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# Sample Preparation & Liquid Scintillation Counting

*From Professionals*
*For Professionals*

**J.Thomson**
**Worldwide C&S Product Manager**

# What is an LSC Cocktail ?

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An LSC Cocktail can be considered as :-

**S**olvent (Aromatic)
**S**cintillators (Fluorescing Agents)

This mixture is sometimes called a

**_Lipophilic Cocktail**

**S**olvent
**S**cintillators
**S**urfactant (Detergent)

This mixture is also called an

**Emulsifying Cocktail.**

# Why is an LSC Cocktail used ?

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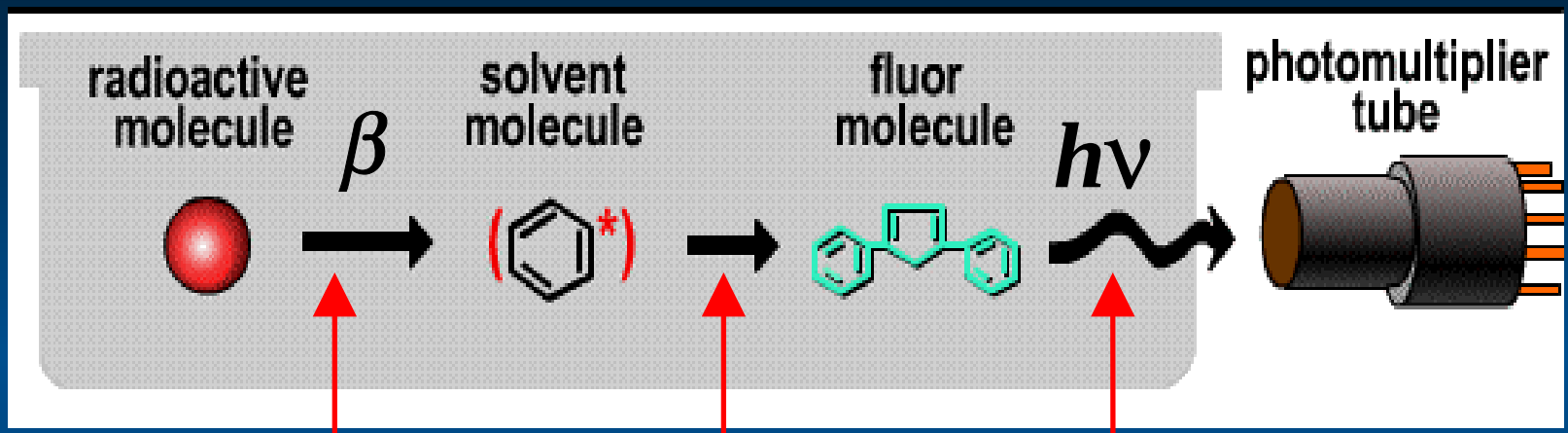
The majority of radioactive species are present in an aqueous form, and as such are not miscible with aromatic solvents.

The presence of surfactants (detergents) in the cocktail enables an aqueous sample to come into intimate contact with the aromatic solvent by forming a stable microemulsion.

# How does an LSC cocktail work ?



# How does an LSC cocktail work ?

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## Scintillation Counting

1

Liquid Scintillation Cocktail

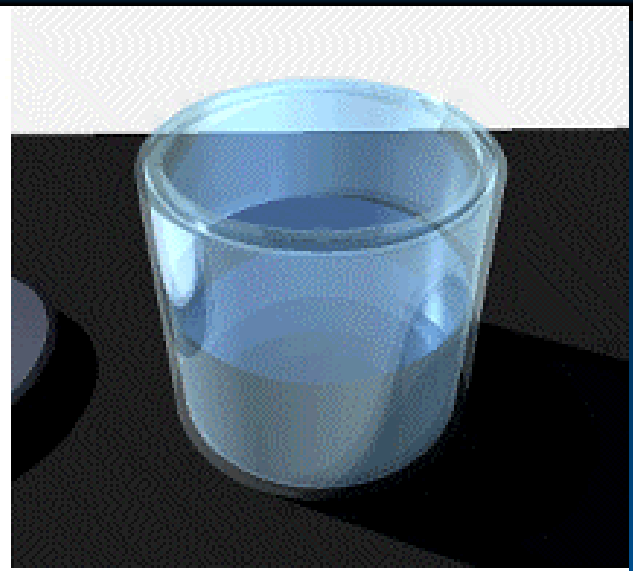
Components:

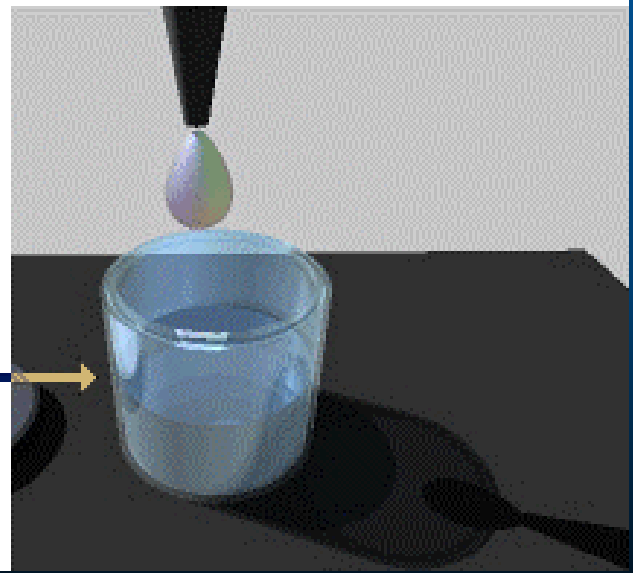
**Solvent:** Typically toluene, xylene pseudodocumene, or an alkyl benzene type solvent.

