Caswell

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
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OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: Overview of Submitted Mutagenicity Studies on Metsulfuron Methyl

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Chemical: Metsulfuron methyl CAS# 74223-64-6 Caswell #419H

This reviewer has been requested to examine and summarize mutagenicity studies concerning metsulfuron methyl 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
CHO/aberrations: positive ± activation
Rat bone marrow/aberrations: negative

Unacceptable studies:

CHO/hgprt gene mutation assay: negative
UDS/primary rat hepatocytes: negative

The following will discuss these studies and then present an overall conclusion.

A. Salmonella assay (Documents #002990, 004055)

The test compound was assayed in Salmonella strains TA98, TA100, TA1535 and TA1537 with and without metabolic activation. A preliminary toxicity experiment with TA1535 with and without activation indicated no revertants observed and decreased background bacterial lawns at concentrations of 50 - 10,000 ug/plate. A subsequent toxicity test with TA100 was performed in which $10^3$ and $10^8$ cells/plate were evaluated. Substantial killing was seen at $10^8$ cells/plate over the range 50 - 2500 ug/plate, but only slight toxicity seen with $10^3$ cells/plate. The report gave no explanation for this differential toxicity; however, since the Salmonella assay is performed with $10^8$ cells, lower concentrations were used. A partial mutagenesis experiment with TA1535 induced a significant drop in revertants at 10 and 50 ug/plate, indicating some toxicity. Therefore, the top concentrations used in the mutagenesis assay were 5 ug/plate without activation and 10 ug/plate with activation.

Negative results were obtained at the concentrations tested. Three trials were performed with each strain with duplicate cultures per trial. Only the first trials tested the top concentration (5 ug/plate without activation, 10 ug/plate with activation) as some reduction in revertants was seen; therefore, for the next two trials, the concentrations were slightly reduced. Results appear adequate to indicate negative activity by the test compound to the tested concentrations.

B. CHO/hgprt gene mutation assay (Documents #002990, 004055)

Cultured Chinese hamster ovary (CHO) cells were exposed to test compound for 18-19 hours without activation and for 5 hours with activation. After exposure to activation conditions, cells were washed and incubated for 21-25 hours before subculturing. Preliminary testing indicated that test compound was soluble in DMSO, but when this dilution was placed into aqueous based culture medium, precipitation was noted at $\geq$ 2.0 mM though the precipitate did not last throughout the treatment period.

For the mutation assay, 3 assays with activation and 2 assays without activation were performed. Two cultures per concentration per trial were used. Concentrations used were 0, 0.5, 1.0, 2.0, 3.5 and 7.0 mM. No concentration dependent increases were seen in mutant frequency. Also, toxicity was not very evident as the lowest survival frequency only reached 50%. This assay is considered unacceptable. The background mutant frequencies are generally too high. A background mutant frequency between 0-20 X $10^{-6}$ clonable cells is the acceptable range. Under non-activated conditions, trial one had a background of about 18 X $10^{-6}$ and trial two about 30 X $10^{-6}$. With activation, these values were about 19 X $10^{-6}$, 36 X $10^{-6}$, and 27 X $10^{-6}$, for the three trials, respectively. These elevated values would preclude identification of a weak genotoxic response if it was present. For example, there are indications

of elevated mutant frequencies, e.g. trial one without activation, at 7.0 mM, obtained about 44 X $10^{-6}$ vs. 18 X $10^{-6}$ background; and with activation trial one, at 2.0 mM, an average frequency of 36 X $10^{-6}$ vs. 19 X $10^{-6}$ background was obtained. It is suggested that the testing laboratory take measures to reduce the variability and elevated values of its spontaneous backgrounds. This experiment should then be repeated to assure that the elevations in mutant frequency in the first trials under either condition are or are not indicative of a potential weak mutagenic response.

The original assessment considered this assay unacceptable (Document #002990) based on inadequate top concentrations that were tested. The registrant responded that the top concentrations were at the limits of solubility, which the original submission appears to substantiate. Document #004055 subsequently upgraded the assessment to acceptable based on this response. However, neither the original review nor the registrant response discussed the high background rates; therefore, this assay should be reassessed as unacceptable.

