Compound Solubility and HTS Screening

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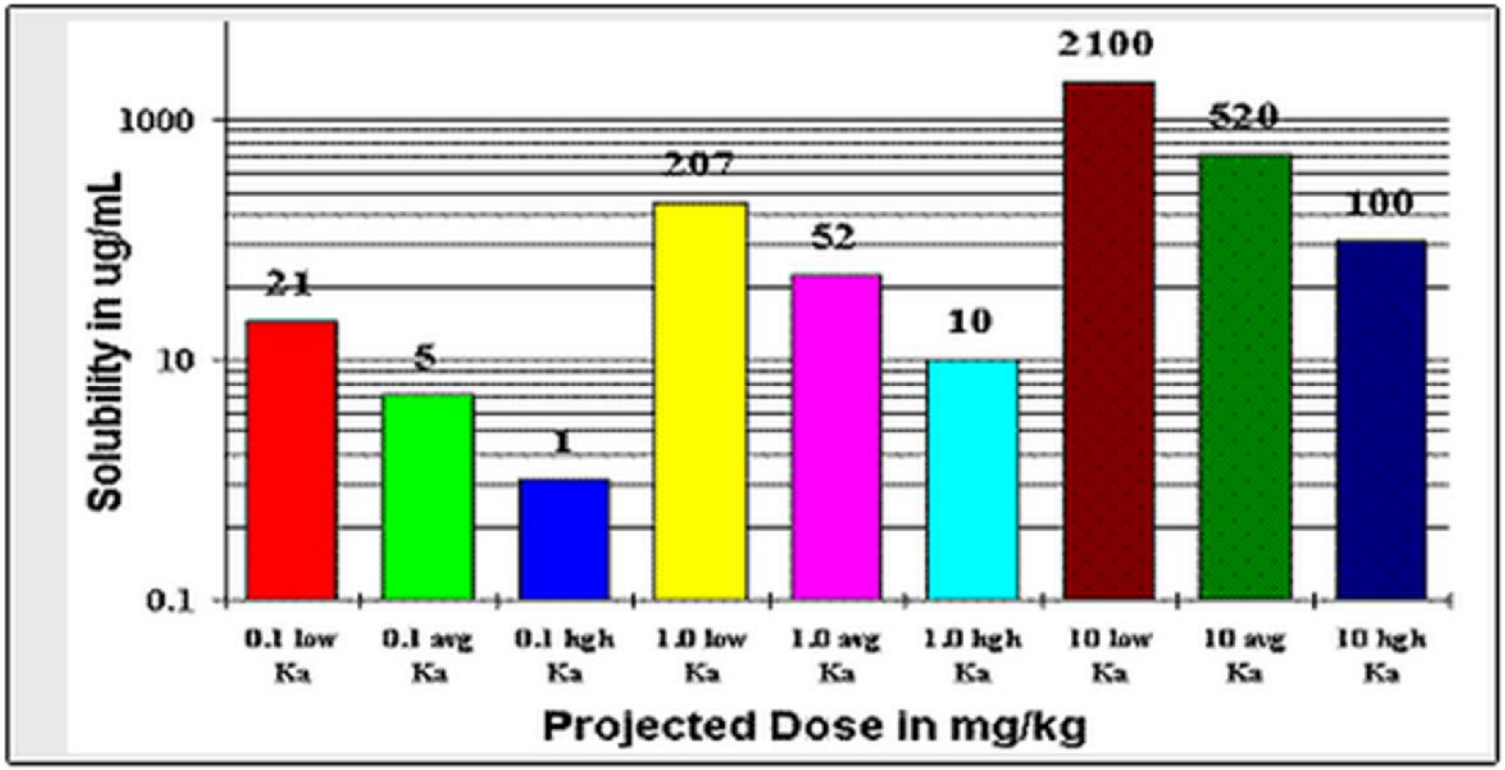
The symptoms to be concerned about

