CHAPTER 3

DI-ISOPROPYLNAPHTHALENE—A New Solvent for Liquid Scintillation Counting

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INTRODUCTION

Although there have recently been a few significant developments in the field of solvents suitable for use in liquid scintillation counting (LSC), the traditional solvents xylene and toluene are still used. A review of the evolution of LSC Solvents (Figure 1) shows that after an initial period of development in the 1950s and '60s very little has happened until quite recently. Toluene and xylene were the initial solvents of choice due to their commercial availability and suitability.[1] Dioxan, on its own and in conjunction with naphthalene, was also widely used at this time due to the ability of this system to accept quantities of aqueous sample, thus expanding the application.

After a brief period of inactivity, the '60s saw the emergence of some alternative solvents of which decalin and aromatic petroleum distillates (C9 to C12) were the most notable. These partly supplanted toluene and xylene because of their lower cost. However, the development of emulsifying cocktails in the '60s further established toluene and xylene as the preferred LSC solvents.

Then, in the 70s changes in market trends saw the introduction of a new type of emulsion cocktail—for Radio-immunoassay—based on pseudocumene.

This change coincided with an increased awareness of safety brought about by the restrictions imposed on the handling and use of certain hazardous solvents. The search was now on for safer alternatives to toluene and xylene that had equivalent or better characteristics with respect to their LSC performance. Another factor which became important at this time was the adverse effect on the environment of waste chemicals. Formulators gradually became aware of these factors and realized that a very different type of solvent would be required to satisfy the changing demands of both the market and the environment. Thus the main driving force behind recent developments in sol-

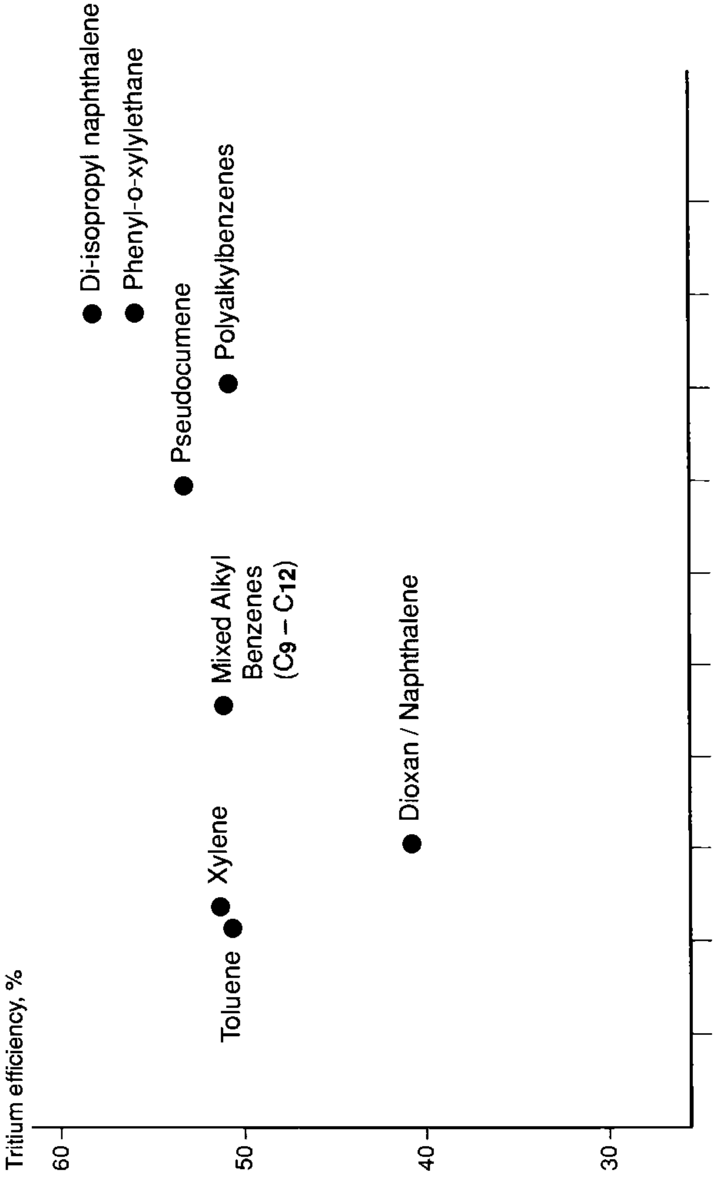

Figure 1. Evolution of LSC solvents.

vents for LSC has not been the search for more efficient solvents, but rather the requirement to substitute less hazardous and more environmentally friendly solvents for those previously used.

In the early '80s the first attempts to resolve these problems involved the use of polyalkylbenzenes which met some but not all of the requirements. The major failings were their low counting efficiency, lack of biodegradability and their toxicity. At Fisons we were active in this field of development. Indeed we used polyalkylbenzene solvents in emulsion cocktails simply because they were the best compromise available. During the course of our investigation about 30 solvents were evaluated for their potential in LSC, but for one or more of the above reasons they all proved to be unsuitable. In due course we became aware of the existence of Di-isopropylnaphthalene (DIN) and quickly realized that here was a solvent that could be ideally suited to LSC.[2] DIN is a mixture of position–isomeric di-isopropylnaphthalenes and is a product of Rutgers-Kureha-Solvents Ltd.

