6-24-86
CASWELL FILE

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

005210

JUN 24 1986

MEMORANDUM

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCE

SUBJECT: The Review of Toxicology and Mutagenicity Reports Pursuant to the Registration of Terbuthylazine.

EPA ID No. 40810-A
Record No. 161818

Project No. 1079
Tox. Chem. No. 125B

TO: John H. Lee (PM Team #31)
Registration Division (TS-767c)

FROM: John E. Whalan, D.A.B.T., Toxicologist
Section II, Toxicology Branch
Hazard Evaluation Division (TS-769c)

John E. Whalan 6-9-86

THRU: Edwin R. Budd, Section Head
Section II, Toxicology Branch
Hazard Evaluation Division (TS-769c)

Budd 6/13/86

6/24/86

The Registrant, Ciba-Geigy Corporation, has submitted the following toxicology and mutagenicity reports in response to a letter from John H. Lee (August 6, 1985):

1. 28-Day Subacute Oral Toxicity Study of GS-13529 (Terbuthylazine) in Rabbits
2. 28-Day Subacute Dermal Toxicity Study of GS-13529 (Terbuthylazine) in Rabbits
3. Dermal Sensitization Study of Gardoprim 500 FW in Guinea Pigs
4. Nucleus Anomaly Test of GS-13529 (Terbuthylazine) in Somatic (Bone Marrow) Interphase Nuclei
5. Mouse Lymphoma Mutagenicity Test of GS-13529 (Terbuthylazine)
6. Autoradiographic DNA Repair Assay of GS-13529 (Terbuthylazine) on Rat Hepatocytes
7. Autoradiographic DNA Repair Assay of GS-13529 (Terbuthylazine) on Human Fibroblasts

The technical reviews of these reports are attached. The mutagenicity reports and the attached reviews were critiqued by Irving Mauer, Geneticist, Toxicology Branch.

There was no identification of Gardoprim 500 FW in the original submission. The Registrant has since confirmed that the Dermal Sensitization Study of Gardoprim 500 FW in Guinea Pigs was performed using a test article containing 44.7% terbuthylazine (ref. Whalan memorandum; 4-18-86).

The Nuclear Anomaly Test and the two Autoradiographic DNA Repair Assays were unacceptable and must be repeated.

1

005210

28-DAY SUBACUTE ORAL TOXICITY STUDY OF GS-13529 (TERBUTHYLAZINE) IN RABBITS

Ciba-Geigy, Ltd.; Report No. 830288; April 19, 1984; Accession No. 259814

PROTOCOL: Groups of 5 male and 5 female New Zealand White rabbits (1800-2325 g; 12-14 weeks old) were randomly assigned to 4 groups. They were housed individually. Food and water were provided ad libitum. The test compound, GS-13529 (Batch No. EN 16727 technical), was formulated by dissolving it in distilled water which contained 0.1% polysorbate 80 and 0.5% carboxymethyl-cellulose. The rabbits were dosed by gavage (5 ml/kg dose volumes) 5 days/week over 28 days at doses of 0 (vehicle control), 5, 20, and 100 mg/kg/day. The rabbits were observed daily for clinical signs, and their body weights were measured weekly. Food consumption was measured twice weekly. The following clinical pathology measurements were made on retroorbital blood of all rabbits on the last day of dosing:

Hematology

- Erythrocytes
- Hematocrit
- Hemoglobin
- Mean corpuscular volume
- Mean corpuscular hemoglobin concentration
- Platelets
- Leukocytes
- Differential leukocyte count
- Reticulocytes

Clinical Chemistry

- Total protein
- BUN
- Total bilirubin
- Creatinine
- Glucose
- Albumin
- Globulin
- Albumin/globulin ratio
- Alkaline phosphatase
- Total cholesterol
- GGT (gamma-glutamyl transpeptidase)
- Aspartate aminotransferase (SGOT)
- Alanine aminotransferase (SGPT)
- Prothrombin time
- Na
- K
- Ca
- Cl
- P

Most of the rabbits were sacrificed on day 28 and examined grossly. Three males and 2 females in the high dose group were retained for 13 recovery days before being necropsied. The following organs were weighed:

- Brain
- Thyroids
- Heart
- Spleen
- Adrenals
- Ovaries
- Testes
- Thymus
- Liver
- Kidneys

The following tissues were examined histopathologically for all rabbits:

2

Brain	Small intestine
Spinal cord	Large intestine
Eye (with optic nerve)	Adrenal gland
Orbital gland [sic]	Pancreas
Extraorbital lacrimal gland	Gall bladder
Pituitary	Liver
Salivary gland	Kidney
Aorta	Urinary bladder
Heart	Ovary
Thymus	Uterus
Thyroid and parathyroid	Testis
Lung (with mainstem bronchi)	Epididymis
Trachea	Prostate
Spleen	Seminal vesicle
Sternum (with marrow)	Mammary area [sic]
Mesenteric lymph node	Femur with joint [sic]
Axillary lymph node	Skeletal muscle
Peripheral nerve (not specified)	Skin
Esophagus	Gross lesions
Stomach	

RESULTS: Eleven high-dose rabbits died between days 2 and 13. A mid-dose rabbit died because of a dosing error. The report's description of clinical signs was barely intelligible, but there appeared to be dose-related signs of sedation, dyspnea, ruffled fur, curved and ventral body positions, diarrhea, and tremor. An undefined set of clinical signs persisted in the high-dose group through the first week of the 13-day recovery period, then reversed during the second week. Body weight gain was markedly decreased in the mid-dose males (nadir, 19%) and high-dose males (nadir, 39%) and females (nadir, 41%), compared to the controls. The high-dose males and females both had sharp weight decreases (compared to their pretest weights) which did not reverse until the recovery period. Dose-related decreases in food-consumption paralleled the decreased weight gain. The high-dose males and females stopped eating for a period during the first week and were anorexic throughout the dosing period. During the recovery period, however, these rabbits ate far more than the controls. Since the test article was administered by gavage, food palatability was probably not a factor.

When the rabbits were evaluated for clinical pathology effects, the high dose males and females were found to be mildly anemic. The males had a 50% reduction, and the females a 21% decrease in leukocytes. The females had a 49% increase in platelets. Other anomalies seen in the high-dose groups included slight hyperkalemia and decreased alkaline phosphatase and inorganic phosphorus; these were the normal physiological consequence of anorexia, and not primary toxic effects.

The test article caused mild to severe decreases in the absolute and relative weights of all measured organs in both sexes in the mid- and high-dose groups. The major reasons for the decreased organ weights were anorexia and the consequent decreases in body weight gain. Even the brain weights were decreased 17% in the high-dose males. males. The most profoundly affected organ was the thymus which was reduced in weight by as much as 94%, compared to the controls. Organ weight changes were reversing in the high dose rabbits after the recovery period, with the exception of ovary weights. Lesser, but never-

3

theless significant changes in the low-dose rabbits included decreased absolute and relative weights for heart, liver, kidney, thymus, and spleen in males, and kidneys in females.

Compound-related gross lesions were seen in the high-dose males and females and included lung consolidation, and thymic hemorrhage, mottling, and diminution. The histopathology data were not evaluable. Because frank toxicity was observed at all dose levels, a NOEL could not be defined.

This study is CORE SUPPLEMENTARY. The doses selected for this study were too toxic to establish a NOEL. This study must be repeated at lower dosage levels in order to define the NOEL. At least 10 animals/sex/dose level should have been used. It was unclear how many animals were used in the high-dose groups. The description of clinical signs in the results was unintellegible, and there were no supporting individual animal data. Gross pathology data for the animals examined after the recovery period were combined with that of the earlier sacrifice. The histopathology data were not evaluable because the summary table failed to report the number of each tissue examined. Also, there was no mention of lesion severities in the summary or individual animal data tables. There were no ophthalmologic examinations. This report did not receive Quality Assurance review.

28-DAY SUBACUTE DERMAL TOXICITY STUDY OF GS-13529 (TERBUTHYLAZINE) IN RABBITS

Ciba-Geigy, Ltd.; Report No. 830287; May, 1984; Accession No. 259814

PROTOCOL: Groups of 5 male and 5 female New Zealand White rabbits (1800-2400 g; 12-14 weeks old) were randomly assigned to 4 groups. Food and water were provided ad libitum. The test compound, GS-13529 (Batch No. EN 16727 technical), was formulated by dissolving it in distilled water which contained 0.1% polysorbate 80 and 0.5% carboxymethylcellulose. The rabbits were dermally dosed 5 days/week during a period of 28 days with 0 (vehicle control), 5, 50, and 500 mg/kg/day of the test article. Gauze patches soaked in the test formulations were applied to the shaved skin of their backs and flanks. The patches were occluded with an impermeable material (not otherwise specified) and held in place with nonirritating adhesive tape. The patches were removed after 6 hours of exposure, and the dosing sites were presumably not rinsed.