**Emulsifier:** A detergent type molecule that ensures proper mixing of aqueous samples.

**Fluor:** A fluorescent solute.

Process: Radioactive Sample is added to scintillation cocktail.





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# How does an LSC cocktail work ?

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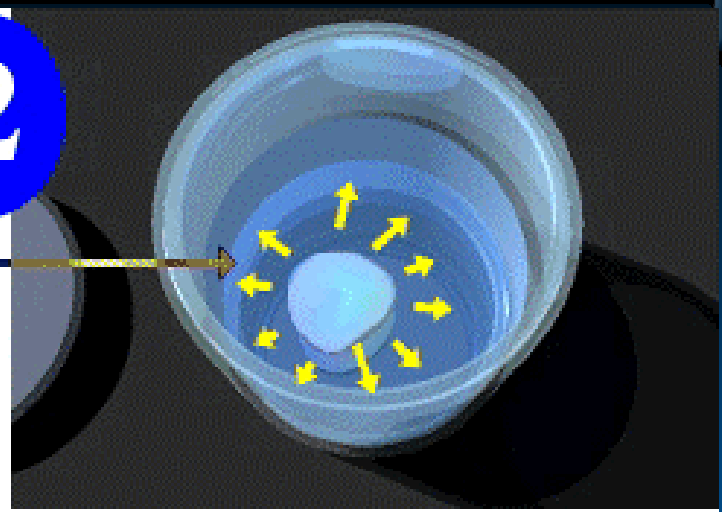
## Scintillation Counting 2

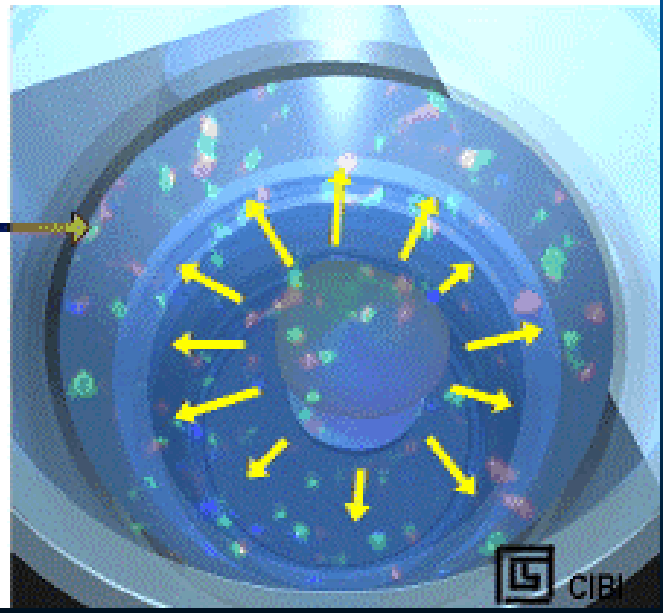
Beta particles are emitted, which cause solvent molecules to become excited.



$$\beta^- \rightarrow (\text{C}_6\text{H}_6^*)_n$$

excited solvent molecules

The energy of the solvent molecules is transferred to the fluor molecules, which in turn emit light.




$$(\text{C}_6\text{H}_6^*)_n \rightarrow (\text{C}_{14}\text{H}_{10}\text{O}^*)_n \sim n$$

excited fluor molecules

light photons

# *Why are these components in an LSC Cocktail?*

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**The aromatic solvent is necessary since it contains a high density of π electrons, necessary for the efficient transfer of the energy of radioactive decay.**

**The surfactant is needed to enable a stable microemulsion to be formed when aqueous samples are present, necessary for stable conditions over the counting period.**

**The scintillators are present to emit a light pulse which is within the optimum detection range of the photomultiplier tube.**

# What Aromatic Solvents are in LSC Cocktails ?

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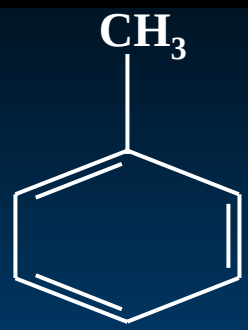
1. Toluene