In order to fully appreciate the benefits afforded by DIN consider an ideal LSC solvent.

DEFINING THE IDEAL

In evaluating solvents for use in LSC our start point was to define the characteristics of an ideal LSC solvent. Based on our own experience and comments by other workers in the field we listed the following essential attributes:

1. high flash point
2. low vapor pressure
3. odorlessness
4. low toxicity and irritancy
5. no permeation through plastics
6. biodegradability
7. good fluor solubility
8. low photo- and chemiluminescence
9. high counting efficiency (tritium)
10. good colour and chemical quench resistance

The ideal LSC solvent would obviously satisfy all these requirements but hitherto no solvent has. The evaluation of DIN against these criteria will indicate how it equates to the ideal LSC solvent. Pseudocumene is used in the comparison because it is typical of the solvents currently in wide use in LSC cocktails.

EVALUATION OF DIN VS THE IDEAL

Flash Point

The 148°C (298°F) flash point of DIN makes it safe to use on the laboratory bench since the flammability risk is minimal. DIN is classified as a nondangerous product in accordance with national and international traffic regulations because the flash point exceeds 100°C. By comparison pseudocumene with a flash point of 52°C (125°F) is classified as flammable and should be handled accordingly.

Vapor Pressure

DIN has a very low vapor pressure (1 mm Hg at 25°C) thus ensuring that there is a low vapor concentration at 25°C. This means that there would be very little build up of DIN vapor in the event of a spillage in an enclosed space. By comparison pseudocumene has a vapor pressure twice (2×) that of DIN.

Odor

DIN is virtually odorless and as such makes it pleasant to work with. Pseudocumene, on the other hand, has a highly aromatic penetrating odor, which, if inhaled in appreciable amounts, can cause headaches and narcosis.

Toxicity and Irritancy

Based on the acute data (LD_{50} = 5600 mg/kg oral rat). DIN is not classified as toxic and imposes no acute health hazard to man. DIN is widely used in the manufacture of carbonless copy paper (NCR paper) and consequently its toxicological properties have been extensively studied. A considerable amount of work (to EEC directives) has been completed on DIN and a summary of the toxicological data shows DIN to have the following reactions:

1. acute toxicity (oral–rat) = nontoxic
2. acute toxicity (dermal–rat) = nontoxic
3. skin irritation = nonirritating
4. eye irritation = nonirritating
5. skin sensitization = nonsensitizing
6. toxicokinetics = nonaccumulative
7. mutagenicity = nonmutagenic
8. cancerogenicity = noncarcinogenic
9. teratogenicity = nonteratogenic
10. acute toxicity to fish = nonharmful to fish
11. bioaccumulation (fish) = nonbioaccumulative

A copy of "Toxicological and Physico-Chemical Studies on Diisopropylnaphthalene" is available from the author upon request. The toxicity of pseudocumene is well known and its principal hazards are that it is a primary skin irritant, is irritating to the eyes, and on inhalation causes respiratory irritation together with central nervous system depression.

Plastic Permeation

In both short- and long-term studies DIN has been shown not to permeate through polyethylene counting vials. Indeed, in a long-term study there was no loss of DIN from a polyethylene vial during a 12 month storage test at room temperature. Pseudocumene, although better than toluene and xylene, is nevertheless steadily lost from polyethylene counting vials. In practice, permeation of the solvent through the vial wall has two effects. Firstly, loss of solvent increases the effect of quench in the vial. Secondly, the solvent and fluors penetrate the vial wall causing the vial to become an additional solid scintillator. This solid scintillator in conjunction with the external standard produces an additional spectrum. This solid scintillator spectrum adds to the "Compton" spectrum produced by the interaction of the external standard with the cocktail solvent. Therefore, there is a gradual change in shape of the overall spectrum. This particularly affects the external standard channels ratio (ESCR) and is manifest as a continual change in the ESCR value. This phenomenon is most evident with ESCR, but the newer parameters (special quench parameter and H-number) are not significantly affected. With ESCR this leads to an underestimate of the efficiency and hence an overestimate of the dpm. The effect is known as the "plastic vial" effect and is shown in Figures 2 and 3.

Biodegradability

By comparison with other aromatic solvents DIN solvent has the remarkable property of being biodegradable in its own right. DIN is described as being greater than 80% biodegraded after 28 d at 4 ppm available oxygen and is therefore classified as biodegradable according to EEC directive 79/831. Annex VII. An independent evaluation by the Severn Trent Water Authority (U.K.) found Optiphase Hi-Safe II (DIN-based emulsion cocktail) to be readily biodegradable by the ISO 7827–1984 (E) method, achieving a degradation level of greater than 80% in 2 d. Greater than 80% degradation in 28 d is the standard necessary for classification as readily biodegradable. A copy of this evaluation is available from the author upon request. However, it is essential that your radiological safety officer and local water authority are consulted to obtain permission for drain disposal before embarking upon any particular course of action.

