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

JAN 9 1989

006987

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: Mancozeb - Mutagenicity Studies Submitted Under MRID Nos. 40810201 to 40810205 and 40778901
EPA ID No. 4581-358

TOX Chem No.: 913A
TB Project No.: 8-1161
RD Record No.: 231070

FROM: Irving Mauer, Ph.D., Geneticist
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THRU: Judith W. Hauswirth, Ph.D., Acting Branch Chief
Toxicology Branch I - Insecticide, Rodenticide Support
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Registrant: Pennwalt Corporation
Agchem Division
Philadelphia, PA

Request

Review and evaluate the following mutagenicity studies, all performed for the registrant by the Huntingdon Research Centre, Cambridgeshire (England):

Vol. 2 - Bacterial DNA Repair Test to Assess the Potential of Mancozeb Technical to Cause DNA Damage. Huntingdon Research Centre Ltd., Project No. CR6376, April 21, 1986. (MRID No. 40778901)

Vol. 3 - Analysis of Metaphase Chromosomes Obtained from CHO Cells Cultured In Vitro and Treated With Mancozeb Technical. Huntingdon Research Centre Ltd., Project ID PWT 38/86855, October 7, 1986. (MRID No. 40810202)

Vol. 4 - An Assessment of the Mutagenic Potential of Mancozeb Technical in a Mammalian Cell Mutation Assay Using the Chinese Hamster Ovary/HPRT Locus Assay. Huntingdon Research Centre Ltd., Project ID PWT 41/861125, February 11, 1987. (MRID No. 40810203)

Vol. 5 - Microbial Metabolic Activation Test to Assess the Potential Mutagenic Effect of Mancozeb Technical. Huntingdon Research Centre Ltd., Project ID PWT 36/86374, February 9, 1988. (MRID No. 40810204)

Vol. 6 - Micronucleus Test on Mancozeb Technical. Huntingdon Research Centre Ltd., Project ID PWT 39/86637, July 21, 1987. (MRID No. 407789-01)

Vol. 7 - Autoradiographic Assessment of Unscheduled DNA Repair Synthesis in Mammalian Cells After Exposure to Mancozeb Technical. Huntingdon Research Center Ltd., Project ID PWT 40/86899, October 22, 1986. (MRID No. 40810205)

[NB: In a cover letter and summary of studies accompanying this submission, the registrant considers all of the in vitro studies (i.e., volumes 2, 3, 4, 5, and 7) to be invalid because:

> . . . Mancozeb was dissolved in dimethyl sulfoxide (DMSO) in which Mancozeb, as well as other EBDCs have been reported to be unstable. (Hence) . . . the actual concentration of Mancozeb used in the studies is uncertain. In addition, several other critical parameters in the various studies are questionable.

However, the registrant provided no documentation for these assertions.]

TB Conclusions

Following are the reported results and our assessments of the submitted studies (detailed reviews are appended):

Volume	Study Type	Reported Results	TB Evaluation
2	Bacterial Repair	Positive for differential toxicity at all doses (50-5000 ug/mL), more severe without activation.	Inconclusive because of data inconsistencies.
3	Chromosome Damage in vitro	Positive for dose-related aberrations in a single assay conducted with/ without activation.	Inconclusive because some procedural details not reported.
4	Gene Mutation in vitro	Negative for induction of HPRT mutants in CHO cells.	Inconclusive because some procedural details not reported.
5	Ames Test	Negative in repeat tests at cytotoxic doses.	Inconclusive because of procedural deficiencies.
6	Mouse Micronucleus	Negative at doses of 10,000 mg/kg, which caused toxicity.	Acceptable
7	DNA Repair in Hepatocytes	Inconsistent and sporadic increased grain counts in replicate trials.	Unacceptable

Attachments

5

Reviewed By: Irving Mauer, Ph.D., Geneticist
Toxicology Branch I - IRS (TS-769C)
Secondary Reviewer: Judith W. Hauswirth, Ph.D., JWHauswirth 1/2/89
Acting Chief, Toxicology Branch I - IRS (TS-769C)

006987

DATA EVALUATION REPORT

I. SUMMARY

TB Project No.: 8-1161
Caswell No.: 913A
MRID No.: 40810201
Shaughnessy No.: 014504

Study Type: Mutagenicity - DNA repair in bacteria (E. coli/ Pol A differential toxicity)

Chemical: Mancozeb

Sponsor: Pennwalt Corporation, Philadelphia PA

Testing Facility: Huntingdon Research Centre (HRC), UK

Title of Report: Bacterial DNA Repair Test to Assess the Potential of Mancozeb Technical to Cause DNA Damage.

Authors: E. Jones, L.A. Farmer, and A.L. Thompson

Study No.: PWT 37/86376

Date of Issue: April 21, 1986

TB Conclusions:

Presumptively positive for the induction of differential toxicity at doses ranging from 50 to 5000 ug/mL, with greater toxicity shown in the absence of metabolic activation.

Classification (Core-Grade):

Inconclusive, because of:

1. Wide variability in response to the test article between repeat assays conducted at identical dosages; and

2. Inconsistency in responses of the controls.

II. DETAILED REVIEW

A. Test Material - Mancozeb technical

Description: Greenish-yellow powder
Batch (Lot): BLI.850930)
Purity (%): (Not stated)
Solvent/carrier/diluent: Dimethylsulfoxide (DMSO)

B. Test Organism - Bacteria

Species: Escherichia coli
Strain: K12, W3110 (Pol A+, wild-type repair proficient); K12, p3478 (Pol A$^-$, repair-deficient)

C. Study Design (Protocol) - A generic protocol was provided and authoritative references listed. Both a Quality Assurance Statement and an assertion of compliance with GLPs were included in the Final Report.

D. Procedures/Methods of Analysis - As described in the protocol (Appendix 1 of the Final Report), parallel suspension cultures of the two strains were exposed to five concentrations of the test article, incubated for 1 hour in the absence or presence of a mammalian metabolic activation system consisting of Aroclor 1254-stimulated rat liver homogenate (S9) plus cofactors (S9 mix), then mixed in soft agar, and mean numbers of survivors (colonies) per treatment group (4 individual plate counts each) assessed after a further 24-hour incubation. Each series of cultures included negative controls (the solvent, DMSO alone, or with S9 mix) and positive controls (methylmethanesulfonate, MMS, in the absence of S9, and 2-aminofluorene, 2-AF, under activated conditions); an additional control for nonactivated cultures was provided by the antibiotic, chloramphenicol (CAP).

