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

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JUN 10 1993

OFFICE OF
PREVENTION, PESTICIDES AND
TOXIC SUBSTANCES

MEMORANDUM:

SUBJECT: **THIDIAZURON:** Review of a general metabolism study in rats and two mutagenicity studies.
EPA DP Barcode: D184498; EPA Submission# S429013; EPA MRID No.s 417611-02 and -03 and 425290-01; EPA Pesticide Chemical Code 120301, Toxicology Chemical Code 659A

TO: Linda Deluise/Tom Myers, PM 52
SRRD (H7508W)

FROM: Stephen C. Dapson, Ph.D. Stephen C. Dapson 6/7/93
Senior Pharmacologist, Review Section I
Toxicology Branch II/HED (H7509C)

THRU: Yiannakis M. Ioannou, Ph.D., D.A.B.T. Y.M. Ioannou 6/7/93
Section Head, Review Section I
and
Marcia van Gemert, Ph.D.
Chief, Toxicology Branch II/HED (H7509C) Marcia van Gemert 6/7/93

Registrant: NOR-AM Chemical Company; 3509 Silverside Road
P.O. Box 7495, Wilmington, DE 19803

Action Requested: Review a general metabolism study in rats and two mutagenicity studies with Thidiazuron.

Recommendations: TB II has reviewed the general metabolism study in rats and two mutagenicity studies with Thidiazuron. The following are the conclusions from the reviews:

MRID# 425290-01: *M13 THIDIAZURON: The Metabolism of ^{14}C-Thidiazuron in Rats*, Huntingdon Research Centre Ltd. for NOR-AM Chemical Company, Laboratory Project I.D. HRC/SMS 254/920450, 10/13/92.

The disposition and metabolism of ^{14}C-Thidiazuron was investigated in male and female rats at a single low oral dose (10 mg/kg), repeated low oral doses (10 mg/kg x 14 days) and a single high dose (1000 mg/kg). Absorption of Thidiazuron was incomplete at both doses, and appeared decreased at the high dose relative to the low dose. Highest concentrations of Thidiazuron derived

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radioactivity at sacrifice were found within the liver, kidneys, thyroid, whole blood, and adrenals at both the 10 and 1000 mg/kg dose. Repeated oral dosing did not significantly affect distribution of Thidiazuron derived radioactivity. Excretion of Thidiazuron derived radioactivity was relatively rapid at the single low dose level, with the urine and feces as significant routes of excretion. Excretion was delayed at the high dose, suggesting saturation of absorption, biotransformation, or both. Repeated low oral dosing did not significantly affect the profile of general disposition.

Identification of urinary and fecal metabolites by TLC and HPLC indicated the presence of one oxidative metabolite in urine (4-hydroxy Thidiazuron), and the presence of sulfate and glucuronide conjugates of 4-hydroxy Thidiazuron. However, insufficient evidence was presented to justify the presence of more than one sulfate and one glucuronide conjugate of 4-hydroxy Thidiazuron in urine. This deficiency does not render the study inadequate, as the other possible oxidative metabolite(s) as candidates for conjugation represent only a minor percentage of metabolized Thidiazuron. In feces, the major metabolites identified were 4-hydroxy Thidiazuron at the low dose, and unmetabolized Thidiazuron at the high dose.

This study is classified as **Core-Minimum Data** and **satisfies** the Guideline requirement (§85-1) for a metabolism study in rats.

MRID# 417611-02: *T59 THIDIAZURON: Gene Mutation Assay in Chinese Hamster V79 Cells in vitro with Thidiazuron Technical (SN 49537);* Cytotest Cell research GmbH & Co, for NOR-AM Chemical Company, Laboratory Project I.D. Schering No. TB 89 083, 7/31/90.

Under the conditions of the gene mutation assay in Chinese hamster V79 cells, Thidiazuron Technical (SN 49537) was tested at concentrations from 6 to 250 µg/ml in two independent assays, with considerable cytotoxicity at 250 µg/ml. While there was an increased number of mutant colonies/10^6 cells (87.0 vs 25.1 for negative controls) at the highest dose level (250 µg/ml) in the absence of S9 in the first assay, a similar increase was not observed in the second assay. It was concluded that Thidiazuron Technical was tested over an appropriate range of concentrations with appropriate controls and did not cause mutations at the HGPRT locus in V79 cells.

This study is classified as Acceptable and satisfies the Guideline requirement (§84-2a) for Category 1, gene mutation test.

