10-17-96

MEMORANDUM

SUBJECT: CHLOROTHALONIL - Reviews of Two Mutagenicity Studies

PC Code: 081901 **DP Barcode:** 217614 **Tox. Chem. No.:** 215B
Submission: S490761 **MRID Nos.:** 43700601 and 43700602

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Special Review and Reregistration Division (7508W)

THRU: Jess C. Rowland, M.S., Acting Section Head
Review Section I, Toxicology Branch II
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and

Yiannakis M. Ioannou, Ph.D., Acting Branch Chief
Toxicology Branch II
Health Effects Division (7509C)

Registrant: ISK Biosciences Corporation, Mentor, OH

REQUEST: Review two mutagenicity studies with CHLOROTHALONIL: in vivo bone marrow cytogenetic assay in the rat (MRID No. 43700601) and in vivo bone marrow cytogenetic assay in the hamster (MRID No. 43700602).

RESPONSE:

Both studies are classified as Acceptable and satisfy the Subdivision F Guideline requirement (§84-2) for in vivo cytogenetic mutagenicity studies. The study in hamsters (MRID No. 43700602) resolved the inconclusive results of a previous cytogenetic assay in hamsters (Acc. No. 00147948). The Exective Summaries are as follows:

cc: Tom Myers (RCAB, HED, 7509C)

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EXECUTIVE SUMMARIES

Five-Day Repeated-Dose Chromosomal Aberration Test In vivo with SB-341 Using Rats - MRID No.: 43700601

In an in vivo bone marrow cytogenetic assay (MRID No.: 43700601), groups of five male Sprague Dawley rats received single oral gavage administrations of 500, 1000 or 2000 mg/kg SB-341 (98.85%) once daily for 5 consecutive days. The test material was delivered to the animals as suspensions prepared in olive oil. Animals were sacrificed 6 and 24 hours following administration of the final dose; bone marrow cells were harvested and examined for the incidence of cells with structural chromosomal aberrations.

No signs of overt toxicity or cytotoxic effects on the target organ were seen in any treatment group. The positive control induced the expected high yield of cells with structural chromosome aberrations. **There was also no indication that SB-341 induced a clastogenic or aneugenic effect at any dose or sacrifice time.**

The study is classified as **Acceptable** and satisfies the requirements for FIFRA Test Guideline 84-2 for in vivo cytogenetic mutagenicity data.

In vivo Bone Marrow Chromosomal Analysis in Chinese Hamsters Following Multiple Dose Administration of Technical Chlorothalonil - MRID No.: 43700602

In an in vivo bone marrow cytogenetic assay (MRID No.: 43700602), groups of ten male Chinese hamsters received single oral gavage administrations of 187.5, 375 or 750 mg/kg CHLOROTHALONIL technical (98.3%) once daily for 5 consecutive days. The test material was delivered to the animals as suspensions prepared in 1% aqueous methylcellulose. Animals were sacrificed 6 and 24 hours following administration of the final dose; bone marrow cells were collected and examined for the incidence of structural chromosomal aberrations.

Overt toxicity was largely confined to the high-dose males and included death, piloerection, hunched posture, ptosis, spasmodic contractions, increased respiratory rate, lethargy, diarrhea and increased salivation. Body weight depression (≈15%) was also noted in the high-dose group. No evidence of a cytotoxic effect was seen on the target organ. The positive control induced the expected high yield of cells with structural chromosome aberrations. **There**

was, however, no indication that the test substance induced a clastogenic effect at any dose or sacrifice time.

The study is classified as **Acceptable** and satisfies the requirements for FIFRA Test Guideline 84-2 for in vivo cytogenetic mutagenicity data.