The entire assay was repeated once.

The mean number of test survivors was compared to control values, and expressed as a survival index (SI), calculated by the ratio:

$$\frac{\% \text{ Survival of Pol A}^-}{\% \text{ Survival of Pol A}^+}$$

According to the authors, a positive response in this assay is indicated by an SI < 0.35, i.e., preferential killing ("differential toxicity") involving the repair-deficient strain, plus a dose-response relationship.

5

Results:

In both the initial and repeat assays, cell survival of the mancozeb-treated pol A^- (repair deficient) strain was less than that of pol A^+ in a dose-dependent fashion, as indicated by the decreasing SIs with increasing dosage, as shown on the page following.

Relative survival was more severely affected at identical dose levels in the absence of activation than with the S9-mix addition; even the LDT (50 ug/mL) elicited a positive response without S9 (SIs of 0.53 and 0.07 in the two assays), compared to activation (SIs near "normal," 0.85 and 0.97).

The authors concluded that the test article demonstrates evidence of DNA-damaging potential in this bacterial system.

TB Evaluation:

Inconclusive (presumptively positive).

Studies of this type in bacterial test systems are difficult to interpret, mainly because the relationship between differential toxicity and DNA-damage and its repair is not well understood. Additionally, the test system employed here is inherently unstable, as shown clearly in this study by: 1) the wide variability in response between assays conducted at identical dosages of the test article; and 2) the inconsistency in response of the controls.

In a company review of this study submitted at the same time, the conclusions of the testing lab were further disputed because mancozeb was dissolved in DMSO, in which all EBDCs are said to be inherently "unstable. [and] Thus, the results of this study are highly uncertain." This reported "instability," however, was not documented by either physicochemical data or [illegible].

Whatever the role of the [illegible] differential survival of cells possessing or lacking the polymerase A_1-based repair system, and despite [illegible] than satisfactory reproducibility in results, dose-related decreases in cell survival SIs were found in repeat assays by these investigators. Thus, we judge that [illegible] performed adequately, and the results are valid. However, the interpretation of what these data mean in terms of toxicity (DNA damage) or mutagenicity (DNA repair) [illegible] results are considered only presumptively positive, and [illegible] Inconclusive.

The Effect of Mancozeb on Cell Survival in *E. coli* Strains Pol A^+ and Pol^-*

Test Substance	Dose (uq/mL)	Test 1 Pol A^+ Mean Colony Count	Test 1 Pol A^+ % Survival	Test 1 Pol A^- Mean Colony Count	Test 1 Pol A^- % Survival	Test 1 SI	Test 2 Pol A^+ Mean Colony Count	Test 2 Pol A^+ % Survival	Test 2 Pol A^- Mean Colony Count	Test 2 Pol A^- % Survival	Test 2 SI
NONACTIVATION (-S9)											
DMSO	100 uL	292	(100)	42	(100)	(1.00)	518	(100)	520	(100)	(1.00)
Mancozeb	50	286	98	22	52	0.53	469	91	29	6	0.07
	150	271	93	13	31	0.33	400	77	2	0.4	0.01
	500	269	92	6	14	0.15	383	74	0	0	0
	1500	154	53	0	0	0	78	15	0	0	0
	5000	0	0	0	0	0	0	0	0	0	0
MMS	0.5	303	104	26	62	0.60	529	102	513	99	0.97
	1.0	270	92	27	64	0.70	487	94	300	58	0.62
	1.5	295	101	25	60	0.59	454	88	291	56	0.64
CAP	0.05	297	102	31	74	0.73	312	60	415	80	1.33
	0.10	281	96	36	86	0.90	363	70	422	81	1.16
	0.20	289	99	33	79	0.80	463	89	415	70	0.79
ACTIVATION (+S9)											
DMSO	100 uL	339	(100)	57	(100)	(1.00)	545	(100)	736	(100)	(1.00)
Mancozeb	50	288	85	41	72	0.85	499	92	655	89	0.97
	150	287	85	33	58	0.68	452	83	485	66	0.87
	500	285	84	13	23	0.27	314	58	183	25	0.43
	1500	186	55	0	0	0	271	50	0	0	0
	5000	0	0	0	0	0	0	0	0	0	0
2-AF	5	329	97	42	74	0.76	659	121	653	89	0.74
	10	325	96	25	44	0.46	531	97	604	82	0.85
	20	327	96	34	60	0.63	444	81	640	87	1.07

*Extracted from Report Table 1 and 2.

% Survival relative to the solvent (-S9) controls.

SI - Survival Index = $\frac{\%\ \text{Survival Pol } A^-}{\%\ \text{Survival Pol } A^+}$

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Secondary Reviewer: Judith W. Hauswirth, Ph.D.,
Acting Chief, Toxicology Branch I - IRS (TS-769C)

006987

DATA EVALUATION REPORT

I. SUMMARY

TB Project No.: 8-1161
Caswell No.: 913A
MRID No.: 40810202
Shaughnessy No.: 014504

Study Type: Mutagenicity - Cytogenetics in vitro (CHO/CA)

Chemical: Mancozeb

Sponsor: Pennwalt Corporation, Philadelphia, PA

Testing Facility: Huntingdon Research Centre, UK

Title of Report: Analysis of Metaphase Chromosomes Obtained from CHO Cells Cultured In Vitro and Treated with Mancozeb Technical.

Authors: J. Allen, P.C. Brooker, A.M. Birt, and A. Howell

Study No.: PWT 38/86855

Date of Issue: October 7, 1986

TB Conclusions:

Presumptively positive for dose-related chromosome aberrations in a single assay conducted both in the presence and absence of metabolic activation at doses up to the toxic range.

Classification (Core-Grade): Inconclusive

Certain modifying factors not accounted for in the conduct of this study, as suggested by the sponsor, might have led to false positive results. The sponsor is requested to supply documentation to support his contentions.

