Sending Us Your Test Article: Considerations & Calculations

Patrick McAnulty
Scientist II, Program Design
XenoTech

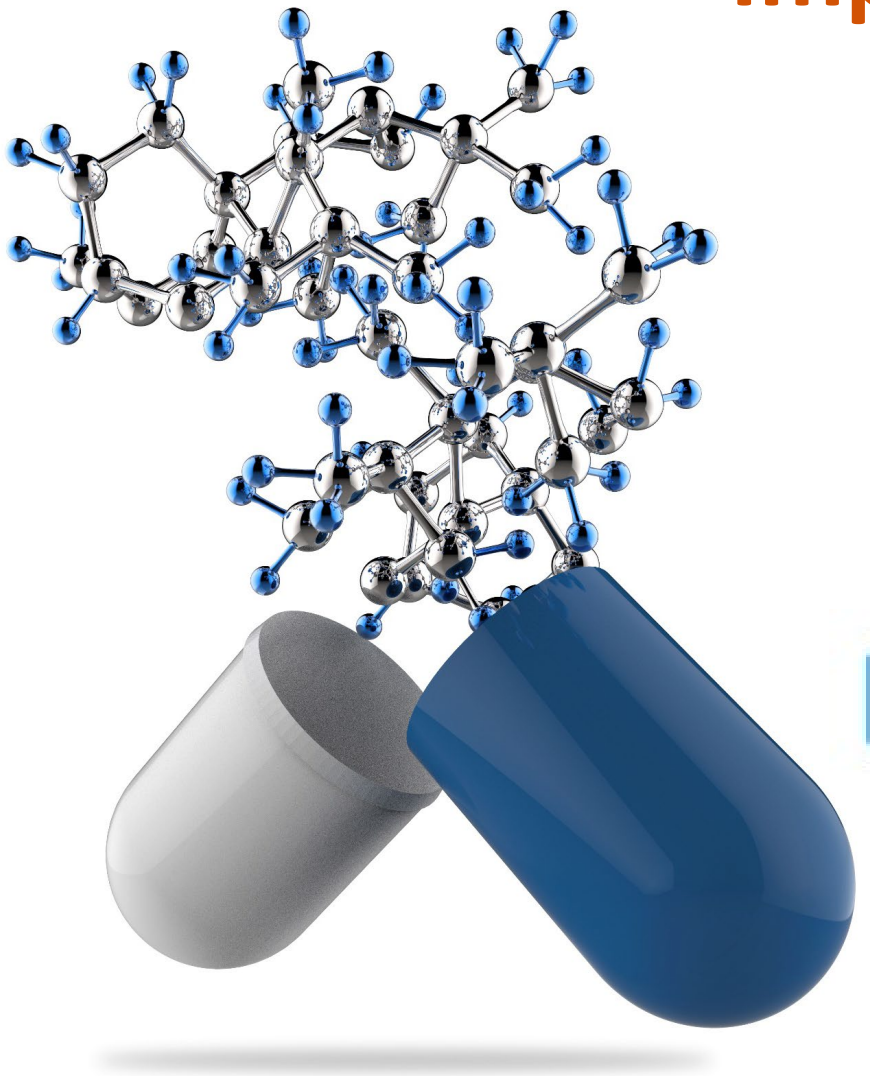
Determining amount of Test Article needed for a Contracted Study

"How much do I need to send??"

#1 most frequently asked question our Protocol Design team receives

Answer may not be as straightforward as it seems…

Impacting Factors:



What are the properties of the test article?

What is the scope of the work?

What concentrations do the guidance documents say need to be tested?

What concentration of stock solution needs to be made?

What solvent percentage can we use in our incubation solutions?

Standard Requests

There are standardized test article amounts that are requested for each study type offered:

Enzyme Inhibition	150 mg
Enzyme Induction	150 mg
Drug Transport	150 mg
Reaction Phenotyping	100 mg
Metabolic Stability	50 mg
Metabolite ID (in vitro)	50 mg
Method Validation	50 mg
Method Qualification	25 mg

These amounts are sufficient for the vast majority of studies run

Based on two assumptions

1) Compound is ~500 g/mol
2) Top treatment concentration of 100 µM

Common concerns:

"My test article is not ~500 g/mol!"

"What if I need to treat at higher than 100 µM (to meet guidance)?"

"My test article availability is extremely limited! What is the minimum amount you can run the study with?"

