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

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OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

008099

MEMORANDUM

SUBJECT: ID. No.CA 02372; 13 - Week Dermal and 13-Week oral Toxicity Studies for Triazine in Rats

Tox. Chem. No.: 481C
Project No.: 01406
Record No. : 265-729

FROM: Melba S. Morrow, D.V.M. MSM 9/11/90
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CONCLUSIONS

Based on the results of the 13 week dermal toxicity study conducted with triazine in rats, the dermal NOEL in both sexes was 5 mg/kg/day and the dermal LEL was 50 mg/kg/day based on the presence of erythema and edema. The systemic NOEL was greater than 250 mg/kg/day.

In the 13 week oral study, the NOEL in male and female rats was 50 mg/kg/day and the LEL was 100 mg/kg/day based on the presence of histological findings which included erosion of the gastric mucosa and prominence of the limiting ridge in males and lymphocytic infiltration in the stomach of females.

Both studies are classified as core minimum.

The sponsor needs to address the significance of the peribronchiolar lymphoid hyperplasia that was present in both treated and control rats in both studies and how this finding relates to the overall health of the test animals.

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ACTION REQUESTED
A review of the 13 week dermal and 13 week oral toxicity studies was conducted in response to a Data Call-In Notice.

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Section II, Tox. Branch I (H7509C)
Secondary reviewer: Marion P. Copley, D.V.M. MPC 9/11/90
Section II, Tox. Branch I (H7509C)

DATA EVALUATION REPORT

STUDY TYPE: 13 Week Dermal -Rat TOX. CHEM. NO.: 481C

MRID NO.: 414830-02

GUIDELINE NO.: 82-3

TEST MATERIAL: Triazine (78.5%)

SYNONYMS: Bioban GK, Grotan

STUDY NUMBERS: LEF/4/89

SPONSOR: Triazine Joint Venture

TESTING FACILITY: Toxicology Laboratories Ltd.
Ledbury, Herefordshire, England

TITLE OF REPORT: Triazine 13 Week Dermal Toxicity Study in the Rat

AUTHOR(S): R.E. Hill and A. J. Newman

REPORT ISSUED: April 26, 1990

CONCLUSION: The dermal NOEL in both sexes was 5 mg/kg/day; the dermal LEL was 50 mg/kg/day, based on the presence of skin lesions. The systemic NOEL was greater than 250 mg/kg/day.

Toxicity category: N/A

Classification: Minimum

MATERIALS: Eighty adult Crl:CD(SD)BR rats (40 males and 40 females) weighing from 197 to 234 grams for the females and from 230 to 276 grams for the males, served as the test animals. Triazine, a clear yellow liquid containing 78.5% active ingredient was the test material. The chemical name for the test material is hexahydro-1,3,5-tris(2-hydroxyethyl)S-triazine.

METHODS: Prior to the start of the study, animals were acclimated for nine days. During this time, all test subjects were weighed, observed for clinical signs of illness and subjected to an ophthalmoscopic examination.

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Afterwards the animals were randomly allotted to one of the following treatment groups:

GROUP	DOSE (mg/kg/d)	VOL. (ml/kg/d)	# ANIMALS	
1	0	2.0	10M	10F
2	5	2.0	10M	10F
3	50	2.0	10M	10F
4	250	.216	10M	10F

The control group received pyrogen free sterile water and the high dose group received undiluted test material.

The dermal application site was clipped three days prior to the start of treatment. During the trial the animals were clipped as necessary to allow for the test material to come in direct contact with the skin. The dosing site was divided into quadrants so that the test substance could be rotated daily. This was done to prevent the development of severe skin lesions that may result from the irritant properties of the compound.

Triazine was applied for 5 consecutive days each week for a duration of 13 weeks. The exposure time for each application was 6 hours. During the exposure period, the application sites were covered with semi-occlusive bandages and animals were fitted with Elizabethan collars to minimize ingestion of the test chemical. The test site was not washed between doses. To account for changes in individual body weights during the study, individual doses were adjusted when necessary.

All rats were observed twice daily for mortality. Daily observations were also conducted to evaluate changes in condition or behavior. Prior to each administration, the application site was evaluated for irritation. All four quadrants were scored for erythema,eschar formation and edema. (See Table I for description of scores).

During the trial, body weights were taken on the first day of dosing and at weekly intervals thereafter. Food consumption was also measured weekly. An additional ophthalmoscopic examination was conducted on week 13 prior to obtaining blood samples. Blood and urine samples were also collected on week 13. Urine was collected under conditions of food and water deprivation. Blood was collected via retro - orbital puncture after an overnight fast.