The rabbits were observed daily for clinical signs, and their body weights were measured weekly. Food consumption was measured twice weekly. The following clinical pathology measurements were made on retroorbital blood of all rabbits on the last day of dosing:

Hematology

- Erythrocytes
- Hematocrit
- Hemoglobin
- Mean corpuscular volume
- Mean corpuscular hemoglobin concentration
- Platelets
- Leukocytes
- Differential leukocyte count
- Reticulocytes

Clinical Chemistry

Total protein	GGT (gamma-glutamyl transpeptidase)
BUN	Aspartate aminotransferase (SGOT)
Total bilirubin	Alanine aminotransferase (SGPT)
Creatinine	Prothrombin time
Glucose	Na
Albumin	K
Globulin	Ca
Albumin/globulin ratio	Cl
Alkaline phosphatase	P
Total cholesterol	

The rabbits were sacrificed on day 28 and examined grossly. An additional 5 males and 4 females were given the high-dose regimen, then retained for an additional 14 recovery days. The following organs were weighed:

Brain	Ovaries
Thyroids	Testes
Heart	Thymus
Spleen	Liver
Adrenals	Kidneys

The following tissues were examined histopathologically for all rabbits:

Brain	Stomach
Spinal cord	Small intestine
Eye (with optic nerve)	Large intestine
Orbital gland	Adrenal gland
Extraorbital lacrimal gland	Pancreas
Pituitary	Gall bladder
Salivary gland	Liver
Aorta	Kidney
Heart	Urinary bladder
Thymus	Ovary
Thyroid and parathyroid	Uterus
Lung (with mainstem bronchi)	Testis
Trachea	Epididymis
Spleen	Prostate
Sternum (with marrow)	Seminal vesicle
Mesenteric lymph node	Mammary area [sic]
Cervical lymph node	Femur with joint [sic]
Sciataic nerve	Skeletal muscle
Esophagus	Skin, treated and untreated

There was no mention of examining gross lesions histopathologically.

RESULTS: There were no deaths during this study. There were no clinical signs observed in the control rabbits. Dose-related signs seen in all groups dermally dosed with the test article included sedation, dyspnea, tremor, curved body posture, and piloerection. These signs were described as ranging from minimal to moderate. In addition, ataxia was observed in the low- (females only), mid- and high-dose groups. The rabbits continued to have sedation, dyspnea, tremor, curved body posture, and piloerection during the first week

5

of the 14-day recovery period, but were asymptomatic during the second week. The rabbits dosed with the test article reportedly had minimal to slight local skin irritation at the dosing sites which included erythema and occasionally edema. This skin irritation was not dose-related, and reversed during the recovery period. The high-dose rabbits had mildly retarded weight gains of 17% for males and 13% for females, compared to the controls. The high-dose rabbits allowed to recover had increased rates of weight gain. Food consumption in the low- and mid-dose groups were similar to that of the controls. The high-dose group had reduced food consumption during the first week of dosing, but resembled the control group by the end of the dosing interval. The high-dose rabbits allowed to recover showed increased consumption after the dosing interval.

There were no clinical pathology anomalies in any group. The test article caused mild decreases in the absolute and relative weights for thymus in high-dose males, and liver, kidney, thymus, ovary, and spleen in high-dose females. These effects appeared to reverse in the recovery period. There were no compound-related gross lesions seen in any groups, including in those allowed to recover. The histopathology data were not evaluable. There were no tumors found in any rabbits. Because frank toxicity was observed at all dose levels, a NOEL could not be defined.

This study is CORE SUPPLEMENTARY. The doses selected for this study were too toxic to establish a NOEL. This study must be repeated at lower dosage levels in order to define the NOEL. At least 10 animals/sex/dose level should have been used. The Study Methods describe dosing the animals for 28 days and having a recovery period of 14 days. The data tables indicate that dosing may have lasted for 29 days, and the recovery period may have been 13 days. It was not reported whether the dosing sites were rinsed; presumably they were not. There were no tables presenting the signs of skin irritation. The histopathology data were not evaluable because the summary table failed to report the number of each tissue examined. Also, there was no mention of lesion severities in the summary or individual animal data tables. There were no ophthalmologic examinations. This report did not receive Quality Assurance review.

