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OPP OFFICIAL RECORD
HEALTH EFFECTS DIVISION
SCIENTIFIC DATA REVIEWS
EPA SERIES 361

OFFICE OF
PREVENTION, PESTICIDES AND
TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: **Isoxaflutole.** Outcome of the review of 3 mutagenicity studies on the isoxaflutole metabolite RPA 203328.

FROM: Alberto Protzel
Branch Senior Scientist
Toxicology Branch I
Health Effects Division (7509C)

7/10/98

TO: Barbara Madden
Risk Characterization and Analysis Branch
Health Effects Division (7509C)

and

Richard Loranger, Chair
Metabolism Assessment Review Committee (MARC)
Health Effects Division (7509C)

Task ID: DP Barcode: ~~D246401~~ D245866
MRID Nos.: 44545301, 44545302, & 44545303

PC Code: 123000
Case No.: 287353
Submission: S541869

cc: Sanju Diwan (HED, 7509C)

An *ad hoc* subgroup of the HED MARC met on March 23, 1998 to re-evaluate the status of the RPA 203328 metabolite of isoxaflutole. The data examined at the meeting included preliminary negative genotoxicity results for a micronucleus assay, a CHO/HGPRT Assay, and an *in vitro* cytogenetics assay. It was concluded at the meeting that, pending submission and review with Acceptable Rating of the above mutagenicity studies, RPA 203328 *does not pose a special toxicological concern as to carcinogenicity at this time.*

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The final reports of the studies have now been submitted and upon review have been found to be **Acceptable. There was no indication of any mutagenic, clastogenic or aneugenic effect associated with RPA 203328 in any of the three studies that were reviewed.**

The executive summaries of the three mutagenicity studies appear in the following paragraphs:

1. Curry, P.T. (1998) Mutagenicity Test on RPA 203328 in the *In Vivo* Mouse Micronucleus Assay; Covance Laboratories, Inc., Leesburg Pike, Vienna, VA; Laboratory Project Identification: Covance 19201-0-455OECD; Study Completion Date: April 23, 1998. (Unpublished) MRID NUMBER: 44545302.

In a mouse micronucleus assay (MRID No. 44545302), groups of six male Crl:CD-1*(ICR)BR mice/dose/sacrifice time were orally dosed with 500, 1000, or 2000 mg/kg RPA 203328 (99%) [RPA 203328 = a metabolite of isoxaflutole] administered in 0.5% methylcellulose at a constant volume of 10 mL/kg. These doses were based on a preliminary range-finding study in which groups of 3 males and 3 females received single oral doses of 200, 500. 800, 1500 or 2000 mg/kg RPA 203328, and no mortality or symptoms occurred. Mice were sacrificed at 24 hours (all dose levels, as well as positive controls) and at 48 hours (vehicle controls and 2000 mg/kg RPA 203328 only) postadministration and harvested bone marrow cells were examined for the incidence of micronucleated polychromatic erythrocytes (MPEs).
No deaths or other signs of toxicity were reported. There was also no evidence of target cell cytotoxicity. The positive control (80 mg cyclophosphamide/kg, administered orally, with a 24-hr sacrifice time) induced the expected high yield of MPEs (only males tested). **However, there was no indication of a clastogenic and/or aneugenic effect associated with administration of RPA 203328 under the conditions of this assay, which included administration of a limit dose (2000 mg/kg) with sacrifice times of 24 and 48 hours.**

The study is classified as Acceptable and satisfies the requirements for FIFRA Test Guideline 84-2 for a micronucleus assay.

2. Cifone, M.A. (1998). Mutagenicity Test on RPA 203328 in the CHO/HGPRT Forward Mutation Assay with Duplicate Cultures and a Confirmatory Assay; Covance Laboratories, Inc., Leesburg Pike, Vienna, VA; Laboratory Project Identification: Covance 19201-0-435 OECD; Study Completion Date: April 24, 1998. (Unpublished) MRID NUMBER: 44545303.]

In two independently performed Chinese hamster ovary (CHO) cell HGPRT forward gene mutation assays (MRID No. 44545303), duplicate cultures of RPA 203328 were assayed at concentrations of 84.5 - 2700 μg/mL -S9 (initial and confirmatory trials) and 338 - 2700 μg/mL +S9 (initial trial) and 675 - 2700 μg/mL (confirmatory trial). The S9 was derived from Aroclor 1254-induced rat (male Sprague-Dawley) livers, and the test material was delivered in dimethyl sulfoxide.

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In the assays, there was no indication of cytotoxicity ±S9 at the highest dose level of 2700 μg/mL, which was 10 mM. **Although there were a few sporadic instances of statistically significant elevations in mutation frequency, these were not dose-related and were generally below the 15 x 10^{-6} required for a positive response except in one case (a value of 15.8 x 10^{-6}). Overall, there was no evidence of any increase in mutation frequency resulting from exposure to RPA 203328.**

The study is classified as **Acceptable** and satisfies the requirements for an in vitro mammalian cell forward gene mutation study (84-2).

3. **Murli, H. (1998). Mutagenicity Test on RPA 203328 Measuring Chromosomal Aberrations in Chinese Hamster Ovary Cells (CHO)**; Covance Laboratories, Inc., Leesburg Pike, Vienna, VA; Laboratory Project Identification: Covance 19201-0-437OECD; Study Completion Date: April 7, 1998. (Unpublished) MRID NUMBER: 44545301.

In an in vitro cytogenetic assay (MRID No. 44545301), Chinese hamster ovary (CHO) cells were analyzed from cultures exposed to RPA 203328 (99.0%) at 931, 1330, 1900 and 2710 μg/mL ± S9 in an initial trial (3-hr exposure, followed by wash and 15-hr incubation, then 2-hr exposure to colcemid, followed by fixation). In the confirmatory trial, cells were exposed to concentrations of 924, 1320, 1890 and 2700 μg/mL ± S9(-S9: 17.8-hr exposure to RPA 203328, followed by 2-hr exposure to colcemid; +S9, same schedule as in the first trial). The S9 mix was derived from Aroclor 1254-induced male Sprague-Dawley rat livers and RPA 203328 was delivered to the test system in dimethylsulfoxide.

The high dose (≈2700 μg/mL) was selected based on the solubility properties of RPA in DMSO (also, 2700 mg/mL was >10 mM concentration of RPA 203328). In the initial trial, there was no indication of any significant effect on the mitotic indices ± S9 at the highest dose level (2710 μg/mL). In the confirmatory trial -S9 (with a 17.8-hr exposure to RPA 203328 as compared to 3 hrs in the initial trial) there was a slight (21%) reduction in the mitotic index at 2700 μg/mL. No effect on mitotic indices was observed at the highest dose level +S9 in either trial. The positive controls induced the expected high yield of cells with chromosome aberrations. **There was, however, no evidence that RPA 203328 induced a clastogenic response at any dose or harvest time.**

This study is classified as **Acceptable** and satisfies the guideline requirements for an in vitro mammalian cell cytogenetic assay (84-2).

