TXR-5992

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

003992

9/20/84

MEMORANDUM

OFFICE OF PESTICIDES AND TOXIC SUBSTANCES

SUBJECT: Review of studies submitted in support of:

1. EPP No. 2217-EUP-G - Experimental Use Permit for the use of the herbicides Trimec-D and Trimec-M on cereal grains and corn.

2. PP No. 3G2863 - Request for the establishment of a temporary tolerance for residues of Mecoprop (MCPP, (2-(2-methyl-4-chlorophenoxy)propionic acid, a. i.), expressed as the acid, on the following food items, from the application of the dimethylamine salt or a mixture of the dimethylamine and the diethanolamine salts of the herbicide:

Small Grains: wheat, barley, oats and rye - 0.1 ppm;

Corn Grain: 0.1 ppm;

Straws of: wheat, barley, oats, and rye - 0.1 ppm.

TO: Richard Mountfort, PM #23
Registration Division (TS-767)

FROM: Karen L. Hamernik, Ph.D. K.L. Hamernik 9/4/84
Review Section I, Toxicology Branch
Hazard Evaluation Division (TS-769)

THRU: David Ritter, Acting Section Head
Review Section I, Toxicology Branch
Hazard Evaluation Division (TS-769) 9/20/84
Tox Chem. 559

Petitioner: PBI/Gordon Corporation
1217 West 12th Street
Kansas City, Missouri 64101

Summary of Data Reviews:

Six studies were submitted by the petitioner for review in answer to recommendations previously made by Toxicology Branch (see C. A. Rodriguez, June 30, 1983, PP 3G2863, EPA No. 2217-EUP-G). The submissions are listed below along with their classifications:

1/9/20

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1. Mecoprop-Seven-Month Feeding Study in Rats. In Report No. 1254 of Boots Pure Drug Co. LTD., England, Feb. 17, 1964. Accession #072279.

Core Classification: Invalid - An insufficient number of animals was used to perform this study. Additional deficiencies were found to exist in the execution and reporting of the study and in the analysis of data.

2. "Subchronic (13-week) Oral Toxicity Study With Mecoprop (MCPP) in Beagle Dogs". Report No. R6105 of the Centraal Instituut Voor Voedingsonderzoek, Zeist, The Netherlands, May 1979. Accession #243171.

Core Classification: Invalid - Individual animal data were lacking for parameters examined and tests conducted. Additional deficiencies were found to exist in the reporting of the study and in the analysis of data.

3. "Mecoprop Oral Teratogenicity Study in the Dutch Belted Rabbit". Report No. 1738R-277/5 of Hazleton Laboratories Europe, Ltd., Harrogate, England, January 1980. Accession #243169.

Core Classification: Minimum.

4. "Evaluation of Herbicides for Possible Mutagenic Properties", Kenneth, J. Anderson, Edith G. Leighty, and Mark T. Takahashi, J. Agr. Food Chem., Vol. 20, No. 3, 1972.

Classification: Not Acceptable - The test design was inadequate to fulfill regulatory requirements.

5. "Significance of Mutagenicity Testing on Pesticides", Yasuhiko Shirasu, The Institute of Environmental Toxicology, Suzuki-cho, Kodaira, Tokyo 187, Japan, 1973.

Classification: Not Acceptable - The test design was inadequate to fulfill regulatory requirements. No data was included in the submission.

6. "Mutagenicity Screening of Pesticides in the Microbial System", Y. Shirasu, M., Moriya K. Kato, A. Furuhashi, and T. Kada, Toxicology Div., Institute of Environmental Toxicology, Suzuki-cho, Kodaira, Tokyo 187, Japan, Mut. Res. 40 (1976) pp. 19-30.

Classification: Not Acceptable - The test design was inadequate to fulfill regulatory requirements. No data was included in the submission.

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Conclusions:

I. Most of the studies had deficiencies in data and the reporting thereof which should be addressed by the petitioner. A No Observed Effect Level (NOEL) or Lowest Effect Level (LEL) could not be established for Mecoprop based on the two subchronic studies submitted and the mutagenicity studies submitted were clearly inadequate. In light of these deficiences and inadequacies, Toxicology Branch cannot recommend that the requested temporary tolerance for Mecoprop on cereal grains and corn be granted.

II. However, Toxicology Branch can recommend that the requested experimental use permit for Trimec-D and Trimec-M on cereal grains and corn be granted, provided that there are crop destruct provisions.

III. The petitioner should also note that for Trimec-D and Trimec-M, [redacted] not been cleared as an inert ingredient in accordance with 180.1001 of the CFR and is not exempt from the requirement for a tolerance on crops. [redacted]

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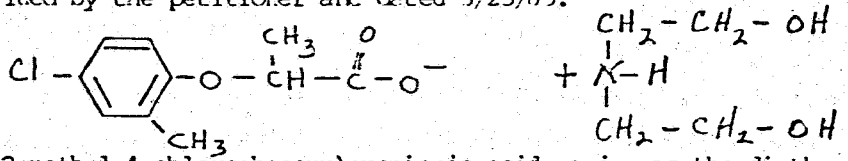
Mecoprop-Seven-Month Feeding Study in Rats.[1] In Report No. 1254 of Boots Pure Drug Co. LTD., England. Feb. 17, 1964. Accession #072279, EPA Registration #2217-FUP-G. Tox. Chem. File #559.