A

II. DETAILED REVIEW

A. Test Material - Mancozeb technical

Description: Greenish-yellow powder
Batch (Lot): BL.1.850930
Purity (%): 88.2
Solvent/carrier/diluent: Dimethylsulfoxide (DMSO)

B. Test Organism - Mammalian cell cultures

Species: Chinese hamster ovary (CHO)
Strain: CHO-K_1 (BH_4 subclone)
Source: BIBRA (British Industrial Biological Research Association)

C. Study Design (Protocol) - A formal protocol was not included, but the study was stated to have been conducted according to OECD Guideline No. 473 (1983), as well as published procedures.

GLP and QA statements were both included.

D. Procedures/Methods of Analysis - Following preliminary range-finding toxicity testing, cell cultures were exposed in duplicate for 20 hours to test compound at final concentrations of 0.15, 0.75, 1.25, and 1.50 uL/mL, or to the solvent DMSO, or to mitomycin C (MMC, 0.4 ug/mL, as positive control). To other sets of cultures were added a metabolic activation system consisting of an Aroclor 1254-stimulated rat hepatic enzyme homogenate (S9) plus appropriate generating cofactors (S9 mix), together with test compound at final concentrations of 2.5, 12.0, 20, or 25 ug/mL, or solvent, or cyclophosphamide (CP, 20 ug/mL, positive control). After 2 hours incubation, the media of such activated cultures were replaced, then incubation continued a further 18 hours without test substance.

At termination, all cultures were treated with colchicine (0.25 ug/mL), harvested 3 hours later, and cells prepared on microscope slides by conventional fixation-staining techniques. One hundred metaphases per culture (25 from each slide) were examined for the presence of chromatid or isochromatid gaps and breaks, exchanges, dicentrics, acentrics ("fragments"), rings, and other more complex rearrangements. Mean percent of cells with chromosomal aberrations in each dose group was compared to solvent control by Fisher's Exact Test, with levels of probability set at $p < 0.05$, $p < 0.01$, and $p < 0.001$.

Results:

In the preliminary range-finding test without S9, mitotic index (MI, a measure of cell cycle delay, and thus indirectly of cytotoxicity) in test cultures fell from control levels (> 7.78%) to zero at concentrations between 1.56 ug/mL (MI = 10.05%) and 3.13 ug/mL, and above (Report Table 1). The initial selection of 2.5 ug/mL as the top dose for analysis in the main nonactivated assay was abandoned, however, since the investigators reported "extreme toxic effects" at this as well as the next lower concentration, 2.0 ug/mL (but no data were presented). Thus, the nonactivated portion of the main study was repeated at the lower dose schedules of 0.15, 0.75, 1.25, and 1.50 ug/mL.

Activated mancozeb-treated cultures apparently fared better, since the highest dose tested, 25 ug/mL decreased MI to 40 percent of control levels (from 14.6% to 5.7%); hence the dose schedule selected for the activated study was 2.5, 12.5, 20, and 25 ug/mL.

Compared to control values ranging between 2 to 3 percent (excluding simple "gaps," nonstaining regions considered by authorities not to represent true aberrations), metaphase analysis of mancozeb-treated cultures revealed dose-related increases in both the extent (mean proportion of aberrant cells) and severity (appearance of more complex chromosomal damage) under both conditions, but more conclusive in the absence of activation (Report Table 2 attached to this DER). Hence, the author concluded that the test substance was positive for chromosomal aberrations in this in vitro cytogenetic test system.

TB Evaluation:

The investigators appeared to have conducted an adequate evaluation of the test agent's capacity to induce chromosomal damage in CHO cells in vitro, resulting in dose-related aberrations with and without metabolic activation. The study, however, can be considered only Inconclusive, and mancozeb only presumptively positive pending:

1. Repetition of the entire assay to confirm the results of this trial, insofar as the induction of chromosome aberrations appears to have occurred at minimally toxic concentrations (using MI as the measure of toxicity). This repeat should also include determinations of cytotoxicity of positive controls.

2. Specification of such cell culture conditions in mancozeb treatment flasks as pH and osmolarity, since extreme variation in both may affect the integrity of chromosomes and other cellular components to produce false positive results.

[NB: In cover documents to this submission, the sponsor suggests just such an explanation for the positive result in this test, and offers several moderating factors, e.g., that: a) cell survival is reduced at cytogenetically effective concentrations (presumably inducing cytotoxicity other than changes in MI). b) Mancozeb as well as other EBDCs are chemically unstable in DMSO, the solvent used in this study (thus, it is unclear to what chemical(s) and concentrations the cells were actually exposed). c) Reported negative results in similar studies by others.

However, no documentation was presented to support any of these assertions.]

Attachment

TABLE 2

Effect of Mancozeb technical on the chromosomes of cultured CHO cells

(a) Without metabolic activation

Culture no	Test agent	Concentration µg/ml	No. cells examined	No. aberrations per 100 cells		Aberrations											No. aberrant cells (excluding gaps)	% Mean	No. aberrant cells (including gaps)	% Mean
				Excluding gaps	Including gaps	IBF	BWF	BF	I	SM	R	D	A	GT	P	ISO	CHR				
83	dimethyl-sulphoxide (solvent control)	10 µl/ml	100	0	1												1	0	1.5	1	3.0
84			100	2	4		2										2	2		4	
85			100	4	5		3					1					1	3		4	
86			100	1	3									1			2	1		3	
87	Mancozeb technical	0.15	100	3	4		3										1	2	1.5	3	2.0
88			100	4	4								4					1		1	
89		0.75	100	5	10		5									1	4	5	**	8	*
90			100	14	27		10		2		2						13	6	5.5	11	9.5
91		1.25	100	26	37	1	14		3	4			2	1	1	1	10	14	***	16	***
92			100	33	42		21	1	6	4					1	1	8	15	14.5	21	18.5
93		1.5	41	66	76		18		4	2					3		4	12	***	12	***
94			36	67	78		14		1	3	1		1	2	2		4	9	27.3	10	28.57
103	sterile water distilled	10 µl/ml	100	2	3		2										1	2	2.0	3	2.5
104			100	2	3		1			1							1	2		2	
105	mitomycin C	0.4	100	13	16		5		1	2			4	1			3	10	**	10	*
106			100	10	11		10										1	6	8.0	6	8.0