EPA Reviewer: Byron T. Backus, Ph.D.
Technical Review Branch
Registration Division (7505C)

Signature: [signature]
Date: 6/11/98

EPA Secondary Reviewer: Nancy McCarroll
Toxicology Branch I
Human Effects Division (7509C)

Signature: [signature]
Date: 6/11/98

012683

DATA EVALUATION REPORT

STUDY TYPE: Mutagenicity: *In vitro* cytogenetics assay in cultured Chinese hamster ovary cells; OPPTS 870.5375 [§84-2]

DP BARCODE: D245866 SUBMISSION NO.: S541869

PC CODE: ------ TOX. CHEM. NO.: MRID NO: 44545301

TEST MATERIAL (PURITY): RPA 203328 (99.0%)

COMPOSITION/SYNONYM(S): CAS 142994-6-7; 2-Methanesulphonyl-4-trifluoromethylbenzoic acid; a metabolite of Isoxaflutole (PC Code 123000)

CITATION: Murli, H. (1998). Mutagenicity Test on RPA 203328 Measuring Chromosomal Aberrations in Chinese Hamster Ovary Cells (CHO); Covance Laboratories, Inc., Leesburg Pike, Vienna, VA; Laboratory Project Identification: Covance 19201-0-437OECD; Study Completion Date: April 7, 1998. (Unpublished) MRID NUMBER: 44545301.

SPONSOR: Rhône-Poulenc, Research Triangle Park, NC 27709

EXECUTIVE SUMMARY: In an *in vitro* cytogenetic assay (MRID No. 44545301), Chinese hamster ovary (CHO) cells were analyzed from cultures exposed to RPA 203328 (99.0%) at 931, 1330, 1900 and 2710 µg/mL ± S9 in an initial trial (3-hr exposure, followed by wash and 15-hr incubation, then 2-hr exposure to colcemid, followed by fixation). In the confirmatory trial, cells were exposed to concentrations of 924, 1320, 1890 and 2700 µg/mL ± S9(-S9: 17.8-hr exposure to RPA 203328, followed by 2-hr exposure to colcemid; +S9, same schedule as in the first trial). The S9 mix was derived from Aroclor 1254-induced male Sprague-Dawley rat livers and RPA 203328 was delivered to the test system in dimethylsulfoxide.

The high dose (≈2700 µg/mL) was selected based on the solubility properties of RPA in DMSO (also, 2700 mg/mL was >10 mM concentration of RPA 203328). In the initial trial, there was no indication of any significant effect on the mitotic indices ± S9 at the highest dose level (2710 µg/mL). In the confirmatory trial -S9 (with a 17.8-hr exposure to RPA 203328 as compared to 3 hrs in the initial trial) there was a slight (21%) reduction in the mitotic index at 2700 µg/mL. No effect on mitotic indices was observed at the highest dose level +S9 in either trial. The positive controls induced the expected high yield of cells with chromosome aberrations. **There was, however, no**

evidence that RPA 203328 induced a clastogenic response at any dose or harvest time.

This study is classified as **Acceptable** and satisfies the guideline requirements for an in vitro mammalian cell cytogenetic assay (84-2).

COMPLIANCE: Signed and dated GLP (p. 3), Quality Assurance (p. 6) and (No) Data Confidentiality Statements (p. 2) were provided.

I. MATERIALS AND METHODS

A. MATERIALS:

1. Test Material: RPA 203328; CAS 142994-06-7
   Description: white powder with small aggregates
   Lot/batch number: NMI874
   Purity: 99%
   Receipt date: Feb. 6, 1998
   Stability: Not reported
   CAS number: 142994-06-7
   Structure: not given (chemical name: 2-Methanesulphonyl-4-trifluoromethylbenzoic acid).
   Vehicle used: Dimethyl sulfoxide (DMSO)
   Other provided information: According to the certificate of analysis (p. 53) the test substance was stored at room temperature. It is stated that a stock concentration of 271 mg/mL in DMSO was prepared for the initial assay. While achieved concentrations were not analytically verified, the report states (p. 18) that "at a treated concentration of 2680 μg/mL, a burst of oily droplets which went into solution was observed..."

2. Control Materials:

   Negative: (20-hour harvest): Untreated cells in McCoy's 5a culture medium supplemented with 10% fetal calf serum (FCS), L-glutamine and antibiotics.

   Solvent/final concentration: DMSO at 1%

   Positive:

   Nonactivation (concentrations, solvent): Mitomycin C (MMC) was dissolved in distilled water; final concentrations of 0.75 and 1.5 μg/mL were used in the initial assay, and 0.08 and 0.10 μg/mL in the confirmatory assay.

   Activation: (Concentration, solvent): Cyclophosphamide (CP) was dissolved in distilled water to yield final concentrations of 5 and 10 μg/mL.

3. Activation: S9 derived from male Sprague-Dawley

_x_ Aroclor 1254	_x_ induced	_x_ rat	_x_ liver
___ phenobarbital	___ noninduced	___ mouse	___ lung
___ none		___ hamster	___ other
___ other		___ other	

The S9 fraction (lots 0742 and 810) was obtained from Molecular Toxicology Inc., Boone, NC. The final concentration of the S9 mix components in cultures is reported as the following:

NADP	1.5 mg/mL
Isocitric acid	2.7 mg/mL
S9 homogenate	15.0 µL/mL

Final concentration of S9 in the reaction mixture was 1.5%.

4. Test Compound Concentrations Used:

(a) Preliminary cytotoxicity assay: There is no indication that any preliminary cytotoxicity assay was conducted.

(b) Cytogenetic assays:

Nonactivated conditions: Doses and harvest times of cultures that were subsequently evaluated for chromosomal aberrations were as follows:

- 931, 1330, 1900, 2710 µg/mL (initial assay: 3-hr cell exposure; harvest at 20 hours).
- 924, 1320, 1890, 2700 µg/mL (confirmatory assay: 17.8-hr cell exposure; harvest at 20 hours).

S9-activated conditions: Doses and harvest times for the S9-activated phase of testing were the same in the initial assay as those in the nonactivated phase. Doses in the confirmatory assay were the same as those in the nonactivated phase, but exposure to RPA 203328 was only for 3 hours, followed by wash, and harvest at 17 hours after this wash.

5. Test Cells: CHO cells were originally obtained from Dr. Sheldon Wolff, Univ. of CA and were grown for ≈24 hours prior to use in McCoy's 5a culture medium supplemented with 10% FCS, L-glutamine and antibiotics.

Properly maintained? Yes.
Cell line or strain periodically checked for mycoplasma contamination? Yes.
Cell line or strain periodically checked for karyotype stability? YES.

B. TEST PERFORMANCE:

1. Cell Treatments:

(a) Cells were exposed to test compound, solvent or positive controls for:
3 hours (nonactivated; initial trial) 3 hours (activated)
17.8 hours (nonactivated; confirmatory trial)

3 hours (activated; confirmatory trial)

(b) Cells were washed and reincubated in complete medium until 20 hours after initiation of exposure to RPA RPA 203328 (nonactivated and S9-activated conditions).