MATERIALS AND METHODS

Test Material: Technical grade Mecoprop* (MCPP) as the diethanolamine salt was used as the test material in this study. This does not appear to be the same technical material described in the statement of formulation of technical Mecoprop provided by the petitioner and dated 3/25/85.



*2-(2-methyl-4-chlorophenoxy)propionic acid, a.i., as the diethanolamine salt (see above figure).

Test Animals: Male and female rats were used. However, no information on the species, age (at onset of treatment), or source of these animals was provided.

Husbandry: Five animals/sex/treatment group were housed in one cage. Descriptive details of the environmental conditions which prevailed during the course of the study were omitted.

Administration of Test Material: Test animals were fed Mecoprop intermixed in their daily diet. Information regarding the composition of the basal diet, the levels of any contaminants therein and the homogeneity and stability over time of the test material in the diet was not provided. In addition, the protocol used in determining the levels of test material in the daily diet was not reported.

Statistical Methods: Use of statistics was minimal in this study, however where statistics were employed to analyze certain data, the specific methods used were neither identified nor discussed. The group means provided were not accompanied by values for standard deviation or standard error of the mean. Furthermore, the numbers of animals used in calculating the group means were never reported.

PROTOCOL

Procedure: Rats were divided into groups consisting of five animals/sex/treatment level and for seven months (28 weeks) were fed diets containing 100, 400, 1000, or 2500 ppm Mecoprop[2]. The ten control animals/sex included in the study were fed the basal diet alone. The method by which animals were assigned to groups was not described. Animals appeared to be uniquely numbered only with respect to their cages. Autopsies were performed on those animals which were killed due to their moribund state or which died prior to the end of the study as well as on those animals which survivied the 28 week test period.

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1 This study as it appears in Boots Report 1254 is listed as a chronic study. However, it was only of seven months duration while "chronic" refers to one-half the life time of the animals or longer.

2 Similarly, other groups of male and female rats were fed diets containing technical MCPA as the diethanolamine salt (2-methyl-4-chlorophenoxyacetic acid, a. i.); however, the results obtained are not reviewed herein since the petition under consideration does not concern this material.

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Parameters Examined: Data on animal body weights, food consumption, hematological indices (erythrocyte count, blood hemoglobin, packed cell volume (PCV), and differential white cell count) and relative organ weights (liver, kidney, spleen) were provided. Tissues samples for histopathological exam were taken from heart, lung, liver, kidney, spleen, suprarenal, stomach, ileum, pancreas, testis, ovary, thyroid, brain, and femoral bone marrow.

RESULTS

Food Consumption: In general, the mean daily food consumption by Mecoprop-treated animals was similar to that of controls except in the highest dose group of male rats. During the first month of treatment, males fed 2500 ppm Mecoprop consumed 28% less food than did controls. A smaller relative decrease was observed in this group during months 2-4. However, in months five through seven, food consumption by the high dose group animals approximated control intakes. Thus the declines observed were most likely due to the initial unpalatalility of the test chemical.

Dosing: The approximate mean daily dose of test material that each group of animals received varied with food intake and tended to decrease over the course of the study as the animals got older.

Body Weight: Body weight changes which occurred over the 28-week study period are presented in Table 1 below. When group means of body weight gains of Mecoprop-treated animals were compared with those of controls, decreases in body weight gains considered by Tox Branch to be significant and related to ingestion of the test material were noted for males fed 1000 ppm Mecoprop and for males and females fed 2500 ppm Mecoprop.

Table 1. Effect of Mecoprop in Daily Diet on Rat Body Weight

	Body Weight Group Means (gms)[1]					
	Male Rats[2]			Female Rats[2]		
Treatment Group	Initial	Week 28	Delta[3]	Initial	Week 28	Delta[3]
Control	108.9	277.4†	168.5	97.7	177.9	80.2
Mecoprop 100 ppm	114.9†	269.4	155.4 (92)[4]	96.0	181.0	85.0 (106)[4]
400 ppm	107.0	288.2	181.2 (107)	87.6	159.8	72.2 (90)
1000 ppm	111.4	225.0††	114.0 (68)	87.4	162.8	75.4 (94)
2500 ppm	103.0	164.3†††	61.3 (36)	86.8	133.3†††	46.5 (58)

[1] Body weight group means were calculated by Tox Branch from individual animal body weight data found in Appendices 1 and 3 of submitted material. All surviving animals were included in the calculations for week 28.
[2] n=10 for control groups and n=5 for Mecoprop-treated groups except where indicated: † n=8; †† n=4; ††† n=3.
[3] Delta equals the difference between group mean body weights at week 28 and at the initiation of the study.
[4] Numbers in parentheses refer to percent of control.

