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

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OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

TOX Chem No.: 352H
TB Project No.: 9-1736
RD Record Nos.: 247,679

MEMORANDUM

SUBJECT: Imazapyr (ARSENAL) - Mutagenicity Data Submitted under Accession No. 260,000
EPA ID No. 241-273

FROM: Irving Mauer, Ph.D., Geneticist
Toxicology Branch I - Insecticide, Rodenticide Support
Health Effects Division (H7509C)

11/03/89

TO: Robert J. Taylor, PM 25
Fungicide-Herbicide Branch
Registration Division (H7505C)

THRU: Karl P. Baetcke, Ph.D., Chief
Toxicology Branch I - Insecticide, Rodenticide Support
Health Effects Division (H7509C)

Karl P. Baetcke 11/07/89

Registrant: American Cyanamid (AC), Princeton, NJ

Request

Review and evaluate the following mutagenicity studies:

Study 1a. Cytotoxicity Pilot Study in Male Albino Rats with AC 243,997, performed by ToxiGenics Inc., Decatur, IL, Project No. 450-1283, Final Report dated August 30, 1983.

Study 1b. (DLT) Dominant Lethal Assay in Male Albino Rats with AC 243,997, performed by ToxiGenics, Inc., Decatur, IL, Project No. 450-1284, Final Report dated January 30, 1984.

Study 2. (UDS) Unscheduled DNA Synthesis Rat Hepatocyte Assay with AC 243,997, performed by Hazleton Laboratories America, Inc., Vienna, VA, Study No. 362-170, Final Report dated January 21, 1984.

Study 3. (CA) Vitro Chromosomal Aberrations in Chinese Hamster Ovary Cells with AC 243,997, performed by Hazleton Laboratories America, Inc., Vienna, VA, Study No. 362-169, Final Report dated February 3, 1984.

Study 4. (HGPRT) Mutagenicity Testing of AC 243,997 in the in vitro CHO/HGPRT Mutation Assay, performed at American Cyanamid, GTOX Volume 4, Number 1, Final Report dated February 17, 1984.

TB Conclusions

Study	Reported Results	TB Evaluation
1. DLT	Although reported as negative, major procedural and reporting deficiences exist	UNACCEPTABLE
2. UDS	Although reported as negative, major procedural and reporting deficiencies compromise the study	UNACCEPTABLE
3. CA	Negative up to a toxic dose, 5000 mcg/mL)	ACCEPTABLE
4. HGPRT	Negative up to toxic doses (5000 mcg/mL +)	ACCEPTABLE

Detailed reviews are appended to this memorandum.

Attachments (DERs)

Reviewed By: Irving Mauer, Ph.D., Geneticist 11/03/89
Toxicology Branch I - IRS (H7509C)
Secondary Reviewer: Karl P. Baetcke, Ph.D., Chief Karl P. Baetcke 11/07/89
Toxicology Branch I - IRS (H7509C)

DATA EVALUATION RECORD

I. SUMMARY

MRID No.: 260,000
ID No.: 241-273
RD Record No.: 247,679
Shaughnessy No.: 128821
Caswell No.: 352H
Project No.: 9-1736

Study Type: Mutagenicity - Chromosome damage in vivo (Rat dominant lethal test, DLT)

Chemical: Imazapyr

Synonyms: ARSENAL; AC 243,997

Sponsor: American Cyanamid, Princeton, NJ

Testing Facility: Toxigenics, Decatur, IL

Title of Report: Dominant Lethal Assay in Male Albino Rats with AC 243,997.

Authors: Dale A. Mayhew, Clare M. Salamon and Peter V. Enloe

Study Number: 450-1284

Date of Issue: January 30, 1984

TB Conclusions:

The test substance appeared to be nontoxic on oral administration, as well as apparently without effect on reproductive (fertility) or mutagenic (induction of dominant lethals) indices at doses up to 1000 mg/kg/day for 5 days.

Classification (Core-Grade)

UNACCEPTABLE, because 1) there was insufficient documentation that the test material was absorbed and transported to target; and 2) the full spermatogenic cycle was not sampled.