MRID# 417611-03: T58 THIDIAZURON: Unscheduled DNA synthesis in primary hepatocytes of male rats in vitro with Thidiazuron Technical (SN 49537); CCR-Cytotest Cell research GmbH & Co, for NOR-AM Chemical Company, Laboratory Project I.D. Schering No. TB 89 084, 7/26/90.

In this UDS study, Thidiazuron Technical (SN 49537) was tested at concentrations ranging from 0.25 to 75 µg/ml in two assays utilizing rat hepatocytes. In both assays, cytotoxicity (reduced cell viability) was evident at 25 µg/ml (highest dose evaluated), and there were insufficient numbers of viable cells for scoring at 75 µg/ml. In both assays, there was no indication of a positive response (a significant increase in mean net grains/nucleus) at any of the evaluated dose levels. The positive controls elicited the appropriate responses. It is concluded that, under the conditions of this assay, there was no evidence of unscheduled DNA synthesis in rat hepatocytes exposed to Thidiazuron.

This study is classified as Acceptable and satisfies the Guideline requirement (§84-4) for Category 3, other genotoxic effects.

I. Toxicology Profile for Thidiazuron (40CFR 158.340)

Technical: Thidiazuron
Use Pattern: food
Action Type: reregistration

This compound is a registered active ingredient. The following data are required for technical Thidiazuron. This chemical is on **LIST D** for reregistration.

THIS INFORMATION DOES NOT NECESSARILY REFLECT THE DATA REQUIREMENTS FOR REREGISTRATION.

	Required	Satisfied
§81-1 Acute oral toxicity in rats	Yes	Yes
§81-2 Acute dermal toxicity in rabbits	Yes	Yes
§81-3 Acute inhalation toxicity in rats	Yes	**NO***
§81-4 Primary eye irritation in rabbits	Yes	**NO**
§81-5 Primary dermal irritation in rabbits	Yes	Yes
§81-6 Dermal sensitization - guinea pig	Yes	**NO**
§82-1(a)90 day feeding study - rat	Yes	No[1]
§82-1(b)90 day feeding study - nonrodent	Yes	Yes
§82-2 21 day dermal - rabbit	Yes	**NO**
§83-1(a)2-year feeding - rodent	Yes	Yes
§83-1(b)1 year feeding - nonrodent	Yes	**NO**
§83-2(a)Carcinogenicity - rat	Yes	**NO**
§83-2(b)Carcinogenicity - mouse	Yes	Yes*
§83-3(a)Teratology - rat	Yes	Yes
§83-3(b)Teratology - rabbit	Yes	Yes
§83-4 Multigeneration reproduction-rat	Yes	Yes*
§84-2(a)Mutagenicity Gene Mutation	Yes	Yes[2]
§84-2(b)Muta - Struct.Chromosome Aberr.	Yes	Yes
§84-4 Muta - Other Genotoxic Effects	Yes	Yes
§85-1 General metabolism - rat	Yes	Yes[2]
§85-2 Dermal Penetration (Absorption)	No	

* = IBT Data
[1] = satisfied by 2-year chronic feeding study in the rat
[2] = see review attached

II. Data Gaps

The database for technical Thidiazuron is not complete, additional data on the following studies are required for the technical database:

§81-3 Acute inhalation toxicity in rats
§81-4 Primary eye irritation in rabbits
§81-6 Dermal sensitization - guinea pig
§82-2 21 day dermal - rabbit
§83-1(b)1 year feeding - nonrodent
§83-2(a)Carcinogenicity - rat

III. Actions Being Taken to Obtain Additional Information or Clarification

None at this time.

IV. Reference Dose

The RfD has not been established, this chemical will be presented to the RfD/Peer Review Committee for consideration . **(NOTE: The acceptability of all IBT studies will be discussed by the RfD/Peer Review Committee at this meeting; additional data gaps in the Thidiazuron data base may result.)**

V. Pending Regulatory Actions

None at this time.

VI: Toxicological Issues Pertinent to this Request

A. New toxicology Data on Thidiazuron

Addressed in this memo.

B. Carcinogenicity

No evidence of carcinogenicity was noted in the mouse carcinogenicity study; the rat study must be repeated at higher doses (MTD not obtained in the submitted study).

C. Toxicology One-liners

One-liners have been ammended to reflect the above discussion.

