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UNITED STATES ENVIRONMENTAL PROTECTION AGENCY

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SUBJECT Lasso Technical, Lasso EC, Lasso II, and Lasso + Atrazine 15G-Addition of Data to Files EPA Registration=524-314,-285,-296,-304, Caswell#[redacted] Shaw#09050

FROM Larry Anderson
Toxicology Branch/HED (TS-769)

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TO Robert Taylor
Product Manager=25

Recommendations:

The acceptability of studies reviewed herein to support registration of the above Lasso formulations is outlined as follows:

Type of Study*	Results (Acceptability)
Mutagenicity (Dominant Lethal) in Mice	NEL = 30 mg/kg (Provisional)
Teratogenicity in Rabbits	NEL < 10 mg/kg/day (No)
Three-Generation Reproduction in Rats	NEL = 300 ppm (No)
Eighteen-Month Carcinogenicity in Mice	Undetermined NEL (No)
Two-Year Chronic Feeding in Dogs	NEL = 1000 ppm (Yes)
Intraperitonal LD_{50} in Mice	LD_{50} = 870 mg/kg (No)
Subcutaneous LD_{50} in Mice	LD_{50} = 1200 mg/kg (No)
Oral LD_{50} in Mice	LD_{50} = 2100 mg/kg (No)
Subcutaneous LD_{50} in Rats	LD_{50} = 3600 mg/kg (No)
Inhalation LC_{50} in Rats[a]	LC_{50} > 3.2 mg/L, 6hrs. (No)
Thirty-Day Inhalation in Rats[a]	NEL = 1.55 mg/L, 6hrs/day, 30 days (No
Intraperitoneal LD_{50} in Rats	LD_{50} = 600 mg/kg (No)
Inhalation LC_{50} in Rats[a]	LC_{50} > 32 mg/L, 1 hr (No)
Mutagenicity (Recombination Assay) in Bacteria	NEL = 1000 µg (Provisional)
Mutagenicity (Reverse Mutation) in Bacteria and Yeast	NEL - 10 µl (nonactivated) and 100 µl (activated) Provi.
Mutagenicity (Host-Mediated) in Mice	NEL = 1000 mg/kg (Provisional)
Mutagenicity (Host-Mediated) in Rats	NEL = 500 mg/kg (Provisional)
Four-Week Subacute Feeding in Mice	NEL = 3000 ppm (No)
Two-Year Chronic Feeding in Rats	NEL = 1000 ppm (Yes)
Repeated Insult Patch Test in Humans	Undetermined Effect (No)
Repeated Insult Patch Test in Humans	Skin Sensitization Found (Yes)

Reasons for or against acceptability of the aforementioned studies are presented in the review.

*Lasso Technical (90-94%) was the test material used in all studies described in the outline except those indicated by an (a).

**No RPAR criteria have been exceeded.

***Studies conducted at Industrial Bio-Test Laboratories, Inc., have been submitted and must be validated by the registrant.

EPA FORM 1320-6 (REV 3-76)

Review:

A. Mutagenicity (Dominant Lethal) Study of Lasso Technical in Albimo Mice (Industrial Bio-Test Laboratories, Inc., IBT No. E1184, 7/26/72, submitted by Monsanto Agricultural Products Co., 8/16/78, Acc.#234630).

1. Procedure:

Forty eight male Charles River mice, 60-70 days old, were divided into 4 groups of 12 animals each which were given 0, 15, or 30 mg/kg of test material or 50 mg/kg of methyl methanesulfonate (MMS; positive controls) intraperitoneally. Controls were given corn oil, the vehicle used in the study, alone. Each mouse was caged with a different group of 3 untreated virgin females each week over a period of 6 weeks. All males were sacrificed at the conclusion of the 6 week mating period. Females were sacrificed at 1 week following mating, and numbers of implantation sites, resorption sites, and embryos were determined. The criterion for pregnancy was the presence of corpora lutea in the ovaries. Mutagenicity was determined by comparing both the proportions of implantations as deciduomata and the numbers of viable embryos between dosage groups.

2. Results:

a) Range-Finding Study: The selection of doses for the mutagenicity study was based on the following results of a range-finding study:

Dose (mg/kg)	No. of Deaths & Mice	Observations
1000	4/4	Tremors, ataxia, hypoactivity
300	0/4	Tremors, ataxia, hypoactivity
100	0/4	Hypoactivity
30	0/4	Hypoactivity

b) Mortality: Two males in the 30 mg/kg group.

c) Toxic Signs: Hypoactivity in males in the 30 mg/kg group.

d) Mating Performance: Unremarkable

e) Sacrifice Data (Implantations, Resorptions, Embyros): Unremarkable differences between control and test groups. Enhancement and reduction in the numbers of early resorption sites and viable embryos, respectively, were noteworthy in the positive control group.

f) Mutagenicity Data:

i) Pre-implantation Losses: Unremarkable

ii) Mutation Rates: Unremarkable according to proportions of implantation sites as decideuomata and numbersof viable embryos.

3. Conclusions:

[redacted]

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a) Classification: Supplementary Data

i) A final conclusion on the validity of the study towards satisfying regulatory requirements is deferred until mutagenicity guidelines are finalized.

ii) A mutagenic effect of the test material was not demonstrated in the present study; however, the dominant-lethal test may not in itself be entirely indicative of mutagenic effects in mammalian cells in vivo.

b) The mutagenic N.E.L. according to the present study is 30 mg/kg.

B. Teratogenic Study of Lasso Technical in Albino Rabbits (Industrial Bio-Test Laboratories, Inc., IBT#J1183, 8/14/72, submitted by Monsanto Agricultural Products Co., 8/16/78, Acc.#234630).