Sign-off date: 10/17/96
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HED DOC Number: 012076
Toxicology Branch: TB2

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MUTAGENICITY STUDIES

Principal Reviewer: Nancy E. McCarroll
Review Section III,
Toxicology Branch II/HED (7509C)

Signature:

Date:

Secondary Reviewer: Byron T. Backus. Ph.D.
Toxicologist, Review Section II,
Toxicology Branch II/HED (7509C)

Signature:

Date:

DATA EVALUATION REPORT

STUDY TYPE: Mutagenicity: In vivo bone marrow cytogenetic assay, OPPTS 870.5385 [§84-2]

DP BARCODE: D217614 SUBMISSION NO.: S490761 MRID NO.: 43700602
PC CODE: 081901 TOX. CHEM. NO.:

TEST MATERIAL (PURITY): Chlorothalonil technical (98.3%)

SYNONYM(S): 2,4,5,6-Tetrachloroisophthalonitrile; SDS-2787-1002; $C_{15}H_{18}N_4O_5$

CITATION: Mizens, M. and J. Laveglia (1995) In vivo Bone Marrow Chromosomal Analysis in Chinese Hamsters Following Multiple Dose Administration of Technical Chlorothalonil; Huntingdon Research Centre, Ltd. (HRC) Cambridgeshire, England/ Ricerca, Inc., Painesville, OH; HRC Study No. RIC 56/941583; Ricerca Study No. 94-0047. Completion Date: June 2, 1995. (Unpublished) MRID NUMBER: 43700602

SPONSOR: ISK Biosciences Corp., Mentor, OH

EXECUTIVE SUMMARY: In an in vivo bone marrow cytogenetic assay (MRID No: 43700602), groups of ten male Chinese hamsters received single oral gavage administrations of 187.5, 375 or 750 mg/kg chlorothalonil technical (98.3%) once daily for 5 consecutive days. The test material was delivered to the animals as suspensions prepared in 1% aqueous methylcellulose. Animals were sacrificed 6 and 24 hours following administration of the final dose; bone marrow cells were collected and examined for the incidence of structural chromosomal aberrations.

Overt toxicity was largely confined to the high-dose males and included death, piloerection, hunched posture, ptosis, spasmodic contractions, increased respiratory rate, lethargy, diarrhoea, and increased salivation. Body weight depression (~15%) was also noted in the high-dose group. No evidence of a cytotoxic effect was seen on the target organ. The positive control induced the expected high yield of cells with structural chromosome aberrations. **There was, however, no indication that the test substance induced a clastogenic effect at any dose or sacrifice time.**
The study is classified as Acceptable and satisfies the requirements for FIFRA Test Guideline 84-2 for in vivo cytogenetic mutagenicity data.

COMPLIANCE: Signed and dated GLP, Quality Assurance and Data Confidentiality statements were provided.

I. MATERIALS AND METHODS

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A. MATERIALS:

1. Test Material: Chlorothalonil technical

Description: Gray powder
Lot/batch number: 1002
Purity: 98.3%
Receipt date: September 7, 1994
Stability: Listed with an expiration date of March 1995
CAS number: 1897-45-6
Structure:

Vehicle used: 1% Aqueous methylcellulose
Other provided information: The test material was stored at room temperature, protected from light. Dosing suspensions were prepared for each phase of testing and stored in the dark at -4°C for up to 13 days. Samples of each preparation were frozen and shipped to Ricerca, Inc. for confirmation of actual concentrations prior to use.

2. Control Materials:

Negative/route of administration: None

Vehicle/final concentration/route of administration: 1% methylcellulose 20 ml/kg by oral gavage

Positive/final dose(s)/route of administration: Cyclophosphamide (CP) was prepared in sterile 0.9% physiological saline and was administered once by oral gavage at a dose of 40 mg/kg.

3. Test Compound:

Route of administration: Oral gavage

Volume of test substance administered: 20 ml/kg

Dose levels used:

(a) Preliminary toxicity study: 500 and 750 mg/kg (8 males/treatment group) received single oral gavage administrations of the test substance once daily for 5 consecutive days.