II. DETAILED REVIEW

A. Test Material - AC 243,997 [nicotinic acid, 2-(4-isopropyl-4-methyl)-5-oxo-2-imidazolin-2-yl].

Description: Light tan powder
Batch (Lot): AC 4391-97
Purity (%): 94 (nitrosamines < 1 ppm)
Solvent/Carrier/Diluent: 0.1% Tween 80 in deionized water (DW)

B. Test Organism - Rodent

Species: Rat
Strain: CD Albino
Age: 68 days
Weights - Males: 390 g
Females: (not provided)
Source: Charles River, Portage, MI

C. Study Design (Protocol) - This study was designed to assess the mutagenic potential of imazapyr when administered by gavage to male rats for 5 days. The procedures employed nominally followed standard techniques as reported in published literature.

Statements affirming compliance with GLPs as well as Quality Assurance measures (inspections/audits) were provided in the Final Report.

D. Procedures/Methods of Analysis - Dose selection for this study was determined by a preliminary toxicity assay in males of the same strain of rat,* which received test compound at oral doses of 125, 250, 300, 1000 or 2000 mg/kg/day for 5 days. Animals were observed for 6 days, weighed on postdose days 1, 2, and 5, then injected with colchicine (for cytogenetic examination of the bone marrow cells) and sacrificed 2 to 4 hours later. Femoral bone marrow was harvested,** and thoracic and abdominal organs examined for gross changes.

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*(Unpublished) Cytotoxicity Pilot Study in Male Albino Rats with AC 243,997, performed by ToxiGenics, Inc., Decatur, IL, ToxiGenics Study No. 450-1283, August 30, 1983.

**Cytogenetic analysis of bone marrow preparations was conducted by Microbiological Associates, Bethesda, MD (see below).

For the dominant lethal study, groups of 10 males each were gavaged with AC 243,997 at levels of 0 (DW), 250, 500 or 1000 mg/kg/day for 5 days, while a fifth group of 10 animals served as positive control, and received the mutagen, triethylenemelamine (TEM, 0.5 mg/kg), as a single ip injection on test day 5 of the study. Two days after the final treatment, males were caged with untreated females (1 male:2 females) for 1 week, then caged sequentially with fresh females weekly for an additional 7 weeks of matings.

Females of each week's matings were sacrificed 10 days following the final day of cohabitation, and uteri examined for implantation sites, viable fetuses, early fetal deaths (deciduomata), and late fetal deaths. Males were killed after completion of mating, and testes (with epididymides) fixed for later histological examination.

The following reproduction parameters were calculated for each treatment group for each mating week:

1. Male fertility index = $\frac{\text{Number of Males Siring at Least 1 Litter}}{\text{Total Number of Males Paired}} \times 100$

2. Female fertility index = $\frac{\text{Number of Pregnant Females}}{\text{Total Number of Females Paired}} \times 100$

3. Preimplantation loss = $\frac{\text{Number of Corpora Lutea - Number of Implantations}}{\text{Number of Corpora Lutea}} \times 100$

4. Mutation rate = $\frac{\text{Number of Deciduomata}}{\text{Number of Implantation Sites}} \times 100$

These four indices were statistically analyzed by Chi-Square; all other enumeration data (numbers of corpora lutea, implants, resorptions, and live fetuses) were analyzed by ANOVA, with any resulting differences further tested (if needed) by Tukey's or Scheffe's analysis for multiple comparisons.

E. Results - In the preliminary toxicity test, salivation was the only recurring clinical observation, especially in 1000 and 2000 mg/kg-dosed animals. Gross necropsy revealed no compound-related alterations, and evaluation of bone-marrow slides for mitotic index (MI) was also negative for significant differences ($p > 0.05$, one-way ANOVA) between test groups (MI ranging from 1.4% at 125 mg/kg to 2.3% at 2000 mg/kg) and the vehicle control (MI = 1.6%) [Report APPENDIX A].

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All males treated in the dominant lethal (main) assay survived the entire study period, without any apparent adverse clinical effects. Final body weights of treated animals were comparable to controls (548, 546 and 549 g for test groups 1, 2 and 3, compared to 544 g for vehicle control) and, except for a small left testis recorded in two animals (one control and one high-dose), no other gross pathologic alterations were found.

In contrast to definitively positive reproductive and mutagenic findings in positive control females mated to males treated with TEM (decreased implants in weeks 1 through 4; increased deciduomata, with concomitant decrease in viable fetuses in weeks 1-5) random significant fluctuations from control values were recorded for the three test groups (Report Tables 2 and 3, data extracts from which are summarized on the page following). These changes were considered by the authors as reflecting strain variance rather than compound-related, since they were sporadic, without consistent direction and unrelated to dose.

No reproductive effects different from control were found in test groups, and fertility was unaffected by imazapyr treatment.