1. Procedure:

New Zealand albino rats, 3.22-4.25 kg, were used. The study was designed as follows:

Compound	Dosage Level (mg/kg/day)	No. of Females Inseminated	No. of Pregnant Animals
None (Controls)	0	17	9
Thalidomide (Positive Controls)	37.5	17	13
Lasso Technical	10.0	17	11*
Lasso Technical	30.0	17	15*

*One animal in each group aborted on day 20 of gestation.

The rabbits received the compounds in gelatin capsules from day 6 through day 18 of gestation, inclusively. Controls were given empty gelatin capsules concurrently. The time of insemination was designated day 0 of gestation. Does were weighed regularly and were observed for toxic signs until sacrifice at day 29 of gestation.

After the rabbits were sacrificed, fetuses were removed by cesarian section, examined externally, and weigned. Fetal viability was determined by respiratory and paw movements observed during a 24-hour period of incubation at 37 °C. All young were examined for internal and skeletal abnormalities. Skeletal examinations were done according to the method of Hurley (1965).

2. Results:

a) Maternal Effects

i) Body Weight Changes: Gain which was similarly reduced in all treatment groups compared to controls during treatment was normal in all groups post-treatment.

ii) Mortality: None

iii) Toxic Signs: Unremarkable

iv) Reproductive Effects (Number of Implantation Sites, Resorption, Live Young, Abortions): The number of resorptions and live young were markedly increased and decreased, respectively, in the positive control and 30 mg/kg groups.

b) Fetal Effects

i) External Development: Unremarkable. Either bilateral ectrodactyly or open fontanel were found in 2 positive control fetuses.

ii) Body Weights: Unremarkable

iii) Viability: Unremarkable

iv) Internal Development: Unremarkable

v) Skeletal Development: Outstanding findings were as follows:

Anomaly	No. of Incidences/No. of Fetuses Control	Positive Control	10 mg/kg	30 mg/kg
Non-ossified sternum	1/52	5/66	10/72	13/73
Thickened ribs	2/52	3/66	5/72	7/73

3. Conclusions:

a) Classification: Invalid Data (Provisional)

i) The signature of Dale Fletcher was entered into the report in lieu of that of Robert Ladd, the designated group leader. This discrepency must be resolved to verify the scientific conduct of the study.

ii) At least 3 dosage levels should have been used.

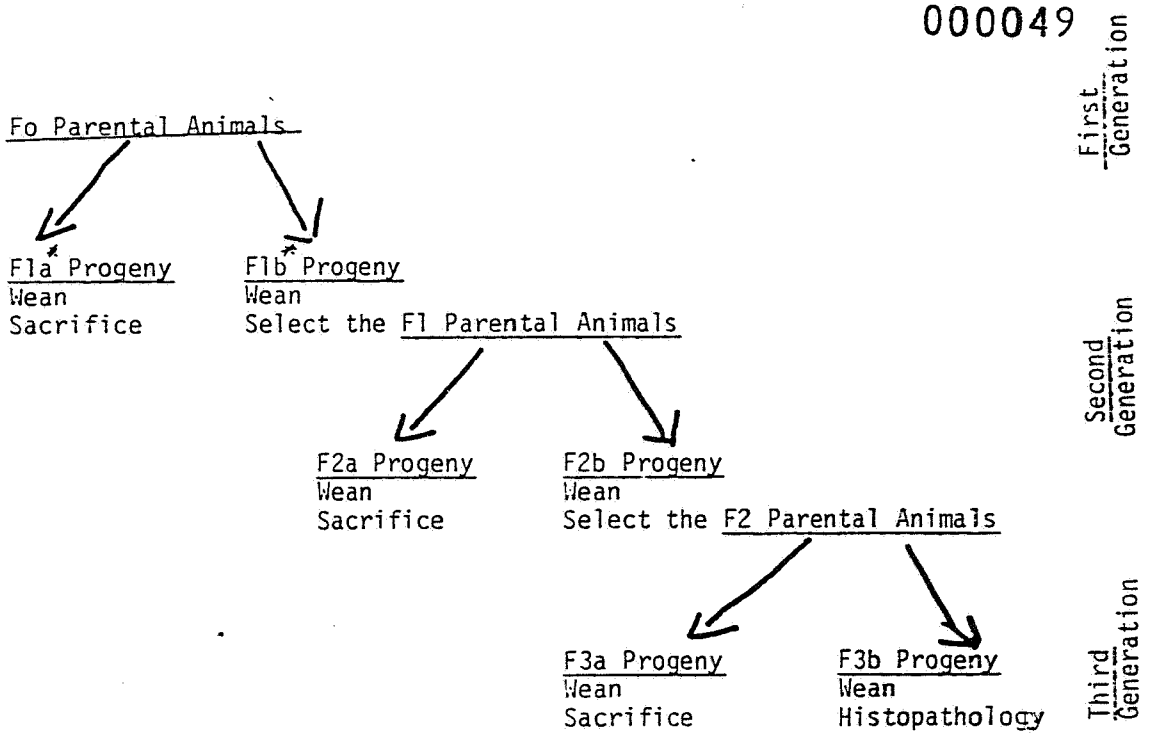
iii) Individual data of internal examinations were not submitted.

b) Based on higher incidences of skeletal abnormalities in the treatment groups, a teratogenic N.E.L. cannot be determined ($<$10 mg/kg/day, lowest dosage level tested).

C. Three-Generation Reproduction Study of Lasso Technical in Albino Rats (Industrial Bio-Test Laboratories, Inc., IBT#622-01185, 4/18/74, submitted by Monsanto Agricultural Products Co., 8/16/78, Acc.#234630).