The following parameters were evaluated:

Hematology:
- x Hematocrit (HCT)
- x Hemaglobin (HGB)
- x Leucocyte count (WBC)
- x Erythrocyte count (RBC)

Serum chemistry:

Electrolytes:
- x Calcium
- x Chlorine
- Magnesium

Hematology (cont.)
x Leucocyte differential
Mean corpuscular hemaglobin
Mean corpuscular hemaglobin concentration
Mean corpuscular volume
x Reticulocytes
Blood clotting measurements:
x Thromboplastin time
Clotting time
x Prothrombin time

Electrolytes (cont.)
x Potassium
x Sodium

Enzymes:
x Creatinine phosphokinase
x Alkaline phosphatase
x Lactic dehydrogenase
x SALT (SGPT)
x SAST (SGOT)
Gamma glutamyl transferase
Glutamate dehydrogenase
Cholinesterase

Other Serum Chemistry Values:
x Albumen
x Albumen: Globulin ratio
x Blood creatinine
x BUN
Cholesterol
x Globulin
x Glucose
x Total Bilirubin
x Total protein
Triglycerides
Serum protein electrophoresis

Urinalysis
x pH
x spec. gravity
x volume
x glucose
x protein
x ketones
x bilirubin
x urobilinogen
x pigments

At the end of the 13 week period, all animals were killed with carbon dioxide. A full gross necropsy was performed on all animals. Brain, liver, kidneys, heart, adrenals, spleen, thymus and testes /ovaries were weighed.

The following CHECKED (x) tissues were collected for histological examination. Weighed organs are designated by (xx)

Digestive system
Tongue
x Salivary glands
x Esophagus
x Stomach
x Duodenum
x Jejunum
x Ileum
x Cecum
x Colon
x Rectum

xx Liver
Gall bladder
x Pancreas

Cardiovasc./Hemat.
x Aorta
xx Heart
x Bone marrow
x Lymph nodes
xx Spleen
xx Thymus

Urogenital
xx Kidneys
x Urinary bladder
xx Testes
x Epididymides
x Prostate
x Seminal vesicle
xx Ovaries

Neurologic
xx Brain
x Periph. nerves
x Spinal cord

Glandular
xx Parathyroids
xx Adrenals
x Thyroid
x Pituitary
x Mammary
x Lacrimal

Other
x Bone
x Skin
x Skel. muscle

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Respiratory	xx Ovaries	x Skel. muscle
x Trachea	Urogenital	Other
x Lung	x Uterus	x All gross lesions
Nose	x Vagina	
Pharynx		
Larynx		

The parathyroid glands were examined if they were identified. The sternum, trachea, skeletal muscle, femur, lacrimal glands and mammary glands were examined microscopically only if indicated by signs of toxicity or organ involvement. Microscopic examinations were performed on tissues from all control and high dose animals, on any gross lesions and on the lungs, liver, kidneys and skin from animals in the low and intermediate dose groups.

QUALITY ASSURANCE: A statement of comliance with GLPs and a statement of quality assurance were submitted in the report.

STATISTICAL ANALYSIS: An analysis of variance was conducted on body weights, hematology values and organ weights. Pairwise t-tests were conducted between control and treated groups where p= 0.05. Kruskall Wallace was used to analyze blood chemistry values. For between group differences where p = 0.05, Wilcoxon rank sum test was applied.

RESULTS: No deaths were reported during the study . The clinical signs observed during the study included periorbital, perianal and cranial fur staining in all groups; yellow staining at the application site in animals from groups 2 and 3; and skin reactions at the application site in animals treated with Triazine at doses of 50 and 250 mg/kg/day. Occasional incidences of erythema were observed in female rats which received 5 mg/kg/day.

In assessing the severity of the dermal lesions, erythema was scored as very slight to severe in both groups 3 and 4. An increase in the numbers of animals affected and an increase in the severity of the reported reactions occured in group 4. Edema was present in animals in group 4 and was characterized as being very severe to moderate. In group 3, there were only isolated incidences of edema reported.

Statistically significant differences were found in blood chemistry values from individual animals. In group 2 the parameters included one male with increased alanine aminotransferase, one female with increased phosphorus and one female with increased chloride. In group 3, blood chemistry parameters were increased in two animals. One female in this group had increased sodium and one male had increased chlorine levels. In group 4, one female had elevated glucose levels and one male had elevated sodium levels. All values were within the

Microscopically, there was an increase in the incidence of epidermal hyperplasia and ulceration of the treated area of the skin in animals treated with 50 or 250 mg/kg/day. Eighty to 100 percent of both the treated and control animals were found to have peribronchiolar lymphoid hyperplasia in the lungs. The significance of this lesion was not discussed.