$C_7H_8$

CAS No. 108-88-3

RPH = 100

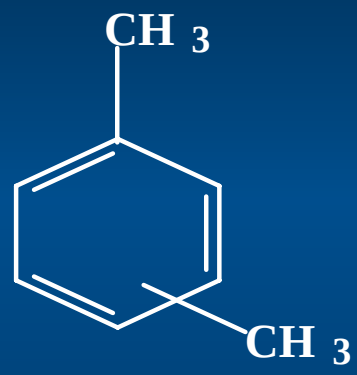



2. Xylene (mixed isomers)

$C_8H_{10}$

CAS No. 1330-20-7

RPH = 110

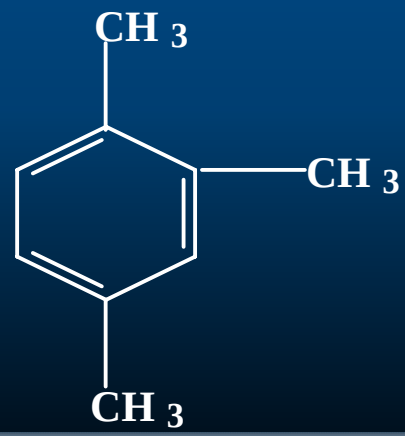



3. Pseudocumene (1,2,4-trimethylbenzene)

$C_9H_{12}$

CAS No. 95-63-6

RPH = 112

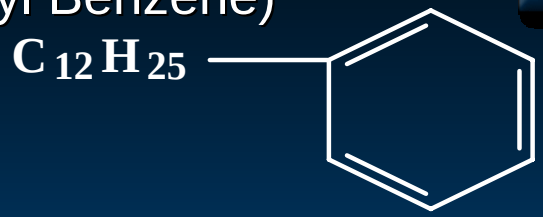


4. Dodecyl Benzene (LAB -Linear Alkyl Benzene)

**$C_{18}H_{30}$**

**CAS No. 123-01-3**

**RPH = 94**

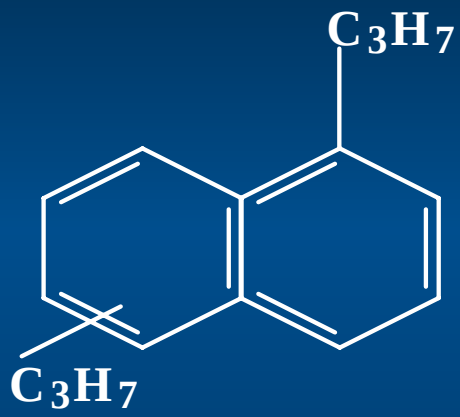



5. Di-isopropylnaphthalene (DIN)

**$C_{16}H_{20}$**

**CAS No. 38640-62-9**

**RPH = 112**

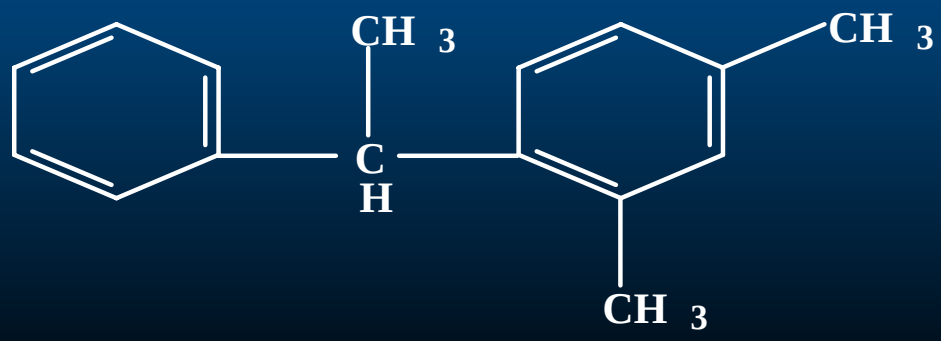



6. 1-Phenyl-1-Xylyl Ethane (PXE)

**$C_{16}H_{18}$**

**CAS No. 6196-95-8**

**RPH = 110**



# What Detergents are used in LSC Cocktails ?

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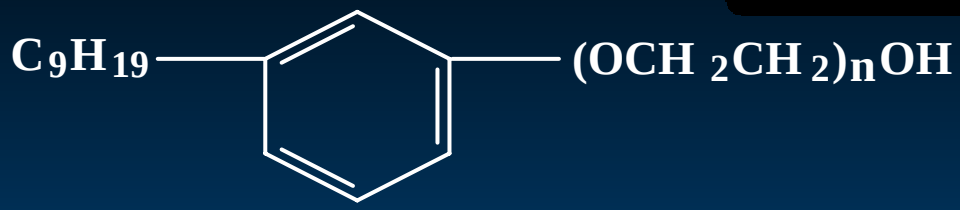
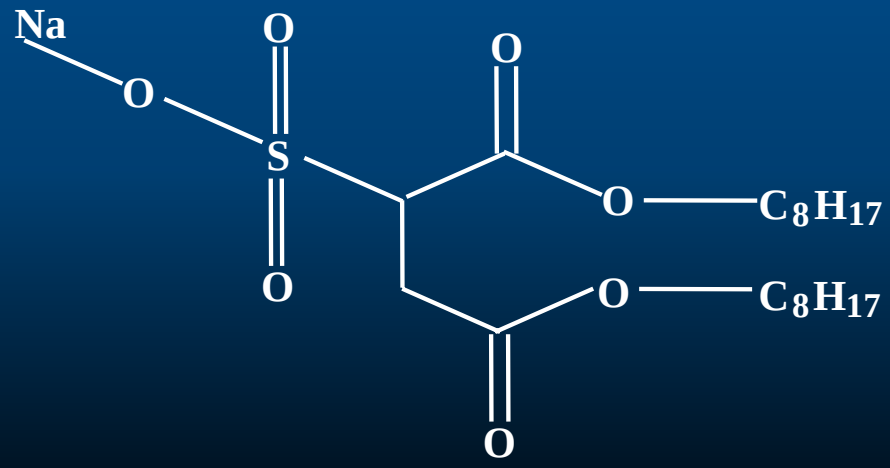
1. Ethoxylated Alkylphenols
   (Non-Ionic detergent)
   CAS No. 9016-45-9