Hence the authors concluded the test substance was not mutagenic in this assay under the conditions designed.

F. TB Evaluation - UNACCEPTABLE because of the following:

1. The authors have not demonstrated absorption from the gi tract, and transport of the test material to target tissue in sufficient concentrations to be effective. The highest dose tested in the main assay, 1000 mg/kg/day, was nontoxic, and did not affect fertility or any reproductive parameters. That the material was apparently not absorbed by the oral route to any great extent was confirmed in the preliminary toxicity trial, where mitotic indices in bone marrow cells were reportedly not impacted by the administration of double the high dose of the main assay, namely 2000 mg/kg/day. The positive control was effective, perhaps because it was given ip, assuring greater availability systemically.

2. Since the spermatogenic cycle in rats ranges up to 10 weeks, the investigators did not sample that portion of the cycle whose elements are probably the most important for risk assessment, namely, spermatogonia.

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Effect of Imazapyr on Reproductive
And Mutagenic Indices in Rats[1/]

Mating Week	Index	Dose Groups (mg/kg) 0	250	500	1000	TEM
1	PI[2/]	4.6	3.7	3.5	6.1	24.2**
	MR[3/]	8.0	2.4**	4.9	4.3	61.7**
2	PI	9.7	12.7	9.7	10.4	46.7**
	MR	5.6	4.4	6.1	4.7	60.5**
3	PI	14.1	8.6	7.6*	10.4	70.9**
	MR	9.1	6.4	6.6	2.4**	100.00**
4	PI	16.2	10.8	12.6	13.5	70.4**
	MR	8.3	2.6**	4.7	4.6	89.6**
5	PI	8.9	10.5	9.0	19.8**	9.0
	MR	10.5	3.7**	5.4*	8.4	46.9**
6	PI	6.6	16.8**	12.3*	15.5**	20.7**
	MR	10.4	4.6*	3.0**	4.7*	9.7
7	PI	14.9	22.7	14.9	14.0	15.5
	MR	8.0	6.4	5.8	2.4**	7.9
8	PI	11.6	12.4	12.5	13.3	14.0
	MR	11.5	6.0*	7.0	7.8	4.8**

*Statistically significant difference, $p < 0.05$.
**Statistically significant difference, $p < 0.01$.
1/Extracted from Tables 2 and 3 of the Final Report.
2/PI, preimplantation loss.
3/MR, mutation rate.

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TB recommends repeating the same assay (subacute treatment followed by 10 weeks of mating) but employing ip administration (following proper dose selection for this route), with the full cycle of mating weeks for rats, in order to a) assure distribution of test material in effective amounts to the target, male germinal epithelium, and b) to sample all stages of the cycle. Alternatively, the investigators may treat males for the full 10 weeks of the spermatogenic cycle, and sample but twice thereafter. This saves animals, while still satisfying the data requirement for this type of mutagenic assay.

Attachments

ATTACHMENT A

Procedures:

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_____ The product confidential statement of formula.

_____ Information about a pending registration action.

✓ FIFRA registration data.

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Reviewed By: Irving Mauer, Ph.D., Geneticist
Toxicology Branch I - IRS (H7509C)
Secondary Reviewer: Karl P. Baetcke, Ph.D., Chief
Toxicology Branch I - IRS (H7509C)

DATA EVALUATION RECORD

I. SUMMARY

MRID No.: 260,000
ID No.: 241-273
RD Record No.: 247,679
Shaughnessy No.: 128,821
Caswell No.: 352H
Project No.: 9-1736

Study Type: Mutagenicity - Foreward gene mutation in mammalian cells (Hypoxanthine-guanine phosphoribosyl transferase in Chinese hamster cells, HGPRT/CHO)

Chemical: Imazapyr

Synonyms: ARSENAL; AC 243,997

Sponsor: American Cyanamid, Princeton, NJ

Testing Facility: American Cyanamid, Princeton, NJ

Title of Report: Mutagenicity Testing of AC 243,997 in the in vitro CHO/HGPRT Mutation Assay.

Authors: E. Johnson and J.S. Allen

Study Number: 0493

Date of Issue: February 17, 1984

TB Conclusions:

Test substance was demonstrated to be negative for inducing forward mutations at the HGPRT locus in CHO cells, when these cells were exposed to concentrations into the toxic range (5000 mcg/mL and higher).

