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

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SEP 12

SEP 12 1990

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: Prometon Mutagenicity Studies

TO: J. Yowell/R. Taylor, PM #25
Registration Division (H7505C)

FROM: Byron T. Backus, Ph.D., Toxicologist Byron T. Backus 8/30/90
Fungicide/Herbicide/Antimicrobial Toxicology Branch
HED (H7509C)

THROUGH: K. Clark Swentzel K. Clark Swentzel 9/6/90
Section Head, Review Section II
Fungicide/Herbicide/Antimicrobial Toxicology Branch
HED (H7509C)

and

Marcia van Gemert, Branch Chief M van Gemert 9/10/90
Fungicide/Herbicide/Antimicrobial Toxicology Branch
HED (H7509C)

EPA Record No. 232965

Project No. 9-0127

EPA Reg. No. n/a

Tox. Chem. 96

Action Requested:

Review three mutagenicity studies on technical Prometon.

Comments and Recommendations:

1. The Ames Salmonella microsome reverse assay (MRID 406364-01) has been classified as not acceptable. While there was no evidence of a mutagenic response, there was also no indication of cytotoxicity at the highest concentration tested (2500 µg/plate) and quantitative data from the preliminary toxicity study with strain TA-100 at concentrations of 5000 and 10000 µg/plate are not reported. Although precipitation occurred at 5000 and 10000 µg/plate, there is still a possibility that the

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technical Prometon could have been tested at a level substantially greater than 2500 µg/plate. In addition, the test material was inadequately described in terms of purity (percentage of active ingredient), batch number, or physical characteristics.

2. The micronucleus test (MRID 406364-02) has been classified as not acceptable. While there was no evidence of a positive mutagenic response, there is insufficient justification given for 648 mg/kg being the highest dose, and no rationale for the use of the rat (rather than the mouse, which is the species primarily used in the development of this assay) is made. The response of the positive controls in the second assay appears to have been relatively weak, particularly when compared to vehicle control findings in the first assay, and the p-values (presumably from the X^2-test) are not reported.

3. The unscheduled DNA synthesis study utilizing rat hepatocytes has been classified as not acceptable. No mutagenic response was observed, but percentages of viable cells at concentrations of 625 µg/ml and higher in the preliminary cytotoxicity testing are not reported (this might possibly be satisfied by a clarification of what is meant by the cells being "in bad condition") and there was no indication of cell viability testing in the primary and/or confirmatory UDS assays. The only reported finding possibly relating to cell viability is that at 400 µg/ml in both the primary and confirmatory UDS assays 3.23% of the cells were in S-phase (interpreted as indicating the cells were sufficiently viable for this activity to occur).

4. Copies of the attached DERs should be made available to the registrant.

000100

Reviewed by: Byron T. Backus Byron T. Backus
Section 2, HFASB (H7509C) 8/08/90

Secondary Reviewer: John H. S. Chen, D.V.M. John H. S. Chen 8/13/90
Section 1, HFASB (H7509C)

Tertiary Reviewer: K. Clark Swentzel K. Clark Swentzel 9/6/90
Section 2, HFASB (H7509C)

DATA EVALUATION REPORT I

STUDY TYPE: Gene Mutation (84-2) Ames Salmonella microsome reverse mutation assay

TOX CHEM NO. 96

MRID NO: 406364-01

TEST MATERIAL: Prometon Technical

SYNONYMS: (none given)

STUDY NUMBER(S): Report 85159

SPONSOR: Ciba-Geigy Corporation

TESTING FACILITY: Research Department
Pharmaceuticals Division
Ciba-Geigy Corporation
Summit, NJ

TITLE OF REPORT: Prometon Technical Salmonella/Mammalian-Microsome Mutagenicity Assay

AUTHOR(S): Lasinski, E. R., Giknis, M. L. A., Arthur, A. T.