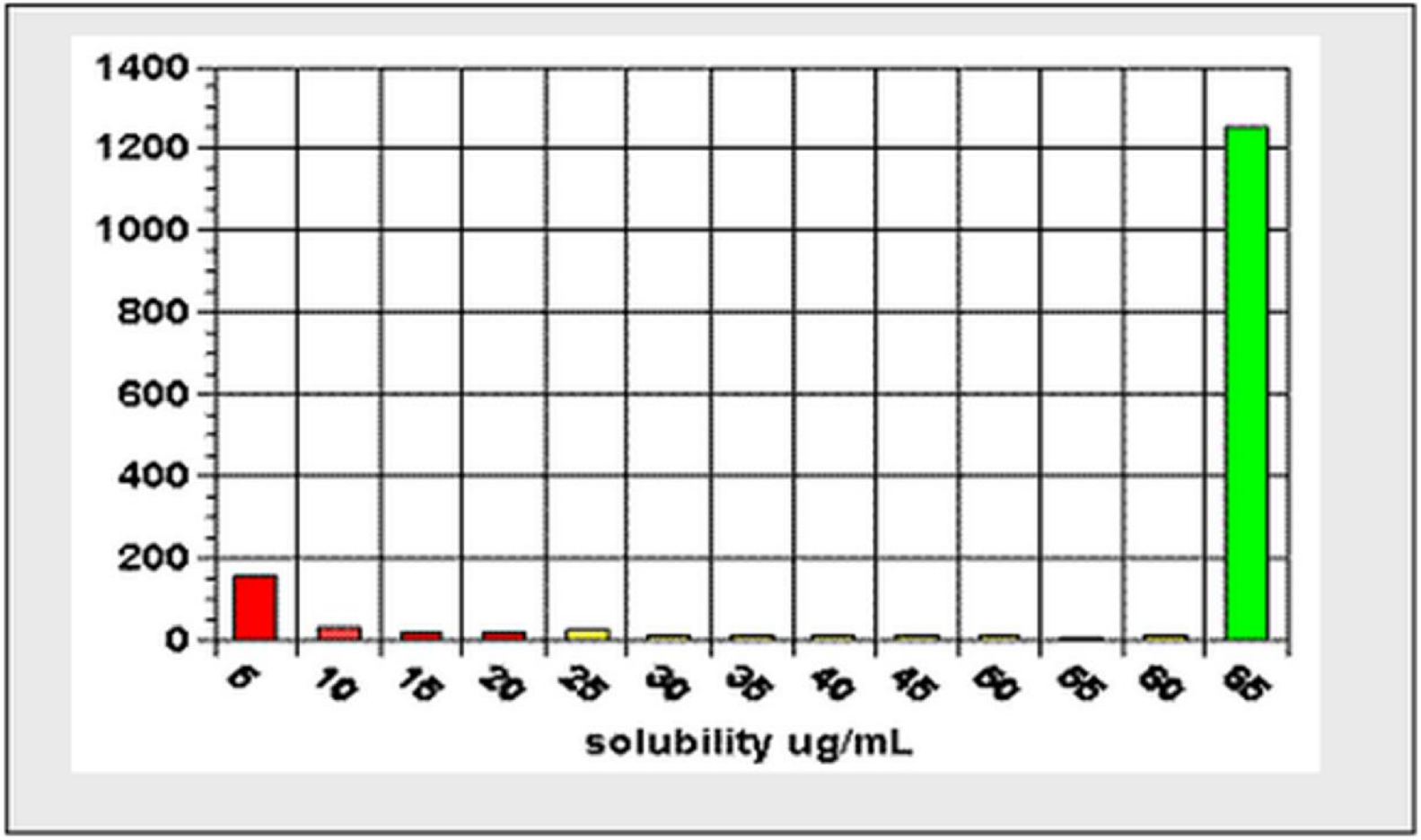
- **Erratic HTS screening results that seem to bear little or no relationship to the biology and maybe even to the chemistry**
  - **erratic rates of primary hits in HTS screens**
  - **erratic re-confirm rates on primary HTS actives**
  - **differences among screening sites**
  - **differences among compound collections**

Minimum Acceptable Solubility in µg/mL Bars shows the minimum solubility for low, medium and high permeability (Ka) at a clinical dose. The critical range for aqueous and DMSO solubility is around 10 uM



