6-24-91

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

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JN 24 91

MEMORANDUM

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

SUBJECT: Amitraz
Chromosomal Aberration Study (84-2b)

HED Project No.: 0-1570
TOX Chem No.: 374A

FROM: Ray Landolt 6/18/91
Review Section I
Toxicology Branch II
Health Effects Division (H7505C)

TO: Dennis H. Edwards, Jr., PM 12
Insecticide-Rodenticide Branch
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THRU: Mike Ioannou, Section Head 6/19/91
Review Section I
Toxicology Branch II
Health Effects Division (H7509C)
and
Marcia van Gemert, Branch Chief 6/19/91
Toxicology Branch II
Health Effects Division (H7509C)

Registrant: Nor-Am Chemical Company, letter of February 21, 1991.

Action Requested: Review "Technical Amitraz: Metaphase Chromosomal Analysis of Human Lymphocytes Cultured in vitro" (MRID 41795101) submitted to fulfill the 84-2b mutagenicity data gap for amitraz.

This study was submitted to satisify mutagenicity data gap identified in Toxicology review (DER 008177) of December 3, 1990 for an acceptable structural chromosomal aberration study.

Conclusions: Amitraz is negative for clastogenic response in cultured human lymphocytes under nonactivated and S-9 activated conditions.

This study is acceptable and satisifies the mutagenic guideline data requirement (84-2b).

The toxicity data required for estimating the RfD are satisifed.

Toxicity Data Requirements for Technical Grade Amitraz

Amitraz is a FIFRA'88 List A chemical for which the Toxicology Chapter to the Registration Standard (DER 005633), issued February 1, 1985, identified the following data in support of food uses for amitraz. These studies were not subjected to current acceptance criteria for guideline data (158.135) requirments. This data base was evaluated by HED in consort with the California Department of Food and Agriculture March 2, 1989. HED (DER 007190) concluded that these studies are acceptable except for (84-2b) a Chromosomal Aberration study was identified as a data gap.

Acute Testing	Study No.	MRID/Acc No.	DER No.
81-1 Acute oral toxicity	TXM 73041	00041539	001116
81-2 Acute dermal toxicity	YM 72011	00040862	001123
81-3 Acute inhalation toxicity	4971/72/406	00029963	001123
81-4 Primary eye irritation	TXM 72037	00112879	001123
81-5 Primary dermal irritation	TXM 72011	00040862	001123
81-6 Dermal sensitization	PM 7101C	00029965	001125
Subchronic Testing			
82-1 90-day feeding-Rat	P 71548	00028712	001124
Oral - Mouse	TX 74016	00028715	001116
Oral - Dog	P 71547	00028716	001124
82-2 21-day dermal	TX 73026	00029972	001124
Chronic Testing			
83-1 Feeding/Carcinogenicity-Rat	TX 73043	00044585	001124
Two year dog feeding	TX 73035	00044586	001124
83-2 Carcinogecicity- Mouse (80wk)	TX 76039	00044484	001116
- Mouse (2yr) *	TX/83/179-93	252098-102	004252
83-3 Teratogenicity- Rat	TX 73028	00029959	001124
	TX 73031	00029960	001124
Rabbit	TX 73029	00029961	001124
83-4 Reproduction-Rat	TX 73036	00029962	001124
Mutagenicity Testing			
84-2(a)Gene mutation	2590	253131	004174
84-2(b)Chromosomal aberration *	TX 88253	41795101	This Review
84-4 Other genotoxic effects	2634	253131	004174
Special Testing			
85-1 General metabolism-Rat	FBC (M66)	253130	004175
Hormone Levels-Female Mice	FBC 179-97	253131	004175
Hormone Levels-Female Rats	PM 72003	253131	001116
Dermal Absorption-Rat	C 71019	0041493	005633

* An acceptable study submitted after the Registration Standard was issued.