REPORT ISSUED: 12/11/85

CLASSIFICATION: Not Acceptable. There was no indication of cytotoxicity at the highest concentration tested (2500 µg/plate) and quantitative data from a preliminary toxicity study with strain TA-100 and concentrations of 5000 and 10000 µg/plate are not reported. Although precipitation is reported to have occurred at 5000 and 10000 µg/plate when the test material suspension was added to top agar, the possibility still exists that Prometon Technical could have been tested at levels substantially greater than 2500 µg/plate. In addition, the test material is inadequately described in terms of purity (percentage of

active ingredient), batch number, or physical characteristics. This study does not presently satisfy the guideline requirements (84-2) for a gene mutation assay (bacteria, reverse mutation).

CONCLUSIONS:

1. No evidence was observed for a mutagenic effect at the histidine locus in any of the Salmonella typhimurium strains (TA 98, TA 100, TA 1535, TA 1537, or TA 1538) at concentrations of 25, 100, 500, 1,000 or 2,500 µg/plate either in the presence or absence of activation (rat S9 mix). The mutagenicity assay was conducted twice (with triplicate plates at each dose level), and the positive controls elicited appropriate responses. However, there was no conclusive indication of any cytotoxicity at the highest concentration tested (2,500 µg/plate). It is stated within the report that concentrations of test article greater than 2,500 µg/plate (specifically, 5,000 and 10,000 µg/plate) exhibited "toxic and inhibitory properties to tester strain TA-100 in the preliminary toxicity assay" and also formed a precipitate when added to the top agar. However, quantitative results from these preliminary findings are not presented, and the possibility exists that Prometon Technical could have been tested at a level substantially greater than 2500 µg/plate.

2. The test material is inadequately described in terms of purity (percentage of active ingredient), batch number and physical characteristics.

A. MATERIALS:

1. Test compound: Prometon Technical. No information is given as to purity, lot number or physical characteristics. The test material was dissolved in DMSO.

2. Positive control compounds: In the absence of S-9 mix, Daunomycin (dissolved in water) at 2 µg/plate was used for TA 98 and TA 1538; sodium azide (dissolved in water) at 3 µg/plate was used for TA 100, and at 0.3 µg/plate for TA 1535; and 9-aminoacridine (dissolved in DMSO) at 40 µg/plate was used for TA 1537. In the presence of S-9 mix, Benzo-(alpha)-pyrene (in DMSO) at 3 µg/plate was used for TA 98, TA 100 and TA 1538; Beta-Naphthylamine (in DMSO) at 10 µg/plate was used for TA 1535; and 3-CH_3-Cholanthrene (in DMSO) was used at 10 µg/plate for TA 1537.

3. Test microorganisms: Salmonella typhimurium strains TA 98, TA 100, TA 1535, TA 1537 and TA 1538, "recommended by and obtained from Dr. Bruce Ames, U. Calif., Berkeley..." All have

mutations in the histidine operon as follows: TA 1535 and TA 100 (base-pair substitution); and TA 1537, TA 1538 and TA 98 (frameshift). All these strains also have a defective lipopolysaccharide coat (making them more permeable to large molecules), and a defect in the repair of ultraviolet-induced DNA damage. TA 98 and TA 100 carry a transfer factor plasmid (pKM101) conferring resistance to ampicillin and also causing an increase in error-prone DNA repair.

4. S9: Refer to appended page 1 for information regarding the S-9 source and preparation of the S-9 mix.

5. Medium: Refer to appended pages 2 and 3.

B. STUDY DESIGN:

1. Mutagenicity assay:

Refer to appended pages 2 and 3. It is stated (see appended page 3) that "concentrations of test article greater than 2,500 ug/plate (5,000 and 10,000 ug) exhibited toxic and inhibitory properties to tester strain TA-100 in the preliminary toxicity assay. The higher concentrations also formed a precipitate when added to the top agar." However, There is no information (such as number of revertants or plate counts) as to how cytotoxicity was assayed. The experiment was conducted twice "which produced six plates at each test chemical concentration."

2. Evaluation:

Refer to appended pages 4 and 5 for the evaluation procedure.