2. Mono-/Di- phosphate ester
   (Anionic detergent)
   CAS No. 298-07-7


3. Sodium di-octylsulphosuccinate
   (Anionic detergent)
   CAS No. 577-11-7



# What Scintillators are used in LSC Cocktails ?

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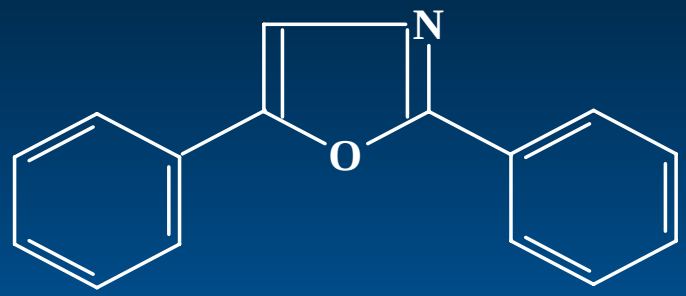
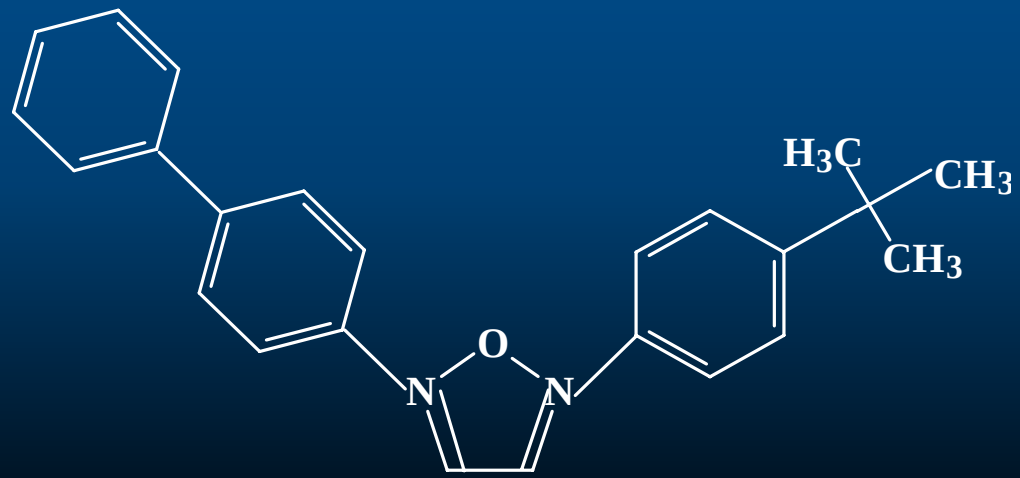
## Primary Scintillators

A. 2,5-diphenyloxazole (PPO)
(Primary Scintillator)
$C_{15}H_{11}NO$
CAS No. 92-71-7




B. Butyl PBD [2-(4-tert-butylphenyl)-5-(4-biphenylyl)-1,3,4-oxadiazole]
(Primary Scintillator)
$C_{24}H_{22}N_2O$
CAS No. 15082-28-7



# What Scintillators are used in LSC Cocktails ?

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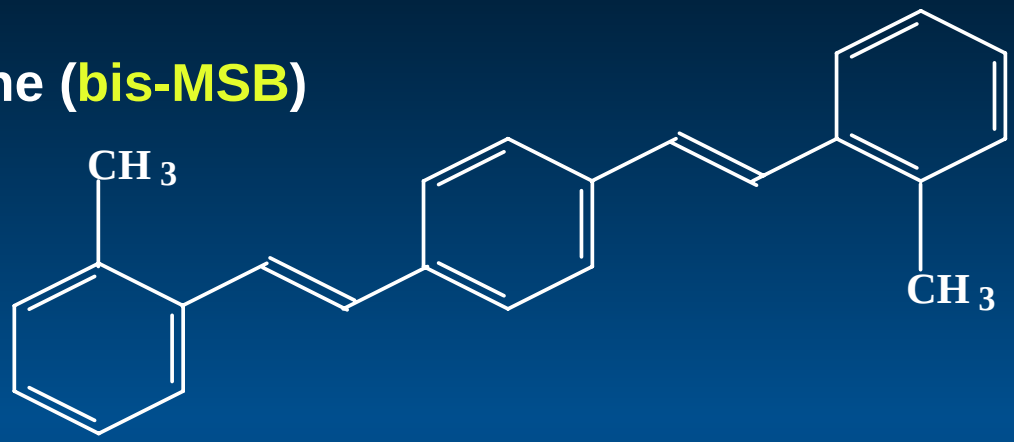
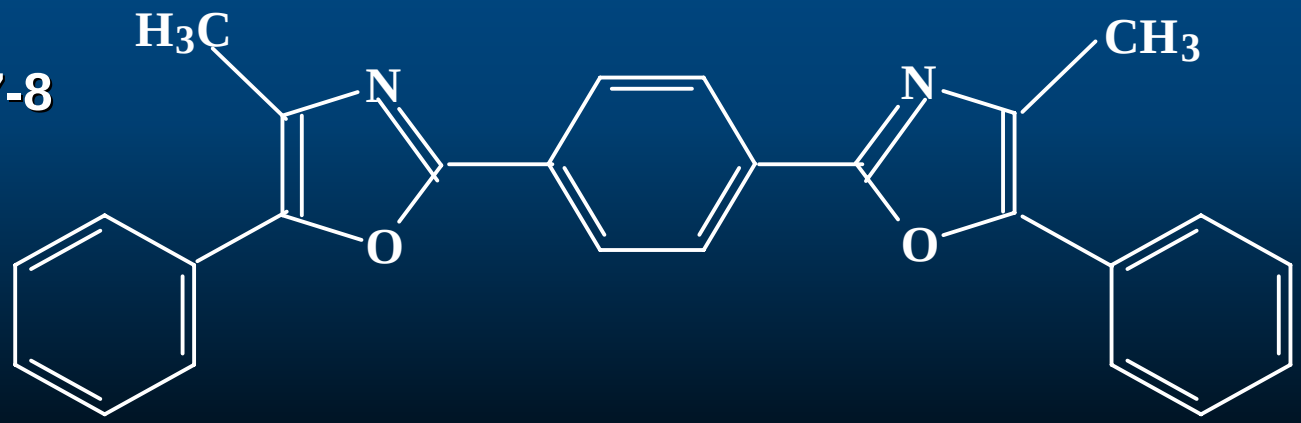
## Secondary Scintillators