Reviewed by: Timothy F. McMahon, Ph.D. 3/19/93
Section I, Toxicology Branch II (H7509C)
Secondary Reviewer: Yiannakis M. Ioannou, Ph.D. 3/23/93
Section I, Toxicology Branch II (H7509C)

Data Evaluation Record

Study type: Metabolism (85-1)

EPA identification numbers:

ToxChem No: 120301
Submission: S429013
DP Barcode: D184498
MRID No: 425290-01

Laboratory Project I.D.: TOX 90463

Test material: N-phenyl-N'-1,2,3-thiadiazol-5-ylurea

Synonyms: ^{14}[C-aniline]thidiazuron; ^{14}[C-thiadiazole]thidiazuron; unlabeled thidiazuron

Testing Facilities: Huntingdon Research Centre, Ltd.
Cambridgeshire, England

Sponsor: NOR-AM Chemical Company
Wilmington, Delaware

Title of reports: M13 Thidiazuron: The Metabolism of ^{14}C-Thidiazuron in Rats

Author(s): D. R. Hawkins, B.C. Mayo, A.B. McEwen, L.V. Newton, W.S. McCombe

Reports issued: October 13, 1992

Conclusions:

The disposition and metabolism of ^{14}C-thidiazuron was investigated in male and female rats at a single low oral dose (10 mg/kg), repeated low oral doses (10 mg/kg x 14 days) and a single high

dose (1000 mg/kg). Absorption of thidiazuron was incomplete at both doses, and appeared decreased at the high dose relative to the low dose. Highest concentrations of thidiazuron derived radioactivity at sacrifice were found within the liver, kidneys, thyroid, whole blood, and adrenals at both the 10 and 1000 mg/kg dose. Repeated oral dosing did not significantly affect distribution of thidiazuron derived radioactivity. Excretion of thidiazuron derived radioactivity was relatively rapid at the single low oral dose level, with the urine and feces as significant routes of excretion. Excretion was delayed at the high dose, suggesting saturation of absorption, biotransformation, or both. Repeated low oral dosing did not significantly affect the profile of general disposition.

Identification of urinary and fecal metabolites by TLC and HPLC indicated the presence of one oxidative metabolite in urine (4-hydroxy thidiazuron), and the presence of sulfate and glucuronide conjugates of 4-hydroxy thidiazuron. However, insufficient evidence was presented to justify the presence of more than one sulfate and one glucuronide conjugate of 4-hydroxy thidiazuron in urine. This deficiency does not render the study inadequate, as the other possible oxidative metabolite(s) as candidates for conjugation represent only a minor percentage of metabolized thidiazuron. In feces, the major metabolites identified were 4-hydroxy thidiazuron at the low dose, and unmetabolized thidiazuron at the high dose.

Core Classification: minimum

This study satisfies the guideline requirements (85-1) for a metabolism study in rats.

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Reviewed by: Virginia A. Dobozy, V.M.D., M.P.H. Virginia A. Dobozy 6/1/93
Section I, Toxicology Branch II (H7509C)
Secondary Reviewer: Byron T. Backus, Ph.D. Byron T. Backus
Section II, Toxicology Branch II (H7509C) 6/2/93

DATA EVALUATION REPORT

STUDY TYPE: Mammalian cells in culture gene mutation assay in Chinese Hamster V79 Cells

EPA I.D. NUMBERS: P. C. CODE: 120301
DP BARCODE: D184498
SUBMISSION: S429013
MRID NUMBER: 41761-02

TEST MATERIAL: THIDIAZURON TECHNICAL

STUDY NUMBER: TB 89 083

TESTING FACILITY: CCR-Cytotest Cell Research GmbH & Co.
Roßdorf, Germany

SPONSOR: Schering AG
Berlin, Germany

TITLE OF REPORT: T58 THIDIAZURON: Gene Mutation Assay in Chinese Hamster V79 Cells in vitro with Thidiazuron Technical (SN 49537)

AUTHOR(S): A. Heidemann

REPORT ISSUED: July 31, 1990

CONCLUSIONS: Under the conditions of the gene mutation assay in Chinese hamster V79 cells, Thidiazuron Technical (SN 49537) was tested at concentrations from 6 to 250 µg/ml in two independent assays, with considerable cytotoxicity at 250 µg/ml. While there was an increased number of mutant colonies/10^6 cells (87.0 vs. 25.1 for negative controls) at the highest dose level (250 µg/ml) in the absence of S9 in the first assay, a similar increase was not observed in the second assay. It is concluded that Thidiazuron Technical was tested over an appropriate range of concentrations with appropriate controls and did not cause mutations at the HGPRT locus in V79 cells.