Manufacturing test article can be expensive and the material is precious– especially for compounds that are very early in development

Don't worry! XenoTech can calculate a more precise amount that is tailored to the properties of the test article if supply is limited or the assumptions in our standard request are not applicable

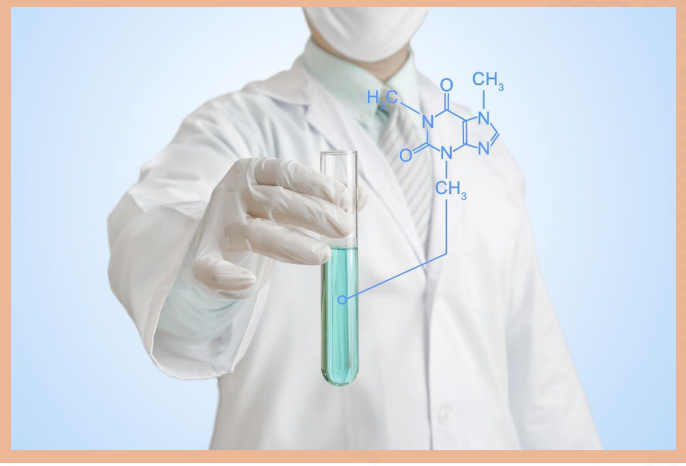
Test Article Properties

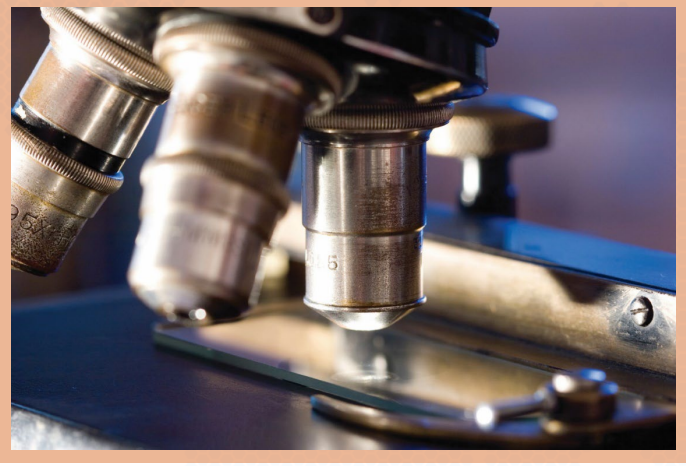
If not already provided, XenoTech will request information on the test article during the protocol drafting phase

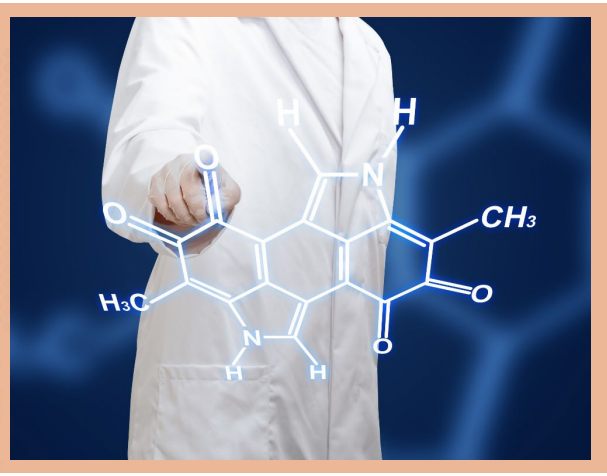
These test article properties generally inform the choice of a treatment range which will meet regulatory guidance

Description	Test Article	Internal Standard
Compound Name	**TEST ARTICLE**	**INTERNAL STANDARD**
Controlled substance (yes/no)		
Molecular weight (free base), g/mol		
Formula weight (salt, if provided), g/mol		
Known solubility (concentration and solvent)		
Stability in solution (concentration, solvent, storage temp, and duration of time)		
Intended indication and mechanism of action (e.g., oncology and kinase inhibitor)		N/A
Route of administration		
Maximum anticipated dose (if oral), mg		
Maximum anticipate intestinal concentration (I_{gut} or I_2)		
Human plasma C_{max} (µM) (specify total or unbound)		
LogP		
Plasma free fraction		

"Why are these properties important?"







- **Molecular weight**—the larger the molecular weight, the more compound you need to prepare a given concentration of solution
- **Route of administration**– Regulatory guidance specifies different parameters that are dependent upon how the test article is administered
- **Dose (if oral)** – more compound ingested results in a higher concentration of exposure in the gut
- **C_{max}** – Maximum drug concentration in the plasma at steady state provides information on the anticipated systemic exposure
- **Plasma free fraction** – Compounds that are more abundantly protein bound have decreased bioavailability