C. 1,4-Bis(2-methylstyryl)benzene (bis-MSB)
(Secondary Scintillator)
$C_{24}H_{22}$
CAS No. 13280-61-0




D. 1,4-Bis(4-methyl-5phenyl-2oxazolyl)benzene (Dimethyl POPOP)
(Secondary Scintillator)
$C_{26}H_{20}N_2O_2$
CAS No. 3073-87-8



# What Vials are best?

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# *Vials*

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**Glass is used because :**

- ✓ It provides unparalleled optical clarity (good visibility)
- ✓ It is chemically inert, making it suitable for use with aggressive reagents and when solvents are used.

**Plastic is used because :**

- ✓ It exhibits lower background levels than glass.
- ✓ It is combustible and therefore easier for waste disposal.
- ✓ It is shatterproof and therefore safer in the laboratory.

# *What size of vials are available ?*

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**Vials (glass and plastic) are available in sizes ranging from <4mL up to 20mL.**

**This maximum of 20mL is fixed due to the dimensions of currently used photomultiplier tubes (2 inch diameter).**

# Liquid Sample Preparation

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## Why is a sample presented in a liquid form ?

**Liquid samples are preferred due to the fact that the scintillation counting process takes place most efficiently in an LSC cocktail (due to 4 π geometry).**

**Since certain liquids are miscible and can therefore mix together rapidly and completely, samples in a liquid medium are preferred.**

# Sample Counting Geometries

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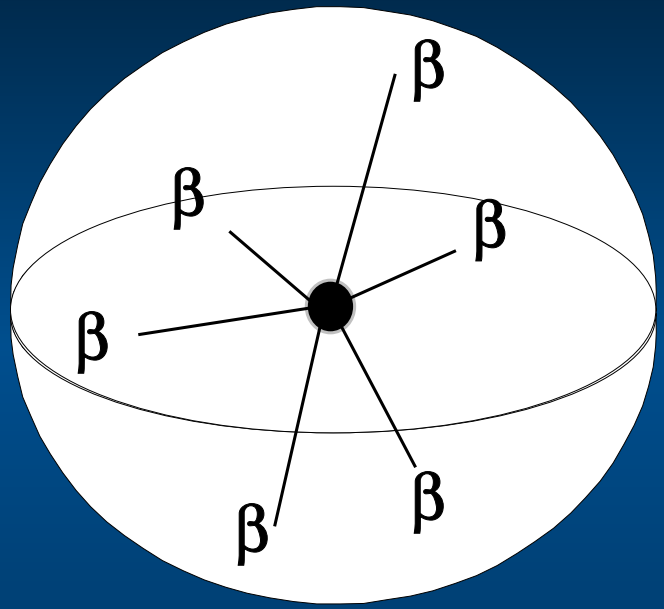
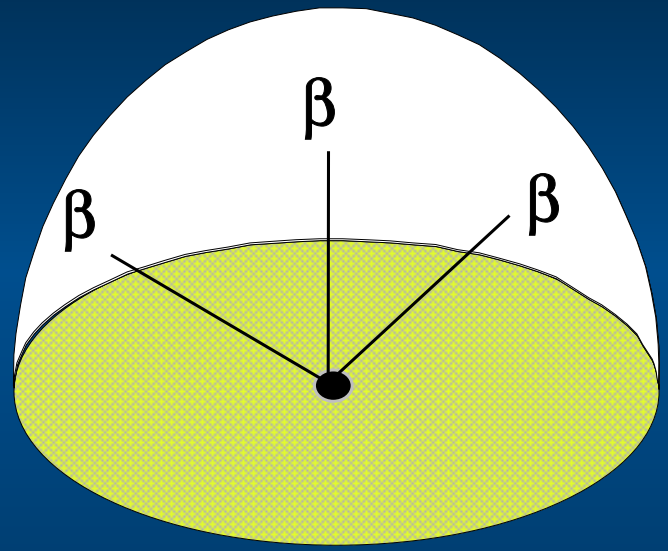
## 4π Geometry (Homogeneous)




Surface Area = $4\pi r^2$

## 2π Geometry (Heterogeneous)




Surface Area = $2\pi r^2$

# *Aqueous Samples*

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## Inorganic Anions

Chlorides, Sulfates, Phosphates, Nitrates, etc.