CONCLUSIONS: Based on the results of this study, the dermal NOEL for Triazine was determined as being 5 mg/kg/day and the dermal LEL was 50 mg/kg/day based on skin lesions observed at this level. The systemic NOEL was found to be greater than 250 mg/kg/day.

The study is classified as minimum. While not expected to affect the results of the study, the sponsor should address the significance of the lung lesions in the treated and control groups as they relate to the overall health of the test animals.

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DATA EVALUATION REPORT

STUDY TYPE: 13 Week Oral -Rat TOX. CHEM. NO.: 481C

MRID NO.: 414830-01

GUIDELINE No.: 82-1

TEST MATERIAL: Hexahydro-1,3,5-tris(2-hydroethyl) S-triazine

SYNONYMS: Triazine, Grotan, Bioban GK

STUDY NUMBERS: LEF/3/89

SPONSOR: Triazine Joint Venture

TESTING FACILITY: Toxicology Laboratories Ltd.
Ledbury, Herefordshire, England

TITLE OF REPORT: Triazine: 13 Week Oral (Gavage) Toxicity Study in the Rat

AUTHOR(S): R.E. Hill/ A.J. Newman

REPORT ISSUED: 4/25/90

CONCLUSION: Females: NOEL = 50 mg/kg/d, LEL = 100 mg/kg/day (lymphocytic infiltration). Males: NOEL = 50 mg/kg day, LEL = 100 mg/kg/day (erosion of the gastric mucosa, and prominence of the limiting ridge of the stomach).

Toxicity category: N/A

Classification: Minimum

MATERIALS: Triazine (78.5 % active ingredients) served as the test material. Physically the test substance was a clear yellow liquid. Fifty male and 50 female Crl: CD(SD)BR rats served as the test animals. Males weighed from 132 to 183 grams and females weighed from 121 to 154 grams.

METHODS: All test animals were acclimated for a period of 10 days during which they were observed for clinical signs of disease. These animals were also subjected to an ophthalmological examination prior to the start of the study.

Animals were randomly assigned to treatment groups as follows:

Group	Dose (mg/kg/d)	Volume	# Animals
1	0	10 ml/kg	10M / 10F
2	10	10 ml/kg	10M / 10F
3	50	10 ml/kg	10M / 10F
4	100	10 ml/kg	10M / 10F
5	250	10 ml/kg	10M / 10F

The test material was administered daily via gavage, using a choke 8 or 10 rubber catheter attached to a disposable syringe. The vehicle for the control group consisted of pyrogen free sterile water.

During the study the stability of the test material was confirmed by analysis on the day of preparation of the test material and on days 8 and 29 of the study. This analysis was conducted after the material had been stored in a dark room. An additional stability trial was conducted on days 1,7 and 14.

Mortality checks were conducted twice daily. Any animal found dead during the treatment period was subjected to a full necropsy. Daily observations were also conducted to assess changes in behavior and condition of the animal. Body weights were recorded on the first day of dosing and at weekly intervals thereafter. Weekly food consumption was also recorded for each rat and the group mean weekly intakes were calculated. Animals in the high dose group underwent an additional ophthalmoscopic examination on week 13 of the study.

Blood and urine samples were collected during week 13. Urine samples were obtained overnight, after rats had been deprived of food and water. Blood samples were obtained by retro- orbital sinus puncture after animals were fasted and lightly anesthetized with ether.

The following checked (x) parameters were examined

Hematology:
- x Hematocrit (HCT)
- x Hemaglobin (HGB)
- x Leukocyte count (WBC)
- x Erythrocyte ccunt (RBC)
- Platelet count
- x Leukocyte differential
- Mean corpuscular hemaglobin
- x Mean corpuscular hemaglobin concentration
- Mean corpuscular volume
- Reticulocytes

Blood clotting measurements:
- x Thromboplastin time
- Clotting time
- x Prothrombin time

Serum chemistry:

Electrolytes:
- x Calcium
- x Chlorine
- Magnesium
- x Phosphorous
- x Potassium
- x Sodium

Enzymes:
- Creatinine phosphokinase
- Alkaline phosphatase
- Lactic dehydrogenase
- x SGPT
- x SGOT
- Gamma glutamyl trns.

