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

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APR 14 1987

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: Mutagenicity study with Telone II
EPA # 464-511 Caswell No. 324 A
EPA Accession No. 262994 Tox Proj. No. 2105

TO: Lois Rossi/William Forrest
Product Manager # 21
Registration Division (TS-767C)

FROM: Quang Q. Bui, Ph.D. Quang Bui 4/13/87
Acting Head, Review Section V
Toxicology Branch/HED (TS-769C)

THRU: Irving Mauer, Ph.D
Geneticist
Toxicology Branch/HED (TS-769C)
and
Theodore M. Farber, Ph.D.
Chief, Toxicology Branch
Hazard Evaluation Division (TS-769C)

Registrant: Dow Chemical Co.,
Midland, Michigan 48640

Action Requested: Review a mutagenicity study with Telone II in Chinese Hamster Ovary cell/HGPRT; Dow Chemical Co., 2/27/86.

RECOMMENDATION

In this gene mutation assay using a mammalian system (CHO/HGPRT), there is no evidence to suggest that Telone II is a mutagen in both presence and absence of metabolic activation. It is recommended that this investigation be classified as Acceptable Data.

However, the registrant is requested to provide information relative to the unindentified 7.9% in the formulation (the technical test material used in this investigation is listed as consisting of 48.9% cis- and 43.2% transl,3-dichloropropene with the remaining 7.9% not identified).

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DATA EVALUATION RECORD

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Study Type: Mutagenicity - gene mutation
Chemical: 1,3-Dichloropropene; Telone II
Test Material: Telone II technical grade
[48.9% cis-1,3-dichloropropene
43.2% trans-1,3-dichloropropene]

Study Identification:

"The evaluation of Telone II soil fumigant in the Chinese Hamster Ovary Cell/Hypoxanthine (Guanine) Phosphoribosyl Transferase (CHO/HGPRT) Forward Mutation Assay"

Testing Facility: Dow Chemical Co.,
Final Report No.: N/A
Report Date: 2/27/86
Author: A.L. Mendrala
EPA Accession No.: 262994

Reviewed by: Quang Q. Bui, Ph.D.
Acting Head, Review Section V
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Approved by: Irving Mauer, Ph.D.
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RECOMMENDATION AND CONCLUSION

Under the conditions of this investigation, there is no evidence to suggest that 1,3-dichloropropene is a mutagen in the CHO/HGPRT assay in both presence and absence of metabolic activation up to and including a dosage level of 200 and 250 uM/dish, respectively. Cytotoxicity was noted at 150 uM and above in both presence and absence of metabolic activation.

It is recommended that this assay be classified as Acceptable Data.

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MATERIALS AND METHODS

A copy of the procedures used is appended.

In summary, Chinese Hamster Ovary (CHO) cells were obtained from Oak Ridge National Laboratory (Oak Ridge, TN). The CHO cells were cultured in Ham's F-12 medium (without hypoxanthine) supplemented with calf serum, thioguanine, and antibiotics. Positive controls used were ethyl methanesulfonate (EMS) for non-metabolic activation assays and 3-methylcholanthrene (MCA) for metabolic activation assays.

Based on the results of the preliminary cytotoxicity experiments, five dose levels of Telone II were selected for the CHO/HGPRT mutation assays.

RESULTS

1. Cytotoxicity Assays

Cytotoxicity assays were conducted with Technical Telone in the presence and absence of metabolic activation. Cell count, cloning efficiency, and cell survival were monitored and the data obtained are as follows:

CYTOTOXICITY ASSAYS WITH/WITHOUT METABOLIC ACTIVATION

Doses	Absolute Cloning Efficiency (%)		Relative Survival (% of control)	
	OMA	MA	OMA	MA
DMSO 0.1%	58(23)	89	100(100)	100
EMS 3 mM	64(3)	-	110(14)	-
MCA 18.6 uM	-	75	-	84
Telone 50 uM	61(28)	88	105(122)	98
Telone 100 uM	32(8)	61	55(37)	69
Telone 125 uM	-	60	-	68
Telone 150 uM	10(3)	43	18(12)	48
Telone 200 uM	2(3)	13	3(11)	14
Telone 250 uM	0(2)	-	<1(9)	-

(WMA) Without metabolic activation; Average of 5 replicate dishes (second trial)
(MA) With metabolic activation; Average of 5 replicate dishes
(-) Not tested

In the non-metabolic assays, cytotoxicity was observed at dose levels of 150 uM and above and dose levels of 50, 100, 150, 200, and 250 uM were selected for the mutagenicity assays. In the metabolic activation tests, both cloning efficiency and relative survival were significantly reduced at the 20 uM dosage level. Based upon the results, dose levels of 50, 100, 125, 150, and 200 uM were selected for the assays with metabolic activation.

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2. Mutagenicity Assays

Mutagenicity assays were conducted in both presence and absence of metabolic activation. Non-metabolic assays were conducted three times and the results were as follows:

MUTAGENICITY ASSAYS/WITHOUT METABOLIC ACTIVATION

Doses	Total Mutant Colonies			Absolute Cloning Efficiency (%)			TG-resistant mutants per 10^6 Clonable Cells		
	(I)	(II)	(III)	(I)	(II)	(III)	(I)	(II)	(III)
DMSO 0.1%	6	16	9	74	61	69	8	27	13
EMS 3 mM	396	271	357	46	96	62	853*	282*	581*
Telone 50 uM	12	8	4	75	73	84	16	11	5
Telone 100 uM	10	17	21	91	98	76	11	17	28
Telone 150 uM	10	7	6	61	102	73	17	7	8
Telone 200 uM	37	15	12	52	102	86	72*	15	14
Telone 250 uM	19	13	20	43	116	86	44*	11	23

(*) Positive response

During the first trial, an apparent positive mutagenic response was observed at the 200 and 250 uM dosage levels. From a spontaneous mutation frequency of 8 mutants per million clonable cells (control value), approximately 72 and 44 mutants per million were observed at, respectively, 200 and 250 uM. The effect was not dose-related. The authors stated that this effect was observed only at dose levels which were associated with extreme cytotoxicity and, hence, the biological significance of this finding is questionable. To ascertain the significance of these findings, the assays with non-metabolic activation were repeated twice (experiment II and III) and no positive mutagenic responses were observed with Telone II up to and including a dose level of 250 uM in the repeated assays (experiment II and III).

In the assays with metabolic activation, no positive mutagenic responses were observed with Telone up to and including a dosage level of 200 uM. However, a positive response was observed with MCA at 18.6 uM.

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DISCUSSION AND CONCLUSION

The soil fumigant, 1,3-dichloropropene (Telone II) as well as the individual cis- and trans-isomer were previously shown to act as direct microbial mutagens that function primarily by base pair substitution.

In this gene mutation assay using a mammalian system (CHO/HGPRT) and under the conditions of the investigation, there is no evidence to suggest that Telone II is a mutagen in the presence and absence of metabolic activation. The positive response observed in the first non-activated assay at 200 and 250 uM did not follow a dose-response and could not be confirmed by the results of the second and third assays.

It is recommended that the results of this experiment be classified as Acceptable Data.

However, the registrant is requested to provide clarification relative to the 7.9% "unidentified" in the formulation tested. The technical test material used in this investigation is listed as consisting of 48.9% cis and 43.2% trans-1,3-dichloropropene with the remaining 7.9% not identified. It is unclear as to whether epichlorohydrin or another chemical was used as stabilizing agent in the formulation tested.

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