Fluor Solubility

All the commonly used fluors are soluble in DIN solvent with PPO having particularly good solubility. Figure 4 shows the solubilities of selected fluors in

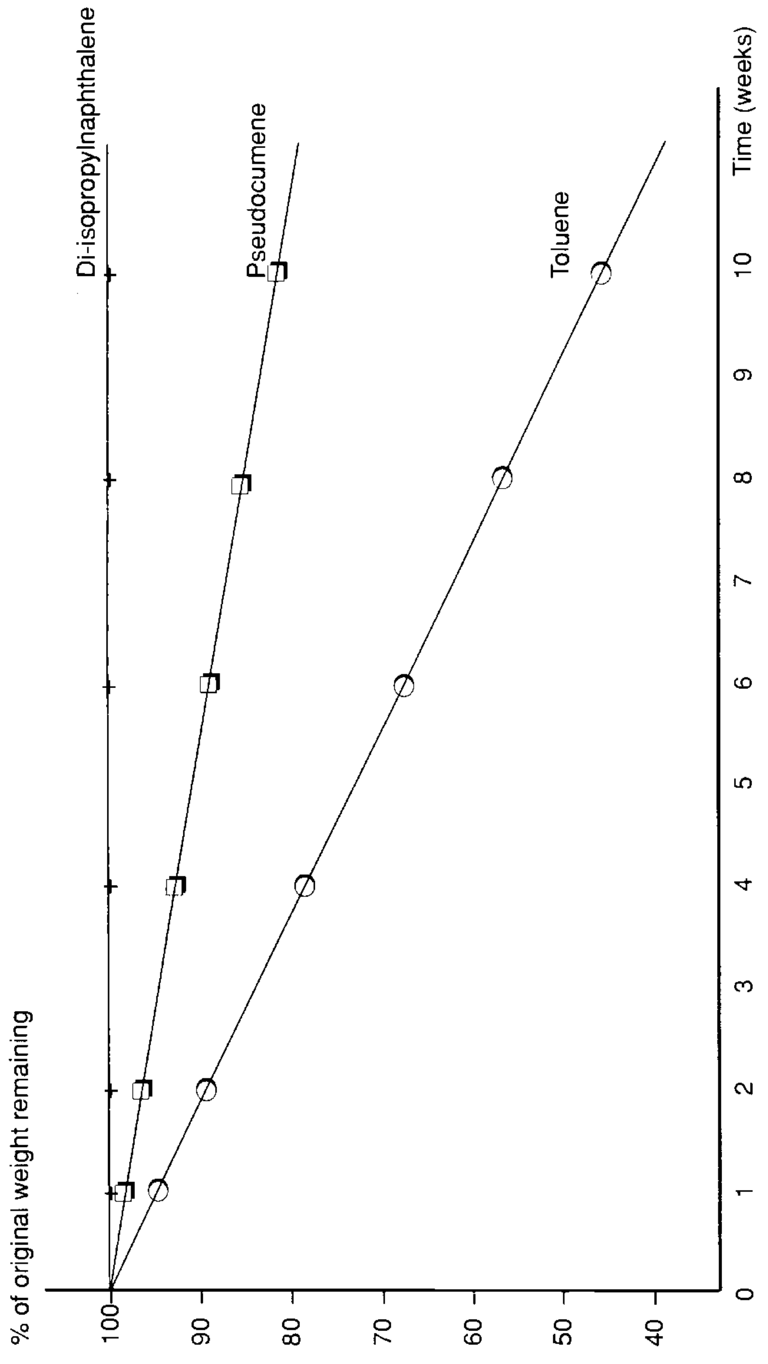

Figure 2. Permeation of various solvents (at 20°C in 20 mL polyethylene vials).

