UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

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OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: FMC Response to EPA Review of a CHO/HGPRT Mutation Assay

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Product Manager 15

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Project No. 8-0868

Tox. Chem. No. 554C (FMC 102032)

Action Requested:

Review of a company response regarding preparation and activity of a rat S9 fraction used in CHO/HGPRT assay.

Background:

The study was previously reviewed (DER II of a memorandum dated March 2, 1988). At that time it was classified as "acceptable without S9 activation, with no evidence of mutagenic activity at levels of up to 1000 ug/ml, the limit of solubility and osmolality for FMC 102032 with acetone as solvent. Not acceptable with S9 activation, as the S9 fraction had been prepared from rats sacrificed two (instead of the usual five) days after administration of Aroclor-1254."

According to information from the performing laboratory (Microbiological Associates) peak metabolic activity (particularly as measured by mutagenic response of TA 100 to 1 ug 2-anthramine in an Ames Assay performed with triplicate plating) is in the range of 10 to 50 ul S9/ml using 5-day Aroclor-induced Sprague-Dawley rats or 2-day Aroclor-induced Fischer 344 rats. However, for 5-day induced Fischer 344 rats it is between 40 and 200 ul S9/ml.

Comments and Recommendations:

1. The Toxicology Branch does not fully accept the rationale provided by the contracting laboratory. The positive control with S9 activation in the CHO/HGPRT assay was Benzo(a)pyrene (BaP), not 2-anthramine or 7,12-dimethylbenz[a]anthracene (used to compare activity "spectrums" of different S9 fractions). In the future, the Toxicology Branch, on a case-by-case basis, may ask for more complete activity spectrum comparisons when the S9 fractions are not derived from 5-day Aroclor-induced Sprague-Dawley rats.

2. The original data from the activated part of the assay included the following (from table 4, p. 19-20):

Treatment	Cloning Efficiency	Total # Mutant Colonies	Total # cells selected ($x10^6$)	Mutants/10^6 Clonable Cells
untreated control A	0.68	11	1.00	16.2
untreated control B	0.71	0	1.00	< 1.4
vehicle (acetone) control A	0.53	3	1.00	5.7
vehicle (acetone) control B	0.63	7	1.00	11.1
BaP (positive control) A	0.47	149	0.69	459.5
BaP (positive control) B	0.29	79	0.61	446.6
FMC 102032				
850 ug/ml (A)	0.99	15	0.81	19.9
850 ug/ml (B)	0.51	0	0.64	< 2.0
1000 ug/ml* (A)	0.78	17	1.00	21.8
1000 ug/ml* (B)	0.77	0	0.78	< 1.3

*Highest dose tested

After reexamination of these data, it is concluded that exposure to the positive control (benzo(a)pyrene at 4 mg/ml) in the presence of the S9 from 2-day Aroclor-induced Fischer 344 rats, elicited an acceptable level of mutagenic response. It is therefore concluded that the assay with S9 activation can be reclassified as acceptable in demonstrating that there was no evidence of mutagenic activity at levels of up to 1000 ug/ml, the limit of solubility and osmolality of FMC 102032.

3. It is noted that acceptance of the findings of this assay in this instance does not preclude the Toxicology Branch from classifying studies with similar deviations as unacceptable in the future.