1. Procedure:

The basic experimental design is outlined as follows:



*a: First litter, b: Second litter

Ninety six weanling Charles River albino rats (Fo generation), 47-50g, were divided into 4 groups of 8 males and 16 females each which were fed 0, 30, 100, or 300 ppm of test material in the diet until sacrifice following weaning of the second litters. Fresh diets were prepared weekly. Parental animals were allowed to mature, mate, and produce 2 litters. Eight males and sixteen females from each second litter were parental animals for each succeding generation until the study was ended following weaning of the F_3 litters.

Mating commenced when parental animals were 100 days old. Two females and one male of each dosage group were caged together. Examinations for copulation were made daily. Males were rotated within their dosage groups at 10-day intervals until either conception was confirmed or each female had been paired with a maximum of 3 males. First litters were weaned at 21 days post-partum. After a 10-day rest female parents were bred to obtain the second litters.

Animals were weighed weekly until mating and at sacrifice. Observations of mortality and toxic signs were made daily. Parental animals were observed also for fertility, gestation period, and lactation performance. Pups were examined for external abnormalities and viability. It should be noted that the number of animals in each litter was reduced to 10 on day 4 of lactation.

Necropsies were done on all surviving males and 8 females from each parental group after the second litters were weaned. Histopathological examinations were done on 5 males and 5 females from each control and 300 ppm parental group. (continued on next page)

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Ten male and ten female pups of the F_3 generation (second litters) were subjected to necropsy at weaning; histopathological evaluation of the F_3 progeny was restricted to weanlings of the control and 300 ppm dosage groups.

Organ, organ/body, and organs/brain weight data for necropsied parental animals include the following organs: liver, kidneys, spleen, gonads, heart, and brain.

The following tissues and organs were examined microscopically:

Heart (right and left ventricles)
Trachea
Lung
Liver
Pancreas
Stomach (cardiac, fundic, and pyloric regions)
Small Intestine (duodenum, jejunum, and ileum)
Caecum
Colon
Spleen
Lymph Node (cervical and mesenteric)
Kidney
Urinary Bladder
Testis
Ovary
Prostate
Pituitary Gland
Adrenal Gland
Salivary Gland (submaxillary)
Thyroid Gland
Parathyroid Gland
Skeletal Marrow (sternum and femu
Bone Marrow (sterum and femur)
Peripheral Nerve (sciatic)
Brain (cerebrum, cerebellum, and pons
Seminal Vesicles
Esophagus
Spinal Cord

2. Results:

a) Parental Observations

i) Body Weight Changes: Unremarkable

ii) Mortality: Unremarkable except that all of the 3 F_1 parental males died. Histopathological examination of tissues from three F_1 males attributed the cause of death to acute respiratory infection.

iii) Toxic Signs: Unremarkable

iv) Necropsy: Unremarkable

v) Organ, Organ/Body, Organ/Brain Weights: Unremarkable

vi) Histopathology: Unremarkable; however, respiratory lesions attributed to chronic murine pneumonia were slightly more severe in some test animals and were found in all but 3 female parental animals examined.

vii) Reproduction Data (Mating, Fecundity, Fertility, Paturition Indices): Unremarkable. Significant differences were sporadic and were not dose-related.

b) Progeny Observations

i) Findings concerning viability, location, survival, body weights, toxic signs, necropsies, and histopathology were all unremarkable. Significant differences were sporadic and were not dose-related.

3. Conclusions:

a) Classification: Supplementary Data

i) Data on histopathological examinations of tissues and organs from all animals in the parental and F_3 (second litters) groups should have been submitted.

ii) At least 20 females and 10 males should have been included with each dosage group.

iii) Gross- and histo-pathological results for F_3b animals were summarized as negative in a sentence; all such findings should be presented individually in tables as was done for parental animals.

iv) The marked incidence of respiratory disease found in all parental animals examined histopathologically suggests the use of unthrifty animals in the present study.

b) A reproductive N.E.L. of 300 ppm of test material has been demonstrated in the present study (Provisional).

D. Eighteen-Month Carcinogenicity Study of Lasso Technical in Albino Mice (Industrial Bio-Test Laboratories, Inc., IBT#621-01182, 7/17/74, submitted by Monsanto Agricultural Products Co., 3/16/78, Acc.#234630).

1. Procedure:

Four hundred eight Charles River albino mice, weights unspecified, were separated into 4 groups of 120 animals each (60 males and 60 females) which received 0, 100, 300 or 1000 ppm of test material in the diet over 18 months. Animals were caged in groups. Fresh diets were preapred weekly. Observations for toxic signs and mortality were made daily, and animals were checked for tumors weekly. Necropsies were performed, and histopathological examinations were conducted as follows on tissues and organs from (10 males and 10 females)/ dosage group at final sacrifice as well as on suspected tumors.

Heart	Urinary Bladder	Skeletal muscle
Liver	Testis	Bone marrow
Lung	Ovary	Peripheral nerve
Pancreas	Prostate	Trachea
Stomach	Uterus	Spinal cord
Small Intestine	Pituitary	Eye
Caecum	Adrenal	Optic nerve
Colon	Salivary Gland	
Spleen	Thyroid	
Lymph node	Parathyroid	
Kidney	Brain (cerebrum, cerebellum, pons).	

2. Results:

a) Mortality at 18 Months:

Total Dead	Control Male	Control Female	100 ppm M	100 ppm F	300 ppm M	300 ppm F	1000 ppm M	1000 ppm F
No. Tested	52/60	21/60	45/60	10/60	43/60	19/60	31/60	9/60

b) Toxic Signs: Unremarkable

c) Necropsy: Unremarkable

d) Histopathology: Unremarkable including tumor findings. However, bilateral focal lymphoid infiltration of the kidney was an outstanding finding in all dosage groups. Amyloidosis was noted for several organs.

