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

004508

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

JUN 27 1985

MEMORANDUM

SUBJECT: Piperonyl Butoxide-Mutagenicity Study submitted in response to Special Review DCI. Accession No. 257430. Registration No: 1021-974. Caswell: 670

TO: T. Gardner/P. Shroeder, PM 17
Registration Division (TS-767)

FROM: Irving Mauer Ph.D., Geneticist
Toxicology Branch
Hazard Evaluation Division (TS-769)

6-26-85

THRU: Jane E. Harris, Ph.D., Head
Section VI, TB/HED (TS-769)

JEH 6/26/85

6/27/85

Action Requested: Review and evaluate the following mutagenicity study, submitted by McLaughlin, Gormley and King: "Evaluation of Piperonyl Butoxide in the CHO/HGRPT Mutation Assay With and Without Metabolic Activation, A Final Report to The Piperonyl Butoxide Task Force, c/o Dr. Frederick J. Preiss, Chairman, Technical Committee McLaughlin, Gormley and King Company 8810 10th Avenue North, Minneapolis, MN 55427." (Submitted by Arthur D. Little, Inc., 25 Acorn Park, Cambridge, MA 02140, ADL Reference: 53906, April 1, 1985). Also: "An Addendum to the Final Report" (submitted by Arthur D. Little, Inc., April 18, 1985).

TB Evaluation: The study is judged ACCEPTABLE (see DATA REVIEW, attached).

TOXICOLOGY BRANCH: DATA REVIEW

Caswell: 670

EPA Chem. # 067501

Chemical: Piperonyl Butoxide

Study Type: Mutagenicity - Gene mutation in mammalian cells in vitro (CHO/HGPRT Assay)

Citation: Evaluation Of Piperonyl Butoxide in the CHO/HGPRT Mutation Assay With and Without Metabolic Activation

Accession No./MRID No.: 257430/na

Sponsor/Testing Lab: McLaughlin, Gormley and King/A.D. Little

Study No./Date: ADL-53906/April 1, 1985

Test Material: Piperonyl butoxide (PB) technical (Ref. #FEG-32), an oily liquid dissolved in dimethylsulfoxide (DMSO) for testing.

Procedures:

Following cytotoxicity testing at nine concentrations ranging from 0.1 through 1000 µg/ml, CHO-K_1 (BH_4 subline) cells were exposed to five levels of test material, in the absence (16-hr exposure) and presence (5 hr) of a metabolic activation system consisting of an Aroclor 1254-induced rat liver microsomal fraction (S-9) plus cofactors. Five replicate plates each from duplicate cultures were sampled 7 to 9 days later for thioguanine-resistant colonies (HGRPT mutants). Parallel assays were performed with the reference mutagens, ethylmethanesulfonate (EMS) and dimethylnitrosamine (DMN). Differences between test and negative control (DMSO) cultures were evaluated by the t-test and linear regression analysis.

Results:

Dose-dependent reduction in cloning efficiency (a measure of cytotoxicity) occurred at concentrations above 30 µg/ml test substance in nonactivated cultures, but only above 300 µg/ml in S-9 cultures.

In the absence of S-9, PB induced a slight but dose-dependent increase in HGPRT mutants, statistically significant ($p < 0.01$), however, only at one concentration (75 µg/ml of control)---see attached tabulation, Table I. Linear regression analysis on the four lower doses (10 to 75 µg ml) showed a correlation coefficient of 0.92.

In the presence of S-9, PB produced no increase in mutants at concentrations up to 500 µg/ml (44% relative toxicity). Both positive controls performed appropriately, registering significant mutant frequencies 200 to 250 times DMSO controls.

Conclusions:

The author concludes that PB was "weakly mutagenic" in this assay but only under nonactivated conditions (absence of S-9).

In an Addendum to the report, dated April 18, 1985, the authors reanalyzed the nonactivation data from a submitted tabulation of all 10 replicate plates per test concentration (5 plates per duplicate test). Linear regression analysis of all five PB concentrations (rather than the four lower) revealed a correlation coefficient of 0.47 (compared to 0.92). Further, there are (normally) discrepancies between the expansion of the original duplicate treatment plates in final mutant colonies derived after the expression period of 7 to 9 days (see Table II), often marked. It is noted that 21 of the 23 mutants at 75 µg/ml were derived from only one of the duplicate cultures originally treated.

