Aqueous samples (0·02–0·05 ml) of ^{3}H-inulin and ^{14}C-3OHphenyltrimethylammonium, previously standardized (dpm/ml) in an homogeneous counting solution (Bray, 1960), were counted in a Triton X-100 scintillant (Toluene: Triton X-100, 2:1 v/v and butyl P.B.D. 6 g/l. containing water 75 ml/l.). The calculated counting efficiency (cpm/dpm) of the polar compounds and hexadecane standards were the same and were predicted by the channels ratio (counting under single labelled conditions) and external standard ratio methods (counting under double labelled conditions). When the water was excluded from the scintillant, different efficiencies for the polar compounds were calculated compared to hexadecane, but these were predicted by the different channels ratio. However, the external ratios were similar and did not detect the difference in efficiency of the ^{14}C isotope. These results are shown in Table 1.

TABLE 1. *The counting efficiencies, channels ratio and external standard channels ratio of two polar compounds and hexadecane in two different triton scintillants*

	Triton-water scintillant		Triton-no water scintillant	
	Efficiency (%)	Channels ratio	Efficiency (%)	Channels ratio
^{14}C-Hexadecane	85	0·31	84	0·23
^{14}C-3OHPTMA*	86	0·30	77	0·30
^{3}H-Hexadecane	31	2·97	39	4·00
^{3}H-Inulin	31	2·92	36	3·66
	Efficiency (%)	External channels ratio	Efficiency (%)	External channels ratio
^{14}C-Hexadecane	60	3·6	57	10·4
^{14}C-3OHPTMA*	60	3·6	47	10·2
^{3}H-Hexadecane	27	3·6	17	10·4
^{3}H-Inulin	27	3·6	16	10·2

* 3-OHPTMA≡3-Hydroxyphenyltrimethylammonium iodide.

The Triton/toluene/water system apparently behaves as a solution (Benson, 1966; Turner, 1968) and both internal and external methods of efficiency determination are suitable. The scintillant without water is apparently heterogeneous and only the internal ratio is satisfactory provided the working standard has the same phase distribution as the compound measured. The homogeneity or heterogeneity of the two scintillants was confirmed by comparison of the pulse height spectra of the polar compounds with the hexadecane standards.

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Screening tests for assessing the relative potency of sensory irritant materials

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Studies on the relative potency of compounds which irritate peripheral nervous elements frequently include, as screening procedures, measurements of threshold concentrations required to produce reflex irritant responses. We have compared the guinea-pig blepharospasm, frog flexor reflex and mouse plethysmography techniques.

Using blepharospasm of the guinea-pig eye as the response to topically applied irritant, the threshold concentration was calculated as the concentration required to produce the response in 50% of a group of animals (TC_{50}). The test was found to be easy to perform, free from tachyphylaxis, to give reproducible values with restricted 95% confidence limits, and to separate irritant materials of relatively close chemical structure.

The flexor response was carried out with decerebrate frogs whose hind limbs were immersed in progressively stronged solutions of irritant. Using withdrawal of the limb as a positive response, the log molar concentration of irritant was plotted against withdrawal time and the curve was extrapolated by fitting a template of a standard curve defined by:

$$C=\frac{1}{1-e^{-t}}$$

where C=concentration of irritant, and t=withdrawal time. In this way the minimum irritating concentration was estimated. This technique was found to be relatively insensitive, the results were variable and there was evidence of tachyphylaxis.

Depression of the respiratory rate of the mouse, measured by whole body plethysmography, was used to assess the response to aerosols of irritants. The peak depression of respiratory rate was measured for several concentrations, and the threshold value calculated as that producing a 50% depression rate. The test is time-consuming but was found to have all the advantages of the blepharospasm test and appeared the most sensitive.

No single animal test is likely to be completely adequate for screening the relative potency of sensory irritants, but the guinea-pig blepharospasm test offers a simple technique for reliable comparative tests. These may require confirmation using mouse plethylsmography and, where appropriate, studies on man.

Results from animal threshold studies may not be a reliable guide to the situation in man, and do not allow the measurement of subjective irritant effects. Thus, for *o*-chlorobenzylidene malononitrile the threshold values were $2{\cdot}2\times10^{-5}$ M by guinea-pig blepharospasm, $6{\cdot}0\times10^{-8}$ M by mouse plethysmography and $9{\cdot}8\times10^{-6}$ M by flexor reflex. In man, we found the TC_{50} for blepharospasm to be $3{\cdot}2\times10^{-6}$ M and for elicited corneal sensation to be $7{\cdot}3\times10^{-7}$ M. The human eye appears about 7 times more sensitive to this irritant than does the guinea-pig eye, using blepharospasm as the response. The TC_{50} for sensation on the human tongue was $6{\cdot}8\times10^{-6}$ M, a concentration 9·4 times that required to elicit corneal sensation. Extrapolation of the data used to obtain TC_{50} values in man in the form of log probit plots may be of value for predicting incapacitating concentrations of irritant materials.

The assessment of β-adrenoceptor blocking compounds in the conscious dog (T)

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