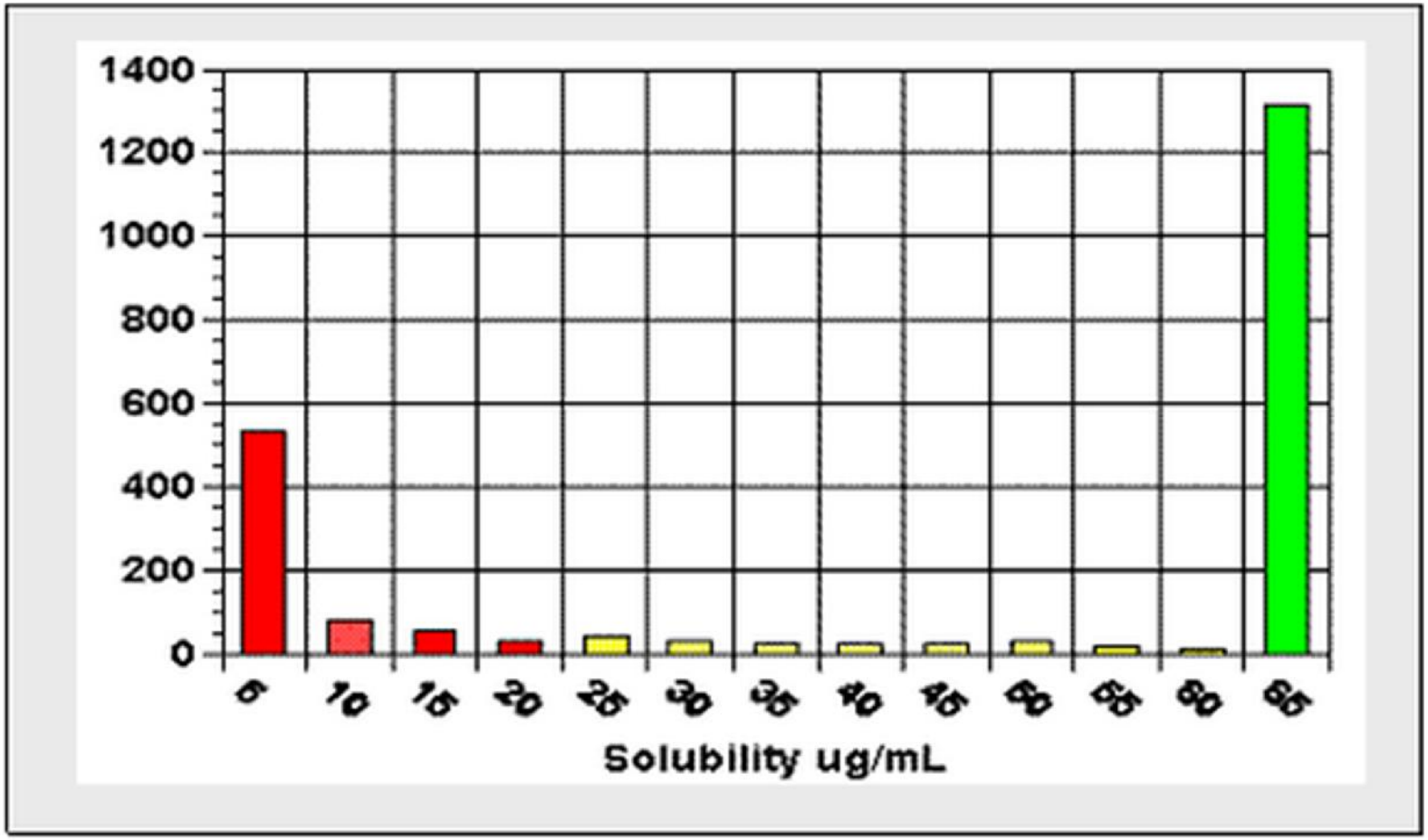
Lipinski LRIG, Mid Atlantic Meeting, January 2004

14.2% of 1597 phase II compounds have low aqueous solubility (<20 µg/mL)