Classification (Core-Grade): ACCEPTABLE

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II. DETAILED REVIEW

A. Test Material - AC 243,997 (Imazapyr)

Description: White powder
Batch (Lot): AC 4391-97
Purity (%): 93
Solvent/Carrier/Diluent: Dimethylsulfoxide (DMSO)

B. Test Organism - Established cell line

Species: Chinese hamster ovary (CHO)
Strain: K_1-BH_4
Source: J.P. O'Neill, University of Vermont

C. Study Design (Protocol) - This study was designed to assess the mutagenic potential of AC 243,997 when administered in vitro to CHO cells with/without metabolic activation. The procedures employed were those established in the literature by expert practitioners.

A statement affirming compliance with Agency GLPs was provided.

A Statement of Quality Assurance measures (inspections/audits) was provided.

D. Procedures/Methods of Analysis - Following preliminary cytotoxicity testing, triplicate cultures of cells were exposed for 5 hours to test substance at each of five concentrations (up to the limit 5000 mcg/mL), in the absence or presence of a mammalian metabolic activation system prepared from Aroclor 1254-induced rat liver (S9 mix). In addition to a solvent control (DMSO), positive controls were run concurrently, respectively the direct-acting mutagen ethylmethane sulfonate (EMS, 200 mcg/mL) for the nonactivated series, and 7,12-dimethylbenz(a)anthracene (DMBA, 7 mcg/mL) in the presence of S9.

One day after transfer to fresh culture medium without test substance, treated cells were subcultured for phenotypic expression (every 2 days until day 9 post-dose), then plated onto selection medium containing 6-thioguanine (TG) for mutant colony enumerations. Mutation frequency (MF) was calculated by dividing the total number of mutant colonies by the number of cells tested (corrected for cloning efficiency) and expressed as mutants per 10^6 surviving cells.

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Concurrent determinations of cytotoxicity and cloning efficiency were made from aliquots of the same treated cultures.

The entire experiment was repeated once at doses up to 12,000 mcg/mL test substance.

E. Results - In the preliminary toxicity test, only the HDT, 5000 mcg/mL, showed evidence of toxicity (Report Tables 1 and 2), moderate in the presence of S9 (56.8% relative survival) but severe in nonactivated cultures (100% lethal). Hence, the initial mutation experiment was performed at five doses of 250, 500, 1000, 2500, and 5000 mcg/mL with S9, but only up to 2500 mcg/mL in the absence of S9.

In this first trial, cloning efficiencies were unaffected by AC 243,997 treatment at any dose, being equivalent to solvent controls (Report Tables 3 to 6), and mutation frequencies were likewise similar to solvent controls (Report Tables 7 and 8, attached to this DER).

In the repeat assay, dose levels up to 12,000 mcg/mL +S9, and up to 10,000 mcg/mL -S9, were used in preliminary cytotoxicity testing, but the highest concentrations proved too severely toxic: In S9-activated cultures 100 percent lethality at 12,000 mcg/mL, 46 percent survival at 9,000 mcg/mL (Table 9); without S9, no cells survived 7500 or 10,000 mcg/mL, but survival was at control levels below that (Table 10). As in the first trial, all dose levels of test substance produced mutation frequencies comparable to solvent control values (Report Tables 13 and 14, attached here).

By contrast, both positive controls performed as expected, inducing MFs from 12 to > 20 times DMSO values.

The authors concluded that AC 243,997 was not mutagenic at the HGPRT locus in CHO cells when assayed in repeat experiments up to toxic concentrations.

F. TB Evaluation - ACCEPTABLE. The test compound was assayed adequately with appropriate controls such that the negative results obtained may be judged valid.

Attachments

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ATTACHMENT

Data Tables

(Report Tables 7, 8, 13, 14)

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Reviewed By: Irving Mauer, Ph.D., Geneticist
Toxicology Branch I - IRS (H7509C)
Secondary Reviewer: Karl P. Baetcke, Ph.D., Chief
Toxicology Branch I - IRS (H7509C)

11-03-89
11/07/89

DATA EVALUATION RECORD

I. SUMMARY

MRID No.: 260,000
ID No.: 241-273
RD Record No.: 247,679
Shaughnessy No.: 128821
Caswell No.: 352H
Project No.: 9-1736

Study Type: Mutagenicity = Chromosome damage in vitro (in Chinese hamster ovary cells, CHO)

Chemical: Imazapyr

Synonyms: ARSENAL; AC 243,997

Sponsor: American Cyanamid, Princeton, NJ

Testing Facility: Hazleton Labs America (HLA), Vienna, VA

Title of Report: In Vitro Chromosomal Aberrations in Chinese Hamster Ovary Cells with AC 243,997.

