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Preparation of the S9 fraction for the third UKEMS collaborative trial

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The rat liver S9 fraction used in this study was prepared at Sittingbourne Research Centre and distributed to the participating laboratories.

Methods

Animals

Fischer F344 male rats (8–12 weeks, 200–250 g) were purchased from OLAC, Shaws Farm, Blackthorn, Bicester, Oxon. The rats were acclimatized for 1 week prior to i.p. injection with a single dose of 500 mg/kg Aroclor 1254, 5 days before killing.

Preparation of S9 fraction

The S9 was prepared according to the methods described by Ames *et al.* (1975). Liver was removed from each animal and washed in ice cold 0.15 M KCl. The liver was then homogenized in ice cold 0.15 M KCl (3 ml/g wet liver) in a Thomas tissue grinder attached to a motor (speed 1425 r.p.m.) using 10 strokes of the pestle (a single homogenizing vessel and pestle was used throughout) and the homogenate centrifuged (9000 *g* for 10 min). The S9 from each animal was pooled and distributed in 2 ml aliquots into storage ampoules. The ampoules were frozen directly in dry ice and stored at −80°C prior to distribution to the participating laboratories.

A total of 280 g of liver was removed from 26 rats yielding 894 ml S9 fraction.

Distribution of S9 fractions

Ampoules of frozen S9 were packed in polystyrene boxes with dry ice and distributed by Datapost within the UK to arrive within 24 h of dispatch.

Reference

Ames,B.N., McCann,J. and Yamasaki,E. (1975) Methods for detecting carcinogens and mutagens with the Salmonella/mammalian microsome mutagenicity test. *Mutat. Res.*, **31**, 347–364.