(c) Colcemid 0.1 µg/mL was added and cells were harvested 2 hours after mitotic arrest (nonactivated and S9-activated conditions).

Note: Cultures treated with the positive controls were also harvested at 20 hours.

2. Preliminary Cytotoxicity Assay: Not done.

3. Cytogenetic Assay:

(a) Exposure: Duplicate cultures/concentration seeded at a cell density of $1.2x10^6$ cells per flask were exposed to the selected test material doses, the solvent (DMSO) or positive controls (MitC -S9; CP +S9) with and without S9 activation for 3 hrs (or 17.8 hrs confirmatory assay -S9 only). Cells were washed, refed fresh complete medium and incubation was continued until harvest at 20 hours after initiation of exposure to RPA 203328. Colcemid was added to all cultures for the final 2 hours of incubation.

(b) Preparation of chromosomes: Cells were trypsinized, collected, centrifuged, treated with 0.56% (0.075M) KCl, fixed with methanol:glacial acetic acid (3:1), dropped onto slides and stained with 5% Giemsa solution. Slides were coded prior to scoring.

(c) Slide analysis: Two hundred well-defined metaphases (100/culture) were scored for structural chromosome aberrations; gaps were counted (and the numbers and types are presented in the report) but the data were only analyzed without gaps. Mitotic indices (MIs) were determined from the number of mitotic cells in 1000 cells per culture.

4. Statistical Analysis: The data (excluding gaps) were evaluated for statistical significance using a Cochran-Armitage test for linear trend and Fisher's Exact Test at $p \leq 0.01$.

5. Evaluation Criteria:

(a) Assay validity: The assay was considered acceptable if: (1) the negative (untreated) and the vehicle control cultures contained less than approximately 5% cells with aberrations; (2) the positive control result is significantly higher (≤ 0.01) than the vehicle controls ["If the positive control result in the test with S9 is adequate in an assay where activation and nonactivation assays are run concurrently, but the positive

control in the nonactivation assay fails, the test need not be repeated because the S9 activation positive control demonstrates the sensitivity of the cells."]

(b) Positive response: The test material was considered positive if it induced a statistically significant increase in the number of cells with chromosomal aberrations at one or more dose levels. "The linear trend test evaluates the dose-responsiveness. If a significant increase is seen at one or more dose levels, a dose-response should be observed."

C. REPORTED RESULTS:

1 Solubility Determinations: RPA 203328 was soluble in DMSO at about 270 mg/mL. This means that the final top concentration of RPA 203328 in this assay was about 2700 μg/mL, or >10 mM [from p. 43: "If the aberration results are negative and there is no significant reduction (approximately ≥50%) in mitotic index, the assay must include the highest applicable dose (a target dose of 10 mM or 5 mg/mL, whichever is lower) or a dose exceeding the solubility limit in culture medium."].

2 Preliminary Cytotoxicity Assay: Not done.

3. Cytogenetic Assay: Doses selected were:

- Initial assay: 18.3, 26.2, 37.4, 53.4, 76.3, 109, 156, 223, 319, 456, 652, 931, 1330, 1900, and 2710 μg/mL (3-hr exposure; then 17-hr incubation before harvest at 20 hours) ±S9. Cultures from 931, 1330, 1900 and 2710 μg/mL ±S9 were analyzed for chromosomal aberrations.

- Confirmatory assay: 317, 453, 647, 924, 1320, 1890 and 2700 μg/mL -S9: 17.8-hr exposure, with harvest 2.2 hrs later; or 647-2700 μg/mL +S9: 3-hr exposure, with harvest 17 hours later. Cultures from 924, 1320, 1890 and 2700 μg/mL were analyzed for chromosomal aberrations.

Summarized data from the nonactivated phase of testing are presented in Tables 1 and 2. As shown, there was no indication of a reduction in the mitotic index (MI) for the high-dose cultures in the initial assay (3-hr exposure). However, there was a 21.3% reduction in MI at the highest concentration in the confirmatory assay (with 17.8-hr exposure to RPA 203328). No significant (or biologically relevant) increases in the frequency of cells with structural chromosome aberrations were observed at any nonactivated dose.

Cytotoxicity was not observed under S9-activated conditions. As with the results under nonactivated conditions, there was no indication of a clastogenic response in CHO cells exposed to RPA 203328. Representative results from both trials are presented in Tables 3 and 4.

In contrast to the negative results with the test material, the positive controls (1.5 µg/mL MMC -S9 in the initial assay, 0.1 µg/mL MMC in the confirmatory assay, with the difference in concentrations reflecting the longer exposure time in the confirmatory assay; 10 µg/mL CP +S9) caused significant ($p \leq 0.01$) increases in the percentages of cells with abnormal chromosome morphology and the average number of aberrations per cell. From the overall results, the study author concluded that RPA 203328 was negative in this *in vitro* test system.

D. REVIEWERS' DISCUSSION/CONCLUSIONS: We agree with the study author's assessment that RPA 203328 did not induce a clastogenic response in cultured CHO cells when tested to an adequate limit dose (>10 mM). Additionally, the results obtained with the positive controls demonstrated that the assay was adequately sensitive to detect a genotoxic effect. We conclude, therefore, that the study provided acceptable evidence that RPA 203328 was not genotoxic in this test system.

E. STUDY DEFICIENCIES: NONE

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IN VITRO CYTOGENETICS (84-2)

TABLE 1. Representative Results of the Nonactivated Chinese Hamster Ovary Cell *In Vitro* Cytogenetic Assay with RPA 203328
Initial Assay with 3-Hour exposure

Substance		Dose per mL	Harvest Time (Hrs.)	No. of Cells Scored	% Mitotic Index	Total No. of Structural Aberrations[1]	No. of Cells with Structural Aberrations[1]	Percent Cells with Structural Aberrations[1]	Biologically Significant Aberrations (No./Type)
Negative Controls									
McCoy's 5a	A	-	20	100	8.8	4	4	4.0	1SB;1QR;2D
	B	-	20	100	9.2	3	3	3.0	1TB;1SB;1R
	A+B	-	20	200	9.0	7	7	3.5	1TB;2SB;1QR;2D;1R
Solvent Control									
DMSO	A	1%	20	100	12.7	2	2	2.0	1D;1DF
	B	1%	20	100	11.4	2	2	2.0	1TB;1ID
	A+B	1%	20	200	12.1	4	4	2.0	1TB;1ID;1D;1DF
Positive Control									
Mitomycin C	A	1.5 µg	20	25	9.2	35	18	72.0	8TB;1SB;6ID;3TR;9QR;5CR;3CI
	B	1.5 µg	20	25	6.2	14	9	36.0	5ID;7QR;2CI
	A+B	1.5 µg	20	50	7.7	49	27	54.0*	8TB;1SB;11ID;3TR;16QR;5CR;5CI
Test Material									
RPA 203328	A	1900 µg	20	100	8.9	1	1	1.0	1D
	B	1900 µg	20	100	9.8	4	4	4.0	1SB;1D;2R
	A+B	1900 µg	20	200	9.4	5	5	2.5	1SB;2D;2R
RPA 203328	A	2710 µg	20	100	10.7	2	1	1.0	2TB
	B	2710 µg	20	100	13.7	4	3	3.0	2SB;2D
	A+B	2710 µg	20	200	12.2	5	5	2.5	2TB;2SB;2D

[1] Gaps excluded.
Results for lower doses (931 and 1330 µg/mL -- 20-hour harvest) did not suggest a clastogenic response.
*Significantly ($p \leq 0.01$) higher than the solvent control.