3. Conclusions:

a) Classification: Invalid Data

i) Histopathological results for all animals on study should be provided.

ii) Results for 10 surviving control males are indicated in the histopathology tables; but the mortality table shows that only 8 control males survived.

b) An NOEL and oncogenic potential cannot be determined from the present study.

E. Two-Year Chronic Oral Toxicity Study of Lasso Technical in Beagle Dogs (Industrial Bio-Test Laboratories, Inc., IBT#C1181, 6/10/74, submitted by Monsanto Agricultural Products Co., 8/16/78, Acc.#234630).

1. Procedure:

Thirty two purebred beagle dogs, 5.4-11.3 kg, were divided into 4 groups of 8 animals each (4 males and 4 females) which received 0, 100, 300, or 1000 ppm of test substance in the diet for 2 years. Before treatment the dogs had been immunized against parasitic infestations. Four dogs/sex/dosage level were housed in each kennel. Preparation of fresh diets and estimation of food consumption were done weekly. Observations of toxic signs were made daily. Hematologic, clinical chemistry, and urine analyses, done initially and at 1.5, 3, 6, 12, 18, and 24 months, included the subsequent parameters:

Hematology: Total leukocyte count, erythrocyte count, hemoglobin, hematocrit, differential leukocyte count.

Clinical Chemistry: Blood urea nitrogen, glucose, alkaline phosphatase, SGOT, SGPT, bilirubin, protein, protein electrophoresis.

Urine Analysis: Albumin, glucose, pH, specific gravity, microscopic elements.

Complete necropsies were done on each dog. The following organs were weighed: Liver, kidneys, heart, brain, spleen, gonad, adrenals, thyroid, pituitary. Microscopic evaluation of the following tissues and organs was reported:

Adrenal Glands
Aorta (thoracic)
Bone Marrow (sternum)
Brain (cerebrum, cerebellum, pons)
Caecum
Esophagus
Gall Bladder
Gonads
Heart
Kidneys
Liver
Lungs
Lymph Nodes (cervical, mesenteric)
Muscle (skeletal)
Pancreas
Peripheral Nerve (sciatic)
Pituitary Gland
Prostate Gland
Salivary Gland (submaxillary)
Small Intestine (duodenum, jejunum, ileum)
Spinal Cord
Spleen
Stomach (cardia, fundus, pylorus)
Trachea
Thyroid Gland
Uterus
Urinary Bladder

In addition, bone marrow differential smears were taken.

2. Results:

a) Mortality: None

b) Toxic Signs: Unremarkable

c) Body Weight Changes: Unremarkable (Gain of 2.0-4.1 kg)

d) Food Consumption: Unremarkable

e) Hematology: Clinical Chemistry, Urine Analysis: Unremarkable

f) Necropsy: Unremarkable

g) Organ, Organ/Body, Organ/Brain Weights: Unremarkable

h) Histopathology: Unremarkable

3. Conclusions:

a) Classification: Core Minimum Data

i) At least 8 dogs/sex/dosage level should have been used.

ii) The highest dosage level did not reveal significant toxicological effects.

iii) Periodic chemical analysis of diets, if done, was not reported.

b) The systemic two-year chronic oral N.E.L. in dogs is 1000 ppm.

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F. Acute Intraperitoneal, Subcutaneous, and Oral LD_{50} Studies of Lasso Technical in Mice and Rats (Younger Laboratories, Nos. Y-76-96 and Y-76-87, 3/30/76 and 3/5/76, submitted by Monsanto Agricultural Products Co., 8/16/78, Acc.#234630).

1. Procedure:

In one study 35 Swiss-Webster albino mice, 23-35g, were separated into 7 groups of 5 animals each (2 or 3 males and 2 or 3 females) which were given 398, 501, 631, 794, 1000, 1260, or 1580 mg/kg of test material intraperitioneally. In another study 35 Swiss-Webster albino mice, 24-34g, were divided into 7 groups of 5 animals each (2 or 3 females and 2 or 3 males) which were administered 631, 794, 1000, 1260, 1580, 2000, or 3510 mg/kg of test compound subcutaneously. In a third study 20 Swiss-Webster albino mice, 22-33g, were separated into 4 groups of 5 animals each (2 or 3 females and 2 or 3 males) which were given 1260, 1580, 2000, or 2510 mg/kg of test material orally. In a fourth study 25 Sprague-Dawley albino rats, 205-215g, were divided into 5 groups of 5 animals each (2 or 3 males and 2 or 3 females) which received 2000, 2510, 3160, 5010, or 6310 mg/kg of test substance subcutaneously. Observations of mortality and toxic signs were continued for 14 days post-treatment in each study. Necropsies were done in all 4 studies.

2. Results:

a) Intraperitoneal Study in Mice

i) Mortality: LD_{50} = 870 (750-1000) mg/kg.
ii) Toxic Signs: Reduction of activity and appetite, weakness, collapse.
iii) Necropsy: Decedents - Lung hyperemia, liver discoloration, gastro-intestinal inflammation; Survivors - Normal.

b) Subcutaneous Study in Mice

i) Mortality: LD_{50} = 1200 (1010-1410) mg/kg
ii) Toxic Signs: Reduction of activity and appetite, weakness, collapse.
iii) Necropsy: Decedents - Lung hyperemia, liver discoloration, gastro-intestine inflammation; Survivors - Normal.

d) Subcutaneous Study in Rats

i) Mortality: LD_{50} = 3600 (3060-4180) mg/kg
ii) Toxic Signs: Reduction of activity and appetite, weakness, tremors, collapse.
iii) Necropsy: Decedents - Hemorrhages in lungs and liver, gastrointestinal inflammation; Survivors-Normal.

3. Conclusions:

a) Classification: The four studies in part F are considered to be Supplementary Data

i) The numbers of males and females in the dosage groups were not consistent.

ii) Body weight changes were not reported.

iii) At least 4 mice/sex/dosage level should have been used in the acute oral LD_{50} study.

iv) Acute intraperitioneal and subcutaneous LD_{50} studies are not applicable for regulatory data requirements.