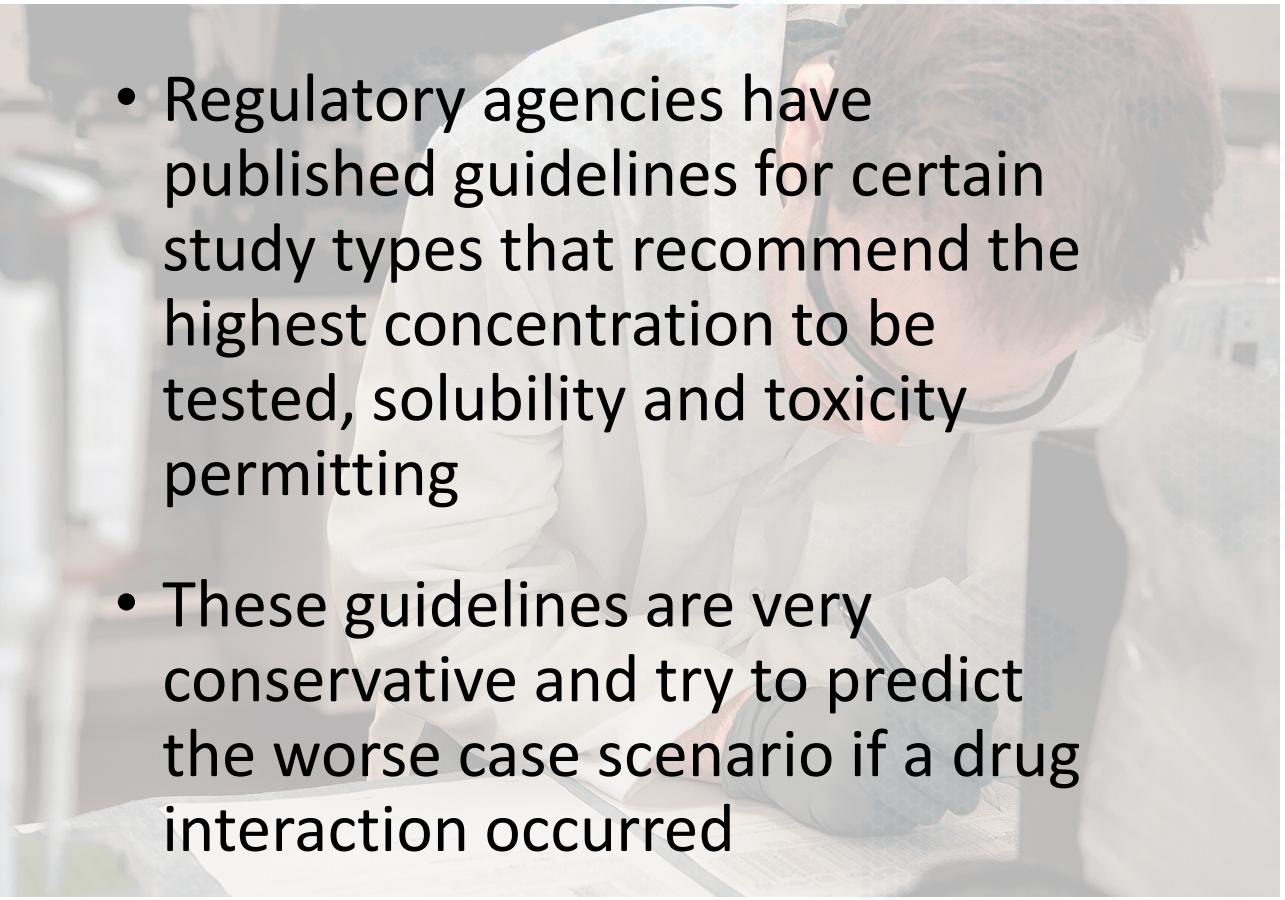
"What if I don't know some of that information?"

- Sometimes a sponsor will not have all the information that is needed to calculate a treatment range that meets guidance recommendations

That is OK.

- Although not preferred, in the absence of human data, concentrations can be determined based on animal data, if available, or solubility limitations
- XenoTech has been running studies that support IND and NDA packages for over 25 years and are experienced in recommending treatment ranges that result in quality data that is accepted by regulatory agencies

"What concentrations do I need to test?"