Abbreviations used:

- TB = Chromatid break
- TF = Chromatid fragment
- TD = Chromatid deletion
- SB = Chromosome break
- SF = Chromosome fragment
- ID = Interstitial deletion
- TR = Triradial
- QR = Quadriradial
- CR = Complex Rearrangement
- D = Dicentric
- DF = Dicentric with fragment
- TC = Tricentric
- R = Ring
- CI = Chromosome Intrachange
- GT = Greater than 10 aberrations in a single cell

Note: Data were extracted from the Study Report Table 1; p. 23.

IN VITRO CYTOGENETICS (84-2)

TABLE 2. Representative Results of the Nonactivated Chinese Hamster Ovary Cell In Vitro Cytogenetic Assay with RPA 203328 Confirmatory Assay with 17.8-Hour exposure

Substance		Dose per mL	Harvest Time (Hrs.)	No. of Cells Scored	% Mitotic Index	Total No. of Structural Aberrations'	No. of Cells with Structural Aberrations'	Percent Cells with Structural Aberrations'	Biologically Significant Aberrations (No./Type)
Negative Controls									
McCoy's 5a	A	-	20	100	7.1	1	1	1.0	1QR
	B	-	20	100	6.9	1	1	1.0	1SB
	A+B	-	20	200	7.0	2	2	1.0	1SB;1QR
Solvent Control									
DMSO	A	1%	20	100	6.3	1	1	1.0	1TB
	B	1%	20	100	5.9	3	3	3.0	1TB;2SB
	A+B	1%	20	200	6.1	4	4	2.0	2TB;2SB
Positive Control									
Mitomycin C	A	0.1 µg	20	25	4.1	9	8	32.0	3TB;2SB;2TR;1QR;1D
	B	0.1 µg	20	25	3.9	13	9	36.0	4TB;5SB;1TR;2QR;1CR
	A+B	0.1 µg	20	50	4.0	22	17	34.0*	7TB;7SB;3TR;3QR;1CR; 1D
Test Material									
RPA 203328	A	1890 µg	20	100	6.6	2	2	2.0	1TB;1D
	B	1890 µg	20	100	6.2	1	1	1.0	1TB
	A+B	1890 µg	20	200	6.4	3	3	1.5	2TB;1D
RPA 203328	A	2700 µg	20	100	5.4	1	1	1.0	1SB
	B	2700 µg	20	100	4.1	1	1	1.0	1SB
	A+B	2700 µg	20	200	4.8	2	2	1.0	2SB

'Gaps excluded.
Results for lower doses (924 and 1320 µg/mL -- 20-hour harvest) did not suggest a clastogenic response.
*Significantly (p≤0.01) higher than the solvent control.

Abbreviations used:

TB = Chromatid break
TF = Chromatid fragment
TD = Chromatid deletion
SB = Chromosome break
SF = Chromosome fragment
ID = Interstitial deletion
TR = Triradial
QR = Quadriradial
CR = Complex Rearrangement
D = Dicentric
DF = Dicentric with fragment
TC = Tricentric
R = Ring
CI = Chromosome Intrachange
GT = Greater than 10 aberrations in a single cell

Note: Data were extracted from the Study Report Table 2; p. 24.

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TABLE 3. Representative Results of the Activated Chinese Hamster Ovary Cell *In Vitro* Cytogenetic Assay with RPA 203328
Initial Assay with 3-Hour exposure

Substance		Dose per mL	Harvest Time (Hrs.)	No. of Cells Scored	% Mitoric Index	Total No. of Structural Aberrations[a]	No. of Cells with Structural Aberrations[a]	Percent Cells with Structural Aberrations[a]	Biologically Significant Aberrations (No./Type)
Negative Controls									
McCoy's 5a	A	-	20	100	9.8	1	1	1.0	1TB
	B	-	20	100	14.8	1	1	1.0	1R
	A+B	-	20	200	12.3	2	2	1.0	1TB; 1R
Solvent Control									
DMSO	A	1%	20	100	10.8	1	1	1.0	1TB
	B	1%	20	100	11.7	1	1	1.0	1SB
	A+B	1%	20	200	11.3	2	2	1.0	1TB; 1SB
Positive Control									
Cyclophosphamide	A	10 μg	20	25	2.0	43	21	84.0	8TB; 2SB; 3ID; 13TR; 4QR; 4CR; 7CI; 2GT
	B	10 μg	20	25	4.0	38	19	76.0	7TB; 5SB; 2ID; 10TR; 6QR; 1CR; 5CI; 2GT
	A+B	10 μg	20	50	3.0	81	40	80.0*	15TB; 7SB; 5ID; 23TR; 10QR; 5CR; 12CI; 4GT
Test Material									
RPA 203328	A	1900 μg	20	100	13.7	4	4	4.0	2SB; 1D; 1R
	B	1900 μg	20	100	12.4	3	3	3.0	1SB; 2D
	A+B	1900 μg	20	200	13.1	7	7	3.5	3SB; 3D; 1R
RPA 203328	A	2710 μg	20	100	13.6	1	1	1.0	1D
	B	2710 μg	20	100	10.4	3	3	3.0	2TB; 1QR
	A+B	2710 μg	20	200	12.0	4	4	2.0	2TB; 1QR; 1D

[a] Gaps excluded.
Results for lower doses (931 and 1330 μg/mL -- 20-hour harvest) did not suggest a clastogenic response.
*Significantly ($p \leq 0.01$) higher than the solvent control.

Abbreviations used:
- TB = Chromatid break
- TF = Chromatid fragment
- TD = Chromatid deletion
- SB = Chromosome break
- SF = Chromosome fragment
- ID = Interstitial deletion
- TR = Triradial
- QR = Quadriradial
- CR = Complex Rearrangement
- D = Dicentric
- DF = Dicentric with fragment
- TC = Tricentric
- R = Ring
- CI = Chromosome Intrachange
- GT = Greater than 10 aberrations in a single cell

Note: Data were extracted from Study Report Table 3, p. 25.