G. Acute Inhalation LC_{50} Study of Lasso Dust (Lasso 15G) in Rats (Industrial Bio-Test Laboratories, Inc., IBT#663-06288, 5/15/75, submitted by Monsanto Agricultural Products Co., 8/16/78, Acc.#234630)

1. Procedure:

Twenty Charles River rats, weights unspecified, were divided into 2 groups of 10 animals each (5 males and 5 females) which were placed into an 80L inhalation chamber and were exposed to 0.93 mg/L or 3.2 mg/L (analytical concentrations) of test material as a dust for 6 hours. The size of 86% of the particles was $\leq$10 u. Observations of mortality, toxic signs, and body weight changes were continued over 14 days post-exposure. Necropsies were done.

2. Results:

a) Mortality: None, $LC_{50} > 3.2$ mg/L, 6 hours

b) Toxic Signs: Hypoactivity, ptosis, endophthalmus, lacrimation, nasal discharge.

c) Body Weight Changes: Reduction of gain in 3.2 mg/L males, no effect in females.

d) Necropsy: Unremarkable

3. Conclusions:

a) Classification: Supplementary Data

i) The nominal concentrations of test material were not reported.

ii) Body weights in conjunction with food intake were not determined daily.

b) TOX Cat: III (Provisional, determined from analytical concentration).

H. Thirty-Day Pilot Dust Inhalation Toxicity Study with Lasso Granular Dust (Lasso 15G) in Rats (Industrial Bio-Test Laboratories, Inc., IBT.#663-06288, 7/24/75, submitted by Monsanto Agricultural Products Co., 8/16/78, Acc.#234630).

1. Procedure:

Ten Charles River COBS rats (5 males and 5 females), 151-249g, were placed into an 80L inhalation chamber and were exposed to 1.55 mg/L (analytical concentration) of test material for 6 hours/day, 5 days/week, 30 days. The size of 5 1,7-69.0% of the particles was $\leq$ 10 u (determined weekly). Observations of mortality and toxic signs were made daily. Body weights were recorded weekly. Necropsies were done.

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2. Results:

a) Mortality: None

b) Toxic Signs: Salivation, diuresis

c) Body Weight Changes: Unremarkable

D) Necropsy: Pink discoloration and emphysema of lungs, hydronephrosis of kidney.

3. Conclusions:

a) Classification: Supplementary Data

i) The nominal concentration of test material was not reported.

ii) At least 10 rats/sex should have been used.

iii) At least 3 dosage groups and a control group should have been used.

b) The subacute inhalation N.E.L. is 1.55 mg/L, 6 hours/day, 5 days/week, 30 days (Provisional).

I. Acute Intraperitoneal LD_{50} Study of CP50144 in Rats (Industrial Bio-Test Laboratories, Inc., IBT#A6265, 6/18/68, submitted by Monsanto Agricultural Products Co., 3/16/78, Acc.#234630).

1. Procedure:

Twelve Sprague-Dawley albino rats, 152-198g, were divided into 3 groups of 4 animals each (2 males and 2 females) which were administered 400, 600, or 900 mg/kg of test material as a corn oil solution intraperitoneally. Observations of mortality, toxic signs, and body weight changes were done over 2 weeks post-treatment. Necropsies were done.

2. Results

a) Mortality: LD_{50} = 600 ± 70.4 mg/kg

b) Body Weight Changes: Unremarkable

c) Toxic Signs: Hypoactivity, weakness, ptosis, tremors, convulsions, ruffled fur, prostration.

d) Necropsy: Unremarkable

3. Conclusions:

a) Classification: Supplementary Data

i) Test material was administered intraperitoneally.

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ii) Body weights in conjunction with food intake were not determined daily.

b. TOX Cat.: III (Provisional)

J. Acute Vapor Inhalation LC_{50} Study of Lasso Emulsifiable Concentrate in Rats (Industrial Bio-Test Laboratories, INc., IBT#N6011, 4/5/68, submitted by Monsanto Agricultural Products Co., 8/16/78, Acc.#234630).

1. Procedure:

Ten Sprague-Dawley rats (5 males and 5 females), 230g av. wt., were placed into a 70L inhalation chamber and were exposed to 32 mg/L of vaporized test material for 1 hour. Observations of mortality, toxic signs, and body weight changes were made during 14 days post-treatment. Necropsies were performed.

2. Results:

a) Mortality: None $LC_{50} > 32$ mg/L (1 hour)

b) Toxic Signs: Unremarkable

c) Body Weight Changes: Unremarkable

d) Necropsy: Unremarkable

3. Conclusions:

a) Classification: Supplementary Data

i) The test material emulsion must be more completely identified to show whether Lasso Technical or a Lasso formulation was being used and the amount of test material in the emulsion.

ii) Body weights in conjunction with food intake were not determined daily.

b) TOX Cat.: IV

K. Recombination Assay of CP-50144 (Lasso Technical, 92.6%) in Two Genotypes of Bacillus subtilis (Marburg Strain) Confirmed by the Reversion Plate Method Using Two Strains of Escherichia coli (Industrial Bio-Test Laboratories, Inc., IBT#8536-08850, 4/20/76, submitted by Monsanto Agricultural Products Co., 8/16/78, Acc.#234630)

1. Procedure:

Culture media consisted of the following ingredients:

a) Based Synthetic (BS) Medium: $MgSO_4 \cdot 7H_2O$, citric acid $\cdot H_2O$, KH_2PO_4 anhydrous, $NaNH_4HPO_4 \cdot 4H_2O$, distilled water. For minimal agar plates 1.5% Bacto Difco agar and glucose were added to BS medium.

b) Overnight Culture Meduim for B. subtilis (TF): Casa amino acids, yeast extract, and each amino acid (l-tryptophan, l-arginine) required by each genotype were added to BS medium.

c) Media for Screening Mutagen (M) and for Determining the Number of Viable Cells (V): Difco nutrient broth and Difco Bacto agar. To avoid contamination streptomycin was added to the agar medium.

d) Storage Culture (TF Potato Agar): Potato and Difco agar in TF medium.