3. A signed and dated Good Laboratory Practice Statement is on p. 5 of the report. There is a Quality Assurance Unit Statement on p. 19.

C. RESULTS:

1. Mutagenicity assay:

Refer to appended pages 6 and 7 for the assay results with and without S-9 mix respectively.

D. DISCUSSION:

There was no indication of a dose-related increased incidence of revertants in any of the five Salmonella typhimurium strains either in the presence or absence of an S-9 activation system. However, there was no indication of any cytotoxicity at the highest exposure concentration used in this assay (2,500 µg/plate). The text of the report states that concen-

trations of the test article greater than 2,500 ug/plate (specifically, 5,000 and 10,000 ug/plate) exhibited "toxic and inhibitory properties to test strain TA-100 in the preliminary toxicity assay" and also formed a precipitate when added to top agar. However, the results of this preliminary assay are not otherwise presented and, in any case, the possibility still exists that Prometon Technical could have been tested at a level substantially greater than 2500 µg/plate.

Additional problems with this report include an inadequate description of the test material in terms of its purity (percentage of active ingredient), manufacture batch number and physical characteristics.

The study is currently classified as unacceptable. In order for the classification of this study to be upgraded, the issues raised above must be satisfactorily addressed.

RIN-0334-94 PROMETON REVIEWS (08080Z)

Page_____ is not included in this copy.

Pages 1 through 13 are not included.

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Reviewed by: Byron T. Backus Byron T. Backus 8/30/90
Section 2, HFASB (H7509C)

Secondary Reviewer: John Chen, D.V.M John H Chen 8/30/90
Section 1, HFASB (H7509C)

Tertiary Reviewer: K. Clark Swentzel K. Clark Swentzel 9/6/90
Section 2, HFASB (H7509C)

DATA EVALUATION REPORT II

STUDY TYPE: Mutagenicity - micronucleus test (in vivo rat)

TOX CHEM NO. 96

MRID NO: 406364-02

TEST MATERIAL: Prometon Technical

SYNONYMS: G 31 435 Technical

STUDY NUMBER(S): 871355

SPONSOR: Ciba-Geigy Corporation

TESTING FACILITY: CIBA-GEIGY LIMITED
Basle, Switzerland
Laboratories of Genetic Toxicology
GU 7, R-1066

TITLE OF REPORT: Micronucleus Test (Rat)

AUTHOR(S): Strasser, F.

REPORT ISSUED: 4/11/88

CLASSIFICATION: Not Acceptable. While there was no evidence of a positive mutagenic response, there is insufficient justification given for 648 mg/kg being the highest dose, and no rationale for the use of the rat (rather than the mouse, which is the species which was primarily used in the development of this assay) is made. The response of the positive controls in the second assay appears to have been relatively weak, particularly when compared to vehicle control findings in the first assay. Animals sacrificed at 16 and 48 hours were tested only at 648 mg/kg. This study does not presently satisfy the guideline requirements (84-2) for a structural chromosomal aberration assay (mammalian in vivo micronucleus test).

CONCLUSIONS:.

1. There was no indication in this assay of a positive mutagenic response involving the test material. However, there are a number of problems in the report presentation, and the study is currently classified as not acceptable.

2. Because mice were utilized in most of the studies upon which the recommended protocol is based, the mouse is the preferred species for performing the micronucleus test (Federal Register, Vol. 50, no. 188/Friday, September 27, 1985). A rationale for the choice of rat as the test animal in this study is not provided.

3. Although the text states that 648 mg/kg was the highest dose at which no deaths occurred in preliminary toxicity testing, this does not fully justify this dose as the highest dose administered in the assay, as no additional information is provided (particularly as to what doses greater than 648 mg/kg were administered, incidences of mortality at these higher doses, and what - if any - symptoms of toxicity were present). It is also noted that there was no significant difference between the highest dose and negative control groups with respect to the PCE:NCE ratio (i.e., no indication of any general activity of the test material on the bone marrow).