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TABLE 4. Representative Results of the Activated Chinese Hamster Ovary Cell *In Vitro* Cytogenetic Assay with RPA 203328 Confirmatory Assay with 3-Hour exposure

Substance		Dose per mL	Harvest Time (Hrs.)	No. of Cells Scored	% Mitoric Index	Total No. of Structural Aberrations[a]	No. of Cells with Structural Aberrations[a]	Percent Cells with Structural Aberrations[a]	Biologically Significant Aberrations (No./Type)
Negative Controls									
McCoy's 5a	A	-	20	100	12.8	1	1	1.0	1D
	B	-	20	100	11.6	4	2	2.0	1TR;1R
	A+B	-	20	200	12.2	5	3	1.5	1TR;1D;1R
Solvent Control									
DMSO	A	1%	20	100	10.4	2	2	2.0	1TB;1SB
	B	1%	20	100	10.2	0	0	0.0	-
	A+B	1%	20	200	10.3	2	2	1.0	1TB;1SB
Positive Control									
Mitomycin C	A	0.1 µg	20	25	5.6	19	11	44.0	8TB;2ID;5TR;3QR;1CI
	B	0.1 µg	20	25	4.9	29	13	52.0	10TB;10SB;2ID;2TR;3QR;1D;1CI
	A+B	0.1 µg	20	50	5.3	48	24	48.0*	18TB;10SB;4ID;7TR;6QR;1D;2CI
Test Material									
RPA 203328	A	1890 µg	20	100	10.1	0	0	0.0	-
	B	1890 µg	20	100	11.1	1	1	1.0	1R
	A+B	1890 µg	20	200	10.6	1	1	0.5	1R
RPA 203328	A	2700 µg	20	100	12.2	0	0	0.0	-
	B	2700 µg	20	100	11.7	0	0	0.0	-
	A+B	2700 µg	20	200	12.0	0	0	0.0	-

[a]Gaps excluded.
Results for lower doses (924 and 1320 µg/mL -- 20-hour harvest) did not suggest a clastogenic response.
*Significantly ($p \leq 0.01$) higher than the solvent control.

Abbreviations used:

TB = Chromatid break
TF = Chromatid fragment
TD = Chromatid deletion
SB = Chromosome break
SF = Chromosome fragment
ID = Interstitial deletion
TR = Triradial
QR = Quadriradial
D = Dicentric
DF = Dicentric with fragment
TC = Tricentric
R = Ring
CI = Chromosome Intrachange
GT = Greater than 10 aberrations in a single cell

Note: Data were extracted from Study Report Table 4; p. 26.

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RPA 203328

MICRONUCLEUS (84-2)

EPA Reviewer: Byron T. Backus, Ph.D.
Technical Review Branch
Registration Division (7505C)

Signature: Byron T. Backus

Date: 5/28/98

EPA Secondary Reviewer: Nancy McCarroll
Toxicology Branch I
Health Effects Division (7509C)

Signature: Nancy E. McCarroll

Date: 5/28/98

012683

DATA EVALUATION REPORT

STUDY TYPE: Mutagenicity: Mouse micronucleus assay; OPPTS 870.5395 [§84-2]

DP BARCODE: D245866 SUBMISSION NO.: S541869

PC CODE: ------ TOX. CHEM. NO.: MRID NO: 44545302

TEST MATERIAL (PURITY): RPA 203328 (99.0%)

SYNONYM(S): CAS 142994-06-7; 2-Methanesulphonyl-4-trifluoromethylbenzoic acid; a metabolite of Isoxaflutole (PC Code 123000)

CITATION: Curry, P.T. (1998) Mutagenicity Test on RPA 203328 in the *In Vivo* Mouse Micronucleus Assay; Covance Laboratories, Inc., Leesburg Pike, Vienna, VA; Laboratory Project Identification: Covance 19201-0-455OECD; Study Completion Date: April 23, 1998. (Unpublished) MRID NUMBER: 44545302.

SPONSOR: Rhone-Poulenc, Research Triangle Park, NC 27709

EXECUTIVE SUMMARY: In a mouse micronucleus assay (MRID No. 44545302), groups of six male Crl:CD-1®(ICR)BR mice/dose/sacrifice time were orally dosed with 500, 1000, or 2000 mg/kg RPA 203328 (99%) [RPA 203328 = a metabolite of isoxaflutole] administered in 0.5% methylcellulose at a constant volume of 10 mL/kg. These doses were based on a preliminary range-finding study in which groups of 3 males and 3 females received single oral doses of 200, 500. 800, 1500 or 2000 mg/kg RPA 203328, and no mortality or symptoms occurred. Mice were sacrificed at 24 hours (all dose levels, as well as positive controls) and at 48 hours (vehicle controls and 2000 mg/kg RPA 203328 only) postadministration and harvested bone marrow cells were examined for the incidence of micronucleated polychromatic erythrocytes (MPEs).
No deaths or other signs of toxicity were reported. There was also no evidence of target cell cytotoxicity. The positive control (80 mg cyclophosphamide/kg, administered orally, with a 24-hr sacrifice time) induced the expected high yield of MPEs (only males tested). **However, there was no indication of a clastogenic and/or aneugenic effect associated with administration of RPA 203328 under the conditions of this assay, which included administration of a limit dose (2000 mg/kg) with sacrifice times of 24 and 48 hours.**

The study is classified as Acceptable and satisfies the requirements for FIFRA Test Guideline 84-2 for a micronucleus assay.

COMPLIANCE: Signed and dated GLP (p. 3), Quality Assurance (p. 6) and Statement of no Data Confidentiality Claims (p. 2) were provided.

I. MATERIALS AND METHODS

A. MATERIALS:

1. Test Material: RPA 203328; CAS 142994-06-7
   Description: white powder with small aggregates
   Lot/batch number: NMI874
   Purity: 99%
   Receipt date: Feb. 6, 1998
   Stability: Not reported
   CAS number: 142994-06-7
   Structure: not given (chemical name: 2-Methanesulphonyl-4-trifluoromethylbenzoic acid).
   Vehicle used: 0.5% methylcellulose
   Other provided information: According to the certificate of analysis (p. 28) the test substance was stored at room temperature. The top stock and subsequent dilutions were apparently prepared shortly before dosing. While achieved concentrations were not analytically verified, the report includes (p. 17) a description of the dosing solutions (200 mg/mL: "Opaque white, slightly viscous suspension with small evenly distributed particles.").

2. Control Materials:

   Negative/Route of administration: None

   Vehicle/Final concentration/Route of administration: 10 mL/kg 0.5% methylcellulose was orally administered.

   Positive/Final concentration/Route of administration: Cyclophosphamide (CP) was dissolved in deionized water and orally administered at a dose of 80 mg/kg.

3. Test Compound:

   Route of administration: Oral

   Dose levels used:

   (a) Range-finding Test: 200, 500, 800, 1500 or 2000 mg/kg (3♂ and 3♀/group)

   (b) Micronucleus assay: 500, 1000 or 2000 mg/kg (5♂, with 24 hr sacrifice only at 500 and 1000 mg/kg; 24 and 48 hr sacrifice at 2000 mg/kg).