Authors: M.G. Farrow and T. Cortina

Study Number: 362-169

Date of Issue: February 3, 1984

TB Conclusions:

The test compound was demonstrated to have no clastogenic activity in CHO cells exposed to concentrations up to 5000 ug/mL, a dose producing some signs of toxicity.

Classification (Core-Grade): ACCEPTABLE

II. DETAILED REVIEW

A. Test Material - AC 243,997

Description: Off-white powder
Batch (Lot): AC 4361-97
Purity (%): 93
Solvent/Carrier/Diluent: Dimethylsulfoxide (DMSO)

B. Test Organism - Established cell line

Species: Chinese hamster (ovary)
Strain: K-1
Source: American Type Culture Collection (ATCC), Rockville, MD (CCL61)

C. Study Design (Protocol) - This study was designed to assess the clastogenic (chromosome-breaking) potential of imazapyr when administered in vitro to CHO cells exposed to limit doses. Standardized procedures were used for this assay.

A statement affirming compliance with Agency GLPs was provided.

A Statement of Quality Assurance measures (inspections/audits) was provided.

D. Procedures/Methods of Analysis - Following preliminary dose-selection testing, monolayer cultures of CHO cells were exposed for 2 hours in triplicate to five concentrations of test substance (50, 170, 500, 1700 or 5000 ug/mL) in the absence or presence of metabolic activation (referred to as "S-9 mix," and prepared from Aroclor 1254-induced rat liver). Control cultures were run concurrently: solvent controls exposed only to DMSO; nonactivated positive controls treated with mitomycin-C (MC, 1.0 ug/mL), and activated cultures with cyclophosphamide (CP, 140 ug/mL).

Cultures were harvested 3, 8 and 12 hours after treatment, following exposure to the metaphase-arresting agent, colcemid, and five microscope slides prepared for each treatment group according to conventional cytological procedures.

One hundred metaphase cells from each group's coded slides (50 per duplicate flask) were scored for number and type of chromosome aberration (according to established convention) and modal number. Mitotic indices were determined from the number of cells in metaphase per 1000 cells counted.

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Data on aberrations were analyzed by Chi-Square (percent aberrant metaphases), and ANOVA with Students t-test (for mean aberrations per cell; and mean modal number). The level of significance was chosen as $p \leq 0.01$.

E. Results - The test compound was apparently nontoxic at dose levels up to 4000 ug/mL, which generated a mitotic index of 5.8 (compared to a solvent control value of 6.4) and relative growth of 91 percent (Report Table 2).

In the main assay the test material did not induce any statistically significant increases in percent aberrant cells or mean aberrations/cell at any dose level up to 5000 ug/mL, or at any sampling time, with or without metabolic activation (Report Table 4, attached to this DER). Both positive controls performed appropriately at the 8- and/or 12-hour sampling.

Modal number analysis revealed statistically significant ($p < 0.01$) differences from the strain mean of 20 chromosomes for nonactivated test groups at 12 hours; however, mean values ranged between 19 and 21 chromosomes (Report Table 6). Since the CHO line can vary in karyotype and chromosome number, the investigators did not consider these differences compound-related.

The test substance produced some decrease in mitotic index, as reflected in reductions in relative growth percentage, in nonactivated cultures. No such trend was evident under activated conditions (Table 7).

The authors concluded that AC 243,997 did not appear to be clastogenic under the conditions of this assay.

F. TB Evaluation - ACCEPTABLE. The test substance appeared to have been assayed in an appropriate manner and with proper controls. Hence the negative result obtained is considered a valid conclusion from the procedures employed.

Attachment

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ATTACHMENT A

Summary Data Table

Page _____ is not included in this copy.

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Toxicology Branch I - IRS (H7509C)

DATA EVALUATION RECORD

I. SUMMARY

MRID No.: 260,000
ID No.: 241-273
RD Record No.: 247,679
Shaughnessy No.: 128,821
Caswell No.: 352H
Project No.: 9-1736

Study Type: Mutagenicity = DNA damage/repair in vitro (Unscheduled DNA Synthesis in rat hepatocytes, HPC/UDS)

Chemical: Imazapyr

Synonyms: ARSENAL; AC 243,997

Sponsor: American Cyanamid, Princeton, NJ

Testing Facility: Hazleton Labs America (HLA), Vienna, VA

Title of Report: Unscheduled DNA Synthesis Rat Hepatocyte Assay with AC 243,997.