Hematological Indices: Hematological indices (erythrocyte counts, blood hemoglobin, and PCV) were determined over the last three treatmen weeks of the study. Statistically significant decreases in at least two of these indices were reported for MCPP-treated groups at all treatment levels of MCPP above 100 ppm. However, considering the rather small number of animals involved, three weeks is considered to be an inordinate amount of time over which to measure these parameters. In addition, it is unclear how and when data for each parameter were collected since no description of the protocol used in conducting the tests was submitted. Clarification of these points is necessary.

Relative Organ Weights (organ wet weights calculated relative to animal body weight): Statistically significant increases in relative kidney weight group means relative to controls were reported for male and female rats at all levels of MCPP tested. Statistically significant increases in relative liver weight group means were also reported for males fed 2500 ppm MCPP and females fed 400, 1000, and 2500 ppm MCPP compared to controls. However, after checking the group means reported for relative kidney and liver weights against the individual animal data appended to the submission, it was evident that values for some animals were excluded from certain control group mean calculations. The reasons for these omissions were not stated. Since addition or deletion of data can alter both groups mean values and the results of statistical analysis performed on these data, clarification of the points presented here is necessary.

Relative spleen weights tended to be slightly higher in the two highest MCPP treatment groups compared to controls, although no statistical changes were reported. These increases were probably related to the effect of MCPP on certain hematological parameters.

Animals Deaths During Study: Seven rats died or had to be killed prior to their designated date of termination at the end of 28 weeks. Results of autopsy and histopathological examinations are summarized in Table 2 below:

Table 2. Animals Fed Mecoprop in the Diet Which Died or Were Killed Before the End of the Study[1]

Treatment	Sex	Week killed (K) or found dead (D)	Gross Pathological or Histopathological Finding
Control	M	K20	Paralysis of hind limbs
	M	D28	Suspected infection of lung and heart
Mecoprop 1000 ppm	M	D6	Bronchiectatic abscess and necrosis of spleen, pancreas, testes[2]
2500 ppm	M	D15	Suspected infection of lung
	M	D26	Autolysis
	F	K24	Face abscess, Swollen liver cells
	F	K26	Abscess anterior to brain, Swollen liver cells, Increase in cells of meninges[3]

1 Summarized from data submitted by petitioner and found in Appendix 10.
2 It was not clear whether tissues listed were truly necrotic or had instead undergone autolysis.
3 It was not clear whether increase was in cell size or number.

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Deaths or animal morbidity considered to be related to the ingestion of the test chemical occurred in the 2500 ppm treatment group wherein 40% of the males and females died prematurely. The finding that members of this group had swollen liver cells correlated well with the increases in liver weight also reported for these animals. Most of the deaths in the 2500 ppm treatment group were associated wiith an abcess or infection. These animals in the highest doe group exibited a weakened resistance to infection which (as suggested by the study authors) could be considered to be compound related.

Animals Killed At Term: A summary of autopsy and histopathological findings reported appears in Table 3 below.

Table 3. Summary Of Reported Autopsy and Histopathological Findings on Animals Killed At Term

Treatment	Sex	# animals affected / # surviving animals in treatment group at wk 28	Gross Pathological Findings	Histopathological Findings
Control	M	2/8		VE Bronchiectasis
	F	2/10		VE Bronchiectasis
	F	1/10		Bronchiectasis with abcess
	F	1/10		Lung granuloma
Mecoprop 100 ppm		-None Reported-		
400 ppm	M	1/5		S Lung inflammation
	F	2/5		S Lung inflammation
	F	1/5		VE Bronchiectasis
	F	1/5	Lung infection	
1000 ppm	M	1/4	Lung infection	
	F	1/5		Suspected Hypo-plastic Bone marrow
	F	2/5	Lung infection	
	F	1/5	Lung infection	S Lung inflammation
2500 ppm	M	1/3	Lung infection	E Bronchiectasis, swollen liver cells
	M	1/3	Lung infection	Liver cells affected*
	M	1/3		Liver cells affected*
	F	1/3	Lung infection	VE Bronchiectasis
	F	1/3	Lung infection	E Bronchiectasis Swollen liver cells*
	F	1/3	Lung infection	S Lung inflammation Swollen liver cells*

Data used in above table was provided by petitioner and found in Appendix II of submitted material.
(VE)= very early; (S)=slight; (E)=early
*Information submitted in report not totally readable.

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Pathological findings that could definitely be attributed to ingestion of Mecoprop were the liver changes that occured in the 2500 ppm treatment group. It was reported that most of the animals in this group (dying prematurely or at term) had swollen liver cells with a homogeneous eosinophil cytoplasm, a condition which represents degenerative changes in the liver cell. In some animals, the amount of liver tissue affected involved many cells in a substantial portion of the lobules. In others, fewer cells were involved.

It can be seen from Table 3 that as the dose of Mecoprop administered increased, the incidence of lung infection as a gross pathological finding also increased. Again, animals exibited a weakened resistance to infection which could be considered to be compound related.