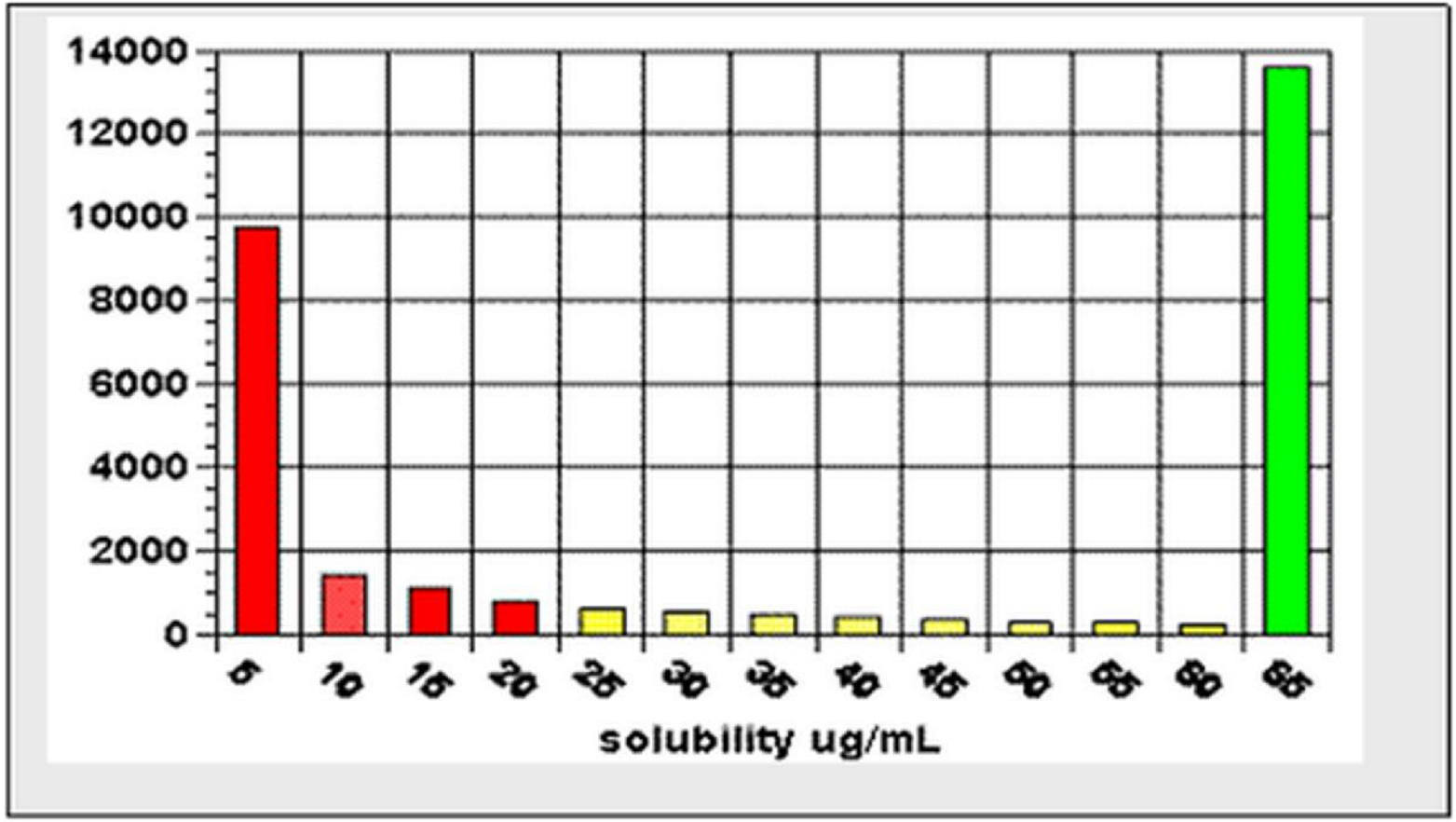
Lipinski LRIG, Somerset NJ, January 13, 2005

31.2%of 2246 commercial compounds have low aqueous solubility (<= 20 µg/mL)



Lipinski LRIG, Somerset NJ, January 13, 2005

39.8%of 33093 medicinal chemistry compounds have low solubility (<= 20 µg/mL)



Lipinski LRIG, Somerset NJ, January 13, 2005

Evidence for 35-40% poor aqueous solubility

- **70,000 compounds screened at Pfizer, Groton**
- **Capsugel Symposium June 2003 Tokyo**
  - **solubility formulation, customer feedback**
- **Collaborator feedback to Matrical**
  - **“Sonicman” HTS plate based sonicator**

Dimension of the solubility problem

- **40% of compounds have poor aqueous solubility**
- **Half the problem due to size / lipophilicity**
- **Half the problem due to crystal packing**
- **DMSO no better than water for compounds insoluble due to crystal packing**
- **Suggests an upper limit of 20% compounds insoluble in dry DMSO at 10uM**
- **Will this limit ever be achieved?**
- **Depends on whether a nucleation event occurs**
- **Depends on number of freeze thaw cycles**

Compounds differ in aqueous and DMSO solubility based on crystalline form

- **Ostwalds "rule of stages"**
- **Sequence of compound batch isolation proceeds towards thermodynamically most stable form**
  1. **amorphous - highest energy solid form**
  2. **highest energy crystalline polymorph**
  3. **lowest energy crystalline polymorph**
- **Amorphous is the highest energy form**
  - **most soluble in water and DMSO**

Early discovery compound purity

- **Pressure on chemistry to increase output**
  - **crystallization has disappeared**
- **Combinatorial compounds are now being purified by automated procedures**
  - **85-95% pure by ELSD or UV**
- **Compounds “appear” more soluble**
  - **amorphous state**
  - **impurities enhance solubility**
  - **crystal growth retardation**

