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

007162

NOV 29 1988

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: Overview of Submitted Mutagenicity Studies on Benefin

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Chemical: Benefin CAS# 1861-40-1 Caswell #130

This reviewer has been requested to examine and summarize mutagenicity studies concerning benefin that have been submitted to OPP. The following is a listing of these studies with their result and classification for acceptance:

Acceptable studies:

Salmonella assay: negative (MRID #160863)
UDS/primary rat hepatocytes: negative (MRID #160865)

Unacceptable studies:

Mouse lymphoma gene mutation assay: negative (MRID #160866)
SCE/Chinese hamster bone marrow: negative (MRID #160864)

The following will discuss these studies as well as additional information and then present an overall conclusion.

185

A. Salmonella assay

Benefin was assayed in the submitted study in Salmonella strains TA98, TA100, TA1535, TA1537 and TA1538 with and without activation. A preliminary toxicity experiment indicated no toxicity at concentrations up to 5000 ug/plate, but some precipitate was evident at >100 ug/plate (without activation) and at >50 ug/plate (with activation). A precipitation test performed without bacteria indicated precipitate at >500 ug/plate (without activation) and at >100 ug/plate (with activation). Therefore, the top concentrations chosen for the mutation assay were 750 ug/plate without activation and 300 ug/plate with activation. Triplicate plates per culture were used. Negative results were obtained with the positive controls appearing adequate. These results are supported by a published assay where benefin was negative in the same five Salmonella strains as well as in the E. coli strain WP2 hcr for mutation (Moriya et al., 1983).

Further support for the negative findings in the Salmonella assay for benefin comes from evidence for the analogue trifluralin. Trifluralin (Caswell #889, [1582-09-8]) has been negative in most Salmonella assays (2 submitted studies to OPP: MRID #249846 and Document #005898; Benigni et al., 1982; Waters et al., 1982; Moriya et al., 1983) as well as in E. coli strain WP2 hcr (Waters et al., 1982; Moriya et al., 1983). Trifluralin was found by the NTP to induce a weak positive response (about 2 fold over background), but only in TA100 in the presence of 10% hamster S9 instead of rat S9 as used in the other studies (Mortelmans et al., 1986). Overall, the weight of the evidence suggests at this time that benefin does not present a mutagenic risk in microbial mutation assays. If any questions about benefin microbial mutagenicity surface in the future, the use of hamster activation may be investigated.

B. Unscheduled DNA synthesis (UDS) in primary rat hepatocytes

Hepatocytes were obtained from male Fischer 344 rats, attached for 2.5 hours and then exposed to test compound for 20 hours. Two separate experiments were performed and 20 cells per concentration were scored. Concentrations of 0.5, 1, 5, 10, 50, 500 and 1000 ug/ml were used. Chemical precipitated at $\geq$500 ug/ml. Cytotoxicity was reported at $\geq$50 ug/ml (cells were unable to be evaluated for UDS). The positive controls appeared adequate. No increases in UDS over DMSO control were noted at concentrations up to 10 ug/ml. Although 50 cells/concentration should have been counted, the repeat experiment appears to substantiate the negative results.

The analogue trifluralin is reported in the published literature to be negative for the induction of UDS in cultured human WI-38 fibroblasts (Waters et al., 1982) and in cultured EUE cells (Benigni et al., 1982).

2

C. Mouse lymphoma gene mutation assay

Test compound was exposed to mouse lymphoma L5178Y cells in culture for 4 hours with and without activation. A preliminary toxicity experiment indicated excessive toxicity at ≥50 ug/ml (no cells) without activation and at ≥250 ug/ml with activation. Therefore, concentrations chosen for the mutation experiment were 40 ug/ml and 100 ug/ml without and with activation, respectively. A precipitation test indicated some precipitate at >50 ug/ml. Under non-activated conditions, 40 ug/ml appeared too toxic. At 30 and 35 ug/ml, survivals were 9 and 8%, respectively, but no mutant frequencies were calculated or tabulated. The next highest concentration, 25 ug/ml, had 35% survival with no increase in mutant frequency compared to spontaneous background. Lower concentrations were also negative. Under activated conditions, concentrations of ≥40 ug/ml were too toxic. At 20 ug/ml, 17% survival was noted with a mutant frequency not significantly different from spontaneous background. Lower concentrations were also negative. Positive controls appear adequate.