Organ Weight Recovery Experiment: Technical MCPP as the diethanolamine salt was administered for three weeks to other groups of rats in order to examine the reversibility of liver and kidney weight increases seen in the seven month sub-chronic study. However, the conclusions reached by the study authors were not well supported by the material submitted in the study report. For example, the number of animals per test group, the total number of test groups, the means by which the test material was administered, and the methods by which data were analyzed were not made clear. In addition, data was inadequately reported: no individual animal data were submitted, no animal body weight data were provided, and a portion of the table listing relative organ weight means (Table 6) was unreadable.

COMMENTS

The 7-month study should have included more animals/treatment group (i.e. at least 10 animals/sex/group). Additional organs and tissues (such as thymus, lymph nodes, pituitary gland, urinary bladder, etc.) should have been been subjected to histopathological exam. Clinical biochemistry tests (in addition to blood chemistry tests) should have been performed. Data obtained from tests assessing kidney and liver function would have been helpful in evaluating the effects of Mecoprop in these target organs.

CONCLUSIONS

This sub-chronic study was performed in order to determine the effects of feeding technical Mecoprop (as the diethanolamine salt) to rats in their daily diet for a seven month period. However, the data submitted are deficient in numerous ways and neither a NOEL nor and LEL could be determined based on the data submitted. In addition, the core-classification of the study cannot be upgraded because an insufficient number of test animals (5 rats/sex/dose) were treated with each dose of MCPP. A minimum of 10 rodents/sex/dose is required for a study of this type.

Information which should have been included in the study report is listed below:

1. Since the test material appears to be different from that described in the Confidential Statement of Formulation (dated 3/25/83) provided by the petitioner, a complete chemical analysis statement of the technical Mecoprop used to perform the study should have been submitted.

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2. The study report should have included the identities of the species and strain of rat used in the investigation, the source of the animals, their age at the onset of treatment, details of how animals were marked and identified from one another, and a description of conditions under which the animals were housed.

3. The study report should have included information and any data necessary to document the composition of the basal diet, the levels of any contaminants therein, the homogeneity and stability over time of the test material in the diet, the method(s) by which the levels of test material were assayed in the daily diet, and how often such determinations were made.

4. A full description of statistical methods used to analyze the data should have been reported, the number of animals used in the calculation of all arithmetic means should have been noted, and when cetain animals were omitted from such a calculation, an explanation should have been given (example: Table 5, relative organ weight data).

5. The protocol used in determining hematological indices should have been described including: methods employed, the time points in the study when each test was performed for each group of animals, how and when each value was obtained for moribund animals or animals killed during the period in which hematological indices were determined, and the reasons why certain determinations (abbreviated by ND in either Appendices 6 or 7) were not made.

The following ambiguities were also noted:

1. A statement on page 9 of the summary section implies that histopathological abnormalities were found in kidney at the 2500 ppm treatment level. However, no abnormalities in the kidney were listed in the histopathological report.

2. The autopsy findings in Appendix 10 for rats 1R2L, cage 233 and 2R2L, cage 236 were unclear for reasons indicated in Table 2 of this review.

3. The abbreviation ND (not done) appeared in some appendices, yet no explanation was given for why determinations were not made.

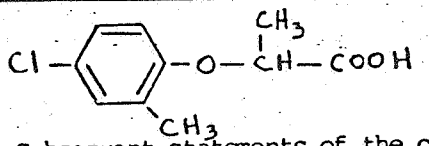
In addition, the presentation of the organ weight recovery experiment protocol and data was inadequate as was indicated earlier in this review. Furthermore, a number of pages in the submission contained information which did not photo-reproduce clearly or from which information was cut off the page (i.e. Table 4, p. 12, Table 6, p. 14, Appenidix 11, p. 41).

CORE CLASSIFICATION: Invalid. An insufficient number of animals was used to perform this study. Additional deficiencies were found to exist in the execution and reporting of the study and in the analysis of data.

"Subchronic (13-week) Oral Toxicity Study With Mecoprop (MCPP) in Beagle Dogs," Report No. R6105 of the Centraal Instituut Voor Voedingsonderzoek, Zeist, The Netherlands, May 1979. Accession #243171, EPA Registration #2217-EUP-G. Tox. Chem. File #559. This study was conducted from Sept. 21, 1977 through Dec. 20, 1977.

Test Material: Upon analysis, a sample of dry technical grade Mecoprop (MCPP), Batch CT 2675E, used in this study was found to consist of the following components:

	% by Weight
2-(2-methyl-4-chlorophenoxy)propionic acid*, a. i.	93.3
[redacted]	

* 

Subsequent statements of the chemical composition of technical Mecoprop are very similar in content to the one presented above (see review by R. Loranger, Chemist, Residue Chemistry Branch, dated April 11, 1984). [redacted]

Test Animals: 16 male and 16 female pure-bred beagle dogs about 4 to 5 months of age, obtained from the Central Institute for the Breeding of Laboratory Animals TNO, Zeist, The Netherlands, were used in this study.

Husbandry: Animals were housed individually in indoor cages. Other details concerning animal husbandry were omitted.