## Potential Obstacles

Di- and Tri- valent anions sometimes cause instability.
Both volume and concentration dependent.
Some metallic salts form colored solutions.

## Remedy

- ✓ Select a cocktail suitable for use with these difficult anions (Ultima Gold and HiSafe series)
- ✓ Dilute sample with water or increase cocktail volume.
- ✓ Use a cocktail which is resistant to color quenching (Ultima Gold and HiSafe series)

# *Aqueous Samples*

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## Mineral Acids and Organic Acids

**Hydrochloric Acid, Nitric Acid, Sulfuric Acid, Phosphoric Acid, TCA, Acetic Acid etc.**

## Potential Obstacles

**Certain strong mineral acids interact with the cocktail components causing color development and performance changes. Conc. sulfuric acid will cause color formation and sulfonation. TCA can result in acid-induced luminescence.**

## Remedy

- ✓ **Do not add concentrated acids to the cocktail.**
- ✓ **Always dilute the acid with water.**
- ✓ **Use a cocktail which has proven performance with acidic samples (Ultima Gold AB & LLT).**

# *Aqueous Samples*

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## Inorganic Alkalis

Sodium Hydroxide, Potassium Hydroxide, etc.

## Potential Obstacles

Alkalis usually cause chemiluminescence.
Prolonged contact can result in color formation.
Conc. potassium hydroxide will give high "backgrounds".

## Remedy

- ✓ Use a cocktail with proven resistance to CLM (**Hionic-Fluor**)
- ✓ Dilute the alkali to reduce the effect.
- ✓ Acidify the cocktail / sample mixture.
- ✓ Do not store for prolonged periods.
- ✓ Change to Sodium Hydroxide; KOH contains $^{40}K$.

# *Samples in Organic Solvents*

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**Potential Obstacles:**

**Quench effect of organic solvent i.e.**
**Nitro > Sulphide > Halide > Amine > Ketone > Aldehyde > Organic Acid > Ester > (Water) > Alcohol > Ether > Hydrocarbon**

**Remedy:**

**1. Change the organic solvent to a less quenching alternative and/or use a more chemical quench resistant cocktail.**
**2. Always check to ensure that the cocktail accepts the mixture.**

# *Biological Samples*

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1. **Direct Counting** (addition of raw sample to a suitable cocktail) is suitable for :
   Urine, Serum, Plasma and Water soluble protein

2. **Solubilization** (dissolution/digestion of sample) is suitable for :
   Blood, Plasma, Serum, Feces, Homogenates & Bacteria

3. **Combustion** (oxidation/burning sample to convert the organic carbon and hydrogen to $^{14}CO_2$ and $^{3}H_2O$ respectively) is suitable for all of the above sample types without exception.

# Problems with Biological Samples

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## Urine :-

**Color quench:**

Reduce sample volume.

Use correct cocktail.

**Precipitation of proteins:**

Arrange to count samples within 24 hours. Correct cocktail selection (Pico-Fluor MI, Ultima Gold LLT)

**Additional information**

Urine from small animals more difficult

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## Recommended LSC cocktails for Urine

| Cocktail | Type | Max. urine* capacity @20°C | Recommended loading |
|---|---|---|---|
| Ultima Gold LLT | Safer | 10.0mL | 2.0mL |
| Ultima Gold | Safer | 8.0mL | 2.0mL |
| Pico-Fluor MI | Classical | 4.0mL | 4.0mL |

* Results based on human urine

Note :-

1. It is possible to add up to 1mL of either ethanol or isopropyl alcohol per 10mL cocktail, to suppress the appearance of the protein precipitate.

2. Urine samples from small animals are usually difficult. With these, either keep sample volume low, or dilute the urine with water.

# Other LSC cocktails for Urine

| Cocktail | Type | Max. capacity at 20°C | Comments |
|---|---|---|---|
| Ultima Gold | Safer | 8.0mL | Slight wispy precipitate appears after 24hours at >2.0mL sample. |
| Ultima Gold XR | Safer | 10.0mL | Slight wispy precipitate appears after 24hours at >3.0mL sample. |
| Ultima Gold AB | Safer | 10.0mL | Slight wispy precipitate appears after 24hours at >2.0mL sample. |
| Ultima Gold LLT | Safer | 10.0mL | Slight wispy precipitate appears after 24hours at >2.0mL sample. |
| Opti-Fluor | Safer | 9.0mL | Slight wispy precipitate appears after 24hours at >3.0mL sample. |
| Emulsifier Safe | Safer | 2.5mL | Slight wispy precipitate appears after 24hours at >3.0mL sample. |
| Pico-Fluor MI | Classical | 4.0mL | Remains clear up to 4.0mL sample |
| Hionic-Fluor | Classical | 1.5mL | >2.0mL sample forms a gel |
| Insta-Gel Plus | Classical | 1.0mL | |
| Pico-Fluor 40 | Classical | 3.0mL | Slight wispy precipitate appears after 24hours at >3.0mL sample. |

# Problems with Biological Samples

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## Plasma/Serum :-

**Sample volume:**

Keep sample volume to <1mL per 10mL cocktail

**Color quench:**

Reduce sample volume.