With metabolic activation

Culture no	Test agent	Concentration µg/ml	No. cells examined	No. aberrations per 100 cells		Aberrations											No. aberrant cells (excluding gaps)	% Mean	No. aberrant cells (including gaps)	% Mean
				Excluding gaps	Including gaps	IBF	BWF	BF	I	C	SM	R	D	A	GT	P	ISO	CHR				
57	dimethyl-sulphoxide (solvent control)	10 µl/ml	100	12	20		6		5		1							8	6	3.0	11	5.5
58			100	3	6		2		1									3	3		5	
59			100	0	3													3	0		2	
60			100	4	7		2				1	1						3	3		6	
81	Mancozeb technical	2.5	100	5	7		4		1									2	5	5.0	6	6.0
82			100	6	8		3		1		1		1					2	5		6	
83		12.5	100	14	19	1	7		3		3						1	4	9	5.5	11	7.0
84			100	2	3		1		1									1	2		3	
85		20	100	15	19	1	10		3							1		4	11	**	12	9.5
86			100	6	12		2		4									6	5	8.0	7	
87		25	91	10	19		5		1		1	2						8	6	**	8	
88			100	13	16		5		6				1					3	9	7.9	11	9.9
89	sterile water distilled	10 µl/ml	100	4	7		2				2							3	4	3.0	5	3.5
90			100	2	2		1					1							2		2	
1	cyclophosphamide	20	94	28	34		7		11	1	4	1		1	1		1	6	18	***	20	***
2			100	50	62	1	22	4	15	1	5		1	1		1	11	31	25.3	32	26.3

Statistical analysis used was Fisher's test
*** P<0.001
** P<0.01
* P<0.05
Otherwise P>0.05

IBF Isochromatid break with fragment
BWF Chromatid break with fragment
BF Chromatid break without fragment
I Interchange
C Complex rearrangement
R Ring
D Dicentric

SM Single minute
A Acentric fragment
GT Greater than 10 aberrations
P Pulverised cell
ISO Isochromatid gap
CHR Chromatid gap

(M) = micronuclei (see Report Table 1)

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Secondary Reviewer: Judith W. Hauswirth, Ph.D.,
A ing Chief, Toxicology Branch I - IRS (TS-769C)

1/3/8987

Judith W. Hauswirth
1/3/89

DATA EVALUATION REPORT

I. SUMMARY

TB Project No.: 8-1161
Caswell No.: 913A
MRID No.: 40778901
Shaughnessy No.: 014504

Study Type: Mutagenicity - Cytogenetics in vivo (Mouse MT)

Chemical: Mancozeb

Sponsor: Pennwalt Corporation, Philadelphia, PA

Testing Facility: Huntingdon Research Centre, UK

Title of Report: Micronucleus Test on Mancozeb Technical.

Authors: J.A. Allen, R.J. Proudlock, and L.C. Pugh

Study No.: PWT 39/86637

Date of Issue: July 21, 1987

TB Conclusions:

The test substance did not induce micronuclei in bone marrow cells (evidence of chromosomal damage) of animals treated orally at 10,000 mg/kg, which caused some clinical effects as well as cytotoxicity.

Classification (Core-Grade): ACCEPTABLE

B

006987

II. DETAILED REVIEW

A. Test Material - Mancozeb technical

Description: Greenish-yellow powder
Batch (Lot): BL.I.850930
Purity (%): 88.2%
Solvent/carrier/diluent: Aqueous 1% methylcellulose (AMC)

B. Test Organism - Rodent

Species: Mouse
Strain: CD-1 (SPF) outbred (Swiss origin)
Age: 35 days
Weights: Males/Females combined - 22 to 24 g
Source: Charles River (UK), Margate

C. Study Design (Protocol) - A formal protocol was not presented, but the procedures were based upon OECD and EEC Guidelines for this type of assay, supported by a reference list of published authorities in the field.

Statements of compliance with FIFRA GLPs and quality assurance were both included in the Final Report.

D. Procedures/Methods of Analysis - Following preliminary toxicity testing at doses up to 8000 mg/kg, mice (15 males, 15 females) were gavaged once orally with the maximum administrable amount of the test compound in methylcellulose, 10,000 mg/kg. A comparable group of animals was given only the AMC vehicle, while 5/sex were administered mitomycin C (MMC, 8 mg/kg) and served as positive control. Groups of 5 males and 5 females each were sacrificed at three times after dosing: 24 hours (including all of the positive group), 48, and 72 hours. Femoral bone marrow smears (2 per animal) were prepared by conventional techniques and 1000 polychromatic erythrocytes (PCE) per animal scored for the presence of micronuclei (representing fragments of broken chromosomes, or lagging whole chromosomes). The ratio of PCE to normochromatic erythrocytes (NCE) in 1000 erythrocytes was also determined, as well as the incidence of micronucleated NCE.

Wilcoxon's sum-of-ranks test was used to assess significant differences ($p < 0.05$) in mean incidences of micronucleated PCE between groups.

Results:

No animals died in any group in either the preliminary test or main test. Clinical toxicity was minimal, consisting

14

mainly of slight and transitory piloerection, hunched posture, and ptosis in high-dose mancozeb animals (4000, 8000, 100,000 mg/kg), which began within an hour of dosing, and disappeared 5 hours later (Report Appendices 1 and 2).

Compared to highly significant increases ($p < 0.001$) in the frequency of micronucleated PCE from animals treated with MMC (16.6/1000 vs. 0.8/1000 in the vehicle control), no significant increases were found at any time period for mancozeb treatment (Report Table 1, attached to this DER, which is a summary of individual animal data presented as Report Tables 2, 3, and 4).

The values recorded in the present experiment were comparable to this laboratory's historical control of results for 2830 animals gathered over the previous 6-year period of an individual animal mean incidence of 0.87/1000 m-PCE, with group means ranging from 0.1 to 2.5 for experiments with 10 animals in the control group (Report Appendix 3).

As anticipated, no increases in the incidence of m-NCE were found in either the mancozeb or MMC group.

Slight but statistically significant decreases from concurrent control values in PCE/NCE ratios were recorded at the 48-hour ($p < 0.05$) and 72-hour ($p = 0.001$) kill times, suggesting bone marrow cell toxicity caused by mancozeb.

However, these mean p/n values for both treated and control groups were within the range of vehicle control values recorded by this lab for 11 other experiments, an overall mean of 0.96, range 0.625 to 2.799. Thus, the authors discounted these decreases as representing evidence of cytotoxicity, since no comparable concurrent decrease was seen at 24 hours, and there was no trend in the ratios with time. A small but statistically significant decrease ($p = 0.001$) in the p/n ratio was also seen in positive controls, 0.664 probably representing the initial stages of cytotoxicity according to the investigators.