Note: Dose selection was based on the findings of an earlier preliminary toxicity study conducted with chlorothalonil treatment levels of 216-512 mg/kg. This study was aborted due to technical problems with dosing suspension preparation and the quality of bone marrow slides; however, the results suggested that the high concentration (512 mg/kg) approached the maximum tolerated dose (MTD).

(b) Cytogenetics assay: 187.5, 375 and 750 mg/kg (10 males/treatment group/sacrifice time)

4. Test Animals:

(a) Species: Chinese hamsters Strain: Not applicable
Age: 6-8 week (at arrival) Sex: Males
Weight(at initiation): 26.6-34.6g--preliminary toxicity test
28.8-45.4g--cytogenetics assay
Source: Wrights of Essex Breeding Centre Ltd., Essex. UK.

Note: The study authors stated that the use of males only was justified "because they are the most sensitive sex to the toxic effects".

(b) Number of animals used per dose:

- Treatment groups and vehicle control: 10 males per sacrifice
- Positive control: 10 males

Note: A secondary group of ten males received the high dose; these animals were used in the event of unscheduled deaths in the primary group. One high-dose male in the secondary dosing group was used in the study.

(c) Properly maintained? Yes.

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B. TEST PERFORMANCE:

1. Treatment and Sampling Times:

Test compound and vehicle control
Dosing: once twice (24 hours apart)
x other (describe): once daily for 5 consecutive days

Sampling (after last dose): x 6 hours 12 hours
x 24 hours 48 hours 72
other (describe):

Positive control
Dosing: x once twice (24 hours apart)
other (describe):
Sampling (after last dose): 6 hours 12 hours
x 24 hours 48 hours 72
other (describe):

Administration of spindle inhibitor

Inhibitor used/dose: Colchicine/4 mg/kg

Administration time: Two hours prior to sacrifice

Route of administration x i.p. other (describe)

Tissues and Cells Examined:

x bone marrow _ other (list):

Number of cells examined per animal, per group: 50.

Slides coded prior to analysis: Yes.

3. Details of Cell Harvest and Slide Preparation: Animals in the treatment and vehicle control groups were sacrificed by cervical dislocation at 6 and 24 hours postexposure to the final administration of the appropriate dose of the test material or vehicle. Animals in the positive control group were sacrificed 24 hours posttreatment. Bone marrow cells were aspirated from both femurs using Hank's balanced salt solution, centrifuged and resuspended in 0.56% KCl. Cells were fixed overnight in methanol:acetic acid (3:1) at 4°C, dropped onto slides, air dried, stained with Giemsa, and mounted.

4. Statistical Methods: The percentage of cells with structural aberrations was evaluated using Wilcoxon's sum of ranks, Kruskal-Wallis' and Jonckheere's tests.

5. Evaluation Criteria: The test material was considered positive if it induced a significant ($p<0.01$) and dose-related increase in the frequency of aberrant cells in the treatment groups compared to the vehicle control.

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C. REPORTED RESULTS:

1. Preliminary Toxicity Assay:

a. Analytical determinations: The analysis of dosing formulations indicated that both dosing suspensions were accurately prepared (within ≥97% of the intended levels).

b. Animal observations: Animals were observed for clinical signs and mortality "regularly during the working day" until sacrifice. Body weights were recorded prior to treatment and at termination. Gross necropsies were performed on all animals that died prior to the terminal sacrifice. No deaths occurred throughout the course of study. Clinical signs noted in both treatment groups included piloerection, increased respiratory rate, lethargy and hunched posture. Piloerection and lethargy persisted until sacrifice. Terminal body weights were decreased by ≈15%-in both groups. Based on these data, the study authors estimated the MTD to be ≈750 mg/kg. Accordingly, doses of 187.5, 375 and 750 mg/kg/day were selected for evaluation in the cytogenetic assay.