EPA No.: 68D80056
DYNAMAC No.: 371-A
TASK No.: 3-71A
June 12, 1991

008421

DATA EVALUATION RECORD

AMITRAZ

Mutagenicity--Mammalian Cells in Culture Cytogenetic Assay in Human Lymphocytes

APPROVED BY:

Robert J. Weir, Ph.D.
Program Manager
Dynamac Corporation

Signature: Robert J. Weir

Date: 6-12-91

Guideline Series 84: **MUTAGENICITY**

EPA No.: 68D80056
DYNAMAC No.: 371-A
TASK No.: 3-71A
June 12, 1991

DATA EVALUATION RECORD

AMITRAZ

Mutagenicity--Mammalian Cells in Culture Cytogenetic Assay in Human Lymphocytes

REVIEWED BY:

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Principal Reviewer
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Signature: Nancy E. McCarroll
Date: 6-12-91

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Signature: Y. M. Ioannou
Date: 6/18/91

MAMMALIAN CELLS IN CULTURE CYTOGENETIC ASSAY

DATA EVALUATION RECORD

Tox. Chem. No.:
EPA File Symbol:

CHEMICAL: Amitraz.

STUDY TYPE: Mutagenicity--Mammalian cells in culture cytogenetic assay in human lymphyocytes.

ACCESSION OR MRID NUMBER: 417951-01.

SYNONYMS/CAS NUMBER: N-methylbis(2,4-xylyliminomethyl)amine; N,N-di-(2,4-xylyliminomethyl)-methylamine.

SPONSOR: Schering Agrochemicals Ltd., Federal Republic of Germany.

TESTING FACILITY: Huntingdon Research Centre, Huntingdon Cambridgeshire, England.

TITLE OF REPORT: T300 Technical Amitraz Metaphase Chromosome Analysis of Human Lymphocytes Cultured in vitro.

AUTHORS: Brooker, P.C., Akhurst, L.C., and Gray, V.M.

STUDY NUMBER: TOX 88253.

REPORT ISSUED: November 22, 1988.

MAMMALIAN CELLS IN CULTURE CYTOGENETIC ASSAY

CONCLUSIONS - Executive Summary:

Under the conditions of the assay, three nonactivated (5, 10, and 20 µg/mL) and three S9-activated (3, 15, and 30 µg/mL) doses of amitraz technical failed to induce a clastogenic effect in cultured human lymphocytes. The highest nonactivated dose induced a moderate cytotoxic effect; under S9-activated conditions, the test material was assayed to the solubility limit. We conclude, therefore, that an appropriate range of nonactivated and S9-activated doses were evaluated and that amitraz technical was found to be negative in this in vitro test system. The study, therefore, satisfies Guideline requirements for genetic effects Category II, Structural Aberrations.

Study Classification: The study is acceptable.

Recommendation: It is recommended that future in vitro human lymphocyte assays be conducted with replicate cultures from different donors or that separate experiments with cells from different donors be performed.

A. MATERIALS:

1. Test Material:

Name:	Amitraz technical.
Description:	Fine, off-white crystalline powder.
Batch/Lot No.:	CR 17612/4.
Purity:	99.5%
Contaminants:	None listed.
Solvent used:	Dry ethanol (ETOH).
Other comments:	The test material was stored at room temperature protected from light and humidity. The test material was dissolved in ETOH immediately prior to use.

2. Control Materials:

Negative: None.

Solvent/concentration: ETOH/10 µL/mL.

Positive: Nonactivation (concentrations, solvent): Ethyl methanesulfonate (EMS) was prepared in dimethyl sulfoxide to yield a final concentration of 1000 µg/mL.

Activation (concentrations, solvent): Cyclophosphamide (CP) was prepared in distilled water to yield a final concentration of 20 µg/mL.

3. Activation: S9 derived from CD male Sprague-Dawley

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

The S9 homogenate was prepared by the performing laboratory and was tested for its ability to metabolize 7,12-dimethyl benz(a)anthracene to a mutagen prior to use.

S9 mix composition:

Component	Volume
0.1 M NADP (sodium salt)	0.04 mL
1.0 M Glucose 6-phosphate	0.005 mL
0.4 M Magnesium chloride	0.02 mL
0.2 M Disodium phosphate (pH 7.4)	0.5 mL
Distilled water	0.335 mL
S-9	0.10 mL

4. Test Compound Concentrations Used:

a. Preliminary cytotoxicity assay: Ten doses (0.06, 0.1, 0.2, 0.5, 0.9, 1.9, 3.8, 7.5, 15.0, and 30.0 µg/mL) were assayed with and without S9 activation for adverse effects on the mitotic index (MI).

b. Cytogenetic assay:

1) Nonactivated conditions: The three concentrations evaluated without S9 activation were 5, 10, and 20 µg/mL.