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Enzymes (cont.)
Glutamate dehydrogenase
Cholinesterase

Other Serum Chemistry Values:
x Albumen
x Blood creatinine
x BUN
  Cholesterol
  Globulin
x Glucose
x Total Bilirubin
x Total protein
  Triglycerides
  Serum protein electrophoresis

Urinalysis:
x Sp. Gravity
x pH
x Glucose
x Protein
x Ketones
x Bilirubin
x Urobilinogen
x Blood pigments

At the end of the 13 week period, all survivors were killed by CO_2 asphyxiation. All animals were subjected to gross necropsies and any abnormalities were recorded.

The following CHECKED (x) tissues were collected for histological examination. Weighed organs are designated by (xx)

Digestive system
  Tongue
x Salivary glands
x Esophagus
x Stomach
x Duodenum
x Jejunum
x Ileum
x Cecum
x Colon
x Rectum

xx Liver
  Gall bladder
  Pancreas

Respiratory
Trachea
x Lung
  Nose
  Pharynx
  Larynx

Cardiovasc./Hemat.
  x Aorta
 xx Heart
  x Bone marrow
  x Lymph nodes
 xx Spleen
 xx Thymus

Urogenital
xx Kidneys
 x Urinary bladder
xx Testes
 x Epididymides
 x Prostate
 x Seminal vesicle
xx Ovaries
x Uterus
  Vagina

Neurologic
xx Brain
 x Periph. nerves
 x Spinal cord
 x Eyes (optic n.)

Glandular
 x Parathyroids
xx Adrenals
 x Thyroid
  Pituitary
 x Lacrimal
 x Mammary

Other
 x Bone
  Skin
 x Skel. muscle
x All gross lesions

Microscopic examinations were conducted on all tissue from control and high dose animals, from any animal dying during the study, and from any observed gross lesion. The liver, kidneys, lungs, and stomachs of animals in the other three groups were also examined microscopically. Parathyroids were only examined

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if they were identified. The femur,lacrimal gland, mammary gland, skeletal muscle and sternum were only examined microscopically if there were signs of toxicity or if there was target organ involvement.

QUALITY ASSURANCE: A signed statement of Quality Assurance and a statement of GLP compliance dated 4/25/90 have been submitted.

STATISTICAL ANALYSIS: An analysis of variance was used to evaluate body weights, hematology and organ weight data. Where "p" = 0.05, pairwise T tests were used between control and treated groups. Blood chemistry parameters were analyzed using the Kruskal Wallis test. The Wilcoxon rank sum test was used to determine significant differences between treated and control groups at "p" = 0.05.

RESULTS:
Mortality
One male(from group 3) and seven females (one for each of groups 1,3 and 4 and four from group 5) were found dead during the study period. The male and the female from group4 both died on day 27 of the study, the female from the control group died on day 70; the female from group 3 died on day 76; and the females from group 5 died on days 44, 49, 56 and 74 of the study. With the exception of one female from the high dose group, the decedents had gross changes in the lung and/or thoracic cavity. The lungs in these animals did not collapse upon opening the thoracic cavity and adhesions or fluid was present in the area. Inflammatory changes and vascular congestion were present in the thoracic viscera.

In addition to the thoracic lesions, all decedents from the high dose group showed a white discoloration and prominence of the limiting ridge of the stomach.

There were no significant differences in the body weight gain and food consumption between treated and control rats. Urinalysis was unremarkable and no treatment related ocular lesions were present.

With regard to organ weights, absolute adrenal weights were increased for females in group 5. The adrenal weights ranged from 54 to 140 mg (average 77 mg) for animals in group 5 as compared to 45 to 70 mg (average 54 mg) for animals in the control group. This increase was not significant. In addition, an increase in splenic weight was observed in all treated males. The average weight for the spleens from control animals was 0.68 grams, whereas the average weight for the spleens from the treated animals 0.80 for groups 3,4 and 5 and 0.82 grams for group 2. The sponsor states that this finding was due to unusually low weights recorded for controls.

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Gross Necropsy
Grossly, a white discoloration and /or prominence of the limiting ridge of the stomach was present in 10/10 high dose males which were sacrificed at the end of the study. This finding was also present in the remaining high dose females which survived the duration of the study. In animals receiving 100 mg/kg, this change occured in 3/10 males and 1/10 females.