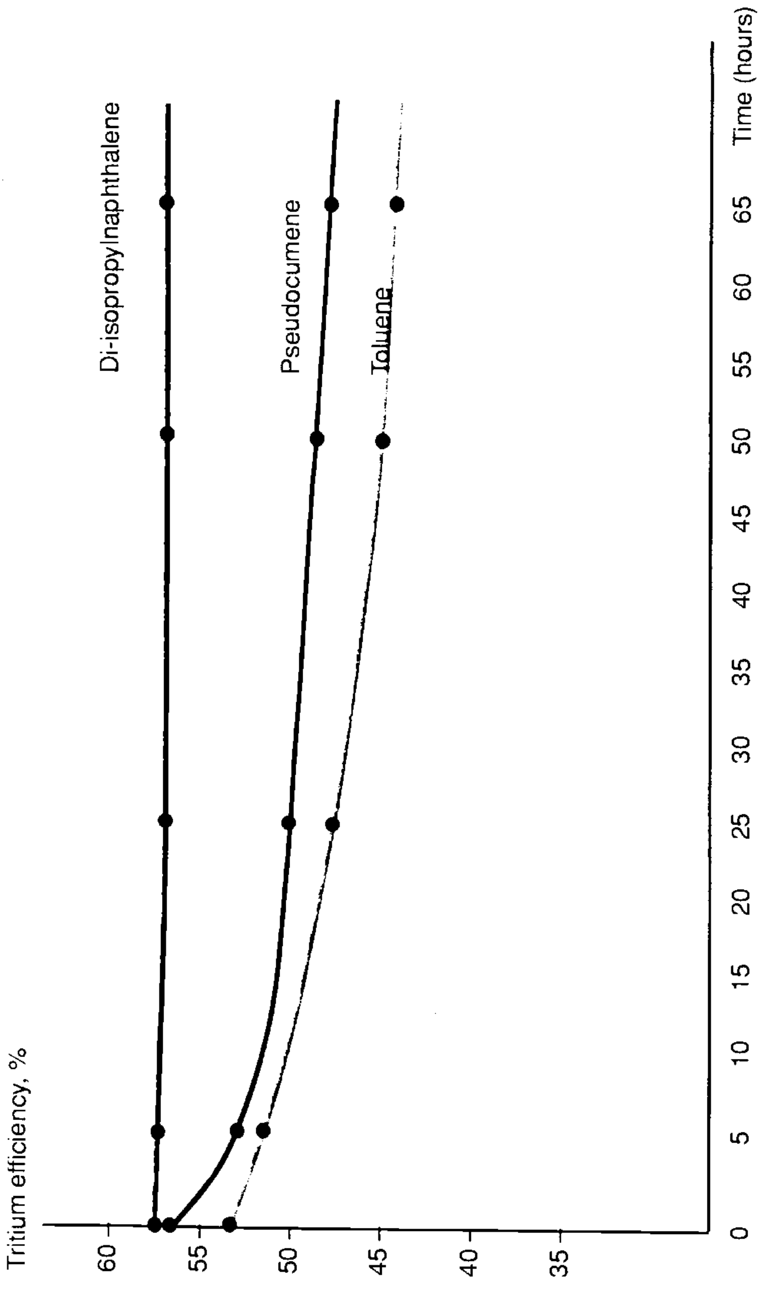

Figure 3. "Plastic vial" effect: variation in tritium efficiency with time for various solvents in 20 mL polyethylene vials.

FLUORS	Max. concentration, g/l
PPO	100
PBD	9.9
Butyl-PBD	40
Bis-MSB	1.8
TP	3.6
POPOP	0.5
Dimethyl-POPOP	1.0

Figure 4. Solubility of various fluors in di-isopropylnapthalene at 15°C.

DIN at 15°C, and easily exceeds the optimum concentration as determined by a simplex optimization. Pseudocumene is also a good solvent for all the common fluors.

Luminescence

DIN solvent is compatible with all the conventional toluene-based alkaline tissue solubilizers and any chemiluminescence should decay within 30 min. Any induced photoluminescence will also decay within 30 min under normal counting conditions. Pseudocumene from some sources will require purification to remove those impurities which can give a color reaction with alkaline tissue solubilizers.

Counting Efficiency (Tritium)

When DIN is used as the solvent in both lipophilic and hydrophilic cocktails then the resulting cocktails exhibit a superior counting efficiency when compared with other types of cocktail. This is especially important when counting to statistical limits since those limits will be reached quicker with DIN based cocktails thus reducing instrument time. The following graphs, Figures 5 through 7, illustrate the superior detection efficiency obtained with tritium-labeled water samples. The DIN based cocktail, Optiphase Hi-Safe II, used in this comparison is a product of Pharmacia–Wallac.