Use correct cocktail (Pico-Fluor MI, Ultima-Flo M)

**Precipitation of proteins:**

Arrange to count samples within 24 hours.

Correct cocktail selection.

(Try adding 10% ethanol or IPA to inhibit protein precipitation) Plasma/Serum from small animals is more difficult.

# Solubilization

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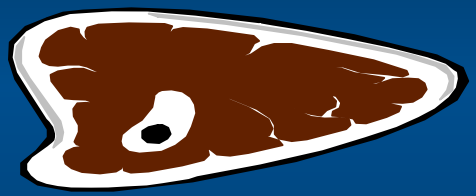
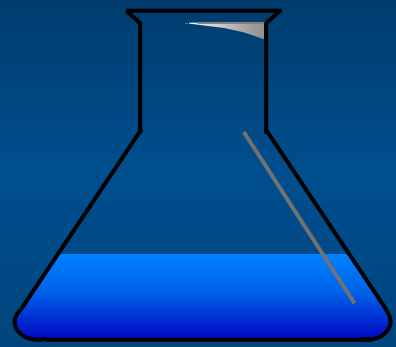
Biological Sample



Solubilizer



# *Solubilization Systems*

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## Alkaline Systems

- ✓Quaternary ammonium hydroxide, NaOH
- ✓Method of action is by ***alkaline hydrolysis*****.**

## Acidic Systems

- ✓Perchloric Acid, Nitric Acid, Perchloric/Nitric Acid Mixture,Perchloric Acid/Hydrogen Peroxide Mixture
- ✓Method of action is by ***acidic oxidation*****.**

## Other Systems

Sodium Hypochlorite

Method of action is by ***oxidative bleaching*****.**

# *Solubilization*

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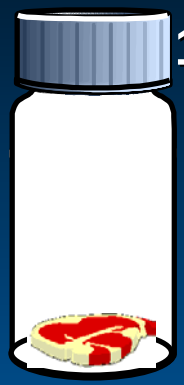
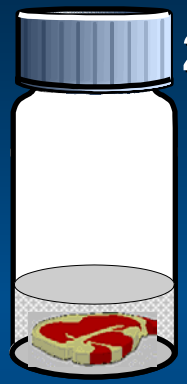
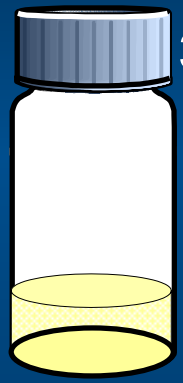
## What happens during Solubilization?



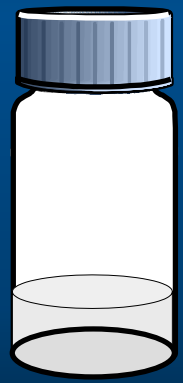
1. **Add sample** to glass vial.



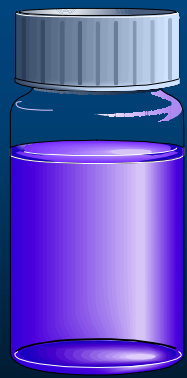
2. **Add solubilizer** and **heat** at ~55°C.



3. **Add peroxide** to decolorize.



4. **Add LSC Cocktail**.



5. **Dark adapt** before **counting**.

# *Solubilizers & Recommended Cocktails*

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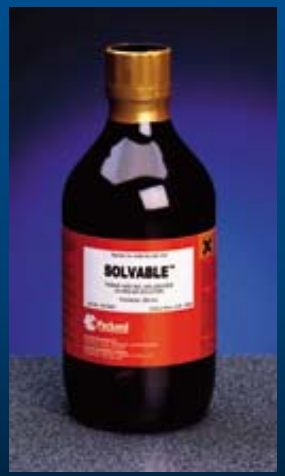
**Soluene-350**

- ✓Organic-based solubilizer, formulated in toluene
- ✓Classical industry-standard solubilizer
- ✓For biological and plant samples
- ✓Corrosive & Flammable

**Recommended cocktail** - **Hionic-Fluor**




**SOLVABLE**

- ✓**Aqueous-based solubilizer (safer)**
- ✓Can substitute for Soluene-350 in most cases
- ✓For biological and plant samples
- ✓Corrosive, non-flammable