4. On page 8 of the report it is stated: "The respective "positive control" experiments with cyclophosphamide (64 mg/kg) yielded an average of 0.81 and 0.24% polychromatic erythrocytes with micronuclei. This is significantly different from the controls (0.17 and 0.09) treated with the vehicle (0.5% CMC) alone." There is no indication within tables 1 or 6 of any significant differences between the vehicle and positive control averages for number of PCEs with micronuclei, and the p values (presumably from the X^2-test) are not reported. It is also noted that the response of the positive controls in the second assay appears to have been relatively weak, particularly when compared with the vehicle control findings in the first assay.

5. Animals sacrificed at 16 and 48 hours were tested only at 648 mg/kg. Assuming that 648 mg/kg can be justified as a highest dose, these animals should have been tested at two lower doses (such as 162 and 324 mg/kg). It is recommended that the second assay be redone, with animal sacrifice at 16, 24 and 48 hours, or 24, 48 and 72 hours (Heddle et al., Mutation Research 123: 61-118, 1983), and dosage at 648 mg/kg (if this can be justified as highest dose) and two lower dosages (such as 162 and 324 mg/kg) for each sacrifice time. The study should also utilize appropriate vehicle and positive controls (the latter using one dose level of CP and sacrifice at 24 hours).

A. MATERIALS:

1. Test compound: G 31 435 technical, batch no. FL 841716, with a purity of 97.9%. According to information on page 5 of the text the "validity" (last date of use of the test substance?) was November 30, 1987 (the last date the test substance was administered was November 4, 1987). The vehicle used was a 0.5% aqueous solution of sodium carboxymethylcellulose (CMC). No information is reported as to the physical nature of the test material (appearance, whether it was a liquid or solid) or how it was stored.

2. Positive control material: Cyclophosphamide (CP or ENDOXAN) at 64 mg/kg (orally administered in 20 ml/kg vehicle).

3. Test animals: Rats (Tif: RAI, SPF; weight (females) 240-320 g, (males) 290-500 g; age (females) 6-10 wks, (males) 4-9 wks).

B. STUDY DESIGN:

1. Animal assignment: From p. 10: "The animals were selected by means of random numbers generated by computer."

2. Preliminary toxicity assay: From p. 10: "A preliminary test was performed to determine the highest dosage of the test substance to be applied in the mutagenicity assay."

"Three groups of four rats (two females and two males) are treated with three different doses, one receiving the maximum dose of 5000 mg/kg, or the highest applicable dose, and the other two doses of 1/5 and 1/25 of that amount respectively. The animals are treated with a single dose. The observation period corresponds to the interval between administration and sacrifice of the animals in the mutagenicity test, plus one day. If all animals in all dose groups die in the first step, a second test is performed in which the highest dose given is 3/4 of the lowest used in the preceding test. If some of the animals in one of the dose groups die, the test is continued with a high dose corresponding to a predetermined fraction of that dose. Depending on the outcome the highest dose causing no deaths is used as the highest in the mutagenicity test, or if necessary the test is repeated with lower doses."

3. Mutagenicity assay: Refer to appended page 1.

4. Preparation of slides: From p. 12: "Bone marrow was harvested from the shafts of both femurs with fetal calf serum. After centrifugation small drops of the sediment mixture were transferred on the end of a slide, spread out with the aid of a glass slide and the preparations were air-dried. Within 24 hours, the slides were stained in undiluted May-Grünwald solu-

tion for 3 min then in May-Grünwald solution/water 1/1 for 2 min. After being rinsed in distilled water, the slides were left immersed in diluted Giemsa solution (16.6%), for 10 min. After rinsing with distilled water and air-drying, the slides were cleared in Xylene and mounted."