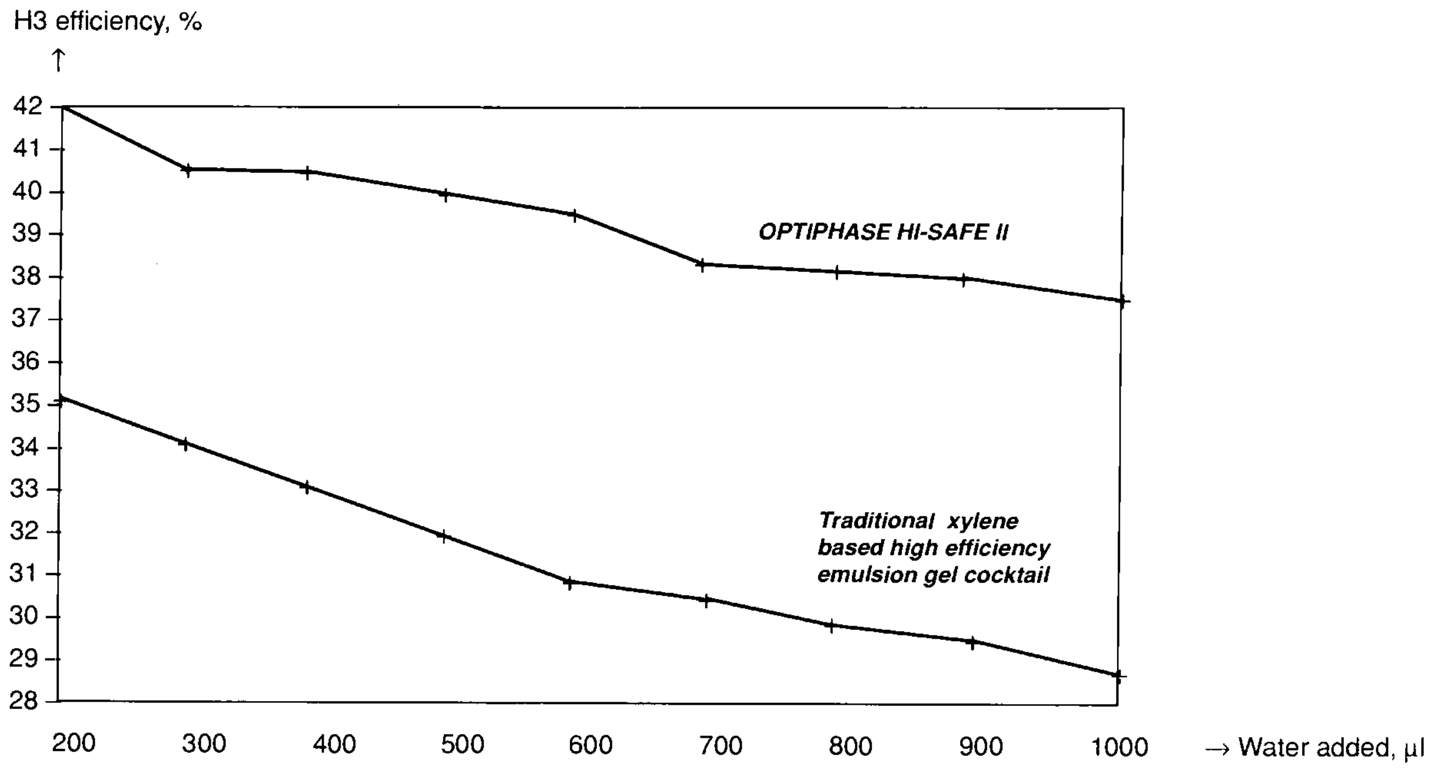

Figure 5. Efficiency vs μL water added (sample added to 5 mL cocktail).

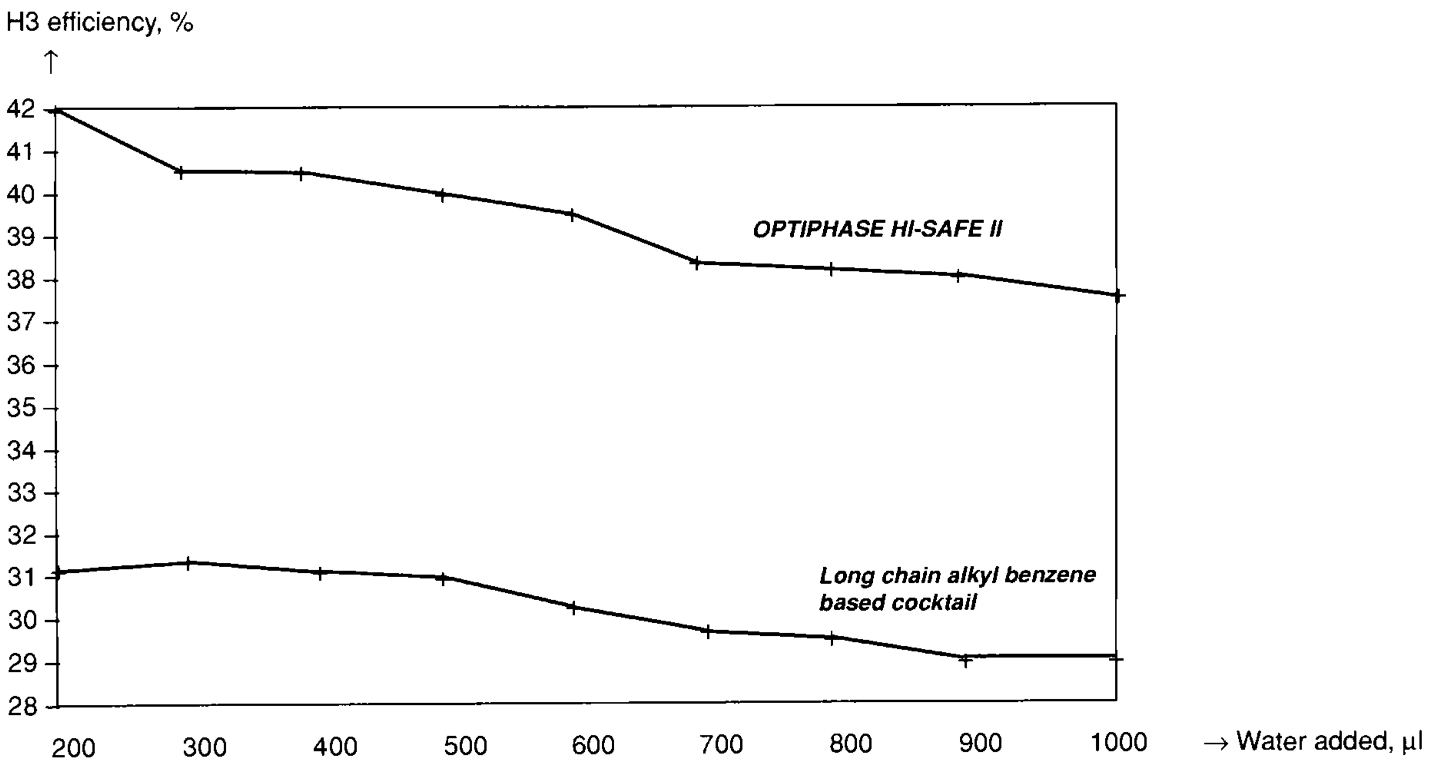

Figure 6. Efficiency vs μL water added (sample added to 5 mL cocktail).