**Recommended cocktail** - **Ultima Gold**

# Conclusion

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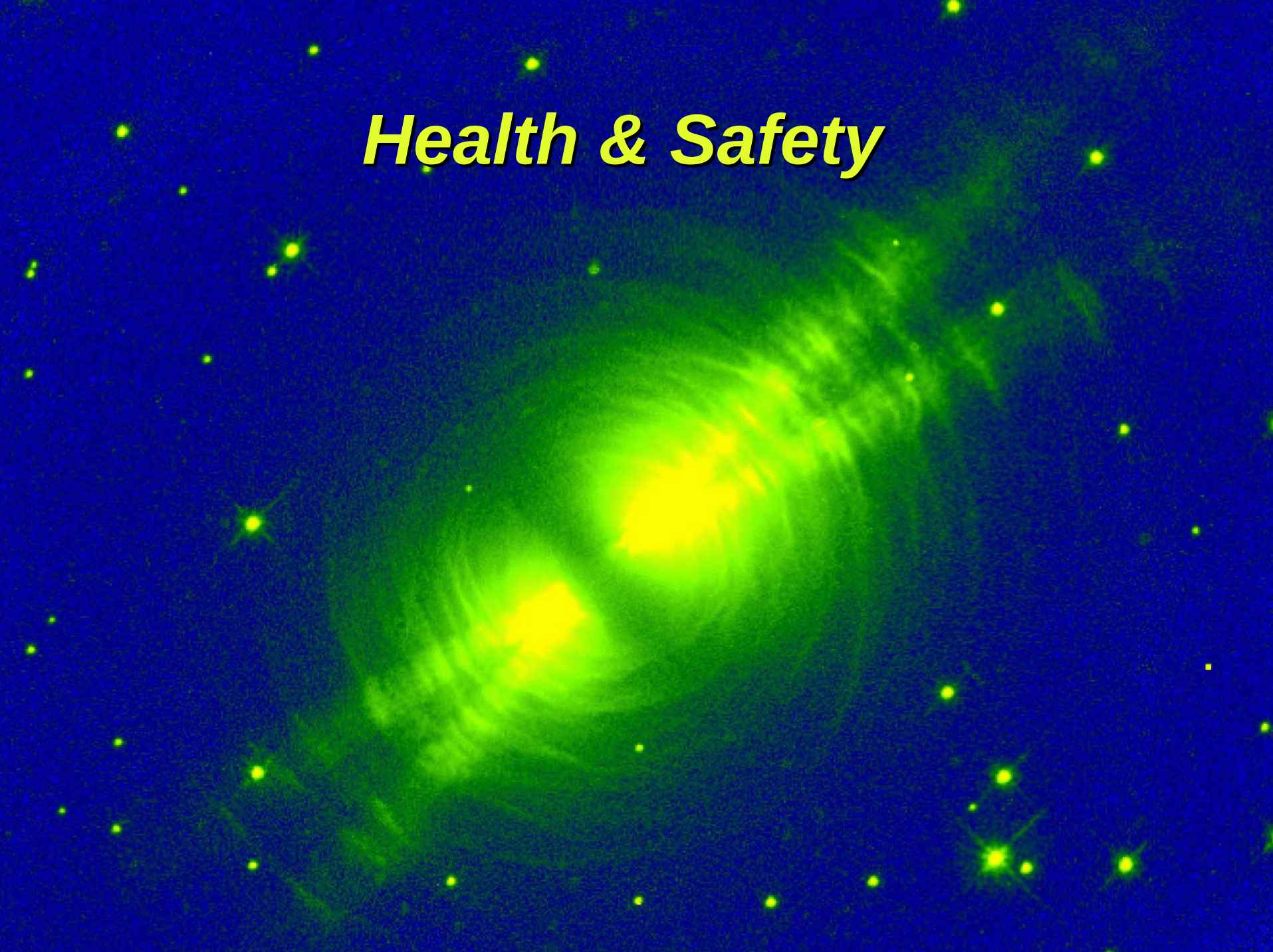
***Good sample preparation***

***=***

***Good LSC counting***

# Health & Safety



# *Health & Safety*

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**The correct term to use with LSC cocktails is SAFER, i.e. safer than the tradition or classical solvents such as Toluene, Xylene and Pseudocumene.**

**NO chemical can be properly classified as SAFE.**

# *Classical vs. Safer LSC Solvents*

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- **Classical Cocktails** - Toluene,Xylene,Pseudocumene
  - ✓ Hazardous by inhalation
  - ✓ Hazardous by skin absorption
  - ✓ Irritating to skin and eyes
  - ✓ High vapor pressure
  - ✓ Low flash point (flammable)

- **Newer Safer Cocktails** - DIN, PXE, LAB
  - ✓ Low toxicity
  - ✓ Classified as harmless (for transport & storage)
  - ✓ Low vapor pressure
  - ✓ High flash point (non-flammable)
  - ✓ Biodegradable

# Safety Phrases (Mandatory)

**Clearly visible hazard warnings & safety phrases**

# *BIODEGRADABLE - What about Cocktails?*

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**PerkinElmer Safer Cocktails:**

**Testing for biodegradability is according to Test Method OECD 301E (equivalent to ISO Method 7827 1984). To pass, the sample must show >70% DOC removal within 28 days.**

**A cocktail which passes this test can be described as "Readily Biodegradable".**

# *Necessary Statements*

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## Disposal Considerations

**Waste products may be disposed of by controlled incineration. National and local legislation concerning the disposal of waste materials must be complied with.**

***"We do not advocate drain disposal of cocktail waste. Biodegradability information on Packard cocktails is provided to ensure that in the event of a small spillage, any waste that ends up in the sewerage system will be biodegraded."***

# A few final thoughts !!!!!

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*Any LSC cocktail will work with any LSC -*
*The trick is to get the best combination.*

*Poor sampling can only produce a bad sample.*

*A badly prepared sample can only produce bad results.*

*No amount of instrument sophistication can ever produce good results from a badly prepared sample.*