CLASSIFICATION: Acceptable - This study satisfies the 84-2(a) gene mutation data requirement (1984 Guidelines).

A. **MATERIALS:**

1. Test Material: Thidiazuron Technical (SN 49537)

Description: Yellow colored solid with "little lumps"
Batch Number: 27 [illegible] B 00000
Purity: 96.9% w/w
Stability: Stable under storage conditions; stability in solvent not indicated by sponsor
Storage Conditions: room temperature, moisture and light protected
Contaminants: None listed
Solvent used: Dimethyl sulfoxide (DMSO)

2. Control Materials:

Negative: yes
Solvent/final concentration: DMSO, 1% v/v
Positive:
Non-activation: Ethylmethanesulfonate (EMS), 1 mg/ml
Activation: 7, 12 dimethylbenz(a)anthracene (DMBA), 15.4 ug/ml

3. Activation: S9 was derived from Aroclor 1254 induced Wistar rat liver. Stock S9 supernatant was thawed and mixed with S9 cofactor solution which was concentrated to yield the following mix:

8 mM $MgCl_2$
33 mM KCl
5 mM glucose-6-phosphate
5 mM NADP
in 100 mM sodium-ortho-phosphate-buffer, pH 7.4

4. Test Cells: mammalian cells in culture

___mouse lymphoma L5178Y cells
___Chinese hamster ovary (CHO) cells
_X_V79 cells (Chinese hamster lung fibroblasts)

Properly maintained: yes
Periodically checked for Mycoplasma contamination? yes
Periodically checked for karyotype stability? yes
Periodically "cleansed" against high spontaneous background? n/a

5. Locus Examined:

____ thymidine kinase (TK)
selection agent: _____bromodeoxyuridine (BrdU)
_____fluorodeoxyuridien (FdU)

_____trifluorothymidine (TFT)

_X_____hypoxanthine-guanine-phosphoribosyl transferase (HPRT)
Selection agent: _____8-azaguanine (8-AG)
11 ug/ml 6-thioguanine (6-TG)

_____Na^+/K^+ ATPase
Selection agent: _____ouabain

6. Test compound concentrations used:

Non-activated and activated conditions: mutation experiments started with six concentrations, 6.0, 15.0, 30.0, 60.0, 100.0, and 250.0 ug/ml. On day 5, four concentrations were selected to proceed with the experiments, 15.0, 30.0, 100.0, and 250.0 ug/ml.

B. TEST PERFORMANCE

1. Cell treatment: A copy of the treatment scheme from the study report is attached to the DER.

2. Protocol: A protocol has not been submitted.

3. Preliminary cytotoxicity assay: In the pre-experiment for toxicity, the following concentrations were tested: 0.10, 1.0, 5.0, 10.0, 30.0, 60.0, 100.0 and 250.0 ug/ml. The plating efficiency of the V79 cells was reduced after treatment at 100.0 and 250.0 ug/ml, both with and without activation.

4. Mutagenicity assay: The following table summarizes the number of mutant colonies per 10^6 cells in the experiments 1 and 2.

Test Substance	Concentration (ug)	S9 Mix	Mutant Colonies per 10^6 Cells	
			Experiment 1	Experiment 2
Negative Control	0.0	-	25.1	29.8
Solvent Control (DMSO)	0.0	-	3.7	18.7
Positive Control (EMS)	1.0 mg	-	765.7	267.2
Test Article	6.00	-	sample discarded	
Test Article	15.0	-	35.9	12.7
Test Article	30.0	-	58.1	16.1
Test Article	60.0	-	sample discarded	
Test Article	100.0	-	27.2	25.6
Test Article	250.0	-	87.0	0.0
Negative Control	0.0	+	11.8	17.6
Solvent Control	0.0	+	13.7	13.8
Positive Control (DMBA)	15.4	+	239.0	190.3
Test Article	6.0	+	sample discarded	
Test Article	15.0	+	9.7	20.0
Test Article	30.0	+	13.7	31.2
Test Article	60.0	+	sample discarded	
Test Article	100.0	+	24.6	20.8
Test Article	250.0	+	11.7	22.3

Extracted from Tables III (page 23) and VI (page 26) of the study report.

The assay met the criteria to be considered acceptable as outlined in the study report (page 18). To be considered positive, the test substance must induce either a significant dose-related increase in mutant colonies/10^6 cells or a reproducible and significant positive response for at least one of the test points. In Experiment 1, the numbers of mutant colonies at the 30.0 and 250.0 ug/ml concentrations were increased over the negative and solvent controls, but this was not observed in Experiment 2.