Dosing: MCPP, intermixed with a basal diet, was administered for 91 days to test animals in doses of 0, 4, 16, or 64 mg/kg body weight/day. The basal diet was prepared at approximately biweekly intervals from raw materials. Analytical methods for determining the concentration and stability of MCPP in the daily diet were not described or referenced and only summary data was provided to show the actual levels of MCPP found in the diet. Although the diet was prepared bi-weekly, MCPP concentrations within it were determined only on days 1, 43, and 84. Dogs were provided water ad libitum.

Protocol: Animals were divided into groups of four animals/sex/dosage level. At the conclusion of the dosing period, animals were terminated and autopsied.

Observations: The following parameters were examined: general health and behavior of animals, body weight changes, food consumption, eyes and eyelids, buccal mucosa, hematological indices, blood biochemistry, urinalysis, fecal blood content, and liver and kidney function.

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At autopsy, gross changes were noted and the following organs were weighed: testicles, ovaries, prostate, liver, kidneys, spleen, lungs, thyroid, adrenals, pituitary, thymus, heart, and brain.

The following were tissues preserved at autopsy. All were examined microscopically except those starred: adrenals, pituitary, testicles, ovaries, prostate, salivary glands (3), liver, kidneys, spleen, bone marrow, jejunum, ileum, caecum, esophagus, stomach, duodenum, colon, urinary bladder, ureter, uterus, thymus, heart, brain, lung, thyroid, nervus ischiadicus, spinal cord, pancreas, diaphragm, gall bladder, buccal mucosa, thoracic aorta, abdominal aorta, skeletal muscle, mammary gland, bone marrow, eye with optic nerve, cervical and mesenteric lymph nodes, trachea*, popliteal lymph nodes*, tongue*, skin*, anal sac*, circumanal glands*, and bone.

Data Analysis and Reporting: No individual animal data was provided for the parameters examined or tests conducted, nor was data documenting animal food consumption submitted. Results were often expressed in the form of group means which were for the most part calculated by lumping male and female data for a given parameter or test together. Group means were not accompanied by a standard deviation nor an n value (number of animals/group).

In general, procedures for the statistical analysis of data were poorly delineated or were not described.

Results: Due to deficiencies in data and the reporting thereof, the results of this study cannot be evaluated at this time.

Conclusions: The data submitted are deficient in numerous way and neither a NOEL nor an LEL could be determined based on data submitted. Before Toxicology Branch can consider upgrading the core classification of the study, it will be necessary for the petitioner to provide the information requested below:

1. Provide individual animal data for all parameters examined and tests conducted (including food consumption). Data should be submitted in an easily readable, tabular form and where applicable, group means and standard deviations should be reported for the particular parameters or tests examined. Animals excluded from calculations or statistical analyses should be noted and an explanation for their omission provided. When used, statistical methods should be thoroughly described and the results of statistical tests should be fully reported.

2. Submit all Appendices which were referenced in the study or which lend support to the study but were omitted from the report including Appendices 1, 2a, 2b, 3, 4, 5, and 6.

3. Delineate the analytical methods and procedures used in determining the concentration and stability of MCPP in the daily diet and submit the actual data derived from such procedures.

4. Describe animal husbandry more fully (i.e. give temperature and humidity ranges and standard deviations, light/dark cycle, and caging details).

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[redacted]

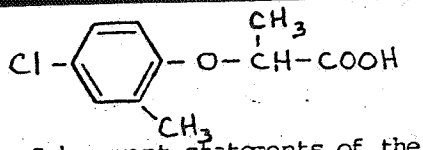
Core Classification: Invalid. Individual animal data were lacking for parameters examined and tests conducted. Additional deficiencies were found to exist in the reporting of the study and in the analysis of data.

"Mecoprop Oral Teratogenicity Study in the Dutch Belted Rabbit". Report No. 1738R-277/5 of Hazleton Laboratories Europe Ltd., Harrogate, England, January 1980. Accession #243169, EPA Registration #2217-FUP-G, Tox. Chem. File #559. This study was conducted from 9/14/78 through 12/8/78.

MATERIALS AND METHODS

Test Material: The test material used in this study was described as a buff-colored, feathery powder labelled as technical Mecoprop (MCPP), Batch CT 2675 Upon analysis, a sample of this material was found to contain (analysis state for this batch was contained in Accession #243171):

	% by Wei
2-(2-methyl-4-chlorophenoxy)propionic acid*, a. i.	93.3
[redacted]	

* 

Subsequent statements of the chemical composition of technical Mecoprop were very similar in content to the one presented above (see review by R. Loranger, Chemist, Residue Chemistry Branch, dated April 11, 1984). [redacted]

Animals: Male and female Dutch Belted rabbits used in the study were obtaine from Goodchilds Ltd., Crawley, Sussex. Prior to the commencement of the study, females were acclimated to the laboratory for at least 18 days and the health status of each animal was assessed by a veterinarian.

Husbandry: Rabbits were individually housed and were maintained under conditions of ambient temperature and humidity and a 14 hour light and 10 hour dark cycle of artificial fluorescent lighting. A commercial pelleted diet (Rabbit diet, B. P. Nutrition (U. K.) Ltd.) and water were made available to the rabbits ad libitum.