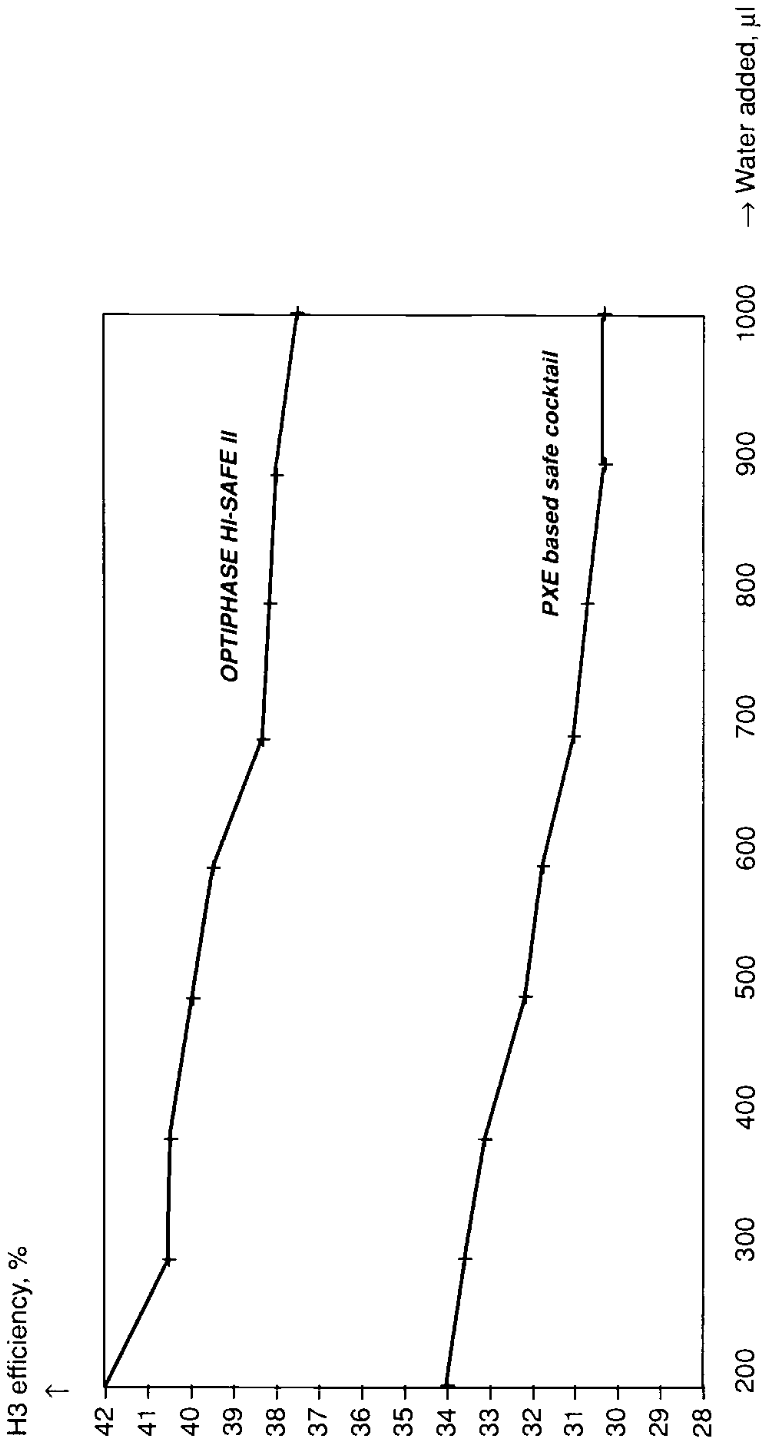

Figure 7. Efficiency vs μL water added (sample added to 5 mL cocktail).

Quench Resistance

DIN exhibits an exceptional resistance to chemical quench as shown in Figure 8. This is important where samples are prepared with acids, solvents etc., which introduce unacceptably high levels of chemical quench with other solvents. DIN also has resistance to color quenching (Figure 9), which is necessary when working with colored samples, e.g., urine and serum. Pseudocumene has good resistance to both chemical and color quench, but has been found to be not as good as DIN.

DIN-BASED EMULSION COCKTAILS

DIN can be formulated into emulsion cocktails which achieve a greater degree of safety without compromising performance. This is aptly demonstrated in the Optiphase Hi-Safe range of cocktails which have seen wide acceptance in the U.K. and Europe over the last few years. Taking a closer look at two of these products:

- **Optiphase Hi-Safe II©**
  This cocktail possesses all of the safety features previously cited about DIN solvent together with the expected very high counting efficiency and the ability to incorporate a diversity of sample types (Figure 10).
- **Optiphase Hi-Safe III©**
  This cocktail combines the capability of accepting large volumes of normal samples with the ability to accept high-ionic strength solutes in moderate to good volume (Figure 11). This makes Optiphase Hi-Safe III almost unique among currently available cocktails. As expected Optiphase Hi-Safe 3 has all the inherent advantages afforded by being a DIN-based cocktail. Optiphase Hi-Safe III has the additional capability of accepting sample types which other cocktails find difficult or impossible to accommodate.

CONCLUSION

Di-isopropylnaphthalene is a significant improvement over existing LSC solvents. Its low vapor pressure, low flammability, low plastic permeability, and low toxicity make it safe and pleasant to handle. It is a more environmentally acceptable product because of its rapid and extensive biodegradability. Lastly and by no means least it is unexcelled in its LSC performance. Therefore DIN comes closest to matching the ideal LSC solvent than any other currently known solvent.