The authors concluded that mancozeb technical was not mutagenic by the oral route in this *in vivo* test.

TB Evaluation: ACCEPTABLE

The study represents an adequate assessment of the lack of potential for mancozeb technical to induce micronuclei in bone marrow cells (evidence of chromosomal damage) of animals

treated orally at a high enough dose (10,000 mg/kg) to cause some clinical toxicity, as well as suggestive evidence of cytotoxicity.

Attachment

TABLE 1

Summary of results - group totals/means for the entire experiment and results of statistical analysis

Kill	Compound & dosage	Ratio p/n		Incidence mnp		Incidence mnn
		Mean	P	Mean °/oo	P	Total °/oo
24 hour	vehicle control	0.911	-	0.8	-	0
	Mancozeb technical (10000 mg/kg)	0.830	0.197	0.6	0.658	0.2
	mitomycin C (8 mg/kg)	0.664	0.001	16.6	<0.001	0.6
48 hour	vehicle control	0.858	-	0.6	-	0
	Mancozeb technical (10000 mg/kg)	0.735	0.045	0.9	0.264	0.2
72 hour	vehicle control	1.089	-	0.8	-	0
	Mancozeb technical (10000 mg/kg)	0.838	0.001	0.6	0.824	0

P Result of statistical analysis using Wilcoxon's sum of ranks test (1-sided probabilities)
p/n Ratio of polychromatic to normochromatic erythrocytes
mnp Number of micronucleated polychromatic erythrocytes observed
mnn Number of micronucleated normochromatic erythrocytes observed
°/oo Number per thousand cells

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Secondary Reviewer: Judith W. Hauswirth, Ph.D.,
Acting Chief, Toxicology Branch I - IRS (TS-769C)

1-3-89

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DATA EVALUATION REPORT

006987

I. SUMMARY

TB Project No.: 8-1161
Caswell No.: 913A
MRID No.: 40810204
Shaughnessy No.: 014504

Study Type: Mutagenicity - Gene mutation in bacteria (Ames Test)

Chemical: Mancozeb

Sponsor: Pennwalt Corporation, Philadelphia, PA

Testing Facility: Huntingdon Research Centre, UK

Title of Report: Microbial Metabolic Activation Test To Assess The Potential Mutagenic Effect of Mancozeb Technical.

Authors: E. Jones, L.A. Fenner, and A.L. Thompson

Study No.: PWT 36/86374

Date of Issue: February 9, 1988

TB Conclusions:

Reportedly negative in repeat tests at doses into cytotoxic levels. However, considered not stable in solvent.

Classification (Core-Grade): Inconclusive

14

006987

II. DETAILED REVIEW

A. Test Material - Mancozeb technical

Description: Yellow/green powder
Batch (Lot): (Not given)
Purity (%): 88.2%
Solvent/carrier/diluent: Dimethylsulfoxide (DMSO)

B. Test Organism - Bacterial cultures

Species: Salmonella typhimurium and Escherichia coli
Strain: S. typhimurium: TA1535, TA1537, TA1538, TA98, TA100; E. coli: WP2uvrA
Source: UCal, Berkeley (Dr. B.N. Ames); and NCIB, Aberdeen, UK

C. Study Design (Protocol) - A study protocol was presented as Appendix I of the Final Report, based on the OECD Guideline #471.

Statements asserting both compliance with FDA GLPs, as well as auditing for quality assurance were included.

D. Procedures/Methods of Analysis - Following dose range-finding tests, plate cultures of bacteria were exposed in triplicate to the test article at five concentrations ranging up to 150 ug/plate, in the absence or presence of a mammalian metabolic activation system consisting of Aroclor 1254-stimulated rat liver microsomal homogenate (S9) plus generating cofactors (S9 mix). After 72 hours incubation, mean numbers of revertent colonies for treatment groups were compared to control values.

19

Positive control substances appropriate for ech bacterial strain and activation condition were employed concurrently.* The entire assay was repeated once.

A compound is considered positive by this lab if statistically significant dose-related increases in mean revertant counts are obtained in two separate experiments. [However, no further details were provided on the statistical analyses employed, nor any bibliographic references.]

Results:

The preliminary range-finding study was conducted at concentrations of 5, 50, 500, and 5000 ug/plate, and cytotoxicity determined by reduction in revertent colony counts and/or absence of a complete background bacterial lawn. No lawn was evident at both of the higher doses in all strains treated in the presence or absence of S9 mix (Report Table 1). Therefore, a concentration of 150 ug/plate was selected as the top dose for the initial mutation assay, together with lower doses of 50, 15, 5, and 1.5 ug/plate.

In this trial, the HDT proved to be too toxic (incomplete bacterial lawn) in nonactivated cultures (only), but at no test concentration (including 150 ug/plate under activation) were any significant increases over solvent control values in revertent colonies recorded under either condition in any of the six bacterial strains (Summary Report Table 2, mean revertent counts, attached to this DER, summarized from individual plate counts in Report Table 3).

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*Positive Controls

With S-9 mix:

2-Aminoanthracene at 2 ug/plate for TA1535 and TA1537.
2-Aminoanthracene at 0.5 ug/plate for TA1538, TA98, and TA100.
2-Aminoanthracene at 80 ug/plate for WP2 uvrA.

Without S-9 mix:

2-Nitrofluorene at 2 ug/plate for TA1538.
2-Nitrofluorene at 1 ug/plate for TA98.
9-Aminoacridine at 80 ug/plate for TA1537.
N-ethyl-N'-nitro-N-nitrosoguanidine at 5 ug/plate for TA1535.
N-ethyl-N'-nitro-N-nitrosoguanidine at 3 ug/plate for TA100.
N-ethyl-N'-nitro-N-nitrosoguanidine at 2 ug/plate for WP2 uvrA.

30

For the repeat assay, 15 ug/plate was chosen as the highest test level, together with lower concentrations of 15, 5, 1.5, and 0.5 ug mancozeb/plate. No toxicity was encountered in this second assay and, as in the first test, revertent colony counts in mancozeb-treated plates were found to be comparable to solvent values (Report Table 5, mean colony counts, attached to this DER; developed from individual plate values comprising Report Table 6).