Overnight cultures of B. subtilis were added to TF medium diluted with BS medium to yield new cultures which were streaked onto plates containing medium M. The bacterial steaks were challenged by placing filter paper soaked with the following appropriate chemical solution onto the end of each streak: 500, 100, or 5000 µg of test material or 100 µg of N-methyl-N'-nitro-N-nitrosoguanidine (MNNG; positive control) or 6000 µg of ethylmethanesulfonate (EMS; positive control) or dimethylsulfoxide (DMSO; solvent control). Chemicals were dissolved in either 0.067 M K_2HPO_4 (pH7) or DMSO. Plates were prepared in triplicate and were incubated for 24 hours at 37 °C. Assays showing greater zones of inhibition in M-45 (Rec^-) than H-17 (Rec^+) plates were repeated.

Escherichia coli strains (B/r WP_2 and B/r WP_2 hcr^-) cultured in V medium were treated as follows: 0, 500, or 1000 µg/µl of test material, 50 or 100 ug/ul of MHHG, or DMSO. Assays were done in triplicate and included estimates of the numbers of revertants and viable cells.

2. Results:

a) B. subtilis Assays: A 3 mm zone of inhibition was found for H-17 streaks exposed to MNNG; inhibition zones of 1 mm, 4-6 mm, and 1 mm were found for M-45 streaks exposed to 5000 µg of test material, MNNG, and EMS, respectively. No inhibition was evident in the remaining assays.

b) E. coli Assays: Considerable reversion was evident for both strains exposed to MNNG. No mutagenic effect was apparent for either strain in the remaining assays.

3. Conclusions:

a) Classification: Supplementary Data

i) A definite conclusion concerning the validity of the present study towards satisfying regulatory requirements is deferred until mutagenic guidelines are finalized.

ii) Although mutagenicity of the test material was indicated in the B. subtilis (H-17) assay, the effect found in the prokacyotic cells may not in itself be predictive of mutagenic effects in mammalian cells in vivo.

b) The mutagenic M.E.L. in the bacterial strains under study is 1000 µg, based on results of the B. subtilis (M-45) assay.

L. Reverse Mutation Studies of CP50144 (Lasso Technical, 92.6%) in Five Salmonella Strains and One Saccharomyces Strain (Industrial Bio-Test Laboratories, Inc., IBT#8536-08852, 6/10/76, submitted by Monsanto Agricultural Products Co., 8/16/78, Acc.#234630).

The D4 strain of Saccharomyces cerevisiae and TA-1535, TA-1537, TA-1538, TA-98, and TA-100 strains of Salmonella typhimurium were used. Enzymic preparations were obtained from the 9000 Xg supernatant fraction of liver homogenate obtained from adult male Sprague-Dawley rats pretreated for 5 consecutive days with 500 mg/kg of Aroclor. The reaction mixture for activation tests included TPN (sodium salt), isocitric acid, Tris buffer (pH 7.4), $MgCl_2$, and 9000 xg supernatant.

Approximately 10^9 cells of each microbial strain were cultured in molten agar supplemented with biotin and histidine. Activation and nonactivation tests were done concurrently in the presence or absence of reaction mixture, respectively. Concentrations of 10^{-3}, 10^{-2}, 10^{-1}, 10^0, 10^1, and 10^2 µl of test material were added to cultures as appropriate. Positive control chemicals included the direct activators methylnitroso-guanidine, 2-nitrofluorene, and quinacrine mustard and the metabolically activated compounds 2-anthramine, 2-acetylaminofluorene, 8-amino-quinoline, and dimethylnitrosamine. Cultures were poured onto agar plates and were incubated for 48-72 hours at 37 °C. Mutagenic effects were based on the number of revertants/plate.

2. Results:

The number of revertants/plate was enhanced markedly in all bacterial strains exposed to positive control chemicals. A noteworthy decrease in the number of revertants/plate was recorded for TA-98 and TA-100 bacterial strains and the D4 yeast strain exposed to 100 µl of test material in the nonactivation test, but the test material was not remarkably effective in the activation test.

3. Conclusions:

a) Classification: Supplementary Data

i) A definite conclusion on the validity of the present study towards satisfying regulatory requirements is deferred until mutagenic guidelines are finalized.

ii) A mutagenic effect of the test material is indicated in both the bacterial and yeast assays which demonstrates a positive response in both procaryotic and eukaryotic cells; however, reverse mutation studies in microbial strains may not in themselves be predictive of mutagenic effects in mammalian cells in vivo.

b) The mutagenic M.E.L. is 10 µl in the nonactivation system and 100 µl in the activated system based on effects in both bacterial and yeast strains.

M. Host-Mediated Assay for the Detection of Mutations Induced by CP50144 (Lasso Technical) in Albino Mice (Industrial Bio-Test Laboratories, Inc., IBT#8533-08849, 8/16/76, submitted by Monsanto Agricultural Products Co., 8/16/78, Acc.#234630).

1. Procedure

Sixteen Charles River albino mice were divided into 4 groups of 4 animals each which received doses of 0, 300, or 1000 mg/kg of test material or 30 mg/kg of N-methyl-N'-nitro-N-nitrosoguanidine (positive controls) as solutions in corn oil. Test material was administered for 5 consecutive days before inoculation of mice. Postive control animals were treated with a single dose of compound on the day of inoculation. Each animal was inoculated with 2 ml of Salmonella typhimurium G46 culture immediately after final dosings.

