was a significant decrease in the incidence of cleft palate ($P = 0.03$).

The doses of primidone used in the dietary experiments were far in excess of those used clinically, so much lower doses (100, 150, 250 mg/kg) were given by gastric intubation. All three doses of primidone produced a similar incidence of cleft palate (9-10%) in 50-60% of the litters.

Blood levels of primidone after 100 mg/kg by gastric intubation were measured in pregnant (day 14) mice. A peak level of 42.7 ± 2.8 μg/ml was attained after 30 min and it was virtually cleared from the blood by eight hours. Thus, although the dose of primidone which was teratogenic in mice was about 10 times the therapeutic dose for humans the blood levels obtained were only three to four times higher than those commonly found in patients on primidone (Kutt, 1974).

References

ELSHOVE, J. (1969). Cleft palate in the offspring of female mice treated with phenytoin. *Lancet*, ii, 1074.

KUTT, H. (1974). Pharmacodynamic and pharmacokinetic measurements of antiepileptic drugs. *Clin. Pharmacol. Ther.*, **16**, 243-250.

MASSEY, K. (1969). Teratogenic effects of diphenylhydantoin sodium. *J. oral Ther. Pharmac.*, **2**, 380-385.

McCOLL, J.D., ROBINSON, S. & GLOBUS, M. (1967). Effects of some therapeutic agents on the rabbit fetus. *Toxicol. Appl. Pharmacol.*, **10**, 244-252.

SPEIDEL, B.D. & MEADOW, S.R. (1972). Maternal epilepsy and abnormalities of the foetus and newborn. *Lancet*, ii, 839-843.

A new approach to the evaluation of the safety of flavouring esters

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Experiments have been performed which are designed to determine whether it is possible to reduce the amount of animal experimentation that is needed to assess the safety of flavouring materials. Many of the flavours are esters which are readily hydrolysed *in vivo* to their component acids and alcohols. It is postulated that if the toxicity of each component is known then it might be possible to extrapolate the results to other esters of a particular series, with a consequent economy in the number of animals used and in the experimental time involved.

A series of allyl esters with a straight, branched or a cyclic chain has been investigated. This series was chosen because many pure allyl esters are available and because allyl alcohol produces an unusual effect on the liver—namely periportal necrosis. Allyl alcohol, acetate, propionate, hexanoate, isobutyrate, isovalerate and 2-ethyl hexoate were administered by daily oral intubation in equimolar doses (based on doses of 5, 25 or 60 mg kg^{-1} day^{-1} of the alcohol) to groups of ten male rats. After 21 days the animals were killed and comparisons of the hepatic effects were made. The lesions were classified into three types according to severity; namely periportal cell enlargement, followed by necrosis, and subsequent fibrosis with bile duct hyperplasia.

The severities of the hepatic effects from the straight chain esters were similar to those produced by allyl alcohol and were more marked than those from the branched chain ones. In terms of the number of animals affected, there was a positive relationship between the dose of ester administered and the degree of periportal damage. The animals given the high dose of the straight chain esters exhibited the most extreme lesions and in many instances the effects were so marked as to obscure any signs of early damage. The results of these *in vivo* experiments are in agreement with the *in vitro* hydrolysis studies. The latter indicate that hydrolysis proceeds about 100 times more slowly with the branched chain than with the straight chain esters.

From the data with this series of model compounds it is suggested that it may be possible to evaluate the toxicity of such a series of esters, if the following information is available: (1) the rate and degree of hydrolysis in the alimentary tract, (2) well established data on the parent alcohols and acids, and (3) results of short-term animal experiments, so that it is possible to correlate the *in vitro* hydrolysis studies with the *in vivo* results.