

CASE

PM

002654

CHEM Chlorsulfuron (formerly DPX-W4189)

BRANCH Toxicology DISC

TOPIC Mutagenicity

FORMULATION Technical (information known to reviewer)

FICHE/MASTER ID

CONTENT CAT

Mutagenicity Evaluation of
12,700 in an
In Vitro Cytogenetic Assay
Measuring Chromosome Aberration
Frequencies in Chinese Hamster
Ovary (CHO) Cells
Amendment

HLO-323-81
MR-0581-920
Genetics Assay No. 5540
Litton Bionetics, Inc.
LBI Project No. 20990
April, 1981
S. M. Galloway

SUBST. CLASS =

OTHER SUBJECT DESCRIPTORS

DIRECT RVW TIME = 3 hours

START-DATE

END DATE

REVIEWED BY: Ladd W. Smith

TITLE: Toxicologist

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DATE: 11/19/81

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DATE:

Conclusion:

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- A. This study is scientifically valid.
 - B. Chlorsulfuron did not induce aberrations in CHO cells at concentrations of up to 5 mg/ml, with or without metabolic activation.
 - C. This study generally conforms to EPA Proposed Guidelines in Sec. 163.84-3 (Federal Register 43: 37391, 8/22/78).

Methods:

Chinese Hamster Ovary (CHO-WBI) cells were grown in McCoy's 5a medium.

Cytotoxicity experiments showed reduced cell growth at 5000 mg chlorsulfuron/ml; test cultures were exposed to concentrations of 16.7 mg/ml to 1.67 mg/ml.

Approximately 3×10^6 cells were exposed to chlorsulfuron for 8 1/2 to 10 hours, with and without metabolic activation (S-9 fraction from livers of, Aroclor-1254-induced male Fisher rats). Negative (medium), solvent (DMSO) and positive (cyclophosphamide, with activation; triethylenemelamine, without activation) controls were tested. Mitosis was inhibited by colcemid and cells were stained with 5% Giemsa.

One hundred cells/dose were scored for:

- estimated number of breaks involved in production of different types of aberrations.
- frequency of cells with more than one aberration.
- evidence for increasing damage with increasing dose.

Statistical analysis by the t-test used the 0.05 and 0.01 levels of probability.

When no preliminary cytotoxicity data are available, the usual procedure is to use a final concentration of 1 mg/ml of test compound, where solubility permits. To achieve a final concentration of about 1 mg/ml without exceeding a 1% concentration of solvent in the cultures, it is usual to make a stock solution at 100 mg/ml and add 0.1 ml of the concentrated stock solution to a 10 ml culture. In the case of test compound 12,700, solubility in DMSO was good, with a maximum solubility of approximately 500 mg/ml. A high dose of 5 mg/ml was selected and doses spanning a range of more than four orders of magnitude, in a half-log series were tested. At the highest two dose levels, 5 mg/ml and 1.67 mg/ml, the test compound formed a precipitate in the culture medium. The compound 12,700 was thus tested up to the limits of solubility.

Because of the variable nature of toxicity, results obtained from counts of surviving cells at short times (10-24 hours) after treatment, dose selection is now based on the availability of mitotic cells. Cell monolayers were observed about eight hours after addition of test compound (about 2 1/2 hours before fixation). The degree of confluence and appearance of cells was noted. This information is shown below. There was marked toxicity at the top doses particularly, at 5 mg/ml, with a reduction in confluence and

appearance of dead cells at many lower doses. A suppression of cell division at high doses was slightly more marked in the test without metabolic activation, in which the treatment period is longer. However, it was possible to obtain scoreable metaphase cells even at 5 mg/ml.

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CELL GROWTH OBSERVATIONS BEFORE CULTURE FIXATION

TREATMENT	% CONFLUENCE (APPROX.)	
<u>CONTROLS</u>	<u>-S9 MIX</u>	<u>+S9 MIX</u>
NEGATIVE	50	50
SOLVENT	50	40
POSITIVE	50	30
POSITIVE	50	40
<u>12,700 (µg/ml)</u>		
5000.00	<10	10
1666.67	30	20
500.00	10	20
166.67	30	30
50.00	40	20
16.67	30	20
5.00	50	20
1.67	50	20
0.50	50	30
0.17	50	30

Results:

Without activation, only 1.67 mg chlorsulfuron/ml produced aberrations. The 3 aberrations noted were within the normal background of 0-1% abnormal cells seen in the negative and solvent controls. With activation, the low frequencies of aberrations were within the 2-5% range seen in the negative and solvent controls. Both positive controls produced expected effects.

Discussion:

The study was conducted by acceptable methods and the collected data support the reported conclusions. 002654

Chromosome gaps were not counted as significant aberrations. Open breaks were considered indicators of genetic damage, as were configurations resulting from the abnormal repair of breaks, such as multiradials, rings and multicentrics.