Administration of Test Material: Various concentrations of MCPP suspended in aqueous solutions of 1% methyl cellulose were administered orally to female rabbits by gavage. Thalidomide, the positive control for the study, was similarly administered as a suspension in an aqueous solution of 2% methyl cellulose and 0.5% Tween 80.

Dose Selection: Dose levels of Mecoprop were selected based on the results of an earlier dose ranging study performed by the same laboratory in which groups of artificially inseminated female Dutch-belted rabbits were gavaged once daily with 1% methyl cellulose (vehicle) or 25 or 100 mg/kg/day MCPP from day 6 to day 27 after insemination. Signs of

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maternal toxicity were noted only in the group of animals receiving 100 mg/kg/day MCPP. Weight loss and reduced food consumption were observed in this group. Only 1 of the 3 pregnant rabbits in the group survived the study. At necropsy, no abnormalities were observed in the two high-dose group animals which died.

PROTOCOL

Procedure: Sexually mature female rabbits generally weighing between 1.8 and 2.7 kg were uniquely numbered with a metal ear tag and were artificially inseminated with semen collected and pooled from male rabbits of proven fertility. Successfully inseminated females were immediatedly injected intraveneously via the marginal vein of the ear with 25 IU of chorionic gonadotrophin. Animals inseminated on a given day were first ranked by ascending order of body weight and were then assigned to treatment groups using a random letter table.

The 30 female rabbits placed in the vehicle control group were dosed with 1% methyl cellulose alone, while the 10 female rabbits in the positive control group were each treated with thalidomide at 200 mg/kg body weight/day. The 15 female rabbits in each group of Mecoprop-treated animals received either 12, 30, or 75 mg/kg body weight/day of the test material.

All animals were gavaged once a day with the appropriate treatment preparation from the sixth day after insemination (the day of insemination was considered to be day 0 of gestation) through the 18th day after insemination. Treatment preparations were administered at dose volumes of 10 ml/kg body weight/day. Adjustments in dose volumes were made based on individual animal body weights as determined on days 6, 9, 12, 15, and 18 after insemination.

The general health and behavior of all females were monitored daily throughout the study. Necropsies were performed on animals which died while the study was in progress, on animals killed due to their moribund condition, and after the termination and dissection of animals still alive on the 28th day after insemination. The ovaries and uteri of necropsied animals were removed and a detailed examination of their contents was conducted.

All fetuses in a litter were examined for external abnormalities. Examinations for visceral and skeletal abnormalities were conducted as follows: the viscera of one-half of the fetuses from each litter were examined after they were dissected. These fetuses were then eviscerated and their skeletons were stained with Alizarin Red S and examined for skeletal defects. The rest of the fetuses from each litter were fixed and partially decalcified in Bouin's solution, then transferred to 70% alcohol, transversely sectioned, and finally examined under 6X magnification for visceral abnormalities. Therefore, while one-half of the fetuses in each litter were examined for skeletal defects, all of the fetuses in a given litter were examined for visceral abnormalities (by means of two different techniques).

Abnormalities were grouped into four categories: major external/visceral, minor external/visceral, major skeletal, and minor skeletal. Major defects were those considered to be rare and/or probably lethal. Minor defects were those considered to be common deviations from normal.

Parameters Examined: Individual maternal body weights were measured on the day of insemination and every three days thereafter. Food intakes were recorded every third day after insemination. For each rabbit, the number of corpora lutea, the number of implantations, the litter weight, the number, sex, position, viability, individual weights, and crown/rump lengths of fetuses in both uterine horns, and the numbers of early and late intra-uterine deaths were determined. An early intra-uterine death was characterized by the presence of decidual or placental tissue only. A late intra-uterine death was characterized by the presence of embryonic or fetal tissue in addition to placental tissue. Pre-implantation losses were calculated as percentages based on the following formula:

$$\% \text{ Pre-implantation loss} = \frac{\text{number of corpora lutea} - \text{number of implantations}}{\text{number of corpora lutea}} \times 10$$

Calculation of Group Means: Group means of # corpora lutea/doe, # implantations/doe, # fetuses/doe, intra-uterine deaths/doe, and pre-implantation losses were calculated based on data from animals with functional corpora lutea in ovaries at day 28 post-insemination. Group means of litter weights, fetal weights, and fetal crown/rump lengths were calculated based on data from animals with live fetuses in utero on day 28 post-insemination.

Statistical Evaluation: Fetal weights and crown/rump lengths were evalutated by Wilcoxon's rank sum test. Pre-implantation losses, implantations, intra-uterine, deaths, and fetal abnormalities were statistically analyzed using Fisher's two-sum randomization (permutation) test with a Monte Carlo simulation for computation of significance levels. The experimental unit for purposes of statistical analysis was the litter.

RESULTS

Effects of Thalidomide: The maternal and fetal effects of thalidomide administration reported were consistant with results obtained from other studies, thus confirming the strains susceptibility to this chemical.