2. Cytogenetic Assay:

a. Analytical determinations: The analysis of dosing suspensions prepared for the cytogenetic assay revealed that actual doses were within 94-97% of the intended levels.

b. Clinical observations: Clinical signs and body weights were monitored as described for the preliminary toxicity test. Results indicated that one male receiving the high dose (750 mg/kg/day) died ≈2 hours prior to administration of the final dose. In addition, three high-dose animals were sacrificed in extremis (2 at 48 hours and 1 at 72 hours after initiation of treatment). The study authors indicated that the necropsy findings for these animals did not reveal any signs of mis-dosing. Clinical observations were largely confined to the high-dose group and consisted of piloerection, hunched posture, ptosis, spasmodic contractions, increased respiratory rate, lethargy, diarrhoea, and increased salivation. The onset of symptoms occurred within the first 2 days of treatment. In agreement with the preliminary results, body weights were depressed by ≈15% in the high-dose group. No effects on body weight were apparent in the mid- (375 mg/kg/day) or the low- (187.5 mg/kg/day) dose groups.

c. Bone marrow cell analysis: Summarized results from the examination of bone marrow cells harvested either 6 or 24 hours after administration of the final low, intermediate or high dose of chlorothalonil technical are presented in Study Report Table 1, p 78 (see attachment). As shown, no significant dose-or time-related increases in the frequency of cells with structural chromosome aberrations were observed at any dose or harvest time. By contrast, the positive control (40 mg/kg CP) induced a significant ($p<0.001$) clastogenic response.

Based on the overall results, the study authors concluded that chlorothalonil technical was not clastogenic in this in vivo hamster bone marrow cytogenetic assay.

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D. REVIEWERS' DISCUSSION/CONCLUSIONS: We assess that the study was properly conducted and we agree with the study authors' interpretation of the data. The administration of multiple doses of the test material for 5 consecutive days resulted in overt toxicity in the high dose group but did not produce a genotoxic effect. There was, however, no evidence of a cytotoxic effect on the target organ. Although less than the intended number of metaphase spreads were available for analysis from several animals, we do not consider the lack of sufficient analyzable cells to be related to treatment with chlorothalonil technical. Animals with either poor quality or an insufficient number of metaphases were noted in the vehicle, low-dose and high-dose groups. The ability of the test system to detect a clastogenic response was adequately demonstrated by the significant ($p<0.001$) results obtained with the positive control (40 mg/kg CP). We conclude, therefore, that the study provided acceptable evidence that chlorothalonil technical is not clastogenic in this in vivo test system. Additionally, it should be noted that the findings of this study resolve the inconclusive results of a previous bone marrow cytogenetic assay in Chinese hamsters (Study Report Nos. 000-5TX-81-0025-004 /625-5TX-83-0014-003; Accession No. 00147948).

E. STUDY DEFICIENCIES: NONE.

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Sign-off date: 10/17/96
DP Barcode: D217614
HED DOC Number: 012076
Toxicology Branch: TB2

MUTAGENICITY STUDIES

Principal Reviewer: Nancy E. McCarroll Signature:
Review Section III,
Toxicology Branch II/HED (7509C) Date:

Secondary Reviewer: Byron T. Backus. Ph.D. Signature:
Toxicologist, Review Section II,
Toxicology Branch II/HED (7509C) Date:

DATA EVALUATION REPORT

STUDY TYPE: Mutagenicity: In vivo bone marrow cytogenetic assay, OPPTS 870.5385 [§84-2]

DP BARCODE: D217614 SUBMISSION NO.: S490761 MRID No.: 43700601
PC CODE: 081901 TOX. CHEM. NO.:

TEST MATERIAL (PURITY): SB-341 (98.85%)

SYNONYM(S): Chlorothalonil; 2,4,5,6-Tetrachloroisophthalonitrile;SDS-2787-1002; $C_{15}H_{18}N_4O_5$

CITATION: Kajiwara, Y., et al. (1994) Five-Day Repeated-Dose Chromosomal Aberration Test In vivo with SB-341 Using Rats; Hita Research Laboratories, Hita, Japan; Study No. K12-0001. Completion Date: September 7, 1994. (Unpublished) MRID NUMBER: 43700601