DERMAL SENSITIZATION STUDY OF GARDOPRIM 500 FW IN GUINEA PIGS

Scantox Biologisk Laboratorium A/S; Report No. 10493; August 30, 1984; Accession No. 259814

PROTOCOL: Forty female Pirbright White guinea pigs (240-280 g) were assigned to two groups of twenty animals each, a treatment group and a control group. Food and water were available ad libitum. Induction doses were applied to the shaved scapular regions of the treatment group by applying 2 x 4 cm patches of Whatman paper containing 0.2 ml of the undiluted test article (44.7% terbuthylazine). The patches were held in place with adhesive tape and occluded with a bandage wrapped around the trunk. Each dose was held in place for 24 hours (there was no mention of washing the dosing sites). A total of three induction doses were applied on days 2, 7, and 9. Immediately before applying the third dose, 0.1 ml of unmodified Freund's complete adjuvant was injected intradermally on each side of the patch. The control group did

not receive induction doses, but they were injected with 0.1 ml of unmodified Freund's complete adjuvant. Three weeks after the first dermal doses were given, all treated and control animals were challenged by dermally applying approximately 0.1 ml of 50% test article in distilled water to the shaved left flanks on 2 x 2 cm Whatman paper. The treated animals were similarly dosed on their right flanks with distilled water. The right flanks of the control animals were dosed with an unspecified compound in conjunction with an unrelated experiment. All patches were secured and occluded as before. The dosing sites were evaluated and scored for skin irritation 24, 48, and 72 hours after the challenge doses were removed. There were no observations of irritation following the induction doses.

RESULTS: A guinea pig in the treatment group died at an unspecified time. The cause of death was not discussed. The water challenge applied to the treatment group was nonirritating. The test article challenge applied to the treatment group caused slight erythema 24 hours after dose removal in 7 of the surviving 19 animals. Nine of the 20 surviving control animals had slight erythema 24 hours after removal of the test article challenge. There was no irritation seen at the 48 and 72 hour intervals in either the treatment or control groups. These findings demonstrate that the test article is not a sensitizing agent in guinea pigs.

This study is CORE MINIMUM. There were no observations of irritation following the induction doses. A guinea pig in the treatment group died at an unspecified time; the cause of death should have been discussed. Positive controls and 0.5 ml dose volumes should have been used. This study received Quality Assurance review.

NUCLEUS ANOMALY TEST OF GS-13529 (TERBUTHYLAZINE) IN SOMATIC (BONE MARROW) INTERPHASE NUCLEI

Ciba-Geigy, Ltd.; Report No. 820910; June 3, 1983; Accession No. 259814

PROTOCOL: Groups of six male (22-32 g) and six female (20-31 g) Chinese hamsters were dosed twice by gavage with GS-13529 over two consecutive days. The test article was dissolved in arachid [peanut] oil and administered at doses of 0 (vehicle control), 750, 1500, and 3000 mg/kg/day. Additional groups were dosed with 128 mg/kg of cyclophosphamide (positive control) in arachid oil. Dose volumes were 20 ml/kg. Twenty-four hours after the second doses, the guinea pigs were sacrificed by cervical dislocation. Bone marrow was harvested from both femurs of each animal with siliconized pipettes wetted with 0.5 ul of rat serum. Bone marrow smears were prepared and scored for 3 animals/sex/group. They were evaluated for the following anomalies:

1. Single Jolly bodies
2. Fragments of nuclei in erythrocytes
3. Micronuclei in erythroblasts
4. Micronuclei in leukopoietic cells
5. Polyploid cells

7

RESULTS: A low-dose female, a mid-dose female, and a mid-dose male died after receiving the second dose; the causes of death were not discussed. The incidence of bone marrow anomalies (mostly single Jolly bodies) in the groups dosed with the test article was similar to that of the vehicle controls. The positive controls had frequent incidence of each of the evaluated anomalies, demonstrating the sensitivity of the system. Thus, the test article did not induce nuclear anomalies in somatic (bone marrow) cells of Chinese hamsters.

This study is UNACCEPTABLE. The data table had numerous unexplained gaps. These gaps either represent the absence of adverse effects, or the failure to evaluate all the parameters for each group - it was impossible to tell which. The rationale for dose selection was not given. The cause of death was not discussed for the three guinea pigs which died after dosing. The marrow cells were evaluated for polyploidy, but there was no mention of chromosome counts. This study did not receive Quality Assurance review.