Hence, the authors conclude that the statistically significant increase at 75 µg/ml originally reported represents an equivocal response in this type of assay.

TB Evaluation:

We agree with the authors that the single point significance represents normal variation during expression, and is not of biological significance. ACCEPTABLE.

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TABLE I: CHO/HGPRT Mutation Assay on Piperonyl Butoxide(PB)*

Treatment	Dose (µg/ml)	S-9	Cytotoxicity (RCE)**	Total Mutant Colonies	Mutant Frequency***
Medium	-	-		4, 3	3.03, 2.54
DMSO	-	-		3	2.11
S-9	-	+		4	3.23
PB	10	-	1.02	4	2.78
	25	+	1.16	12	11.11
	25	-	0.92	11	7.53
	50	+	1.17	10	6.76
	50	-	0.56	10	6.41
	100	+	1.28	(C)	(C)
	75	-	0.28	23	14.56(S)
	250	+	1.15	2	1.49
	100	-	0.11	7	4.12
	500	+	0.44	11	8.09
EMS	248	-	0.29	626	763.41
DMN	500	+		283	589.58

* Compiled from Tables 3 and 4 of original report (April 1, 1985).
** Relative Cloning Efficiency (controls = 1.00).
*** Expressed as mutants per 10^6 clonable cells.
(S) = Statistically significant by t-test ($p<0.01$).
(C) = Contaminated, and not subcultured.

TABLE II: Distribution of CHO/HGPRT Mutants from PB Treatment in the Absence of S-9 Metabolic Activation*

Treatment	Dose µg/ml	Test Plate**	Mutant Colonies***	Total Mutants
Medium	-	A B	1,0,0,0,0 2,1,0,0,0	4
DMSO	-	A B	0,0,0,0,0 1,2,0,0,0	3
PB	10	A B	1,2,0,1,0 0,0,0,0,0	4
	25	A B	3,3,3,1,1 0,0,0,0,0	11
	50****	A_1 A_2	0,0,2,0,1 1,1,1,1,3	10
	75	A B	1,1,0,0,0 4,3,4,5,5	23
	100	A B	1,2,0,1,1 1,1,0,0,0	7
EMS	248	A B	59,49,50,68,50 65,70,62,64,89	626

* Adapted from Table A of the ADDENDUM (April 18, 1985).
** A & B plates indicate the original duplicate cultures exposed.
*** The 5 replicate plates after the expression period per test duplicate culture originally treated.
**** Original duplicate culture A was expanded, since plate B was lost to contamination.

Rev. 4/14/82 .icology Branch/HED Review

Caswell file

MAR 28 1985

Caswell No(s).: 670

To: Jeff Gardiner PM-17 (KD-TS767)

Registration No(s).: 4F16-72

Pesticide Petition No(s).: n/a

Chemical(s): Piperonyl Butoxide

Requested Action(s): Review protocol: (ADL Reference 1-1683)
TITLE: "THE EVALUATION OF "Piperonyl Butoxide" IN THE CHO/HGPRT MUTATION ASSAY WITH AND WITHOUT METABOLIC ACTIVATION." (Submitted

Recommendation: March 5/85, EPA Com. # 35707)
↳ Protocol appears to be adequate (but see "COMMENTS" - below)

Inert(s) cleared 180.1001: n/a

% of ADI occupied: Existing: n/a Resulting: n/a

Resulting % increase in TMRC: n/a

Data considered in setting the ADI: n/a

Attached (?): ADI printout: YES/NO; TOX "one-liner": YES/NO; DER: YES/NO

Existing regulatory actions against registration: "Notice of Intent to Suspend"

RPAR status: Decision Document, 9/30/81; and DCI Notice, 5/12/82

New Data: No

Data gaps: Mutagenicity / Chronic Feeding / Oncogenicity / Reproduction / Metabolism (Teratology ?)

Comments: The protocol appears to be adequate, except I would recommend that if no cytotoxicity seen @ 1000, go to "limit dosing" (5000), or limit of solubility.

Reviewer: IRVING MAUER PH.D. Date: 3/26/85

Section Head: Jane E. Harris 3/26/85 Branch Chief: [illegible] 3/27/85

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