5. Slide scoring: From p. 13: "Prior to analysis the slides are coded. Thereafter the quality of staining is evaluated. The slides of five animals from each sex showing the best differentiation between mature and polychromatic erythrocytes are selected for later scoring. In the first experimental part the slides of five female and five male animals each of the negative control group and of the dosage group sacrificed at 16, 24 and 48 hours post treatment were examined. From the animals of the positive control group which were sacrificed 24 hours after application, the slides of five female and five male animals were scored. In the second experimental part, due to insufficient quality of slides from some animals of the control group and of the 324 mg/kg dosage group, not all slides could be taken for analysis. Thus, five female and only two male animals of the negative control group and the slides of only three female and two male animals of the 324 mg/kg dosage group were chosen for scoring. At the dosage groups of 162 and 648 mg/kg the slide of five female and five male animals sacrificed at 24 hours post treatment were examined. From the animals of the positive control group which were sacrificed 24 hours after application, the slides of five female and five male animals were scored."

6. Statistics: From p. 13: "The significance of difference was assessed by x^2-test."

7. Result evaluation and acceptance criteria: From p. 13: "A test substance is considered to be active in this test system if a statistically significant increase in the number of polychromatic erythrocytes with micronuclei in comparison with the negative control occurs at any dose and sampling time respectively."

   "The quality of the slides must allow a clear differentiation between polychromatic and normochromatic erythrocytes."

   "The result obtained with the positive control has to fulfill the criteria given for a positive response."

8. There is a "Certification of Good Laboratory Practices" statement on page 3 of the report, and a "Quality Assurance Statement" on page 7.

C. RESULTS:

1. Preliminary toxicity assay: No observations of either symptoms or mortality are reported. According to the text (p. 14): "In the tolerability test the dose of 648 mg/kg of G 31 435 tech. proved to be the highest causing no deaths in a group of four animals. Therefore, this dose was selected as the highest in the mutagenicity test."

   "In the first part of the mutagenicity experiment, in the 648 mg/kg dosage group one female and one male animal died within the treatment period of 16 hours."

   "In the second part of the mutagenicity experiment, in the 324 mg/kg dosage group one female animal died within the treatment period of 24 hours."

2. Mutagenicity assay findings: Refer to appended pages 2 through 10. There was no indication of a positive response following administration of the test material.

D. DISCUSSION:

   While there was no indication of a positive mutagenic response in this assay, there are a number of problems in the report presentation.

   Because mice were utilized in most of the studies upon which the recommended protocol is based, the mouse is the preferred species for performing the micronucleus test (Federal Register, Vol. 50, no. 188/Friday, September 27, 1985). A rationale for the choice of rat as the test animal in this study is not provided.

   Although the text states that 648 mg/kg was the highest dose at which no deaths occurred in the preliminary test, no additional information is given, particularly as to what doses greater than 648 mg/kg were administered, incidences of mortality at these higher doses, whether one sex may have been more susceptible than the other, and what (if any) symptoms of toxicity were present. This information should be reported. It is also noted that there was no significant difference between the highest dose and negative control groups with respect to the PCE:NCE ratio (i.e., no indication of any general activity of the test material on the bone marrow).

   On page 8 of the report it is stated: "The respective "positive control" experiments with cyclophosphamide (64 mg/kg) yielded an average of 0.81 and 0.24% polychromatic erythrocytes with micronuclei. This is significantly different from

the controls (0.17 and 0.09%) treated with the vehicle (0.5% CMC) alone." There is no indication within tables 1 or 6 of any significant differences between the vehicle and positive control averages for number of PCEs with micronuclei, and the p values (presumably from the X^2-test) are not reported. It is also noted that the response of the positive controls in the second assay appears to have been relatively weak, particularly when compared with the vehicle control findings in the first assay. These points should be addressed.

Animals sacrificed at 16 and 48 hours were tested only at 648 mg/kg (in the first assay). Assuming that 648 mg/kg can be justified as a highest dose, these animals should have also been tested at two lower doses (such as 162 and 324 mg/kg).