SPONSOR: SDS Biotech K.K., Tokyo, Japan; Submitted by: ISK Biosciences Corp., Mentor, OH

EXECUTIVE SUMMARY: In an in vivo bone marrow cytogenetic assay (MRID No: 43700601), groups of five male Sprague Dawley rats received single oral gavage administrations of 500, 1000 or 2000 mg/kg SB-341 (98.85%) once daily for 5 consecutive days. The test material was delivered to the animals as suspensions prepared in olive oil. Animals were sacrificed 6 and 24 hours following administration of the final dose; bone marrow cells were harvested and examined for the incidence of cells with structural chromosomal aberrations.

No signs of overt toxicity or cytotoxic effects on the target organ were seen in any treatment group. The positive control induced the expected high yield of cells with structural chromosome aberrations. **There was also no indication that SB-341 induced a clastogenic or aneugenic effect at any dose or sacrifice time.**

The study is classified as Acceptable and satisfies the requirements for FIFRA Test Guideline 84-2 for in vivo cytogenetic mutagenicity data.
COMPLIANCE: Signed and dated GLP, Quality Assurance and Data Confidentiality statements were provided.

I. MATERIALS AND METHODS

A. MATERIALS:

1. Test Material: SB-341

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Description: Gray powder
Lot/batch number: EC26M2
Purity: 98.85%
Receipt date: Not reported
Stability: Reported to be stable at "ordinary temperature"
CAS number: 1897-45-6
Structure:

Vehicle used: Olive oil
Other provided information: The test material was stored in a dark and cool place (temperature not specified). Dosing suspensions were prepared within 2 hours of use; actual concentrations were not verified analytically.

2. Control Materials:

Negative/route of administration: None

Vehicle/final concentration/route of administration: Olive oil was administered once daily for 5 days; the dosing volume was 10 mL/kg.

Positive/final dose(s)/route of administration: Mitomycin C (MitC) was prepared in sterile 0.9% physiological saline and was administered once by oral gavage at a dose of 15 mg/kg.

3. Test Compound:

Route of administration: Oral gavage

Volume of test substance administered: 10 mL/kg

Dose levels used:

(a) Preliminary toxicity study: 31.25, 62.5, 125, 250, 500, 1000 and 2000 mg/kg/day (3 males/treatment group) --single oral gavage administrations of the test substance once daily for 5 consecutive days.

(b) Cytogenetics assay: 500, 1000 and 2000 mg/kg (5 males/treatment group/sacrifice time)

4. Test Animals:

(a) Species: Rat Strain: Crj:CD(SD)Age: 7 weeks (at arrival) Weight (at initiation): 293.6-311.9 g--preliminary toxicity test
296.4-336.4 g--cytogenetics assay
Sex: Males Source: Charles River Japan. Inc..

Note: The use of males only was not justified. However, a previously conducted bone marrow cytogenetic assay (Study No. 0005TX-81-0025; Accession No. 071539) and a micronucleus assay (Study No. 000-5TX-81-0024; Accession No. 071539) also employed only males and were classified as acceptable studies.

(b) Number of animals used per dose:

- Treatment groups and vehicle control: 5 males per sacrifice
- Positive control: 5 males

(c) Properly maintained? Yes.

B. TEST PERFORMANCE:

1. Treatment and Sampling Times:

Test compound and vehicle control
Dosing: once twice (24 hours apart)
other (describe): once daily for 5 consecutive days

Sampling (after last dose): x 6 hours 12 hours
x 24 hours 48 hours 72
other (describe):

Positive control
Dosing: x once twice (24 hours apart)
other (describe):
Sampling (after last dose): 6 hours x 18 hours
24 hours 48 hours 72
other (describe):

Administration of spindle inhibitor

Inhibitor used/dose: Colchicine/5 mg/kg

Administration time: Two hours prior to sacrifice

Route of administration x i.p. other (describe)

2. Tissues and Cells Examined:

x bone marrow other (list):

Number of metaphases examined per animal, per group: 50.