2) S9-activated conditions: The three concentrations evaluated with S9 activation were 3, 15, and 30 µg/mL.

5. Test Cells: Human lymphocytes were collected and diluted with RPMI 1640 culture medium; no information on the donor was provided. Lymphocytes were separated on a Histopaque-1077 gradient, washed, resuspended at a density of 1×10^6 cells/mL in RPMI 1640 containing 2% phytohemagglutinin (PHA) and 20% fetal calf serum, dispensed in 1-mL volumes into multiwell culture dishes, and incubated at 37°C for ≈48 hours.

Properly maintained? Yes.

Cell line or strain periodically checked for mycoplasma contamination? Not applicable.

Cell line or strain periodically checked for karyotype stability? Not applicable.

B. TEST PERFORMANCE:

1. Cell Treatments:

a. Cells exposed to test compound for:
22 hours (nonactivated) 2 hours (activated)

b. Cells exposed to positive controls for:
22 hours (nonactivated) 2 hours (activated)

c. Cells exposed to negative and/or solvent controls for:
22 hours (nonactivated) 2 hours (activated)

2. Protocol:

a. Preliminary cytotoxicity assay: Duplicate cultures of PHA-stimulated lymphocytes were exposed to 10 nonactivated and 10 S9-activated concentrations of the test material (0.06 to 30.0 µg/mL); four replicate cultures were exposed to the solvent (ETOH). Under nonactivated conditions, cells were exposed for 22 hours; in the presence of S9-activation, cells were dosed for 2 hours, rinsed, resuspended in fresh medium, and reincubated. Twenty-two hours posttreatment, colchicine (0.25 µg/mL) was added. After 2 hours, metaphase cells were collected, treated with a hypotonic solution, fixed in acetic acid:methanol (1:3), and stained with Giemsa. The mitotic index (MI) was determined from 1000 cells scored from each culture.

b. Cytogenetic assay:

1) Treatment: Duplicate cultures, prepared as described, were exposed to the selected nonactivated and S9-activated test material doses or the positive controls (1000 µg/mL EMS -S9 or 20 µg/mL CP +S9). Quadruplicate cultures were prepared for the nonactivated and S9-activated solvent control (ETOH). The assay was conducted as described for the preliminary cytotoxicity assay.

2) Metaphase analysis: Slides were coded prior to scoring. One hundred metaphase cells/culture for the test and the control groups were scored for structural aberrations; MIs were determined.

3) Statistical methods: The data were evaluated at $p < 0.001$ by Fisher's test.

4) Evaluation criteria: No criteria to establish the validity of the assay or the biological significance of the results were provided.

C. REPORTED RESULTS:

1. Preliminary Assay: The report indicated that final concentrations of the test material >30 µg/mL were not soluble in culture medium; therefore, this level was considered to be the solubility limit of amitraz in this test system. Accordingly, 10 doses of the test material ranging from 0.06 to 30.0 µg/mL +/-S9 were evaluated for adverse effects on the MI. In the absence of S9 activation, the two highest doses (15 and 30 µg/mL) caused a marked reduction in mitotic cells compared to the solvent control. At these levels, the percentage MI was 6.1% at 15 µg/mL and 3.7% at 30 µg/mL. Below 15 µg/mL, there was no appreciable effect on mitosis. MIs for cells exposed to all S9-activated doses of the test material were slightly higher than the solvent control. Based on these findings, the doses selected for the cytogenetic assay were 2, 10, and 20 µg/mL -S9 and 3, 15, and 30 µg/mL +S9.

2. Cytogenetic Assay: The report indicated that the highest nonactivated dose (20 µg/mL) was excessively cytotoxic; therefore, the nonactivated cytogenetic assay was repeated with six doses (1, 5, 7.5, 10, 15, and 20 µg/mL). Results for the initial assay were not presented. The study authors stated that in the repeat nonactivated test, sufficient metaphases were available for the analysis of cultures exposed to 20 µg/mL; accordingly the 5-, 10-, and 20-µg/mL treatment groups were evaluated for chromosome aberrations.