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4. Test Animals:

(a) Species Mouse Strain Crl:CD-1^{s}(ICR)BR Age 8 weeks
Weight range: M: 31.1-34.4 g; F: 21.8-26.0 g (dose selection study)
M: 30.6-37.1 g (micronucleus assay)
Source: Charles River Laboratories, Raleigh, NC

(b) No. animals used per dose:

(1) Range-finding test: 3 males; 3 females

(2) Micronucleus assay: 6 males; 0 females (per sacrifice time) (only 5 animals/dose level/sacrifice time were evaluated for miconuclei)

(c) Properly maintained? YES

B.. TEST PERFORMANCE:

1. Treatment and Sampling Times:

(a) Test compound, vehicle and positive control:
Dosing: x once ____ twice (24 hr apart)
N/A other (describe): ________

(b) Sampling (after last dose): ________ 6 hr ________ 12 hr
x 24 hr x 48 hr (high dose and controls) ______ 72 hr

2. Tissues and Cells Examined:

x bone marrow N/A others (list):
Number of polychromatic erythrocytes (PCEs) examined per animal: 2000
Number of normochromatic erythrocytes (NCEs, more mature RBCs) examined per animal: "The frequency of PCE:NCE ratio was determined by scoring the number of PCEs and NCEs observed in the optic fields while scoring at least the first 200 erythrocytes on the slide."

3. Details of Slide Preparation: At 24 (and, for high-dose and vehicle control groups: 48) hours after administration of the test material, vehicle or positive control, the appropriate groups of animals were sacrificed by CO_2 inhalation. "The hind limb bones (tibias or femurs) were removed from the first 5 surviving animals for marrow extraction. The marrow was flushed from the bone and transferred to centrifuge tubes containing 3-5 mL bovine serum (one tube per animal). Animals in excess of the first 5 survivors were euthanized but no marrow was extracted. Following centrifugation to pellet the tissue, the supernatant was removed by aspiration and portions of the pellet were spread on slides and air-dried. The slides were fixed in methanol, stained in May-Grünwald Solution followed by Giemsa, and protected by mounting with coverslips. For control of bias, all slides were coded prior to analysis."

4. Statistical Methods: "Assay data analysis was performed using an analysis of variance...on untransformed proportions of cells with micronuclei per animal and on untransformed PCE:NCE ratios when the variances were homogeneous. Ranked proportions were used for heterogeneous variances." Statistical significance was established at $p \leq 0.05$.

5. Evaluation Criteria: "The criteria for a positive response was the detection of a statistically significant positive response for at least 1 dose level and a statistically significant dose-related response. A test article that did not induce both of these responses was considered negative. Statistical significance was not the only determinant of a positive response, the study director also considered the biological relevance of the results in the final evaluation."

C. REPORTED RESULTS:

1. Range-Finding Test: Groups of 3 male and 3 female mice received single oral dosages of 200, 500, 800, 1500 or 2000 mg/kg and were observed for 2 days after dosage. "All animals appeared normal immediately after dosing and remained healthy until the end of the observation period..." Accordingly, doses selected for the micronucleus assay were the limit dose (2000 mg/kg) and ½ and ¼ of this dose (1000 and 500 mg/kg, respectively). Only males were used in the micronucleus assay, presumably because there was no indication of any difference in susceptibility between sexes in the range-finding assay.

2. Micronucleus Assay: No deaths or other clinical signs of toxicity were reported. Representative results presented in Table 1 show that there was no significant effect on the PCE:NCE ratio in any of the groups. Similarly, no significant increase in the frequency of MPEs was observed under any of the experimental conditions in males treated with the test material. By contrast to the uniformly negative results with the test material, the positive control (80 mg/kg CP) induced a clear increase in the frequency of MPEs in males sacrificed at 24 hours.

Based on the findings, the study authors concluded that RPA 203328 was not genotoxic in this in vivo mouse micronucleus assay.

D. REVIEWERS' DISCUSSION/CONCLUSIONS: We assess that while only males were tested in the micronucleus assay, the lack of any mortalities and/or symptoms in both males and females in a preliminary dose-finding study at doses up to and including 2000 mg/kg RPA 203328 indicates that it is highly unlikely that significant sex-related differences exist in the metabolization of this compound. In the micronucleus assay, there was no indication that RPA 203328 induced a clastogenic or aneugenic effect in males. We conclude, therefore, that RPA 203328 was assayed to a limit dose level of 2000 mg/kg, and failed to elicit a genotoxic response in treated animals. The results obtained with the positive control (80 mg/kg CP) demonstrate that the sensitivity of test system to detect a positive effect

was adequate. Hence, the study provides acceptable evidence that RPA 203328 was negative in this in vivo assay.

E. STUDY DEFICIENCIES: Only males were tested, but see the discussion above.

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TABLE 1. Representative Results of the Micronucleus Assay in Mice Treated with RPA 203328

Substance	Dose per kg	Exposure Time[a] (hours)	Animals Analyzed per Group	MPEs/2000[b] PCEs ± S.E.	RATIO PCE:NCE MEAN ± S.E.
Vehicle Control 0.5% methylcellulose	10 mL	24 48	5 5	0.02 ± 0.01 0.04 ± 0.02	0.52 ± 0.05 0.42 ± 0.05
Positive Control Cyclophosphamide	80 mg	24	5	3.74 ± 0.26*	0.39 ± 0.05
Test Material RPA 203328	500 mg	24	5	0.03 ± 0.01	0.35 ± 0.04
	1000 mg	24	5	0.05 ± 0.02	0.47 ± 0.05
	2000 mg	24 48	5 5	0.02 ± 0.02 0.03 ± 0.01	0.47 ± 0.06 0.43 ± 0.06

[a]Time after administration of the test material, vehicle or positive control by gavage.
[b]A total of 10000 PCEs were examined per group (2000 PCEs/animal).

Abbreviations:
PCE = Polychromatic erythrocyte
MCE = Micronucleated polychromatic erythrocyte
NCE = Normochromatic erythrocyte

Note: Data were extracted from the Study Report, Table 1, p. 23.

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RPA 203328 CHO/HGPRT FORWARD MUTATION ASSAY (84-2)

EPA Reviewer: Byron T. Backus, Ph.D. Signature: [signature]
Technical Review Branch
Registration Division (7505C) Date: 6/11/98

EPA Secondary Reviewer: Nancy McCarroll Signature: [signature]
Toxicology Branch I
Human Effects Division (7509C) Date: 6/11/98

DATA EVALUATION REPORT

STUDY TYPE: Mammalian cells in culture gene mutation assay in Chinese hamster ovary cells (CHO/HGPRT)

DP BARCODE: D245866 SUBMISSION NO.: S541869

PC CODE: ------ TOX. CHEM. NO.: MRID NO: 44545303

TEST MATERIAL (PURITY): RPA 203328 (99.0%)

COMPOSITION/SYNONYM(S): CAS 142994-6-7; 2-Methanesulphonyl-4-trifluoromethylbenzoic acid; a metabolite of Isoxaflutole (PC Code 123000)

CITATION: Cifone, M.A. (1998). Mutagenicity Test on RPA 203328 in the CHO/HGPRT Forward Mutation Assay with Duplicate Cultures and a Confirmatory Assay; Covance Laboratories, Inc., Leesburg Pike, Vienna, VA; Laboratory Project Identification: Covance 19201-0-435 OECD; Study Completion Date: April 24, 1998. (Unpublished) MRID NUMBER: 44545303.