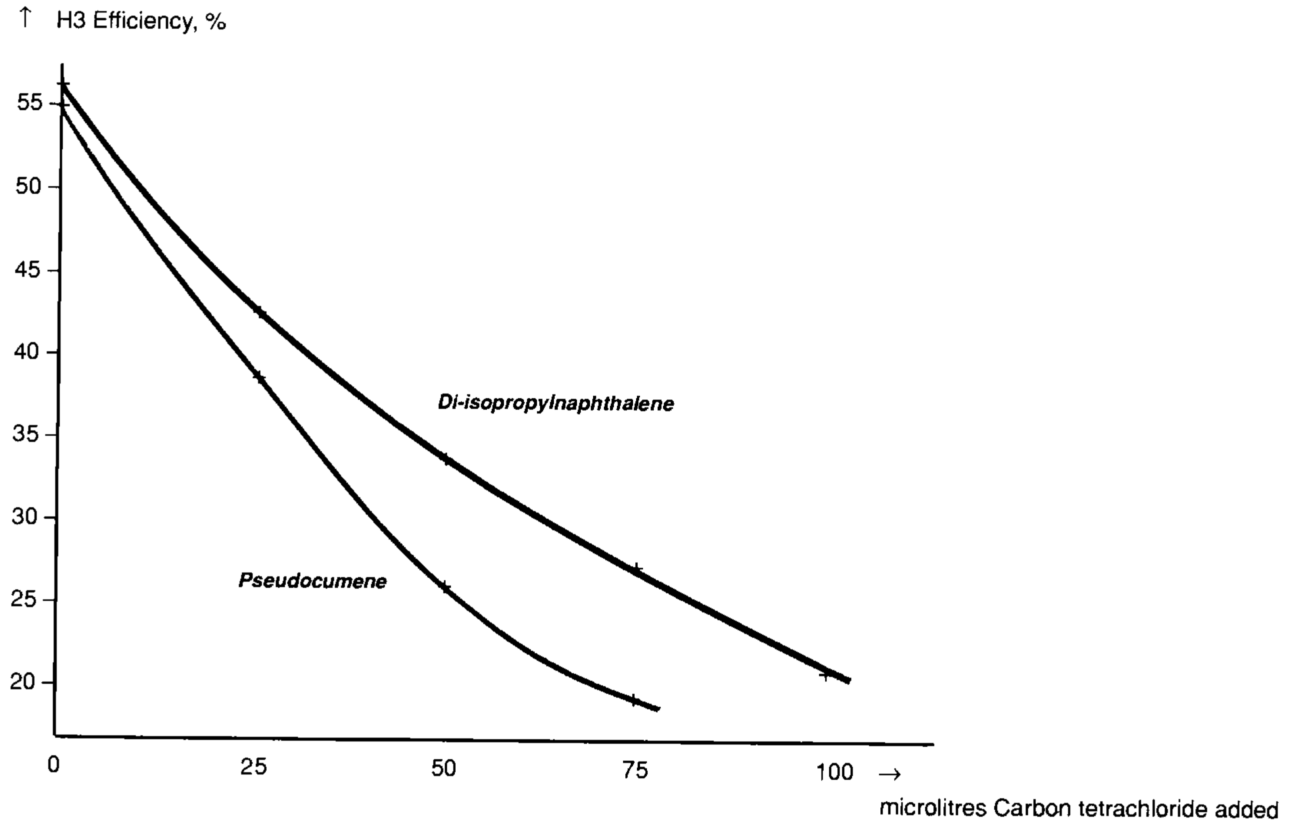

Figure 8. Chemical quench comparison for di-isopropylnapthalene and pseudocumene (μL carbon tetrachloride added to 10 mL cocktail, cocktails = solvent + 4 g/L PPO/0.1 g/L bis-MSB).