- Regulatory agencies have published guidelines for certain study types that recommend the highest concentration to be tested, solubility and toxicity permitting
- These guidelines are very conservative and try to predict the worse case scenario if a drug interaction occurred

- There are four primary calculations that inform the top treatment concentration for our inhibition, induction and transporter studies:

$0.1 \times I_{gut}$
50 x Unbound C_{max} at steady state
10 x Total C_{max} at steady state
$25 \times I_{u,in,max}$

Calculations that define the top treatment concentration

$0.1 \times I_{gut}$

I_{gut} = Apparent intestinal luminal concentration

Concentration equal to the maximum dose in 250 mL (a glass of water)

Applicable for enzymes and transporters that are highly expressed in the intestine compared to other tissues within the body

E.g., CYP3A4/5, P-gp, BCRP

50 x Unbound C_{max} at steady state

Cmax = Maximal drug concentration in the plasma

If fraction unbound is unknown a conservative approach is taken (assume 100% unbound)

Applicable for systemic concentrations, ubiquitously expressed enzymes or renal uptake transporters

E.g., CYP1A2, OAT1, OCT2

Calculations that define the top treatment concentration

10 x Total C_{max} at steady state

C_{max} = Maximal drug concentration in the plasma

Total includes both bound and unbound. Equivalent to assuming 100% unbound

Applicable for systemic concentrations (non-oral delivery) for efflux transporters

- *Ex. P-gp, BCRP*

25 x $I_{u,in,max}$

$I_{u,in,max}$ = the estimated unbound maximum plasma inhibitor concentration at the inlet to the liver

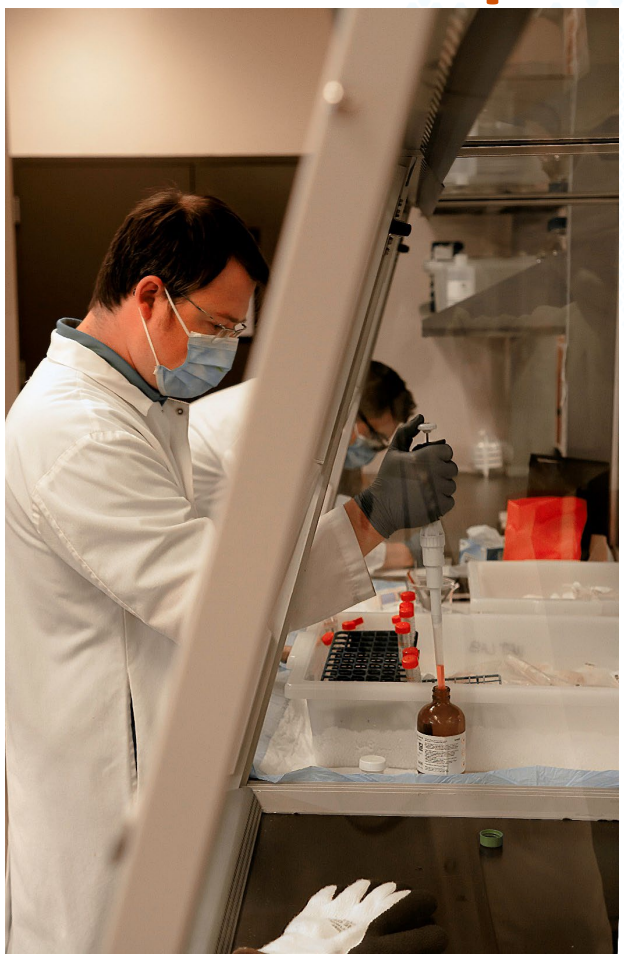
If fraction unbound is unknown a conservative approach is taken (assume 100% unbound)

Applicable for hepatic uptake

- *Ex. OATP1B1 or OATP1B3*

Once these values are defined, the top treatment concentration is established and a log scale is used to define the remaining treatment concentrations

"So you need enough compound to make solutions equal to the top treatment concentration?"



- Sometimes sponsors see the top treatment concentration and cannot reconcile that with the amount of test article requested
- Some organic solvents can inhibit or activate enzymes at high concentrations
- FDA guidance states that the sponsor should keep organic solvents at low concentrations (<1% v/v and preferably <0.5% v/v)
- XenoTech limits all organic solvent percentages to 0.1 to 1% v/v
  - test article solubility in the test system permitting

"What does that mean for me?"

- The stock solution XenoTech makes (and then dilutes accordingly) needs to be significantly higher than the top treatment concentration

  Example:

  If the top treatment concentration is 100 µM and optimal assay conditions call for a final concentration of organic solvent at 0.1% (v/v) then a stock solution of 100 mM is required

- XenoTech will need to calculate the amount of test article based on this stock solution concentration

- If the solution stability is unknown:
  - Fresh preparations will be made on the day of the experiment which can lead to more compound being used

Summary

To determine a test article specific amount of compound needed to run a study, XenoTech needs the Test Article property information as early as possible

This is used to calculate top treatment concentrations that will meet regulatory guidance recommendations

Stock solution concentrations are determined based on the percentage of organic solvent targeted in the top treatment concentration

From this information, total amount of compound to be requested is calculated

Thank you for watching!

Please contact your regional account manager if you are interested in a placing a contracted study investigating your drug's metabolism or DDI potential