Pregnancy Status: In the control and 12, 30, and 75 mg/kg/day MCPP-treatment groups, 24/30, 15/16, 15/17, and 11/15 inseminated animals became pregnant and of these, all survived to 28 days post-insemination except for one doe in the 12 mg/kg/day group and one doe in the 30 mg/kg/day group.

Maternal Body Weight and Food Consumption: Maternal body weight gain group means were calculated for pregnant animals surviving to day 28 post-insemination. Body weight gain means determined for MCPP-treated groups from day 0 post-insemination to day 28 post-insemination were within 10% of the control group mean calculated for this period. However, during the dosing period (post-insemination days 6-18), the average body weight gain in the highest dose group was only about 56% of the corresponding control value suggesting a slight degree of maternal toxicity at this dose not apparent at lower doses. Estimation of food consumption was hampered by the excessive wastage of food by the test animals.

Maternal Health: No clinical changes nor abnormalities at necropsy were reported for the majority of pregnant adults in all groups. One rabbit in the 12 mg/kg/day group was euthanized on day 7 after insemination due to bilateral conjunctivitis. Another animal in this group (#311) became seriously ill and had symptoms of gastric and intestinal distress. Although it survived till the end of the study, all six implantations contained in utero were early intra-uterine deaths. One rabbit in the 30 mg/kg/day group died (respiratory system abnormalities found) on day 9 after insemination. None of the abnormalities, illness, nor deaths indicated in the study were reported to be attributable to administration of the test material.

Other Maternal Parameters: Group means of both the number of corpora lutea per MCPP-treated doe and the number of implantations per MCPP-treated doe differed no more than about 10% from control group means of 8.92 and 7.29 respectively. Although, a pre-implantation loss group mean of 25.6% in the 30 mg/kg/day MCPP group was somewhat higher than the control value of 18.2%, losses in the 12 and 75 mg/kg/day MCPP groups were lower than controls, with group mean values of 14.8% and 11.4% respectively.

Losses due to intra-uterine deaths were totaled and separated into early and late categories (Table I).

TABLE I Intra-Uterine Deaths (Group Totals) and Post-Implantation Losses In Pregnant MCPP-Treated Animals and Control Animals Surviving To Day 28 Post-Insemination[a]

	Control (1% methyl cellulose)	Daily Dose of MCPP (mg/kg)		
		12	30	75
Early Intra-uterine deaths per surviving does	1/24	8/14	1/14	0/11
Late Intra-uterine deaths per surviving does	1/24	1/14	4/14	2/11
Total Intra-uterine deaths	2	9[b,c]	5	2
Post-Implantation Loss (%)[d]	1.14	9.18	5.20	2.56

a Summarized from data submitted by petitioner.
b Significantly different from control group ($p < 0.05$ by Fisher's test).
c Six of nine intra-uterine deaths were in same doe (#311). See "Maternal Health".
d (Number of intra-uterine deaths/number of implantations) x 100

Although the total number of intra-uterine deaths in the 12 mg/kg/day group was found to be statistically different from controls, this was not found to be the case in the other dose groups and no dose-related pattern of post-implantation losses was observed.

Fetal Parameters: No dead fetuses were reported to be found in utero in any study group 28 days post-insemination. The average number of fetuses per doe was slightly lower than the control group mean of 7.21 by approximately 12, 10, and 4% respectively in the 12, 30, and 75 mg/kg/day MCPP groups. In addition, compared to the control group mean of 243.7 gm, slight decreases in litter weight group means of about 9, 15, and 7% were noted in the 12, 30, and 75 mg/kg/day MCPP-treated groups respectively. The study authors suggested that the relative decreases seen in group mean litter weights of MCPP-treated animals might be associated with the slightly lower number of fetuses per doe observed for these groups.

However, fetal weight group means and fetal crown/rump length group means in MCPP-treated groups were within 3% of the respective control group mean values of 34.2 gm and 94.6 mm. The ratio of female to male fetuses was slightly higher in the 30 and 75 mg/kg/day dose groups compared to controls (30 mg/kg/day - 1.22:1 and 75 mg/kg/day - 1.53:1 vrs. control 1.16:1) but this was not considered to be treatment related in context of other data presented in this study.

Fetal Abnormalities: When controls were compared with MCPP-treated groups, no statistically significant differences were reported in the incidences of major and minor external/visceral and major and minor skeletal defects when the litter was used as the experimental unit. In addition, when the incidences of the various categories of defects were analyzed on the basis of the number of fetuses in a particular test group, it was reported that no treatment-related effects were found.

Three out of 24 control group litters each contained a fetus with a major external/visceral defect (omphalocele, arthrogryposis - one forelimb, or rectal stenosis). No major skeletal defects were observed in control group fetuses. One out of fourteen 12 mg/kg/day MCPP-treated group litters contained a fetus with major external/visceral abnormalities (unilateral kidney agenesis and hydronephosis), although no major skeletal defects were found in this group. All 30 mg/kg/day MCPP-treatment group litters were reported to be free of major defects, while two litters in the 75 mg/kg/day MCPP treatment group each had a fetus with major external/visceral or major skeletal abnormalities (1 fetus - omphalocele, thoracogastroschisis, unilateral forelimb amelia and hemimelia; 1 fetus - lateral ventricles of brain enlarged).