C. CHO/aberrations (Document #004055)

Chinese hamster ovary (CHO) cells were exposed to test compound for 10 hours under non-activated conditions and for 2 hours with metabolic activation. After exposure under activated conditions, the cells were washed and incubated for an additional 8 hours. Colcemid was added to all cultures for the final two hours of incubation. A preliminary toxicity experiment indicated a top concentration of 7.9 mM was to be used (toxicity ranges of 52.4 - 78.5% relative survival without activation and of 45 - 70% relative survival with activation were seen in the preliminary toxicity test).

One trial was performed under non-activated conditions and a significant concentration-related trend with significant increases in aberrations at 7.9 mM was observed, e.g. at 7.9 mM, an increase to 0.21 aberrations/cell vs. 0.07 aberrations/cell background was noted. Mostly chromatid type breaks and exchanges were found. Under activated conditions, 3 trials were performed. Trials 1 and 2 indicated concentration-related positive trends with positive increases at 2.63 mM, 5.3 mM and 7.9 mM, e.g. at 7.9 mM, the combined trial results indicated an increase to 0.39 aberrations/cell vs. 0.18 aberrations/cell background. However, in these first two trials, there was a temperature control problem during the cell harvest procedures. Trial 3 was performed with proper temperature control and a similar response was seen at 7.9 mM. Therefore, it appears that the test compound is positive in this aberration assay with and without activation.

D. Rat bone marrow/aberrations (Document #002990)

Sprague-Dawley rats were exposed to doses of 500, 1000 and 5000 mg/kg of test compound orally in corn oil. Five

animals/sex/dose/kill time were used and were killed at 6, 12, 24 and 48 hours (animals were injected with colchicine two hours before termination). There were no clinical effects attributed to the test compound and no alterations in mitotic index noted. A small dose related positive trend was seen at the 12 hour kill, but the values were not significantly different from control values. The positive controls appear adequate. This assay appears negative with dosing performed at an acceptable maximum (5000 mg/kg). It should be noted that the compound appears fairly non-toxic.

The original review (Document #002990) rated this study as unacceptable, mainly due to the lack of toxicity at the highest tested dose. The registrant responded to this classification and suggested that the 5000 mg/kg dose was adequate. This is acceptable as a maximum dose and the study should be upgraded to acceptable.

E. UDS/primary rat hepatocytes (Documents #002990, 004055)

Primary hepatocytes from Sprague-Dawley rats were obtained, seeded for 2 hours, washed, and then exposed to test compound for 18 hours. Two different experiments with two different rats were performed. Two slides/concentration and 25-50 nuclei/slide were scored. The concentrations used ranged from 1 X $10^{-5}$ mM to 1.0 mM. No cytotoxicity was noted. Results were negative for unscheduled DNA synthesis (UDS); however, the test compound was not tested up to high enough concentrations (the top concentration should elicit some signs of toxicity). The classification should be reassessed to unacceptable.

The original review (Document #002990) agreed with the current assessment. The registrant responded by stating that they tested to the limit of solubility. However, this was not reported in the original report and no substantiation was provided. Other studies (e.g. CHO/hgprt assay above) indicated that 1.0 mM may be approaching the concentration(s) where precipitate may be seen. However, without noting it in this experiment and no toxicity apparent, this study should remain unacceptable. In Document #004055, this study was upgraded to acceptable in response to the registrant; however, this should be reversed as stated above.

F. Overall Conclusions

Metsulfuron methyl has been tested adequately in assays that satisfy two of the three areas for mutagenicity testing in support of a mutagenicity assessment - gene mutations (Salmonella assay) and structural chromosomal aberrations (in vitro aberrations and in vivo aberrations). Metsulfuron methyl needs to be further tested to satisfy the requirement for a test in the third area - other genotoxic effects.

Based on the submitted information, metsulfuron methyl does not induce gene mutations in the microbial assay utilized.

Metsulfuron methyl appears to have clastogenic activity with and without activation as evidenced by the activity in the cultured mammalian cells. However, this activity does not appear to be found in the bone marrow of treated rats. It is possible that the bone marrow may not be the appropriate target for possible in vivo clastogenic activity. A final conclusion on the potential genotoxic activity of metsulfuron methyl will be made when all required testing, and possible additional testing, has been submitted to the Agency.