SPONSOR: Rhône-Poulenc Ag Company, Research Triangle Park, NC 27709

EXECUTIVE SUMMARY: In two independently performed Chinese hamster ovary (CHO) cell HGPRT forward gene mutation assays (MRID No. 44545303), duplicate cultures of RPA 203328 were assayed at concentrations of 84.5 - 2700 µg/mL -S9 (initial and confirmatory trials) and 338 - 2700 µg/mL +S9 (initial trial) and 675 - 2700 µg/mL (confirmatory trial). The S9 was derived from Aroclor 1254-induced rat (male Sprague-Dawley) livers, and the test material was delivered in dimethyl sulfoxide.

In the assays, there was no indication of cytotoxicity ±S9 at the highest dose level of 2700 µg/mL, which was 10 mM. **Although there were a few sporadic instances of statistically significant elevations in mutation frequency, these were not dose-related and were generally below the 15 x 10^{-6} required for a positive response except in one case (a value of 15.8 x 10^{-6}). Overall, there was no evidence of any increase in mutation frequency resulting from exposure to RPA 203328.**

The study is classified as **Acceptable** and satisfies the requirements for an in vitro mammalian cell forward gene mutation study (84-2).

I. MATERIALS AND METHODS

A. MATERIALS:

1. Test Material: RPA 203328; CAS 142994-06-7
Description: white powder
Lot/batch number: NBI874
Purity: 99%
Receipt date: Feb. 6, 1998
Stability: Not reported
CAS number: 142994-06-7
Structure: not given (chemical name: 2-Methanesulphonyl-4-trifluoromethylbenzoic acid).
Vehicle used: DMSO
Other provided information: According to the certificate of analysis (p. 34) the test substance was stored at room temperature. From p. 14: "The vehicle was dimethyl sulfoxide... The test article solution was prepared immediately before use." Doses used in the study were not verified analytically.

2. Control Materials:

Negative: None

Solvent/final concentration: DMSO/1%

Positive: Nonactivation (concentration, solvent): 5-Bromo-2'-deoxyuridine (BrdU) was prepared in an unspecified solvent to yield a final concentration of 50 μg/ml.

Activation (concentration, solvent): 20-Methylcholanthrene (20-MC) was prepared in an unspecified solvent to yield a final concentration of 5 μg/ml.

3. Activation: S9 derived from male Sprague-Dawley

x	Aroclor 1254	x	induced	x	rat	x	liver
	phenobarbital		noninduced		mouse		lung
	none				hamster		other
	other				other		

The S9 homogenate (lot number 0797) was purchased from Molecular Toxicology, Inc. "The S9 fraction was purchased commercially and each lot was tested for its activity prior to an assay."

S9 mix composition:

Component	Final Concentration in Cultures
NADP (sodium salt)	0.8 mM
Glucose-6-phosphate	1.0 mM
Calcium chloride	2.0 mM
Potassium chloride	6.0 mM
Magnesium chloride	2.0 mM
Phosphate	10.0 mM
S9 homogenate	10.0 µl/ml

4. Test Cells: Mammalian cells in culture

_____ mouse lymphoma L5178Y cells
__x__ Chinese hamster ovary (CHO) cells
_____ V79 cells (Chinese hamster lung fibroblasts)
_____ other (list):

Properly maintained? Yes.
Periodically checked for mycoplasma contamination? Yes.
Periodically checked for karyotype stability? Yes (with banding)
Periodically "cleansed" against high spontaneous background? Yes.

5. Locus Examined:

_____ thymidine kinase (TK)
Selection agent: (give concentration)
_____ bromodeoxyuridine (BrdU)
_____ fluorodeoxyuridine (FdU)

__x__ hypoxanthine-guanine-phosphoribosyl transferase (HGPRT)
Selection agent: (give concentration)
_____ 8-azaguanine (8-AG)
__4 µg/mL__ 6-thioguanine (6-TG)
_____ methotrexate

_____ Na^+/K^+ATPase
Selection agent: (give concentration)
_____ ouabain

6. Test Compound Concentrations Used:

(a) Preliminary cytotoxicity assay: Ten doses (5.3, 10.6, 21.2, 42.3, 84.5, 169, 338, 675, 1350 and 2700 µg/mL) were evaluated in triplicate with and without S9 activation.

(b) Mutation assays:

- Nonactivated conditions: There were two trials; doses used

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were as follows:

Trial 1 and 2: Six concentrations (84.5, 169, 338, 675, 1350 and 2700 μg/mL) were assayed using duplicate cultures. Cells exposed to all six concentrations were plated to determine mutation frequency (MF).

- S9-activated conditions: There were two trials; doses used were as follows:

Trial 1: Seven concentrations (338, 675, 1350, 1600, 1900, 2300 and 2700 μg/mL) were assayed with duplicate cultures. Cells exposed all concentrations were plated to determined mutation frequency (MF).

Trial 2: Ten concentrations (169, 338, 675, 1350, 1600, 1800, 2000, 2300, 2500 and 2700 μg/mL) were initiated; the eight highest concentrations were evaluated as described for Trial 1.

B. TEST PERFORMANCE:

1. Cell Treatments:

(a) Cells were exposed to the test compound, solvent, or positive controls for:
 4 hours (nonactivated) 4 hours (activated)

(b) After washing, cells were cultured for 7 days (phenotypic expression period) before cell selection.

(c) After expression, cells seeded at $2x10^5$ cells/dish (12 dishes/culture) were cultured for 7-10 days in mutant selection medium to determine numbers of mutants, and cells seeded at 200 cells/dish (3 dishes/culture) were cultured for 7-10 days in normal culture medium (non-selection medium) to determine cloning efficiency (CE).

2. Evaluation Criteria:

(a) Assay validity: The assay was considered valid if (1) the average absolute CEs of the solvent controls was between 50% and 115%; (2) the average mutation frequency (MF) of the pooled solvent control cultures was $\leq 15x10^{-6}$; and (3) the positive controls induced a significant ($p \leq 0.01$) increase in the MF over the concurrent solvent control value.

(b) Positive response: The test material was considered positive if

it induced a concentration- or cytotoxicity-related statistically significant ($p \leq 0.05$) increase in the MF. In addition, the increased MF must exceed 15 mutants/10^6 clonable cells.

3. Statistical Analysis: "Statistical significance was determined using the Fischer Exact Test to determine if the mutant frequencies in each treated culture were significantly elevated compared to the mutant frequencies of the concurrent negative controls at the 95% or 99% confidence levels..."