Animals were killed at 3 hours post-inoculation and were immediately given 1 ml of saline intraperitoneally. Each peritoneal cavity was opened, and as much fluid as possible was removed. Peritoneal fluids were serially diluted, and diluted and undiluted samples were added to molten agar in the presence or absence of histidine, respectively. The preparations in agar were incubated in duplicate in petri plates at 37 °C for 48 hours. Colony counting data for 3 animals/group were reported.

2. Result:

a) Mutation Rates: Significantly increased in samples from positive control animals; otherwise unremarkable.

b) Chemical Effects On Mice: Unremarkable

3. Conclusions:

a) Classification: Supplementary Data

i) A definite conclusion on the validity of the present study towards satisfying regulatory requirements is deferred until mutagenic guidelines are finalized.

ii) A mutagenic effect of the test substance was not demonstrated in the present study; however, the host-mediated assay may not in itself be indicative of mutagenic effects in mammalian cells in vivo.

b) A mutagenic NEL of 1000 mg/kg is evident in the host-mediated assay in mice.

N. Host-Mediated Assay for the Detection of Mutation Induced by CP50144 (Lasso Technical) in Albino Rats (Industrial Bio-Test Laboratories, Inc., IBT#8533-0885, 8/16/76, submitted by Monsanto Agricultural Products Co., 8/16/78, Acc.#234630).

1. Procedure

Sixteen Charles River albino rats were divided into 4 groups of 4 animals each which were given doses of 0, 150, or 500 mg/kg of test material or 100 mg/kg of dimethylnitrosamine (positive controls) by gavage. Test material was administered for 5 consecutive days prior to inoculation of the rats. Positive control animals received a single dose of chemical on the day of inoculation. Chemicals were administered as corn oil solutions.

Immediately following final dosings, animals were inoculated with 5 ml of Salmonella typhinurium G46 intraperitoneally. Rats were killed at 3 hours post-inoculation and were administered 1 ml of saline intraperitoneally. Peritoneal cavities were opened, and fluid was removed. Serial dilutions of peritoneal fluid were made, and diluted and undiluted sample were cultured in molten agar in the presence or absence of histidine, respectively. The cultures were incubated in duplicate in petri plates at 37 °C for 48 hours. Colony counting data for 3 rats/dosage group were reported.

2. Results

a) Mutation Rates: Significantly increased in samples from positive control animals; otherwise unremarkable.

b) Chemical Effects on Rats: Unremarkable.

3. Conclusions:

a) Classification: Supplementary Data

i) A definite conclusion on the validity of the present study towards satisfying regulatory requirements is deferred until mutagenic guidelines are finalized.

ii) A mutagenic effect of the test substance was not demonstrated in the present study; however, the host-mediated assay may not in itself be indicative of mutagenic effects in mammalian cells in vivo.

b) A mutagenic NEL of 500 mg/kg is evident in the host-mediated assay in rats.

O. Four-Week Subacute Oral Toxicity Study of Lasso Technical in Albino Mice (Industrial Bio-Test Laboratories, Inc., IBT#B1182, 10/3/72, submitted by Monsanto Agricultural Products Co., 8/16/78, Acc.#234630).

1. Procedure:

One hundred Charles River albino mice, 16.8-21.8g, were divided into 5 groups of 20 animals each (10 males and 10 females) which received 0, 300, 1000, 3000, or 10,000 ppm of test material in the diet for 4 months. Observations of mortality and toxic signs were made daily. Body weights and food consumption were estimated weekly. Fresh diets were prepared each week.

2. Results

a) Mortality: All males fed 10000 ppm of test material died during the first week.

b) Toxic Signs: Unremarkable

c) Body Weight Changes: Loss in females fed 10000 ppm test compound; otherwise unremarkable.

d) Food consumption: Unremarkable.

3. Conclusions:

a) Classification: Supplementary Data

i) Necropsies were not done.

ii) Histopathological examinations were not done.

iii) Only 10 mice/sex/dosage level were used.

iv) The study was done in mice.

v) Periodic chemical analysis of diets was not indicated.

b) The subacute NEL in mice is 3000 ppm (Reviewer).

P. Two-Year Chronic Oral Toxicity Study of Lasso Technical in Albino Rats (Industrial Bio-Test Laboratories, Inc., IBT#621-01180, 9/16/77, submitted by Monsanto Agricultural Products Co., 8/16/78, Acc.#234629).

1. Procedure:

Four hundred eighty Charles River albino rats, 139-165g, were divided into 4 groups of 120 animals each (60 males and 60 females) which received 0, 100, 300, or 1000 ppm of test material in the diet for 2 years. Two hundred eighty rats were housed individually, and 200 rats were group-housed (3 rats/cage). Body weights were recorded weekly during the first 3 months and monthly thereafter. Food consumption was estimated weekly during the first 11 months and 1 week/month thereafter. Animals were observed for mortality and toxic signs daily. Blood and urine from 10 rats/sex in the control and 1000 ppm groups were analysed at 3, 6, 12, 18, and 24 months. Blood from 10 rats/sex in the 100 and 300 ppm group and urine from 10 males in the 100 and 300 ppm groups were evaluated at 24 months. Hematologic, clinical chemistry, and urine analyses were based on the following parameters:

Hematology: Total leukocyte count, erythrocyte count, hemoglobin concentration, hematocrit value, differential leukocyte count.

Clinical Chemistry: Glucose, blood urea nitrogen, SAP, SGPT, protein, bilirubin, A/G ratio.

Urine Analysis: Glucose, albumin, pH, specific gravity, microscopic elements, bilirubin.