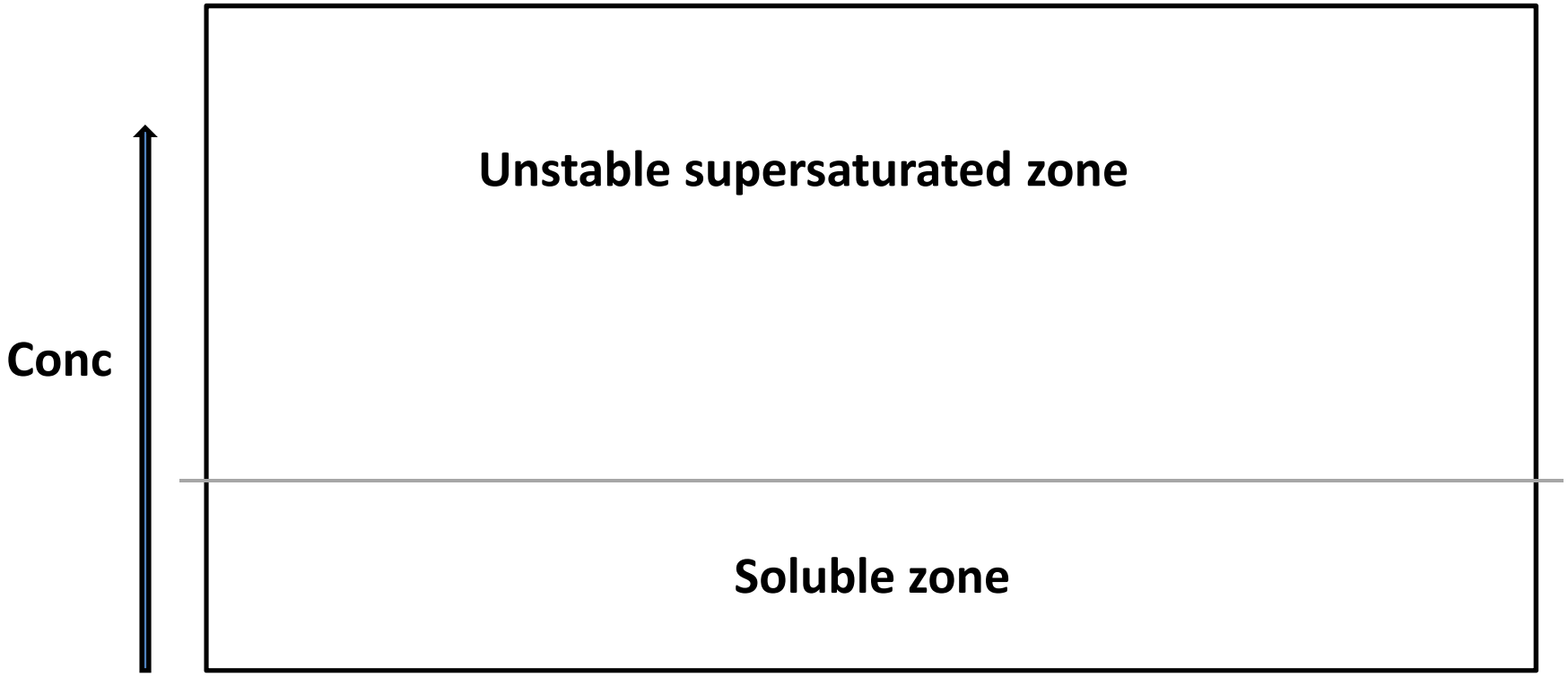
Fraction of Pfizer Groton compounds having melting point field information