This assay is considered unacceptable as there was not an adequate level of toxicity seen with the highest concentrations with reported mutant frequencies under non-activated conditions. It would be useful to have the mutant frequencies at the 30 and 35 ug/ml concentrations without activation. These data might provide some insight to whether there may be a positive trend in the data or not. If the data appear unquestionably negative, then further testing may not be necessary and this assay upgraded to acceptable.

The analogue trifluralin was tested in the mouse lymphoma assay in a submitted study to OPP (MRID #249846). The reviewer considered the study acceptable and trifluralin negative with and without activation up to cytotoxic concentrations.

D. Sister chromatid exchanges (SCE) in Chinese hamster bone marrow

Bromodeoxyuridine tablets were placed subcutaneously into adult female Chinese hamsters. Five to six hours later, test compound was given orally in 10% aqueous acacia solution; 19 hours later, a mitotic spindle inhibitor was administered and bone marrow obtained 2 hours later. Doses used were 200, 300, 400 and 500 mg/kg. Three animals per test dose were used and 25 cells per animals were scored. No increases in SCE were observed at doses up to 500 mg/kg. Only very slight toxicity was observed; a slight shift from second division cells to first division cells. However, in the absence of general toxicity to the animals, an approximate 50% shift to first division cells is necessary (not seen in this experiment). Therefore, this assay is unacceptable as toxicity levels were not adequate; also, at least 5 animals/sex/dose group should be used.

The analogue trifluralin was tested for SCE in Chinese

3

hamster bone marrow in a submitted study to OPP (MRID #249846). The reviewer considered the study acceptable and trifluralin negative for SCE induction at oral doses up 500 mg/kg.

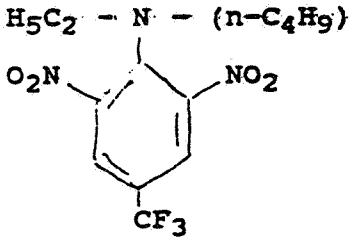
E. Overall conclusions

Benefin mutagenicity testing has fulfilled two of the three categories for mutagenicity testing. The Salmonella test satisfies the requirement for a gene mutation test and the UDS assay satisfies the requirement for other genotoxicity tests. However, the category of structural chromosomal aberrations has not been satisfied. Before a final analysis of the total evidence for benefin is performed, a test for this category is necessary. Based on the limited evidence on trifluralin below, an in vivo cytogenetics assay would be appropriate.

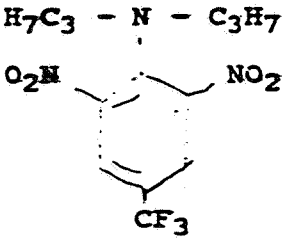
It may be important to examine the potential for structural chromosome aberrations for benefin. There are mixed reports for the clastogenicity of the analogue trifluralin. While trifluralin was reported negative in cultured Chinese hamster ovary cells for aberrations and SCE (Galloway et al., 1985) and for dominant lethal effects in a study submitted to OPP (MRID #250598), a trifluralin formulation has been reported positive for inducing chromosomal alterations in mouse spermatogonia as well as for aberrations in F_1 embryos after parental treatment (Nehez et al., 1980).

Another aspect that may require further consideration is the potential for aneuploidy induction as trifluralin is reported positive for aneuploidy in Neurospora (Griffiths et al., 1986) and weakly positive in Aspergillus for malsegregation (Kafer et al., 1986).

F. Structures



Benefin



Trifluralin

4

G. References

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