In both experiments, the representative positive controls responded as expected, with revertent counts ranging from 2.5 to 23 times solvent controls (Report Tables 4, 7).

The authors concluded that mancozeb technical was not mutagenic in this bacterial test system.

TB Evaluation:

This study appears to have been conducted under adequately controlled conditions, and the negative mutagenic response can be considered valid. Solvent control counts were within the range of published spontaneous values, and all positive controls responded appropriately.

Hence, this study could be judged ACCEPTABLE were it not for the disclaimer made by the sponsor about this series of in vitro assays. Pennwalt questions the validity of these assays because the test chemical was dissolved in DMSO, "in which mancozeb as well as other EBDCs have been reported to be unstable;" thus, neither the actual test concentrations of mancozeb nor the identities of substances under test can be verified. However, this assertion about the stability and possible chemical breakdown of mancozeb in DMSO, was not documented.

Attachments

006987

TABLE 2

Test 1

Mancozeb Technical - mutation test
Mean revertant colonies obtained

Material	Test concentration (µg/plate)	With or without S-9	Reverse mutation (number of colonies/plate)#					
			Base pair exchange type			Frame shift type		
			TA 100	TA 1535	WP2 uvrA	TA 98	TA 1537	TA 1538
Solvent control		-	103	17	55	22	17	15
Mancozeb Technical	150	-	-	-	-	-	-	-
	50	-	67	14	38	16	13	8
	15	-	104	13	53	20	17	10
	5	-	94	17	62	17	12	12
	1.5	-	99	15	60	24	16	11
Solvent control		+	121	14	69	25	19	15
Mancozeb Technical	150	+	100	9	44	19	16	12
	50	+	97	15	48	16	21	12
	15	+	115	9	66	23	20	13
	5	+	120	15	71	24	20	16
	1.5	+	124	14	67	24	20	13
Positive controls	Name	Not requiring S-9 mix	ENNG	ENNG	ENNG	NF	9AC	NF
	Concentration (µg/plate)		3	5	2	1	80	2
	Number of colonies/ plate		470	327	1273	71	X	64
	Name	Requiring S-9 mix	AA	AA	AA	AA	AA	AA
	Concentration (µg/plate)		0.5	2	80	0.5	2	0.5
	Number of colonies/ plate		238	98	245	119	78	95

ENNG N-ethyl-N'-nitro-N-nitrosoguanidine
AA 2-aminoanthracene
9AC 9-aminoacridine
NF 2-nitrofluorene
Values are the mean of 3 plates, for individual plate data see Tables 3 and 4.
X Too many colonies for accurate counting

27

006987

TABLE 5

Test 2

Mancozeb Technical - mutation test
Mean revertant colonies obtained

Material	Test concentration (µg/plate)	With or without S-9	Reverse mutation (number of colonies/plate)#					
			Base pair exchange type			Frame shift type		
			TA 100	TA 1535	WP2 uvrA	TA 98	TA 1537	TA 1538
Solvent control		-	125	16	59	22	12	10
Mancozeb Technical	50	-	102	10	57	14	14	9
	15	-	108	14	52	22	11	12
	5	-	99	14	59	20	14	9
	1.5	-	101	14	44	15	16	9
	0.5	-	120	12	44	22	11	9
Solvent control		+	133	14	64	27	15	21
Mancozeb Technical	50	+	112	11	55	15	12	12
	15	+	116	7	57	23	14	12
	5	+	136	10	58	23	15	10
	1.5	+	104	7	54	19	16	17
	0.5	+	128	12	62	21	16	15
Positive controls	Name	Not requiring S-9 mix	ENNG	ENNG	ENNG	NF	9 AC	NF
	Concentration (µg/plate)		3	5	2	1	80	2
	Number of colonies/ plate		495	283	1322	67	X	56
	Name	Requiring S-9 mix	AA	AA	AA	AA	AA	AA
	Concentration (µg/plate)		0.5	2	10	0.5	2	0.5
	Number of colonies/ plate		433	100	174	162	78	134

ENNG N-ethyl-N'-nitro-N-nitrosoguanidine
AA 2-aminoanthracene
9 AC 9-aminoacridine
NF 2-nitrofluorene
Values are the mean of 3 plates. For individual plate data see Tables 6 and 7.
X Too many colonies for accurate counting

23

Reviewed By: Irving Mauer, Ph.D., Geneticist 1-3-89
Toxicology Branch I - IRS (TS-769C)
Secondary Reviewer: Judith W. Hauswirth, Ph.D., 1/3/89
Acting Chief, Toxicology Branch I - IRS (TS-769C)

006987

DATA EVALUATION REPORT

I. SUMMARY

TB Project No.: 8-1161
Caswell No.: 913A
MRID No.: 40810203
Shaughnessy No.: 014504

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

Chemical: Mancozeb

Sponsor: Pennwalt Corporation, Philadelphia, PA

Testing Facility: Huntingdon Research Centre, UK

Title of Report: An Assessment of the Mutagenic Potential of Mancozeb Technical in a Mammalian Cell Mutation Assay Using the Chinese Hamster Ovary/HPRT Locus Assay.

Authors: L.M. Henderson, S.J. Banks, S.J. Ransome, H.J. Bosworth, and C.E. Brabbs

Study No.: PWT 41/861125

Date of Issue: February 11, 1987

TB Conclusions:

Negative for the induction of mutants in cell cultures at cytotoxic doses under nonactivation conditions, as well as in activated cultures exposed up to the limit of solubility.

Classification (Core-Grade):

Cannot be classified as to acceptability until assertions made by sponsor are clarified or documented.

II. DETAILED REVIEW

A. Test Material - Mancozeb technical

Description: Greenish-yellow powder
Batch (Lot): BL.1.850930
Purity (%): 88.2
Solvent/carrier/diluent: Dimethylsulfoxide (DMSO)

B. Test Organism - Mammalian cell line

Species: Chinese Hamster Ovary (CHO)
Strain: CHO-K1-BH_4 ($HGPRT^+$)
Source: BIBRA

C. Study Design (Protocol) - No formal protocol was presented, however, the study was stated to have been based upon recommended guidelines of both the EPA and OECD, as well as published procedures of experts in the field.