Lipinski LRIG, Mid Atlantic Meeting, January 2004

Consequences of amorphous compounds

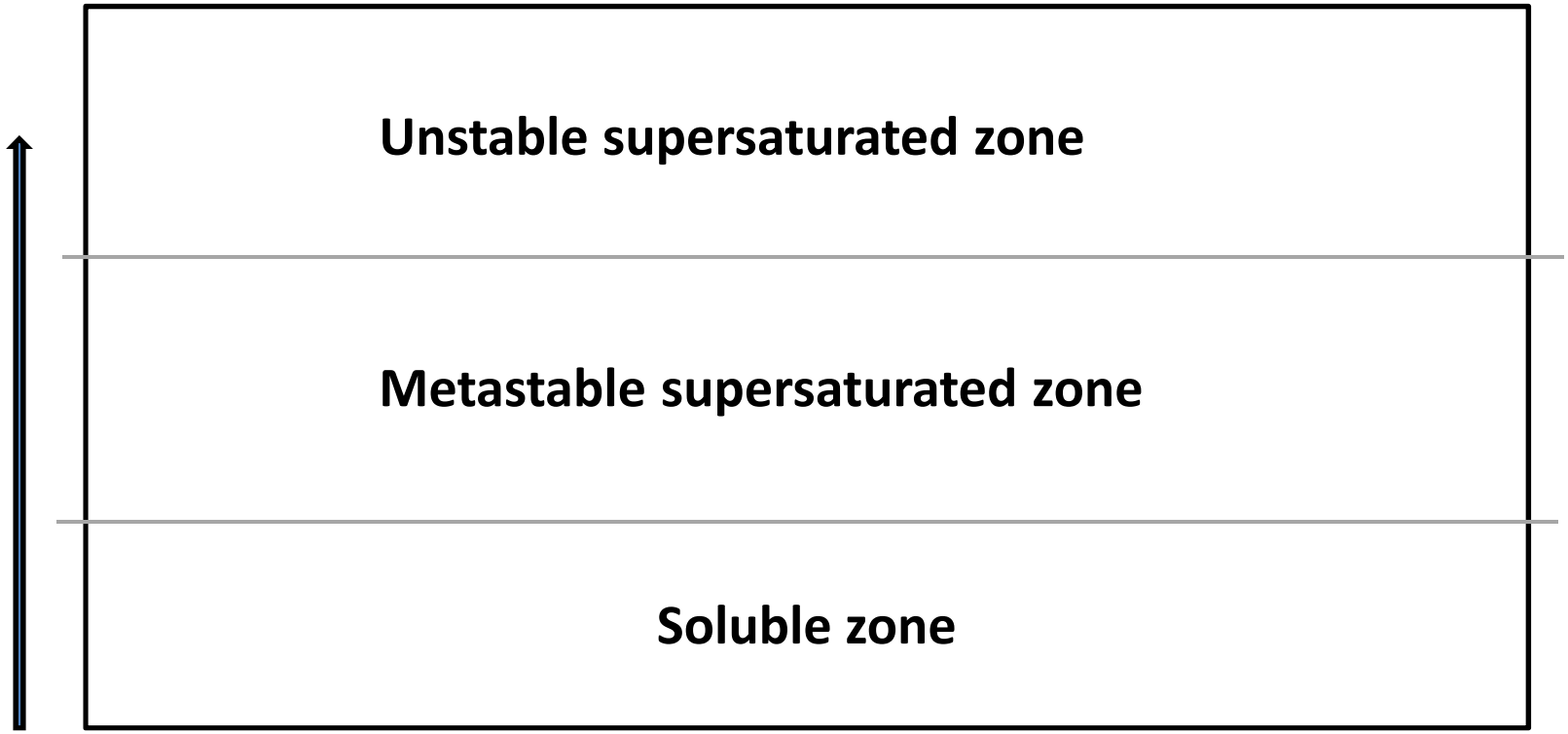
- **Amorphous DMSO solubility is always higher than when compound is crystalline**
- **Amorphous compounds from combichem or medchem initially easily dissolve in DMSO**
  - **allows preparation of DMSO stocks**
- **Sets stage for later precipitation problems**