Control and MCPP-treated groups all had fetuses with minor external/visceral or minor skeletal defects. The most prevalent minor external/visceral defect was post caval lung lobe agenesis, while the most common minor skeletal defects involved delayed bone ossification (i.e. phalanges incompletely ossified, 5th/6th sternebrae incompletely/not ossified) and extranumery ribs (i.e. 13 pairs of ribs, 13 ribs on one side).

Historical Controls Data: Historical control data appended to the submission was inadequately presented.

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CONCLUSIONS

[redacted]

2. Although some slight maternal toxicity was indicated at the highest dose of technical Mecoprop tested in this study (75 mg/kg/day), no evidence was presented in the reporting of the results of this study to indicate that the test material was fetotoxic or teratogenic in Dutch-Belted rabbits at the dose levels administered and under the study conditions used. Therefore, based on the dose levels of technical Mecoprop tested in this study, the No Observed Effect Level (NOEL) for teratogenicity and fetotoxicity, with the Dutch-Belted rabbit as the test species, is 75 mg/kg/day technical Mecoprop and the NOEL for maternal toxicity is 30 mg/kg/day.

3. Because 75 mg/kg/day was also the highest dose tested, no Lowest Effect Level (LEL) for teratogenicity and fetotoxicity could be established based on the results of this study. However, the LEL for maternal toxicity in this study was 75 mg/kg/day.

CORE CLASSIFICATION

Minimum.

Review and Evaluation of Mutagenic Activity of Mecoprop from Three Mutagenicity Study Reports, Accession #071501, EPA Registration #2217-EUP-G, Tox. Chem. File #559.

REPORTS:

I. "Evaluation of Herbicides for Possible Mutagenic Properties", Kenneth J. Anderson, Edith G. Leighty, and Mark T. Takahashi, J. Agr. Food Chem., Vol. 20, No. 3, 1972.

Results: The test compound, Mecoprop (1-5 ul/plate), was found to be non-mutagenic to the eight mutants of Salmonella typhimurium in the spot test assay described by Ames and Whitefield (1966).

Evaluation: The test design of the mutagenicity study, which incorporated only the spot test technique, was inadequate to fullfil regulatory requirements. The study was not conducted according to procedures acceptable for the performance of the Salmonella/mammalian microsome mutagenicity test (Ames et al., 1975) as a general mutagenesis screening assay.

Classification: Not Acceptable. The test design was inadequate to fulfill regulatory requirements.

II. "Significance of Mutagenicity Testing on Pesticides", Yasuhiko Shirasu, The Institue of Environmental Toxicology, Suzuki-cho, Kodaira, Tokyo 187, Japan, 1973.

Results and Evaluation: This is compiled information about the mutagenicity of 165 pesticides in three microbial spot test systems (Rec assay using B. subtilis H17 Rec and M45 Rec strains and Reverse mutation assay using Ames TA1535, TA1536, TA1537, TA1538 S. typhimurium strains and E. coli WP2 strain). Data obtained from the testing of Mecoprop for mutagenic activity was not contained in the submission, although Mecoprop was not reported to be mutagenic. Because of the limited usefulness of the spot test technique and because a mammalian metabolic activating system was not incorporated in the study protocol, this study and conclusions thereof are not considered to meet regulatory requirements.

Classification: Not Acceptable. The test design was inadequate to fulfill regulatory requirements. No data was included in the submission.

III. "Mutagenicity Screening of Pesticides in the Microbial System", Y. Shirasu, M. Moriya, K. Kato, A. Furuhashi and T. Kada, Toxicology Div., Institute of Environmental Toxicology, Suzuki-cho, Kodaira, Tokyo 187, Japan, Mut. Res. 40 (1976) pp. 19-30.

Results and Evaluation: This is compiled information about the mutagenicity of 166 pesticides in three microbial spot test systems (Rec assay using B. subtilis H17 Rec and M45 Rec strains and Reverse mutation assay using

Ames TA1535, TA1536, TA1537, TA1538 S. typhimurium strains and E. coli WP2 strain). Data obtained from the testing of Mecoprop for mutagenic activity was not contained in the submission, although Mecoprop was not reported to be mutagenic. Because of the limited usefulness of the spot test technique and because a mammalian metabolic activating system was not incorporated in in the study protocol, this study and the conclusions thereof are not considered to meet regulatory requirements.

Classification: Not Acceptable. The test design was inadequate to fulfill the regulatory requirements. No data was included in the submission.

RECOMMENDATIONS:

When an application for an Experimental Use Permit is accompanied by a request for a temporary tolerance for residues of a pesticide on a food crop, a battery of three mutagenicity studies are required, using the technical material, to assess gene mutation, chromosome abberrations, and primary DNA damage (See 40 CFR §158.135 and FIFRA GUIDELINES Series 84-1).

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