Both QA and GLP statements were included.

D. Procedures/Methods of Analysis - During the week prior to treatment, CHO cells were exposed to a culture medium containing hypoxanthine, A-methopterin, and thymidine (HAT), to reduce the number of spontaneous mutants ($HPRT^+$ ---> $HPRT^-$).

Following range-finding and preliminary cytotoxicity tests up to the limit of compound solubility, duplicate cell cultures were exposed for 4 hours to concentrations of mancozeb technical ranging up to 15 ug/mL, or to the DMSO solvent, in both the absence and presence of a metabolic activation system consisting of an Aroclor 1254-stimulated microsomal enzyme homogenate (S9) plus generating cofactors (S9 mix). The mutagens, ethylmethanesulfonate (EMS, 250 ug/mL) and 2-methylcholanthrene (MC, 5 ug/mL) served as positive controls for nonactivated and activated assays, respectively.

Following treatment, all cultures were subcultured in fresh nontreatment media at least twice over the next week (to allow expression of the mutant phenotype), then harvested and exposed for 7 days to culture medium containing the selective agent, 6-thioguanine (TG, 10 ug/mL) in which only mutant colonies ($HPRT^-$) can survive.

Two independent assays with and without exogenous activation were conducted.

35

The following assessments of data were made:

Cytotoxicity (% relative cell survival) =

$$\frac{\text{No. viable control colonies}}{\text{No. viable test colonies}} \times 100$$

Mutant Frequency (MF x 10^{-6}) =
No. mutant (TG-resistant colonies per 10^6 viable cells

$$= \text{Total no. mutant colonies (5 dishes)} \times \frac{600}{\text{Total no. viable colonies (3 dishes)}}$$

Statistical significance of concentration vs. MF was assessed using linear regression analysis.
A response (compound) was deemed positive when all the following criteria were satisfied:

1. Statistically significant, dose-related increases in MF at test compound concentrations results in greater than 10 percent cell survival.

2. Reproducibility.

3. Mean MF in treated cultures at least twice the acceptable mean spontaneous background MF of this lab, which is $15/10^6$.

Results:

In preliminary screening tests, cell survival in nonactivated cultures was comparable to solvent control at all concentrations up to 2.5 ug/mL, then steadily decreased in a dose-related manner up to the limit of solubility, 15 ug/mL, as follows: 72 percent of control at 5 ug/mL, 29 percent at 7.5 ug/mL, and 6 percent at 10.0 ug/mL (Report Table 1). The highest dose cultures was discarded because of compound precipitation. In the presence of S9 mix, no cytotoxicity was apparent at any concentration, cell survival, even at the HDT, 15 ug/mL, being 98 percent of control.

006981

More compressed dose schedules were employed in the ensuing definitive cytotoxicity assessments from which were selected the final test concentrations for both sets of repeat assays, as follows:

Test	S9	Mancozeb Concentrations (ug/mL)
1	-	2, 4, 6, 7, 8, 9, 10, 11
	+	10, 11, 12, 13, 14, 15
2	-	4, 5, 6, 7, 8, 9, 10
	+	10, 11, 12, 13, 14, 15

In both initial (Test 1) and repeat (Test 2) mutation assays, moderate to severe cytotoxicity was observed in nonactivated cultures exposed to mancozeb concentrations of 6 ug/mL and above, whereas no cytotoxicity was apparent under activated conditions up to the limit of solubility (= 15 ug/mL) of the test chemical in DMSO (Report Tables 3, 5, 7, and 9, summarized on the page following). However, no evidence of any significant increase in MF was found in either assay, according to the criteria adopted by this laboratory (data extracted from Report Tables 4, 6, 8, and 10, summarized in this DER). By contrast, both positive control substances induced highly significant and reproducible increases in MF, 20 to 30 times background (ibid.).

57

Effect of Mancozeb Technical in Chinese Hamster Ovary Cells*

Dose (ug/mL)	Test 1 -S9 % Surv	Test 1 -S9 MF	Test 1 +S9 % Surv	Test 1 +S9 MF	Test 2 -S9 % Surv	Test 2 -S9 MF	Test 2 +S9 % Surv	Test 2 +S9 MF
0 (DMSO)	(100	9	(100)	4	(100)	5	(100)	3
2	126	9	NT	NT	NT	NT	NT	NT
4	71	3	NT	NT	84	NT	NT	NT
5	NT	NT	NT	NT	78	NT	NT	NT
6	30	5	NT	NT	76	1	NT	NT
7	16	7	NT	NT	57	13	NT	NT
8	13	12	NT	NT	29	16	NT	NT
9	9	NT	NT	NT	25	2	NT	NT
10	1	NT	119	NT	26	12	125	NT
11	2	NT	100	7	NT	NT	126	1
12	NT	NT	112	2	NT	NT	117	6
13	NT	NT	115	7	NT	NT	111	4
14	NT	NT	96	9	NT	NT	111	6
15	NT	NT	108	3	NT	NT	137	8
EMS (250)	81	324	NT	NT	74	416	NT	NT
MC (5)	NT	NT	83	493	NT	NT	101	439

*Extracted form Tables 3 through 10 of the Final Report.
-S9, in the absence of metabolic activation.
+S9, in the presence of metabolic activation.
% Surv, mean percent survival, relative to control (DMSO).
MF, Mean mutant frequency per 10^6 viable cells.

The authors concluded that mancozeb technical showed no evidence of mutagenic potential in this in vitro test system.

TB Evaluation:

The authors have conducted a comprehensive evaluation of the test compound in this test system (CHO/HPRT), according to accepted procedures for this type of assay, and the interpretation of their data appears to be correct, i.e., that mancozeb technical was not mutagenic under conditions of these assays.

Hence, this study would be judged ACCEPTABLE in demonstrating that mancozeb has no potential to induce TG-resistant mutants in CHO cells, were it not for the disclaimer made by the sponsor about this series of in vitro assays. Pennwalt considers this assay (as well as other in vitro studies in this series from the same lab) "invalid," because the test chemical was dissolved in DMSO, "in which

mancozeb as well as other EBDCs have been reported to be unstable;" thus, neither the actual test concentrations of mancozeb nor the identities of substances under test can be verified.

This assertion about the stability and possible chemical breakdown of mancozeb in DMSO, however, was not documented.