Histopathology
A treatment related distribution of lesions was in the stomach and to a lesser degree in the liver. In the stomach, erosion of the glandular mucosa was observed in 4/10 males and 2/10 females in the 250 mg/kg group. This finding was also present in one male from group 4 (100 mg/kg). Lymphocytic infiltration was reported in rats from group 4 (1/10 males and 2/10 females) and from group 5 (4/10 males and 6/10 females). Epithelial hyperplasia was reported in three animals from group 5 (1/10 males and 2/10 females) and in the controls (1/10 females), but there was no statistical significance for this finding. (See Table I).

In the liver, the incidence of hepatocyte vacuolation was increased in male rats from group 5 when compared to other groups. On the other hand, margination of hepatocyte cytoplasm was more pronounced in animals from the control groups and from group 2 (10 mg/kg) than in rats from groups 3,4 and 5. (See Table II).
A high frequency of peribronchiolar lymphoid hyperplasia was reported for all groups. The incidence of this finding ranged from 70 to 100 percent in both sexes. While this finding is not believed to be treatment related, the sponsor has not addressed its significance.

A statistically significant reduction in hematocrit was found in all treated males when compared to controls. Additionally, males in group 5 demonstrated a statistically significant decrease in hemoglobin concentration. Although these findings were statistically significant, they were within the expected laboratory range.

Significant differences in blood chemistry values included reduced bilirubin in all treated groups, reduced total protein in both sexes of rats which received 250 mg/kg, and a statistically significant increase in sodium concentration for all treated males and for females in groups 3,4 and 5. The bilirubin and total protein values were within the normal range and were not considered to be biologically significant.

DISCUSSION: The sponsor states that several of the deaths were related to the administration of the compound. Gross necropsy and microscopic examinations revealed that the test material had entered the lungs in six of the seven decedents. The failure to

correctly administer the test compound probably resulted in an aspiration pneumonia.

The stomach lesions were reported to be due to the corrosive nature of the test material. A treatment related response was clearly present in males, but not as apparent in females when the incidence of mucosal erosion is considered. The incidence of lymphocytic infiltration appeared to also be treatment related and showed a dose related response in both sexes of rats.

Although the sponsor states that the liver is also a target organ, based on the lesions found in the liver, the only finding that could be considered as being treatment related is the increase in the incidence of cytoplasmic vacuolation (60%) observed in the males receiving 250 mg/kg/d of triazine.

Elevations in sodium levels may have resulted if the samples were collected after a period of fasting and water deprivation. Additionally, upon examination of the sodium levels in treated and controls, the individual values ranged from high normal to slightly higher than normal. This parameter did not appear to be related to the chemical.

The presence of peribronchiolar lymphoid hyperplasia in a high percentage of control and treated animals may be associated with some process occuring elsewhere in the body. The sponsor should address the significance of this lesion.

CONCLUSION: Based on the data from this study the NOEL is 50 mg/kg/d and the LEL is 100 mg/kg/d based on gastric lesions (erosion of the mucosa and lymphocytic infiltration) in males and females.

The study is classified as minimum. While not expected to affect the outcome of this study, the sponsor should address the significance of the high incidence of peribronchiolar lymphoid hyperplasia as it relates to the overall health of the test animals.

TABLE I
STOMACH LESIONS IN MALE AND FEMALE RATS RECEIVING TRIAZINE BY GAVAGE

GROUP:	1	2	3	4	5
DOSAGE (mg/kg)	0	10	50	100	250
MALES					
micro. lesions					
erosion of mucosa	0	0	0	1/10	4/10
lymphocytic infil.	0	0	0	1/10	4/10
epith. hyperplasia	0	0	0	0	1/10
gross lesions					
white disc.	0	0	0	1/10	5/10
abn. shape (prominence of limiting ridge)	0	1/10	1/10	4/10	10/10
FEMALES					
micro. lesions					
erosion of mucosa	0	0	0	0	2/10
lymphocytic infil.	0	0	0	2/10	6/10
epith. hyperplasia	1/10	0	0	0	2/10
gross lesions					
white discol.	1/10	0	0	1/10	5/10
abn. shape (prominence of limiting ridge)	2/10	0	1/10	1/10	9/10

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TABLE II
LIVER LESIONS IN MALE AND FEMALE RATS RECEIVING TRIAZINE BY GAVAGE

GROUP	1	2	3	4	5
DOSAGE (mg/kg)	0	10	50	100	250
MALES					
micro.lesions					
marg. cytoplasm	7/10	7/10	0	1/10	1/10
cytopl. vac.	3/10	3/10	3/10	2/10	6/10
chronic inflam.	0	1/10	2/10	1/10	1/10
FEMALES					
chronic inflam.	0	0	0	0	2/10