

Letter to the Editor

Metabolizing systems for *in vitro* genotoxicity tests

Dear Editor,

The paper of Callander *et al.* (1995) on the evaluation of phenobarbital (PB)/ β -naphthoflavone (β NF) as an alternative S9-induction regime to Aroclor 1254 in the rat for use in *in vitro* genotoxicity assays is welcome. The authors, using a series of bacterial mutation, mammalian cell (L5178Y) gene mutation and *in vitro* cytogenetic assays, showed that the magnitude of the mutagenic/clastogenic effects of some well-known indirect-acting genotoxins observed between both the two induction regimes was similar.

The presented findings are in line with the deliberations of a working party set up by the United Kingdom Environmental Mutagen Society (UKEMS) to investigate alternatives to Aroclor 1254-induced S9 for mutagenicity purposes (Elliott *et al.*, 1992). Since the pioneering work of Matsushima *et al.* (1976), which proposed for the first time the combination of PB/ β NF as a safe substitute for Aroclor [reported to be highly toxic and carcinogenic by Alvares *et al.* (1973)], a great number of reports have shown the usefulness of such an induction procedure.

Over the past two decades, however, although much progress in the characterization and purification of novel isoenzymatic forms of cytochrome P450 has been reported, a concomitant interest and effort in the application of such knowledge to the field of genetic toxicology has not occurred. It is in fact well known that apparently distinct isoforms of P450 may be induced by using different classes of inducing agents. Consequently, because different P450 isozymes metabolize different classes of chemical carcinogens (e.g. Guengerich, 1988), it would be desirable to obtain metabolizing S9 fractions with a multiplicity of P450-induced forms. In other words, it should be kept in mind that for genotoxicity studies it is essential to have the broadest spectrum of induced P450s, including, ideally, all isoenzymatic forms able to metabolize structurally different precarcinogens (Paolini *et al.*, 1991a).

We wish, therefore, to point out the necessity of adopting in the future metabolizing systems containing a wide spectrum of induced P450s. Recently, five inducers of different P450 families, including both classical PB/ β NF and isosafrol (IS; inducers of class 1A2 P450), ethanol (EtOH, 2E1) and pregnenolone 16- α -carbonitrile (PCN; 3A, the most representative isoform present in human liver), were used concomitantly to achieve 'superinduced' S9 fractions (Paolini *et al.*, 1991b). The enzymatic characterization of such fractions showed a great increase of metabolic potential when compared with traditional ones, both in terms of enzymatic activity levels and unspecificity of the induced P450s. To test the effectiveness of the novel biosystem, the induced P450 isoforms were then correlated with the metabolism of selected precarcinogens

biotransformed by these enzymes. The five specific precarcinogens elicited a higher mutagenic response in the presence of 'superinduced' S9 fractions (PB, β NF, IS, EtOH and PCN) than the classically induced fraction (PB and β NF) when tested on *Salmonella typhimurium* [cyclophosphamide (P450 2B1), benzo[*a*]pyrene (1A1), 2-naphthylamine (1A2) and dimethylnitrosamine (2E1)] or *Saccharomyces cerevisiae* strain D₇ [diethylstilboestrol (3A)].

One of the most crucial issues in detecting human carcinogens by using short-term mutagenicity tests is the presence of non-genotoxic carcinogens (NGCs). The new multiple-inducer agent protocol increasing the effectiveness of the genetic tests, could reduce the occurrence of 'false negative' results and consequently the appearance of 'false NGCs'.

The availability of more data on a wide array of premutagens/precarcinogens, in the future could fully validate the procedures for preparation of 'superinduced' S9 fractions.

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