Reviewed By: Irving Mauer, Ph.D., Geneticist
Toxicology Branch I - IRS (TS-769C)
Secondary Reviewer: Judith W. Hauswirth, Ph.D.,
Acting Chief, Toxicology Branch I - IRS (TS-769C) Judith W. Hauswirth 1/3/89

006987

DATA EVALUATION REPORT

I. SUMMARY

TB Project No.: 8-1161
Caswell No.: 913A
MRID No.: 40810205
Shaughnessy No.: 014504

Study Type: Mutagenicity - DNA repair in mammalian cells in vitro (HeLa/UDS)

Chemical: Mancozeb

Sponsor: Pennwalt Corporation, Philadelphia, PA

Testing Facility: Huntingdon Research Centre, UK

Title of Report: Autoradiographic Assessment of Unscheduled DNA Repair Synthesis in Mammalian Cells After Exposure to Mancozeb Technical.

Authors: J.A. Allen and R.J. Proudlock

Study No.: PWT 40/86899

Date of Issue: October 22, 1986

TB Conclusions:

Inconsistent and sporadic increases in silver grain counts (indicative of repair due to unscheduled DNA synthesis) in replicate trials make interpretation of the reported negative difficult to accept.

Classification (Core-Grade): UNACCEPTABLE

30

II. DETAILED REVIEW

A. Test Material - Mancozeb technical

Description: Greenish-yellow powder
Batch (Lot): BL.I.850930
Purity (%): 88.2%
Solvent/carrier/diluent: Dimethylsulfoxide (DMSO)

B. Test Organism - Mammalian cell line

Species: Human, HeLa
Strain: S3
Source: Flow Laboratories, Ltd. (UK)

C. Study Design (Protocol) - A formal protocol was not presented; however, the study was asserted to have been conducted according to OECD Guideline GEN 85.4 (1985).

Statements of compliance with FDA GLPs as well as for quality assurance were both included in the Final Report.

D. Procedures/Methods of Analysis - Following incubation in low-serum, arginine-deficient medium (to inhibit normal replicative, i.e., scheduled DNA synthesis), coverslip cell cultures were then exposed to radioactive (6^3H)-thymidine (5 uCi/mL) together with 11 serial dilutions of test article (top dose, 2048 ug/mL), in both the absence and presence of metabolic activation provided by Aroclor 1254-stimulated rat hepatic microsomes (S9), plus appropriate generating cofactors (S9 mix).

After 3 hours incubation in this cocktail, the coverslips were attached to microscope slides, washed, fixed, stained, and processed for autoradiography using stripping film (Kodak AR-10) by conventional (referenced) techniques; exposure was 13 days.

After development and fixing in standard photographic solutions, 100 cells per [illegible] were scored under oil immersion microscopy for silver grains over nuclei as well as comparable areas of the cytoplasm. The number of nonphase nuclei having [illegible] 3 net grains per slide was recorded (% labeled nuclei).

Cultures treated with DMSO [illegible] as negative (solvent) control, while others were treated with either 4-nitroquinoline-1-oxide (NQO) without S9 mix, or 2-aminoanthracene (AA) in the presence of S9 mix, to serve as positive controls. The entire assay was repeated once at the same dose levels.

3

Data from both tests were analyzed statistically by one-way ANOVA.

Results:

According to the investigators, increasing (dose-related) turbidity and precipitation in test cultures caused excessive silver grain development at higher dose levels making quantification difficult to impossible; apparent increases at lower concentrations were considered too difficult to interpret. Although small but statistically significant increases over concurrent control values were recorded in both tests, these were random, non-dose-related and non-reproducible (see tabulation on page following, extracted from Report Tables 1 and 2).

By contrast, the positive control substance NQO induced consistent dose-related and significant increases in grain counts in both tests. The positive control for activated cultures, AA, produced less striking responses.

The authors concluded that ". . . Mancozeb technical has failed to show any clear and reproducible evidence of mutagenic potential in this in vitro test for unscheduled DNA synthesis, although interpretation of results was hindered by the interference of the test agent with the autoradiographic processes invoived."

TB Evaluation: UNACCEPTABLE

This study was apparently carried out according to standard procedures under adequately controlled conditions. The sporadic and equivocal responses, however, indicate problems of solubility and/or adsorption of test agent, probably in the solvent selected.

In a cover document to the submission of these studies, the registrant suggested that mancozeb was unstable in DMSO, making actual exposure to the parent compound uncertain in vitro. Although documentation was not provided for this assertion, the equivocal results generated in this assay require a repeat under presumably "stable" conditions.

Hence, the author's conclusions that mancozeb was negative in this assay are not accepted.

37

Evaluation of Mancozeb for DNA Repair in HeLa Cells[1/]

Dose (ug/mL)	Test 1				Test 2			
	-S9		+S9		-S9		+S9	
	% Net[2/]	% Nuclei[3/]	% Net	% Nuclei	% Net	% Nuclei	% Net	% Nuclei
0 (DMSO)	5	0.6	13	0.5	18	0.4	8	1
Mancozeb:								
1	24*	1.5	5	1.0	1	0	11	0
2	23*	0.5	23	1.5	12	0.5	3	0.5
4	13	0.5	31	0	-1	0.5	17	0
8	35**	1.5	39	0.5	56*	2.5	20	0.5
16	19	0.5	26	0.5	31	1.0	49**	3.0
32	40***	2.0	10	0	39	0.5	14	1.0
64	43**	1.0	37	1.0	77**	3.0	25	1.0
128	(C)	-	28	0.5	82*	1.0	4	1.5
256	(C)	-	49*	2.0	(C)	-	40*	1.5
512(P)	(C)	-	40	0	(C)	-	(C)	-
1024(P)	(C)	-	(C)	-	(C)	-	(C)	-
2048(P)	(C)	-	(C)	-	(C)	-	(C)	-
NQO (0.04-0.32)	321-1732***	41-98	-	-	529-1351***	68-99	-	-
AA (2.5-40.0)	-	-	32-53**	1.0-2.5	-	-	26-88***	0-8

1/Extracted from Tables 1 and 2 of the Final Report.
2/% Net, Net grains per 100 nuclei.
3/% Nuclei, Number of nuclei with $\geq$ 3 net grains per 100 nuclei.
P, Dose-related precipitation.
C, Excessive silver grain development.