Note: 500 cells/animal (6-hour sacrifice) were scored to determine the mitotic index (MI).

Slides coded prior to analysis: Yes.

3. Details of Cell Harvest and Slide Preparation: Animals in the treatment and vehicle control groups were sacrificed by CO2 asphyxiation at 6 and 24 hours postexposure to the final administration of the appropriate dose of the test material or vehicle. Animals in the positive control group were sacrificed 18 hours posttreatment. Bone marrow cells were washed with physiological saline and centrifuged. Supernatants were discarded and cell pellets were resuspended in 0.075 M KCl, fixed in methanol:acetic acid (3:1), spread on slides, air dried, and stained with 2% Giemsa.

4. Statistical Methods: The percentage of cells with structural aberrations was evaluated using Fisher's exact test.

5. Evaluation Criteria:

a. Assay validity: The assay was considered valid if the frequency of aberrant cells in the vehicle control group was within the range of background data (unspecified historical control ranges) and the positive control induced a significant $p<0.05$ clastogenic effect.

b. Positive Response: The test material was considered positive if it induced a significant ($p<0.05$) and dose-related increase in the frequency of aberrant cells in the treatment groups compared to the vehicle control.

C. REPORTED RESULTS:

1. Preliminary Toxicity Assay: Animals were observed for clinical signs and mortality at unreported intervals; body weights were recorded prior to treatment and at termination. MIs were also determined 24 hours postadministration of the final dose. No deaths occurred throughout the course of study and no signs of clinical toxicity were reported. The report indicated that decreased body weights were observed in all males receiving the high dose (2000 mg/kg/day) and in 1/3 males at 500 and 1000 mg/kg/day. There was no evidence of mitotic depression at any dose. Based on these data, the study authors estimated the maximum tolerated dose (MTD) to be ~2000 mg/kg. Accordingly, doses of 500, 1000 and 2000 mg/kg/day were selected for evaluation in the cytogenetic assay.

2. Cytogenetic Assay:

a. Clinical observations: No deaths or signs of compound toxicity were reported. The report did not indicate whether body weight was affected by treatment.

b. Bone marrow cell analysis: Results from the evaluation of bone marrow cells harvested either 6 or 24 hours after administration of the final low, intermediate or high doses of SB-341 are presented in Study Report Tables 2 and 3; pp 21 and 22, respectively (see attachment). As shown, MIs for the treatment groups at the 6-hour harvest were comparable to the vehicle control. No significant dose-or time-related increases in the frequency of cells with structural chromosome aberrations were observed at any dose or harvest time. Similarly, the test material had no effect on the incidence of polyploid cells. By contrast, the positive control (15 mg/kg Mit C) induced a significant ($p<0.01$) clastogenic response.

Based on the overall results, the study authors concluded that SB341 was not genotoxic in this in vivo rat bone marrow cytogenetic assay.

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D. REVIEWERS' DISCUSSION/CONCLUSIONS: We assess that the study was properly conducted and were agree with the study authors' interpretation of the data. SB-341 was not overtly toxic to the test animals, cytotoxic to the target organ or induced a clastogenic or aneugenic response in male rats following 5 daily oral administrations of doses up to 2000 mg/kg/day. The combined high dose (10,000 mg/kg) exceeded the limit dose for this assay system. The ability of the test system to detect a clastogenic response was adequately demonstrated by the significant ($p<0.01$) results obtained with the positive control (15 mg/kg MitC). We conclude, therefore, that the study provided acceptable evidence that SB-341 is not clastogenic in this in vivo test system.

E. STUDY DEFICIENCIES: Although the number of animals recommended for evaluation in whole animal cytogenetic assays (10/dose/sacrifice time) was not employed, it appears unlikely that this deficiency altered the outcome of the study. Similarly, the.lack of testing in females did not compromise the findings since there appears to be no sex specific toxicological effect associated with exposure to chlorothalonil.

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HED DOC Number: 012076
Toxicology Branch: TB2

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