Thermodynamic aqueous solubility



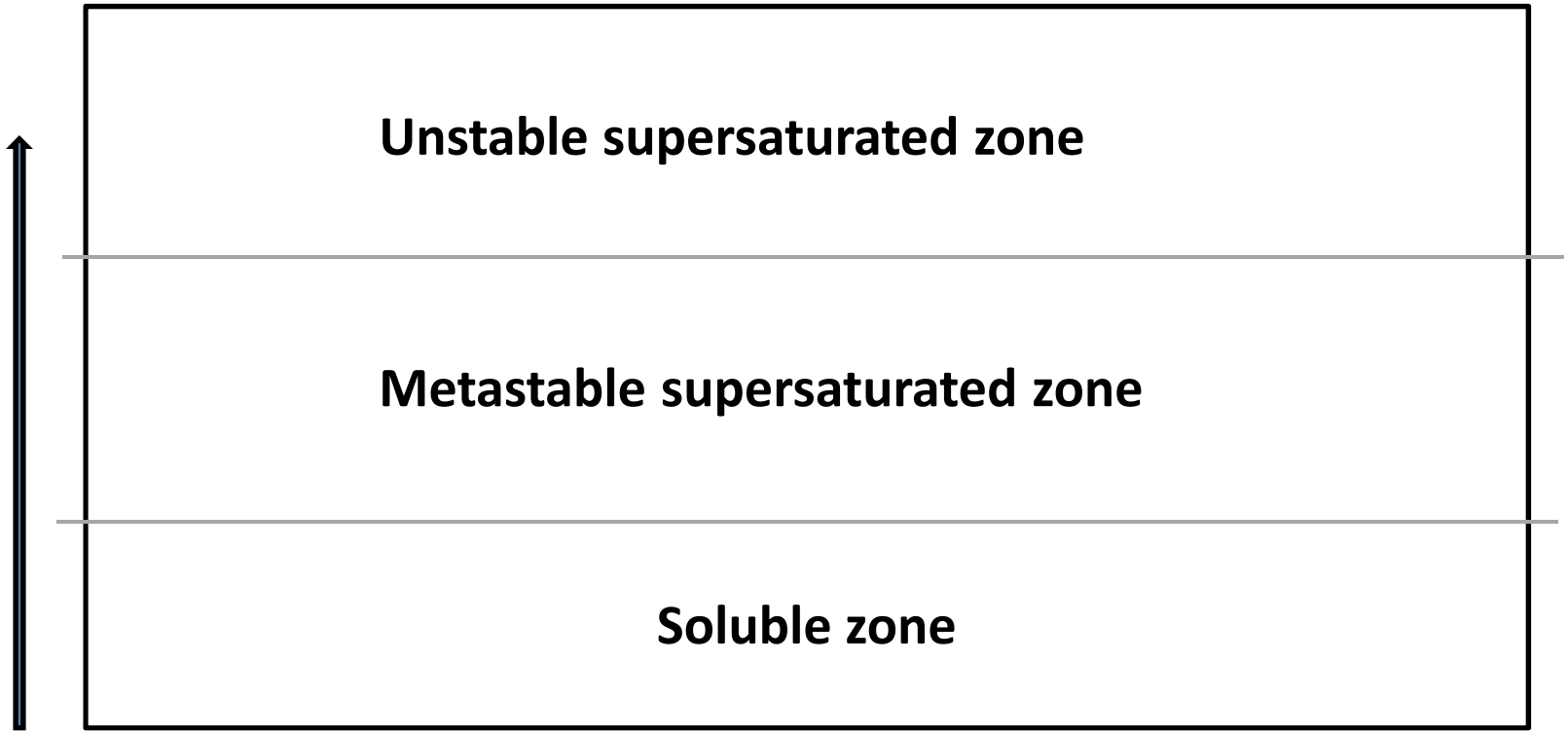
If solid is present, crystallization (precipitation) will occur if concentration rises into the supersaturated zone

Metastable supersaturated zone



If solid is not present, precipitation cannot occur from the metastable supersaturated zone even though the solution is thermodynamically supersaturated

Unstable supersaturated zone



If solid is not present, precipitation can occur from the unstable supersaturated zone. One way to enter the unstable zone is through freeze thaw cycles in wet DMSO

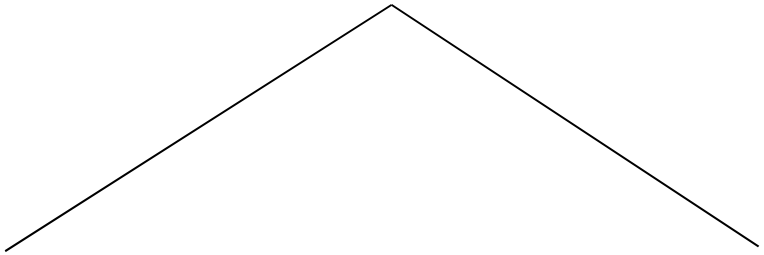
Water and DMSO solubility

- **Large, lipophilic compound**
  - **aqueous insoluble**
  - **DMSO greatly helps aqueous solubility**
- **Very crystalline compound**
  - **may show no computational problem**
  - **No “rule of 5” violation**
  - **aqueous insoluble**
  - **high melting point**
  - **strong intermolecular crystal lattice**
  - **DMSO does not help aqueous solubility**

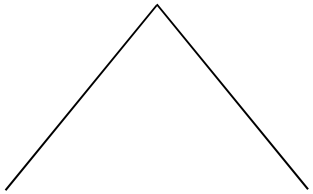
DMSO really helps aqueous solvation problems when the problem is size / lipophilicity

- **Compound has to make a "hole" in DMSO to dissolve**
- **Easier to do this in DMSO than in water**
  - **no H-bond donor / acceptor networks to disrupt**
- **DMSO has a high dielectric constant**
- **Solvates compound dipoles**
  - **almost all drugs have dipoles**