C. REPORTED RESULTS:

1. Preliminary Cytotoxicity Assay: RPA 203328 was prepared at a primary stock concentration of 270 mg/mL in DMSO. "The test article did not alter the pH of the treatment medium outside the range that was compatible with cell growth." Accordingly, ten doses of the test material (5.30-2700 µg/mL) were evaluated with and without S9 activation. Results indicated that without metabolic activation RPA 203328 was noncytotoxic at all concentrations tested. In the presence of S-activation, the test article was noncytotoxic from 5.30 to 1350 µg/mL, and was lethal at 2700 µg/mL. Based on these findings, a top concentration of 2700 µg/mL was chosen for both nonactivation and activation, as "2700 µg/mL is 10 mM, the testing limit for this assay."

3. Mutation Assays: Two trials of the nonactivated and S9-activated mutation assay were conducted. The concentration of RPA 203328 in the nonactivated assays ranged from 84.5 to 2700 µg/mL; in the activated assays it ranged from 338 µg/mL to 2700 µg/mL (first assay) and from 675 to 2700 µg/mL (second assay). The results were as follows:

(a) Initial trial: Representative data from the initial trial are presented in Table 1. As shown, there was no indication of cytotoxicity at the top concentration (2700 µg/mL), even with S9 activation (unlike the results in the preliminary cytotoxicity assay). There was no indication of any genotoxicity, as all MFs were below 15×10^{-6}.

(b) Repeat trial: As in the initial trial, there was no indication of cytotoxicity at even the top concentration. One of the duplicate cultures at 2300 µg/mL +S9 gave an MF of 15.8×10^{-6}, but this has to be considered a sporadic occurrence as the other duplicate culture at this concentration gave a value of 6.8×10^{-6} and all MF values at the two higher concentrations +S9 in this trial were below 15.0×10^{-6}.

D. REVIEWERS' DISCUSSION AND INTERPRETATION OF RESULTS: We agree with the study author that RPA 203328 was not genotoxic under the conditions of

this assay. We note that there was no analytical confirmation of the concentrations in the top dosing solution; however, it is stated in an in vitro chromosomal aberration assay conducted in this laboratory on RPA 203328 (MRID 44545301) which involved dilution of the test material in DMSO) that: "at a treated concentration of 2680 µg/mL, a burst of oily droplets which went into solution was observed..." Hence, we conclude that RPA 203328 was investigated up to the recommended high concentration (10 mM) for this test system and was found to be negative in an acceptable assay.

E. STUDY DEFICIENCIES: NONE

CHO/HGPRT FORWARD MUTATION ASSAY (84-2)

TABLE 1. Representative Results of the Initial Chinese Hamster Ovary (CHO) Cell Forward Gene Mutation Assay with RPA 203328

Substance	Dose/mL	S9 Activation	Relative % Survival (after treatment)[a]	Total Mutant Colonies[b]	Cloning Efficiency (at selection)[a]	Mutation Frequency/ 10^6 cells[a,c]
<u>Solvent Control</u>						
Dimethyl sulfoxide	1%	-	100.0	39	1.21	6.7
	1%	+	100.0	12	0.74	3.4
<u>Positive Controls</u>						
5-Bromo-2'-deoxyuridine	50 µg	-	75.2	738	1.17	131.8*
20-Methylcholanthrene	5 µg	+	95.4	413	0.69	125.6*
<u>Test Material</u>						
RPA 203328	338.0 µg[d]	-	104.6	22	1.09	4.4
	675.0 µg	-	96.0	18	1.11	3.4
	1350.0 µg	-	95.2	36	1.13	6.6
	2700.0 µg	-	98.2	28	1.10	5.3
	1600.0 µg[d]	+	90.0	33	0.85	8.1
	1900.0 µg	+	103.4	6	0.76	1.6
	2300.0 µg	+	106.8	26	0.72	7.6
	2700.0 µg	+	105.6	10	0.72	2.9

[a] Average of two cultures for the solvent control samples, the test material and positive control samples.
[b] Total of 24 dishes/2 cultures

[c] Mutation Frequency (MF) = $\frac{\text{Total Mutant Colonies}}{\text{Number of Dishes (24)} \times \text{Cloning Efficiency} \times 2 \times 10^5 \text{ cells}} \times 10^6$; calculated by our reviewers.

[d] Findings for lower doses (84.5 or 169 µg/mL -S9; 338, 675 or 1350 µg/mL) did not indicate a mutagenic effect.

*Reported as a significant increase: $p \leq 0.01$ (Fischer Exact Test) with MF $\geq 15 \times 10^6$

Note: Data were extracted from the Study Report, Tables 3 and 5, pp. 28 and 30.

CHO/HGPRT FORWARD MUTATION ASSAY (84-2)

012683

TABLE 2. Representative Results of the Confirmatory Chinese Hamster Ovary (CHO) Cell Forward Gene Mutation Assay with RPA 203328

Substance	Dose/mL	S9 Activation	Relative % Survival (after treatment)[a]	Total Mutant Colonies[b]	Cloning Efficiency (at selection)[a]	Mutation Frequency/ 10^6 cells[a,c]
Solvent Control						
Dimethyl sulfoxide	1%	-	100.0	23	0.96	5.0
	1%	+	100.0	37	1.11	7.0
Positive Controls						
5-Bromo-2'-deoxyuridine	50 µg	-	97.8	439	0.81	113.5*
20-Methylcholanthrene	5 µg	+	84.5	441	1.10	83.5*
Test Material						
RPA 203328	338.0 µg[d]	-	122.4	13	0.90	3.0
	675.0 µg	-	107.4	8	0.70	2.4
	1350.0 µg	-	105.6	5	0.84	1.3
	2700.0 µg	-	103.0	20	0.86	4.9
	2000.0 µg[d]	+	117.8	50	1.21	8.6
	2300.0 µg	+	158.0	57	1.05	11.3**
	2500.0 µg	+	127.9	53	0.94	11.8
	2700.0 µg	+	143.7	48	1.07	9.3

[a]Average of two cultures for the solvent control, the test material and positive control samples.
[b]Total of 24 dishes/2 cultures.

[c]Mutation Frequency (MF) = $\frac{\text{Total Mutant Colonies}}{\text{Number of Dishes (24)} \times \text{Cloning Efficiency} \times 2 \times 10^5 \text{ cells}} \times 10^6$; calculated by our reviewers.

[d]Results for lower dose levels (84.5 and 169 µg/mL -S9; 675, 1350, 1600 and 1800 µg/mL +S9) did not indicate a mutagenic effect.

*Significant increase with $p \leq 0.01$ by the Fischer Exact Test and with a MF $\geq 15 \times 10^{-6}$.

** One of the two duplicate cultures gave a MF of 15.8×10^{-6}, suggestive of a positive response, but the other culture gave a MF of 6.8×10^{-6}.

Note: Data were extracted from the Study Report, Tables, 4 and 6, pp. 29 and 31.

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