It is recommended that the assay be redone, with animal sacrifice at 16, 24 and 48 hours (or 24, 48 and 72 hours), and dosage at 648 mg/kg (if this can be justified as highest dose) and two lower doses (such as 162 and 324 mg/kg) for each sacrifice time. The study should also utilize appropriate vehicle and positive controls (the latter using only one dose level of CP and sacrifice at 24 hours).

The study is currently classified as not acceptable.

RIN-0334-94 PROMETON REVIEWS (08804)

Page_____ is not included in this copy.

Pages 20 through 29 are not included.

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____ Sales or other commercial/financial information.

____ A draft product label.

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Reviewed by: Byron T. Backus Byron T. Backus 8/21/90
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Section 1, HFASB (H7509C)

Tertiary Reviewer: K. Clark Swentzel K Clark Swentzel 9/6/90
Section 2, HFASB (H7509C)

DATA EVALUATION REPORT III

STUDY TYPE: 84-4 Unscheduled DNA Synthesis - Rat Hepatocytes

TOX CHEM NO. 96

MRID NO: 406364-03

TEST MATERIAL: Prometon Technical

SYNONYMS: G 31 435 Technical

STUDY NUMBER(S): 871354

SPONSOR: Ciba-Geigy Corporation

TESTING FACILITY: CIBA-GEIGY LIMITED
Basle, Switzerland
Laboratories of Genetic Toxicology
GU 7, R-1066

TITLE OF REPORT: Autoradiographic DNA Repair Test on Rat Hepatocytes.

AUTHOR(S): Hertner, T.

REPORT ISSUED: 11/11/87

CLASSIFICATION: Not acceptable. Percentages of viable cells at doses of 625 µg/ml and higher concentrations in the preliminary cytotoxicity testing are not reported (this might possibly be satisfied by a clarification of what is meant by the cells being "in bad condition") and there was no indication of cell viability testing in both the primary and confirmatory UDS assays. The only reported finding possibly relating to cell viability is that at 400 µg/ml in both the primary and confirmatory UDS assays 3.23% of the cells were in S-phase (interpreted as indicating that the cells were sufficiently viable for this activity to occur). It is therefore concluded that there is

insufficient evidence that 400 µg/ml was a sufficiently high enough maximum concentration. In order for the study classification to be upgraded to acceptable, additional information and/or data has to be submitted to demonstrate that there was some evidence of cytotoxicity at 400 µg/ml, or that this dose level was sufficiently close to one at which unequivocal cytotoxicity occurs. This study does not currently satisfy the guideline requirements (84-4) for an unscheduled DNA synthesis study in rat hepatocytes.

CONCLUSIONS:

1. There was no evidence of induced UDS as a result of exposure to the test material at 2, 10, 50, 100, 200, or 400 µg/ml. The net nuclear grain counts for the two negative controls in both the primary and subsequent confirmatory UDS assays were well below one grain per nucleus (as were all cultures exposed to the test material at concentrations ranging from 2 to 400 µg/ml). The positive controls elicited the appropriate responses.

2. Actual percentages of viable cells at doses of 625 µg/ml and above in the preliminary cytotoxicity testing are not reported (although cells are described as being "in bad condition" with no further clarification as what this means). Also, there is no indication that there was any cell viability testing in the primary and/or confirmatory UDS assays. The only reported finding possibly relating to cell viability is that 3.23% of the cells were in S-phase (interpreted as indicating that the cells were sufficiently viable for this activity to occur). It is therefore concluded that there is insufficient evidence that 400 µg/ml was a sufficiently high maximum concentration in the UDS assays, and the study is currently classified as unacceptable. In order for the classification to be upgraded to acceptable, additional information and/or data has to be submitted to demonstrate that there was some evidence of sufficient cytotoxicity at 400 µg/ml, or that this dose level is sufficiently close enough to one at which unequivocal cytotoxicity occurs.

A. MATERIALS:

1. Test compound: G 31 435 technical, batch no. FL 841716, with a purity of 97.9%. According to information on page 7 of the test the expiration date was October 31, 1987 (the study was completed October 9, 1987). The vehicle used was DMSO. No information is reported as to the physical nature of the test material (its appearance or whether it was a liquid or solid) or how it was stored.