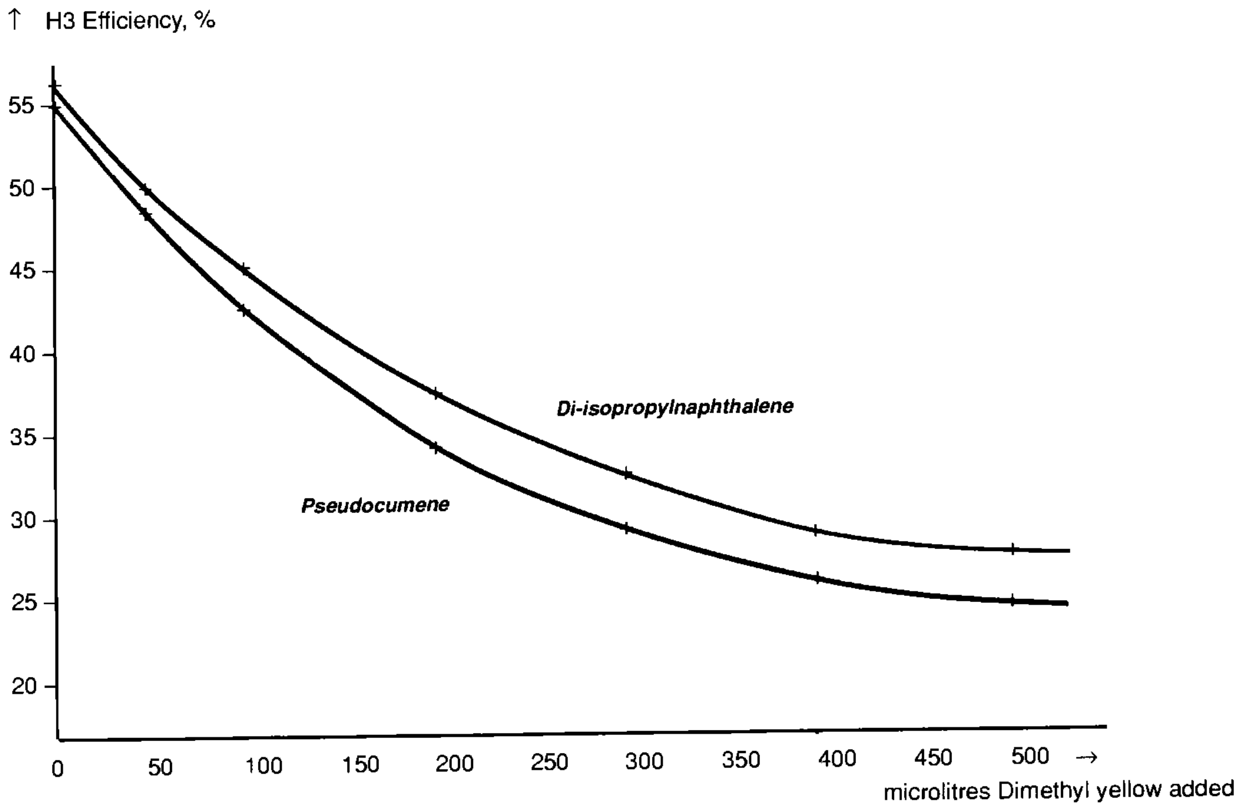

Figure 9. Color quench comparisonf or di-isopropylnapthalene and pseudocumene (μL of 0.001% dimethyl yellow solution added to 10 mL cocktail, cocktails = solvent + g/L PPO/0.1 g/L bis-MSB).

SAMPLE	*Temperature, °C*			
	10	*15*	*20*	*25*
Water	2.5	2.5	2.6	2.6
0.15M Sodium chloride	3.4	3.4	3.8	3.8
Phosphate saline buffer 0.01M	3.4	3.4	3.4	3.4
0.05M Tris-HCl	3.4	3.4	3.4	3.4
0.1M Hydrochloric acid	9.0	10.0	10.0	10.0
10% Sucrose	3.8	3.8	3.8	3.8
0.2M Sodium hydroxide	4.0	4.6	5.0	5.0
0.1M Sodium hydroxide	4.0	4.0	4.0	4.4
Urine	2.2	2.2	2.2	2.2
Serum - canine	1.0	1.0	1.0	1.0
20% Sucrose	6.6	6.6	5.8	5.8
0.1M Ammonium sulphate	3.4	3.4	3.4	3.6
0.05M Sodium acetate	5.8	5.6	5.0	5.0
0.04 Disodium phosphate	8.0	8.0	8.6	8.6
5mM Hepes	2.8	2.8	2.8	2.8
0.1M Tris-50mM EDTA	5.0	5.2	5.8	5.8
0.2M Ammonium acetate	2.4	2.4	2.6	2.8
1.0M Sodium hydroxide	2.0	2.0	2.2	2.4
10% Trichloroacetic acid	2.2	2.3	2.3	2.3
8.0M Urea	1.1	1.1	1.1	1.1
Optisolve	1.0	1.0	1.0	1.0

Figure 10. Optiphase Hi-Safe II sample acceptance (values [mL] determined by addition of sample to 10 mL of cocktail).

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SAMPLE	*Temperature, °C*			
	10	*15*	*20*	*25*
Water	10.0	10.0	9.0	9.0
Hepes	10.0	10.0	9.5	7.5
Tris HCl 50mM	10.0	10.0	10.0	10.0
Ringers	10.0	10.0	8.5	8.0
0.5M Phosphate saline buffer	9.0	7.0	6.5	5.5
0.1M Phosphate saline buffer	3.0	3.0	8.0	8.0
Sea water	3.0	3.0	7.5	7.5
1.0M Sodium acetate	7.0	6.5	6.0	5.5
4.0M Sodium hydroxide	1.9	1.9	1.9	2.0
40% Caesium chloride	6.5	6.0	5.5	5.0
10% Trichloro acetic acid	3.5	6.5	6.5	6.5
Urine	3.0	3.5	8.0	8.0
4.0M Ammonium formate	2.5	2.5	2.0	1.75
1.0M Ammonium formate	7.5	6.0	5.5	5.0
1.0M Sodium chloride	3.0	7.5	7.5	7.5
2.0M Nitric acid	7.5	6.0	4.0	4.0
0.5M Potassium di-hydrogen orthophosphate	7.5	6.0	5.0	5.0
8.0M Urea	3.25	7.0	7.0	7.5
0.5M Ammonium sulphate	5.0	3.75	3.5	3.25

Figure 11. Optiphase Hi-Safe III sample acceptance (values [mL] determined by addition of sample to 10 mL of cocktail).

REFERENCES

1. Birks, J.B. *The Theory of Practice of Scintillation Counting* (Pergamon Press, 1964), pp.272–279.
2. Thomson, J. "Scintillation Counting Medium and Counting Method", U.S. Patent 4,657,696, April 14, 1987; European Patent 0176281, September 11, 1985."