MOUSE LYMPHOMA MUTAGENICITY TEST OF GS-13529 (TERBUTHYLAZINE)

Ciba-Geigy, Ltd.; Report No. 820911; April 15, 1983; Accession No. 259814

PROTOCOL: This study was performed in two parts, a preliminary cytotoxicity test and a mutagenicity test, using L5178Y/$TK^{+/-}$ mouse lymphoma cells. In the preliminary test, cells in the exponential phase of growth were exposed for four hours to the test article dissolved in 1% DMSO at concentrations ranging from 15.63 to 1000.0 ug/ml. Additional cultures were dosed with DMSO. The cells were maintained on F_{10P} medium (Fischer's medium plus antibiotics and 10% horse serum) without metabolic activation. After four hours of dosing, the cells were washed and further incubated for 3 days. At the end of three days, the cultures were evaluated for cytotoxicity.

The mutagenicity test was performed according to the method of Clive and Spector (1975). Spontaneous $TK^{-/-}$ mutant clones were eliminated by exposing the cells to THMG (a medium containing thymidine, hypoxanthine, methotrexate and glycine) for 24 hours. The cells were further exposed (for an unspecified reason) to THG medium (thymidine, hypoxanthine, and glycine) for 3 days. Populations of 3×10^5 cells were treated for 4 hours with 0 (solvent control), 62.5, 125, 250, 500, and 1000 ug/ml of the test article in round bottomed flasks. They were treated in the presence and absence of Aroclor 1254 induced rat liver S-9 mixture. Additional cells were untreated (negative controls) or treated with the positive control articles (ethylmethane sulfonate, EMS, for the nonactivated systems; dimethylnitrosamine, DMN, for the activated systems). After four hours of exposure, the cells were washed with F_{10P} medium, resuspended in F_{10P} medium, and cultured for 3 days to permit expression of $TK^{-/-}$ mutant clones.

Twelve aliquots of each culture were then added to culture tubes containing semi-solid agar cloning medium. To eight of these tubes for each concentration (containing 0.005% BUdR) were added 4×10^5 cells; they were incubated for 14 days and used for mutant selection. To the other 4 tubes (which did not contain BUdR) were added 200 cells; these tubes were for viability control, and were incubated for 10 days. At the end of the incubation periods, the cultures were counted for colonies. The mutagenicity data were adjusted based

8

on the viability control data (i.e. calculated to represent 100% viability of the cells seeded in cultures in the mutagenicity test). The performing scientists used a factor of >2.5 to establish a significant mutagenic effect when comparing a colony count to the solvent control.

RESULTS: In the preliminary test, cell toxicity reportedly remained above the desired 85% reduction, so the 5 highest doses used in the preliminary test were also used in the mutagenicity test. In the mutagenicity test, the percent of relative growth (an indication of cytotoxicity) was significantly reduced in the 1000 ug/ml groups in the nonactivated and activated systems, relative to the negative and solvent controls. The mutant frequency for the negative and solvent controls and the cultures dosed with the test article were similar in both the nonactivated and activated systems. The positive controls had 53-fold and 19-fold increases in mutant frequency for the nonactivated and activated systems, respectively, compared to the negative controls; this demonstrating the sensitivity of the system. Thus, no increases in mutant frequency were seen in the nonactivated or activated systems.

This study is ACCEPTABLE. The source of the mouse lymphoma cells was not reported. In the Autoradiographic DNA Repair Assay on Human Fibroblasts, precipitation occurred at concentrations of >62.5 ug/ml, yet there was no mention of precipitation in this study despite the use of doses ranging from 62.5 to 1000 ug/ml. Different test article formulations may have been used in these studies. This study did not receive Quality Assurance review.