Authors: M.G. Farrow and R.C. Sernau

Study Number: 362-170

Date of Issue: January 26, 1984

TB Conclusions:

The test substance was reported as negative for inducing unscheduled DNA synthesis in hepatocytes from a male Sprague-Dawley rat treated in vitro up to 5000 ug/mL, as measured by nuclear grain counts.

Classification (Core-Grade):

UNACCEPTABLE, due to a number of major procedural and reporting deficiencies (see TB Evaluation at F).

II. DETAILED REVIEW

A. Test Material - AC 243,997

Description: Off-white powder
Batch (Lot): AC 4361-97
Purity (%): 93
Solvent/Carrier/Diluent: Dimethylsulfoxide (DMSO)

B. Test Organism - Rodent hepatocytes

Species: Rat
Strain: Sprague-Dawley
Age: "Adult"
Weights - Males: (not given)
Females: (not used)
Source: Charles River, Kingston, NY

C. Study Design (Protocol) - This study was designed to assess the genotoxic potential of imazapyr when administered in vitro to primary rat hepatocyte cultures.

A copy of the procedures employed is appended to this DER (from the investigator's FINAL REPORT).

A statement affirming compliance with Agency GLPs was provided as well as a Statement of Quality Assurance measures (inspections/audits).

D. Procedures/Methods of Analysis - From preliminary cytotoxicity testing, 5000 ug/mL was selected as the highest dose to be assayed, based on minimal toxicity and solubility considerations. Accordingly, primary monolayer cultures of fresh hepatocytes from an SD male were established on coverslips, and exposed (in triplicate) to solvent control (DMSO) or to six concentrations of test material (10, 50, 100, 500, 1000 and 5000 ug/mL), together with 10 uCi/mL tritiated thymidine (^{3}H-TdR). A series of positive control cultures were treated with 2-acetylaminofluorene (2AAF) at levels of 0.05, 0.10, or 0.5 ug/mL. After 24 hours incubation, cytotoxicity was determined by trypan blue exclusion, and the remaining treated cultures fixed, mounted on microscope slides and treated with photographic emulsion for the development of silver grains. Following 4 days storage in the dark at refrigerator temperatures, the slides were treated with D-19 developer, fixed, then stained with 1:25 (v/v) Giemsa:Dulbecco's saline.

4/3

Nuclei of 50 morphologically normal cells on coded slides from each treatment were scored for silver grains under oil immersion by an automated colony counter, and net nuclear grain count (NNGC) calculated for each cell by subtracting the mean background cytoplasmic count.

For statistical purposes, "zero" was adopted for any calculated NNGC less than zero. The criterion for a significant (positive) test result was considered to be a mean NNGC greater than three standard deviations of the solvent control value, preferably at two or more consecutive doses.

E. Results - Compared to the definitively positive response of the 2AAF control cultures (over 38 times the vehicle control), at no concentration up to 5000 ug/mL did the test substance increase grain counts above the stated criterion for a positive, namely, three times the SD plus control value = 1.62 x 3 + 0.65 = 5.51 (Report Table 1, appended to this DER as ATTACHMENT B).

Hence, the authors concluded that AC 243,997 was negative for UDS in this rat HPC assay.

F. TB Evaluation - This purported negative study was not conducted according to currently recognized procedures of expert practitioners. The following deficiencies compromise the acceptability of the study results:

1. Storage time of the photographic emulsion-covered slides (to allow the appearance of sufficient silver grains to discriminate a positive response) was only 4 days, insufficient compared to the usual 7 to 10 days employed by some experts, and up to 2 weeks by others.

2. The tabulation provided (Report Table 1 see Appendix B to this DER) does not reflect the method of displaying grain count data as stated in the Methods section. "Mean net nuclear grain counts" were to be obtained, but Table 1 shows "mean nuclear grain count," as though background cytoplasmic counts were not taken into account.

3. Further absent are tabulations of:

   % of cells in repair
   % of cells with > 20 grains
   % cells in replicative (scheduled) DNA synthesis (SDS)

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4. Finally, the assay was not repeated (preferably with heptatocytes isolated from a female rat), to confirm the presumptive negative.

Attachments

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ATTACHMENT A

Procedures

Page _____ is not included in this copy.

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