t_{1/2}$ (min)	61	47	52	69	64	67
Vd (mL/kg)	417	260	348	317	275	240
Cl (mL/min/kg)	4.73	3.96	4.8	3.20	2.96	2.47

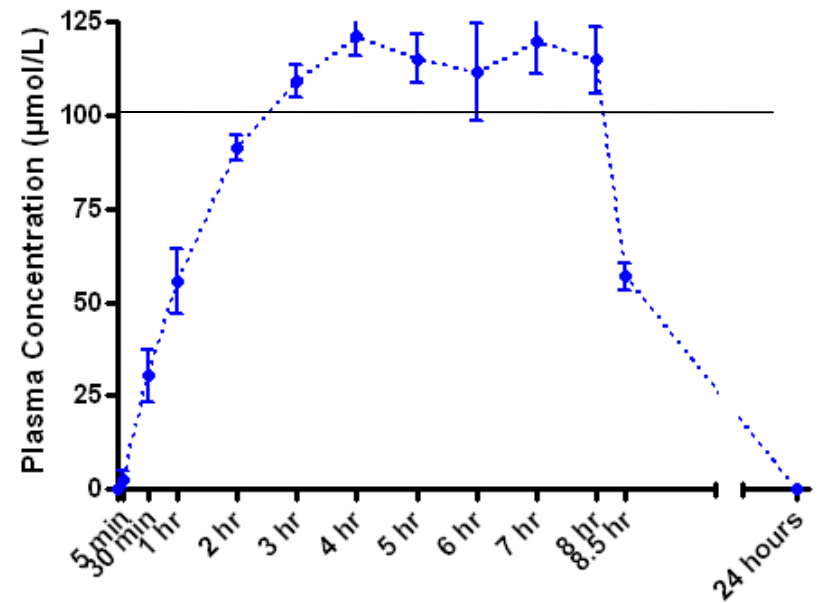


HI-6 Infusion (8 hr) – Guinea Pig

HI-6 2Cl Infusion



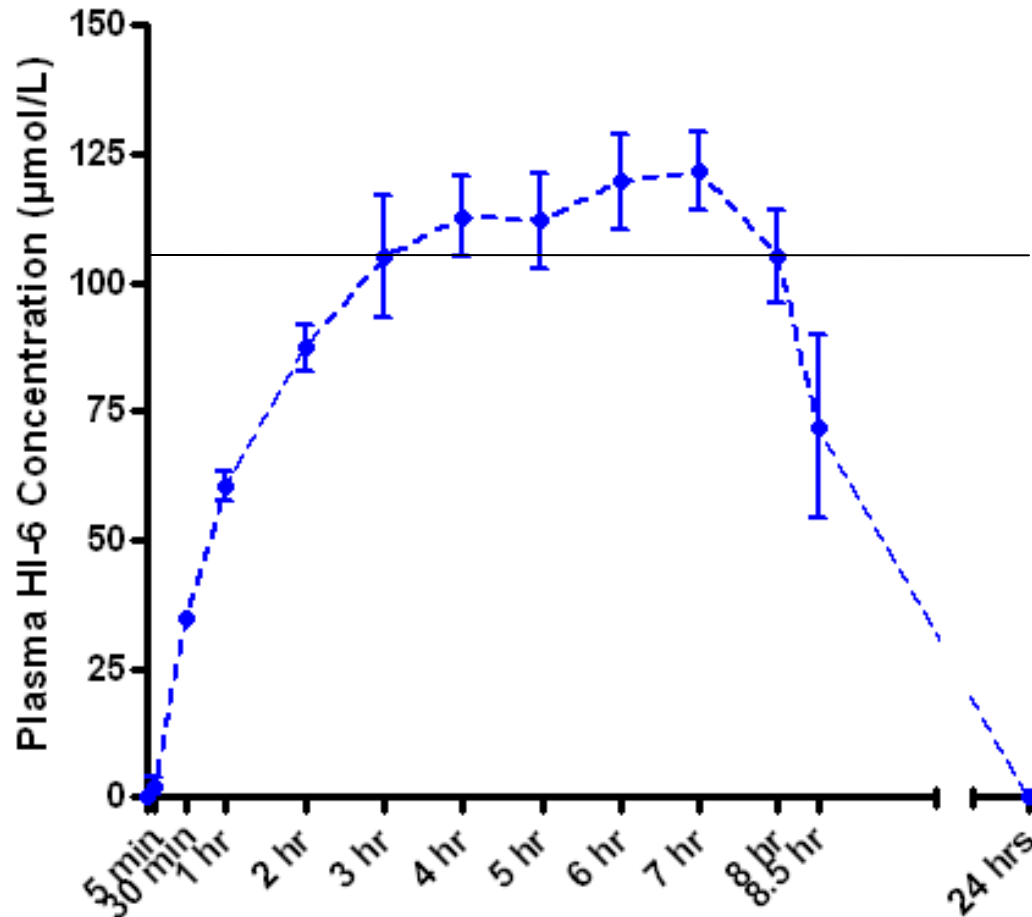
HI-6 DMS Infusion





HI-6 and Atropine Infusion – Guinea Pig

HI-6 DMS & AS Infusion





Conclusions

- Paired-ion chromatography effectively resolves HI-6 and 2-PAM in small plasma samples
- HI-6 pharmacokinetics in anesthetized guinea pigs are similar to those in anesthetized swine
- These kinetics are sufficient to formulate infusion rates to sustain target plasma concentrations for up to 8 hours
- Efficacy experiments need to be conducted (3Q 2010)

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