5. Reviewer's discussion/conclusions

Thidiazuron Technical (SN 49537) was tested to cytotoxic concentrations in two independently performed assays and did

not meet the criteria for a positive response. While the numbers of mutant colonies observed at some concentrations of the test substance without S9 were noticeably elevated over those observed in the negative and solvent controls in Experiment 1, there was no indication of a similar effect in Experiment 2. Results with the positive controls indicated that the test system was sufficiently sensitive to detect a positive response. We conclude that Thidiazuron Techical did not cause gene mutation at the HGPRT-locus in V79 cells.

6. Was test performed under GLPs? yes

7. CBI appendix attached? no

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X DETAILED METHODOLOGY

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Reviewed by: Virginia A. Dobozy, V.M.D., M.P.H. Virginia A Dobozy 6/3/93
Section I, Toxicology Branch II (H7509C)
Secondary Reviewer: Byron T. Backus, Ph.D. Byron T. Backus 6/2/93
Section II, Toxicology Branch II (H7509C)

DATA EVALUATION REPORT

STUDY TYPE: Mutagenicity: *In vitro* unscheduled DNA synthesis assay in primary rat hepatocytes

EPA I.D. NUMBERS:
P. C. CODE: 120301
DP BARCODE: D184498
SUBMISSION: S429013
MRID NUMBER: 417611-03

TEST MATERIAL: THIDIAZURON TECHNICAL

STUDY NUMBER: TB 89 084

TESTING FACILITY: CCR-Cytotest Cell Research GmbH & Co.
Roßdorf, Germany

SPONSOR: Schering AG
Berlin, Germany

TITLE OF REPORT: T58 THIDIAZURON: Unscheduled DNA Synthesis in Primary Hepatocytes of Male Rats *in vitro* with Thidiazuron Technical (SN 49537)

AUTHOR(S): R. Fautz

REPORT ISSUED: July 26, 1990

CONCLUSIONS: In this UDS study, Thidiazuron Technical (SN 49537) was tested at concentrations ranging from 0.25 to 75 µg/ml in two assays utilizing rat hepatocytes. In both assays, cytotoxicity (reduced cell viability) was evident at 25 µg/ml (highest dose evaluated), and there were insufficient numbers of viable cells for scoring at 75 µg/ml. In both assays, there was no indication of a positive response (a significant increase in mean net grains/nucleus) at any of the evaluated dose levels. The positive controls elicited the appropriate responses. It is concluded that, under the conditions of this assay, there was no evidence of unscheduled DNA synthesis in rat hepatocytes exposed to Thidiazuron.

CLASSIFICATION: Acceptable - This study satisfies the 84-4 other genotoxic effects data requirement (1984 Guidelines).

A. MATERIALS:

1. Test Material: Thidiazuron Technical (SN 49537)

Description: Yellow colored solid with "little lumps"
Batch Number: 27 1006 B 00000
Purity: 96.9% w/w
Stability: Stable under storage conditions; stability in solvent not indicated by sponsor
Storage Conditions: room temperature, moisture and light protected
Contaminants: None listed
Solvent used: Dimethyl sulfoxide (DMSO)

2. Indicator Cells: Primary rat hepatocytes were obtained by in situ perfusion of the livers of male Wistar rats purchased from SAVO-Ivanovas, Kisslegg, Germany.

3. Control Substances: DMSO at a final concentration of 1% was the solvent control; the positive control was 2-acetylaminofluorene (2-AAF) prepared at 2.23 ug/ml in DMSO.

4. Medium: Williams' medium E with 100 ul/ml fetal calf serum, 0.29 mg/ml glutamin, antibiotics and insulin.

5. Test Compound Concentrations Used:

Two separate experiments were done with each using the following concentrations of the test substance: 0.03, 0.08, 0.25, 0.75, 2.50, 7.50, 25.00 and 75 µg/ml. There were insufficient numbers of viable cells for scoring at 75 µg/ml; doses of 0.25 to 25 µg/ml were evaluated for UDS.

B. STUDY DESIGN:

1. Cell Preparation:

(a) Perfusion techniques: The male rat was narcotized with Hypnodil. The liver was perfused with modified Seglen's medium supplemented with collagenase. Isolated hepatocytes were washed with the perfusion solution without collagenase and then filtered through a stainless steel mesh to yield a single cell suspension. Recovered cells were stained to determine the quality of the perfusion. The washed hepatocytes were centrifuged and transferred into the Williams' medium E.