Sample storage in DMSO



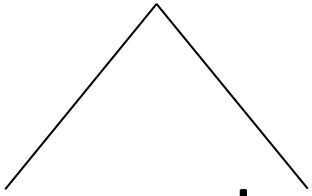
Centralized storage
Business rules enforced
Long storage times



solubility

chemical stability

End user handling / storage
No business rules / chaotic
Short storage times



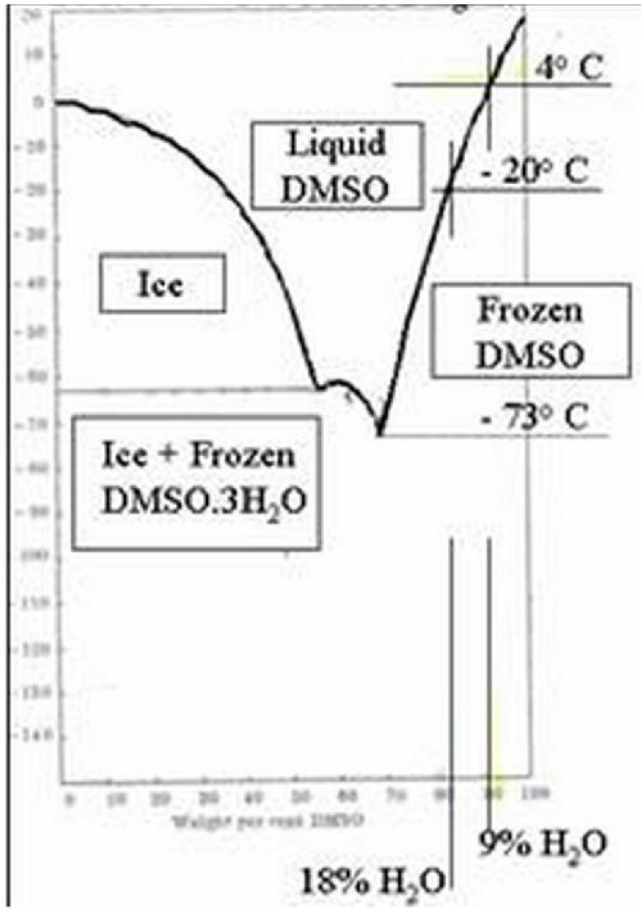
solubility

chemical stability

Sample lifetime. What is the key issue?

- **Compound disappears from DMSO solution**
- **What is the explanation?**
- **Chemical integrity**
  - **Keep cold and frozen**
  - **Avoid oxygen**
  - **Keep dry**
- **Compound solubility**
  - **Cold and / or frozen is the worst choice possible**
  - **Avoid freeze thaw cycles**

DMSO – water phase diagram



DMSO containing 9% water is unfrozen in the typical lab refrigerator

Very bad practice. Typical biology procedure of storing samples in DMSO in the non-freezer part of a lab refrigerator

Nature (1969), 220, 1315-1317

Timing Factor in Compound DMSO Solubility

- **Once a compound crystallizes from DNSO it will not easily re-dissolve**
  - **Crystallized compound is in a lower energy less DMSO soluble form**
- **Narrow working window (time window) for keeping most compounds dissolved in DMSO**
  - **1 to 2 days at room temperature**
  - **Explains why compounds are active when freshly made but not when stored**
- **Freeze thaw cycles increase the probability of crystallization**

Summary

- **Crystalline state is important to aqueous solubility**
- **Crystalline state is important to DMSO solubility**
  - **be alert for compound precipitation from DMSO**
  - **do not store liquid DMSO stocks in the refrigerator**
  - **minimize time once DMSO stocks are diluted**
  - **expect erratic HTS screening results dependent on minor compound handling differences**
  - **minimize freeze thaw cycles**
- **Poor DMSO solubility is here to stay**

Recent Developments in DMSO solubility

- **New Software**
- **DMSO solubility prediction software**
- **Pharma Algorithms**
  - **http://ap-algorithms.com/dmso.html**
- **Chemical Diversity Labs**
  - **http://www.currentdrugdiscovery.com/pdf/2003/500632.pdf**
- **New Hardware**
- **Matrical**
  - **“SonicMan” plate based sonicator**
    - **http://www.matrical.com/Literature/SonicManFlier.pdf**

Acknowledgements

The generous support of Pfizer Global R&D, Groton Labs in my post retirement activities is gratefully acknowledged