Representative results from the repeat nonactivated assay and the S9-activated assay are presented in Table 1. As shown, the MI for the highest nonactivated dose (20 µg/mL) was lower than the control, indicating that this level induced a moderate cytotoxic effect. No chromosome aberrations were seen at this level or at the intermediate level (10 µg/mL). However, one cell with >10 aberrations

TABLE 1. Representative Results of the Human Lymphocyte in vitro Cytogenetic Assay with Amitraz

Substance	Dose/mL	S9 Activation	No. of Cells Scored	Average Mitotic Index	Total[a] No. of Aberrations	No. of[a] Cells with Aberrations	Percent[a] Cells with Aberrations	Biologically[b] Significant Aberrations (No./Type)
Solvent Control								
Ethanol	10 µl	-	400	11.3	1	1	0.25	1TBF
	10 µl	+	400	19.0	10	10	2.50	3TBF; 1SM; 6AF
Positive Control								
Ethylmethane sulfonate	1000 µg	-	200	ND[b]	>132[c]	38	19.00*	72TBF 10I; 4SM; 36AF; 1GT
Cyclophosphamide	20 µg	+	200	ND[b]	>192[c]	92	46.00*	61TBF; 7TB; 16I; 37SM; 1R; 36AF; 3GT; 4P
Test Material								
Amitraz	20 µg[d]	-	200	6.3	0	0	0.00	--
	30 µg[d]	+	200	20.0	2	2	1.00	2SM

[a]Gaps excluded.

[b]Abbreviations used:

TBF - Chromatid break with fragment
TB - Chromatid break without fragment
SM - Single minute
AF - Acentric fragment
I - Interchange
R - Ring
GT - >10 Aberrations/cell
P - Pulverized cell.
ND - Not determined

[c]Value differs from the reported value; our reviewers counted GT as >10 aberrations, while the study authors counted GT as one aberration.

[d]Highest assayed level; results for lower doses (5 and 10 µg/mL-S9 and 3 and 15 µg/mL +S9) did not suggest a clastogenic effect.

*Significantly higher ($p < 0.001$) that the solvent control as determined by Fisher's test.

was scored in the low-dose (5 µg/mL) cultures. This finding in one of 200 cells was not considered by our reviewers to be sufficient evidence of potential clastogenicity.

In the presence of S9 activation, amitraz was neither cytotoxic nor clastogenic. By contrast, highly significant ($p < 0.001$) increases in percentage of aberrant cells were scored from cultures exposed to the nonactivated (1000 µg/mL EMS -S9) and the S9-activated (20 µg/mL CP +S9) positive controls.

Based on the overall results, the study authors concluded that amitraz technical was not clastogenic in this in vitro test system.

D. REVIEWER'S DISCUSSION/CONCLUSION:

We assess that the results of this study provide no indication that amitraz was clastogenic in this human lymphocyte cytogenetic assay. We further assess that the highest nonactivated dose induced a cytotoxic effect and that under S9-activated conditions, the test material was assayed to the limit of solubility.

In addition, the sensitivity of the test system to detect a clastogenic response was adequately demonstrated by the results achieved with the positive control.

We conclude, therefore, that technical amitraz was assayed over an appropriate range of nonactivated and S9-activated concentrations and failed to induce a clastogenic response.

Based on the limited information we assume that the lymphocyte cultures were derived from a single donor. Guidelines do not specifically require that human lymphocyte cytogenetic assays be conducted with replicate cultures from different donors or that separate assays with different donor cells be performed; however, this is a prudent and recommended approach.

E. QUALITY ASSURANCE MEASURES: A quality assurance statement was signed and dated November 14, 1988.

F. CBI APPENDIX: Appendix A, Materials and Methods, CBI pp. 12-15.

APPENDIX A

Materials and Methods
(CBI pp. 12-15)

AMITRAZ Reviews

Page _____ is not included in this copy.

Pages 13 through 16 are not included in this copy.

The material not included contains the following type of information:

___ Identity of product inert ingredients

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___ Description of the product manufacturing process

___ Description of product quality control procedures

___ Identity of the source of product ingredients

___ Sales or other commercial/financial information

___ A draft product label

___ The product confidential statement of formula

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