AUTORADIOGRAPHIC DNA REPAIR ASSAY OF GS-13529 (TERBUTHYLAZINE) ON RAT HEPATOCYTES

Ciba-Geigy, Ltd.; Report No. 831174; June 18, 1984; Accession No. 259814

PROTOCOL: This study was performed in two parts, a cytotoxicity test and a DNA-repair assay. In the cytotoxicity test, fresh hepatocytes taken from a male rat were cultured in Williams' Medium E which contained 10% fetal bovine serum. The cells were then allowed to attach to cover slips over a period of 1.5 to 2 hours. The attached cells were washed and cultivated overnight in renewed medium. The following day, 20 ul volumes of the test article formulations in DMSO (15.625-1000 ug/ml) and the vehicle (vehicle control) were added to 2 ul aliquots of the hepatocyte cultures. The cultures were incubated for 5 hours, after which the medium was removed, and the cells washed with BSS and stained with trypan blue solution. The cells were fixed, then examined for the percentage of unstained cells/100 cells. The doses to be used in the DNA repair assay were based on cell viability, condition for examination, and the ability to adhere to a coverslip.

In the DNA repair assay, fresh hepatocytes taken from a male Tif.RAIf (SPF) rat (185 g) were prepared as in the cytotoxicity test. Hepatocyte cultures were dosed with 0 (vehicle control), 1, 5, 25, and 125 ug/ml of the test article, or 100 mM of dimethylnitrosamine (positive control). There was also an untreated (negative) control. Immediately after dosing with the test articles, ^{3}H-thymidine (specific activity 23.8 Curies/mmol) was added to each mixture. At the end of the 5 hour incubation period, the cells were

9

prepared for autoradiographs. A total of 150 nuclei from each treatment/control group were scored for incidence of DNA repair by counting the number of silver grains/nucleus.

RESULTS: The cytotoxicity test established the doses used in the DNA repair assay. Precipitation occurred at concentrations of ≥125 ug/ml. The incidence of repair in the DNA repair assay was similar for the negative and vehicle controls and the cultures treated with the test article. The positive control had DNA repair incidence 14 times that of the negative control, demonstrating the sensitivity of the system. Thus, the test article did not initiate DNA repair in rat hepatocytes.

This study is UNACCEPTABLE. It should have been repeated at least once, preferably with hepatocytes from a female rat. In the Autoradiographic DNA Repair Assay on Human Fibroblasts, precipitation occurred at concentrations of ≥62.5 ug/ml, yet precipitation in this study occurred at doses ≥125 ug/ml. Different test article formulations may have been used in these studies. This study did not receive Quality Assurance review.

AUTORADIOGRAPHIC DNA REPAIR ASSAY OF GS-13529 (TERBUTHYLAZINE) ON HUMAN FIBROBLASTS

Ciba-Geigy, Ltd.; Report No. 831175; June 18, 1984; Accession No. 259814

PROTOCOL: This study was performed in two parts, a cytotoxicity test and a DNA-repair assay. In the cytotoxicity test, human fibroblasts (CRL 1121 from The American Type Collection, Rockville, Md.) were cultured in Dulbecco's Minimal Essential Medium which contained 10% fetal bovine serum. They were then allowed to attach to cover slips overnight. The following day, 10 ul volumes of the test article formulations in DMSO (15.625-1000 ug/ml) and the vehicle (vehicle control) were added to 1 ul aliquots of the fibroblast cultures. The cultures were incubated for 5 hours, after which the medium was removed, and the cells washed with BSS and stained with trypan blue solution. The cells were fixed, then examined for the percentage of unstained cells/100 cells. The doses to be used in the DNA repair assay were based on cell viability, condition for examination, and the ability to adhere to a coverslip.

In the DNA repair assay, fibroblasts were prepared as in the cytotoxicity test. Fibroblast cultures were dosed with 0 (vehicle control), 1, 5, 25, and 125 ug/ml of the test article, or 5 uM of 4-nitroquinoline-N-oxide (positive control). There was also an untreated (negative) control. Immediately after test article dosing, ^{3}H-thymidine (specific activity 23.8 Curies/mmol) was added to each mixture. At the end of the 5 hour incubation period, the cells were prepared for autoradiographs. A total of 200 nuclei from each treatment/control group were scored for incidence of DNA repair by counting the number of silver grains/nucleus.

RESULTS: The cytotoxicity test established the doses used in the DNA repair assay. Precipitation occurred at concentrations of ≥62.5 ug/ml. The incidence of repair in the DNA repair assay was similar for the negative and vehicle controls and the cultures treated with the test article. The positive control

10

had DNA repair incidence 60 times that of the negative control, demonstrating the sensitivity of the system. Thus, the test article did not initiate DNA repair in rat hepatocytes.

This study is UNACCEPTABLE. This study should have been performed using metabolic activation. It did not receive Quality Assurance review.

11