All survivors and all decedents not extensively autolysed were subjected to necropsy. Weights of brains, gonads, hearts, kidneys, livers, and spleen were estimated. Histopathological examination of the following tissues and organs from all survivors in the control and 1000 ppm groups as well as animals found dead or killed while moribund was done:

Heart	Small and large intestine	Prostate
Lungs	Kidneys	Uterine horns
Trachea	Urinary bladder	Brain
Liver	Pituitary	Spinal cord
Spleen	Thyroid	Peripheral nerve
Lymph nodes	Parathyroid	Eye
Pancreas	Adrenals	Optic nerve
Stomach	Gonads	Salivary glands
Skeletal muscle	Bone marrow	

Additionally, neoplasms found during necropsy were examined microscopically.

2. Results:

a) Mortality at 2 Years:

No. Dead / No. Tested	Control		100 ppm		300 ppm		1000 ppm	
	M	F	M	F	M	F	M	F
	33/60	32/60	26/60	32/60	38/60	34/60	36/60	42/60

b) Toxic Signs: Unremarkable

c) Body Weight Changes: Unremarkable

d) Food Consumption: Unremarkable

e) Hematology, Clinical Chemistry, Urine Analysis: Unremarkable

f) Necropsy: Unremarkable

g) Organ, Organ/Body, Organ/Brain Weights: Unremarkable

Significant differences appear to be marginal and not dose-related.

h) Histopathology: Unremarkable, including tumor findings.

3. Conclusions:

a) Classification: Core Minimum Data

i) The rationale for housing some animals singly and others in groups is unclear. Nonetheless, it is concluded that, considering the range of observations and the results obtained therefrom, the study is adequate to estimate the chronic oral toxicity of the test material in rats.

b) The two-year chronic oral NEL in rats is 1000 ppm.

Q. Repeated Insult Patch Test of CP-50144 (Lasso Technical) in Humans (Industrial Biology Laboratories, Inc., No. SH-67-9, 1/31/68, submitted by Monsanto Agricultural Products Co., 8/16/78, Acc.#234629).

1. Procedure:

Test material was applied onto the skin of 50 humans at a dose of 0.2 ml/ 0.25 sq. in. of skin under occlusive dressing. Dressing was removed at 24 hours post-application. Contact sites were graded for irritation and sensitization during 24 hours following removal of dressing. Test material was again applied to the same site, if no irritation was evident, or to a different site, if irritation was observed, at the end of the 24-hour rest period. The weekly routine was to apply test material for 24-hour exposures on Monday, Wednesday, and Friday and to allow 24-hour rest periods on Tuesday and Thursday and a 48-hour rest period on Saturday and Sunday. Initial contact sites were challenged with test material for 24 hours at 14 days following the first treatment. Challenge areas were reexamined at 24 and 48 hours post-treatment.

2. Results:

Treatment was discontinued after 4 applications because of the severe skin reaction (erythema and edema at and extending beyond the test sites) elicited by concentrated test material. Testing with a 1/40 aqueous emulsion was not attempted since the preparation was heterogeneous.

3. Conclusions

a) Classification: Supplementary Data

i) The marked irritancy of the test material precluded an adequate evaluation of skin sensitization.

ii) The human subjects should have been more fully described to include, for example, sex, age, and race.

b) Skin sensitization potential of the test substance cannot be determined.

R. Repeated Insult Patch Test with Lasso Technical Emulsifiable Concentrate in Humans (Industrial Bio Laboratories, Inc., No. M-7, 3/5/68, submitted by Monsanto Agricultural Products Co., 8/16/78, Acc.#234629).

1. Procedure:

The method described in part Q.1. was used. Aliquots of 0.1 ml of a 1:40 emulsion of test material/sq, cm. of skin were applied to 28 male and 28 female humans. Subjects received 8 applications of test substance.

2. Results:

No reactions to test material were found in 24 subjects. Extreme irritation, including erythema, edema, vesiculation, and ulceration, at and extending beyond the contact sites was observed in 19 subjects after the second application.

In 18 of 19 responsive subjects, new contact sites selected for subsequent applications showed delayed reactions of similar severity. During the post-treatment observation period, 5 additional subjects exhibited similar delayed reactions. Irritation was reduced in severity with medication.

3. Conclusions:

a) Classification: Core Guidelines

i) The results clearly define the irritant and sensitization potential of the test material on human skin.

b) The test material is a skin sensitizer.

S. Acute studies of Lasso Technical and various Lasso formulations are presented by reference only and previously have been submitted to support registration of these products; therefore, these studies are not reviewed herein.

T. Toxicity studies of Lasso formulations in fish and wildlife have been submitted but are not reviewed within; however, the results are briefly summarized as follows:

a) 96-hour TL_{50} of Lasso Tech. in bluegill $>$ 0.75 mg/L

b) 96-hour LC_{50} of Laso Tech. in bluegill sunfish = 2.8 mg/L

c) 96-hour LC_{50} of Lasso Tech. in rainbow trout = 1.8 mg/L

d) 4-day TL_m of Lasso Tech. in rainbow trout = 1.0 ppm, in bluegill = 5.6 ppm

e) 4-day LC_{50} of Lasso EC in catfish = 6.5 ppm, in crayfish = 19.5 ppm

f) 4-day TL_{50} of Lasso/atrazine in rainbow trout = 14.7 ppm, in bluegill = 59 ppm

g) Biodistribution study of ^{14}C Lasso in bluegill showed that ^{14}C was more quickly taken up into and more quickly released from nonedible compared to edible tissue.

h) 11-week LC_{50} of Lasso 15G in bobwhite quail $>$ 5620 ppm

i) Oral toxicity of Lasso 10G in bobwhite quail = 10 g/kg

TOX/HED:th:G.Whitmore:9/21/78