(b) Hepatocyte culture preparation: Aliquots of 2.5 ml of isolated hepatocytes (1.0 x 10^5 cells/ml) in complete culture medium were placed into 35 mm six-well cluster

dishes containing one gelatinized 25 mm round plastic coverslip per well. Cells were allowed to attach to the coverslip in a 95% air/5% CO_2 humidified incubator at 37°C for 1.5 hours. Then the cell layer was rinsed to remove unattached cells.

2. UDS Assay:

(a) Treatment: The dissolved test article together with 5 µCi/ml ^{3}HTdR in 2.0 ml Williams' medium E were added to the hepatocyte cultures. Positive and negative controls were treated similarly; all were tested in triplicate. The hepatocytes were exposed for 18 hours, after which they were rinsed twice to remove the radiolabel. Cultures were treated with a hypotonic solution of 1% sodium citrate for 10 minutes, fixed in three changes of methanol:acetic acid (3:1 v/v) for 15 minutes each, rinsed with 96% ethanol and air dried.

To evaluate toxicity with the neutral red absorption, two additional cultures for each concentration were treated as described above with the omission of the ^{3}HTdR.

(b) Preparation of autoradiographs/grain development: The coverslips were mounted cell surface up on glass slides and coated with ILFORD K-2 photographic emulsion in the dark. They were stored in light-proof boxes in the presence of a drying agent for 7 days at 4°C, then developed with D19, fixed, and stained with aceto orcein.

(c) Grain counting: Slides were coded and counted under oil immersion. At least two slides per dose level and 50 cells per slide were counted. Heavily labelled S-phase cells were not counted. The number of silver grains above the nucleus were counted using the ARTEK 880 counter. Additionally, the mean number of grain counts of one nuclear-sized cytoplasm area adjacent to the nucleus was determined.

3. Evaluation Criteria:

(a) Assay validity: Not stated.

(b) Positive response: The test article was considered positive if it induced either a statistically significant dose-related increase in radiolabel incorporation expressed as grains per nucleus or a reproducible and statistically significant positive response for at least one of the test points. If these conditions are not met, the test article is considered non-effective in this system. Statistical significance was evaluated by the non-parametric Mann-Whitney test.

4. Protocol: A protocol was not provided.

C. REPORTED RESULTS:

1. UDS Assay: In a pre-experiment, ten concentrations ranging from 0.25 to 250 ug/ml were tested (concentrations higher than 250 ug/ml precipitated in the culture medium). In this study, exposure to doses of 8.33, 16.67 and 25.0 µg/ml were associated with some reduction in viability with complete (or nearly complete) cytotoxicity at doses ≥ 83.33 µg/ml.

In experiment 1, there was an increased mean nuclear grain count at 2.5 µg/ml, however, there was an accompanying increase in mean cytoplasmic grain count, so the mean net nuclear grain count was essentially the same as those observed in the negative and solvent controls; similar increases were seen at 0.25 and 7.5 µg/ml in experiment 2. In both experiments, there were slightly increased mean net nuclear grain counts at 25 µg/ml (experiment 1: -1.89 mean net nuclear grains vs. -5.81 for solvent controls; experiment 2: 0.73 vs. -5.69 for solvent controls), but these were neither statistically nor biologically significant findings. These increases may have been due to reduced cell viability (or increased cytotoxicity) at this dose level, which might have then resulted in increases in nuclear grain counts to the background (cytoplasmic) level. The positive control, 2-AAF at 2.23 µg/ml, showed highly significant increases in mean net nuclear grain counts. Summaries of the data from Experiments 1 and 2 from the study report are attached to the DER.

D. REVIEWERS' DISCUSSION/CONCLUSIONS: Thidiazuron Technical (SN 49537) was tested to cytotoxic concentrations in two independently performed assays and did not meet the criteria for evaluation as a positive test. While there were sporadic increases in nuclear grain counts, the net grain counts were comparable to the solvent control. One increase in net grain count was not reproducible. Results with the positive control, 2-AAF, indicated that the test system was sufficiently sensitive to detect a positive response. We conclude that Thidiazuron Technical was not genotoxic in this test system.

E. QUALITY ASSURANCE MEASURES: Was the test performed under GLPs? yes

F. CBI APPENDICES: CBI appendix attached? no

THIDIAZURON - TDXR 010318

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