2. Positive control material: 4-Aminobiphenyl, 4-ABP, at 25 and 50 µM.

3. Indicator cells: Refer to appended page 1 for the preparation and culture of the rat hepatocytes used in this study.

B. STUDY DESIGN:

1. Cytotoxicity assay: Refer to appended page 2 for the procedure for this assay. The concentrations of test material in the cytotoxicity assay were 0 (vehicle only), 4.9, 9.8, 19.5, 39.1, 78.1, 156.3, 312.5, 625, 1250, 2500 and 5000 µg/ml.

2. UDS Assay and evaluation: As a result of the findings of the cytotoxicity assay the test material was assayed at 2, 10, 50, 100, 200 and 400 µg/ml, both in the original and subsequent confirmatory assay. In addition to the test material, the positive control material (4-ABP) was tested at 25 and 50 µM, and two negative controls containing 1) the DMSO vehicle and 2) the culture medium were also assayed. Refer to appended pages 3, 4, and 5 for procedures for the UDS assay and its evaluation.

3. A signed Quality Assurance Statement, which includes the dates of QA inspections, is provided on p. 8 of the original report. There is also a signed compliance statement with FIFRA Good Laboratory Practice Standards on page 95 of the report.

C. RESULTS:

1. Cytotoxicity assay: At concentrations from 4.9 to 312.5 µg/ml cells were "in good condition," with viability between 76 and 83%. At 625 µg/ml and higher concentrations cells were "in bad condition" (no further details are given; it is uncertain whether this refers to poor cellular morphology, whether most or all cells stained with trypan blue etc.) and no percentages for cell viability are reported (uncertain whether the "/" means 0% or simply less than 70% viability). At 2500 and 5000 µg/ml precipitate was observed. Refer to appended p. 6.

2. Assay: There was no indication in either the primary or subsequent confirmatory assay of a dose-related increase in mean net nuclear grain counts, nor (from the percentage distribution of nuclear grain counts) any evidence of an increase in cells with either ≥ 6 or ≥ 20 net nuclear grain counts. The positive controls elicited the appropriate responses. Refer to appended pages 7 through 14. Historical control data for negative (medium and vehicle) and positive test substances (4-ABP, usually at 50 µm/ml) are also provided (pages 28-30 of the report).

D. DISCUSSION:

There was no evidence of induced UDS as a result of exposure to the test material. The net nuclear grain counts for the two negative controls in both the primary and subsequent confirmatory assays were well below one grain per nucleus (as were all cultures exposed to the test material at concentrations ranging from 2 to 400 µg/ml. The positive controls elicited the appropriate responses (mean net nuclear counts: 8.26, 8.40, 4.79 and 6.27).

However, actual percentages of viable cells at doses of 625 µg/ml and above in the cytotoxicity testing are not reported (this might possibly be satisfied by a clarification of what is meant by the cells being "in bad condition") and (most importantly) there was a lack of cell viability testing in both the primary and confirmatory UDS assays. Ideally, at the highest concentration of test material in both the UDS assays there would have been substantially reduced cell viability; with this finding it would not have been necessary to do grain counts for that concentration. However, the only reported finding possibly relating to cell viability is that at 400 µg/ml in both the primary and confirmatory assays 3.23% of the cells were in S-phase (which is interpreted as indicating that the cells were sufficiently viable for this activity to occur). Therefore, it is concluded that there is insufficient evidence that 400 µg/ml was a sufficiently high enough maximum concentration, and the study is currently classified as unacceptable. In order for the study classification to be upgraded to acceptable, additional information and/or data has to be submitted to demonstrate that there was some evidence of cytotoxicity at 400 µg/ml, or that 400 µg/ml is sufficiently close to a concentration at which unequivocal cytotoxicity occurs.

RIN-0334-94 PROMETON REVIEWS (08080Z)

Page_____ is not included in this copy.

Pages 34 through 47 are not included.

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