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

001995

MEMORANDUM

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

DATE: March 29, 1982

SUBJECT: Review of Toxicology Studies Submitted by Union Carbide on 2-(2,4 Dichlorophenoxy)-Propionic Acid. [Caswell No. 320]

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C. F. Chaisson

This memorandum is to convey our toxicological evaluations of toxicological data concerning 264-231,-222,-307,-308, and -309.

Twenty-one studies are reviewed herein which include six acute, four subchronic, four chronic, and six genetic toxicity tests, plus one preliminary metabolism study.

The results of these tests are summarized in the Summary, followed by an Introduction to present current considerations on 2,4, DP, and then followed by the individual reviews in the following order: acute, subchronic, and chronic studies. Each individual study may be located by refering to the Table of Contents.

The present submission from Toxicology Branch represents reviews of all pending toxicological data on 2,4, DP acid or Weedone® that have been submitted to the Toxicology Branch of HED.

For more specifics than in the Summary, RD is urged to refer to the individual study reviews where expanded conclusions and discussion of results are presented.

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TABLE OF CONTENTS OF TOXICOLOGY STUDIES ON 2-[2,4 DICHLOROPHNEOXY]-PROPIONIC ACID

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* Definitions of Classifications:

G -------- core guidelines

M -------- core minimum

S -------- supplemental data

I -------- invalid, according to Minimum Gene-Tox Program Standards (1982).

V -------- valid, surpasses Minimum Gene-Tox Program Test Study requirements, (1982).

Dash (-) indicate Core scores are not applied to this type of study.

SUMMARY OF TOXICOLOGICAL TESTS ON 2,4 DP

Acute Studies

The acute dermal application to rabbits of 2,4 DP showed only mild erythemia (P.I.I.=0.48) and was temporary (<48 hours) at the highest dose tested 2 g/kg. The LD_{50} > 2g/kg which places 2,4 DP is Category III (Dermal). Direct application of 0.5 ml of Weedone® did not produce any dermal irritation.

Primary eye irritation studies in the rabbit showed no corneal or Iris involvement when treated with 0.1 ml of technical 2,4 DP. Some hyperemia and chemosis was observed in 4/9 rabbits at 24 hours post-treatment, decreasing to 2./9 at 48 hours, and 1/9 at 72 hours; the P.I.I. at these respective times were 1.78, 0.89, and 0.44. Weedone® (0.1 ml) showed only very mild redness in one-half of the tested rabbits which subsided within 5 hours. These observations place 2,4 DP acid and Weedone in Category III (Eye).

An acute inhalation study on 2,4 DP was submitted to the Agency but review showed this study to be invalid because of a number of deficiencies (see page 3). No conclusions were made from this inhalation study which was the only inhalation study submitted by Union Carbide. Therefore, information on inhalation toxicity of 2,4 DP is lacking at the present time.

Oral toxicity of Weedone® was tested in rats (strain not specified) and the LD_{50} = 2200 ± 350 mg/kg body weight. Clinical signs included early (<4 hours) depression, excessive salivation, reduced motor activity, and ataxia. Rat which did survive the first few days of the acute oral test recovered completely. No necropsy results were presented.

Oral toxicity of 2,4 DP acid was also tested in rats. The LD_{50} = 532 mg/kg (95% range, 446-633 mg/kg), and the LD_{10} = 344 mg/kg (95% range, 247-479 mg/kg), and the LD_{90} = 823 mg/kg (95% range, 616-1099 mg/kg). Clinical signs show early (2 hours) hypersensitivity, decreased motor activity, abnormal gait, decreased grip, and ptosis. At higher doses (≥ 600 mg/kg) labored breathing, ataxia loss of righting, loss of body tone were observed all of which lead to an ingravescent course. Rats surviving the first three days recovered completely. Autopsied rats which died showed intestinal distention and gastrointestinal irritation.

The rat oral toxicity for Weedone® is 2200 mg/kg and for 2,4 DP is 532 mg/kg. The relative toxicity comparision shows Weedone® is four times less toxic in the rat than 2,4 DP by the oral route and the slopes of the respective lethality curves were similar. Both Weedone® and 2,4 DP are classified as Category III (Oral).

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Oral toxicity of 2,4 DP also tested in the mouse (Blue Spruce CF Strain). The LD_{50} = 650 mg/kg (95%, 484-872), and LD_{10} = 247 mg/kg(95%,144-423), and LD_{90} = 1709 mg/kg (95%, 887-3293). The slopes for the lethality curve in mice is 0.4 of the slope of the rat lethality curve indicating mice are less sensitive to 2,4 DP than rats. Mice showed similar clinical signs to rats. Acute oral toxicity in the mouse is Category III (Oral).

For comparision to the present results the following reslts were abstracted from the Registry of Toxic Effects of Chemical Substances (1976):

Compound	Oral LD_{50} (mg/kg)	Tested Species
Dichloroprop (2,4 DP)	800	rat
2,4 D	375	rat
2,4,5, T	300	rat
2, Dichlorophenol	580	rat
Present 2,4 DP	532	rat
Present 2,4 DP	650	mouse
Present Weedone®	2200	rat

Subchronic Studies

Subchronic feeding (13 weeks) for 2,4 DP acid was fed to SPF Wistar Rats at 5, 25, and 125 mg/kg. Survivals were normal among the dose and sex groups during the 13-week study.

At 125 mg/kg (high dose groups) body weights, food consumption, Hb PCV, RBC's, alkaline phosphatase, SGOT, SGPT, sodium and potassium (blood and urine), kidney and liver weights were all affected. At 125 mg/kg, the MTD is exceeded.

At 25 mg/kg, H6, PCV, blood sodium, and kidney weights were affected although to a lesser degree than as 125 mg/kg. The 25 mg/kg is set as the LEL for rat oral studies.

At 5 mg/kg, no effects of 2,4 DP were manifest and NOEL = 5 mg/kg.

In a subchronic beagle dog study at 8, 20, and 32 mg/kg. At the high dose significant effects were seen: lowering of Hb, hematocrit, RBC's, SGPT and thymus weight. SGPT and thymus weight reductions were seen at the 20 and 8 mg/kg dose levels. Because of the small number of dogs/group (2 males and 2 females) and the thymus weight reductions even in the 8 mg/kg group, a NOEL and a MTD cannot be assigned. The dog study data is considered supplemental.

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A range-finding teratogenic study was performed in rats at 0, 25, and 100 mg/kg. Body weights were suppressed in the 100 mg/kg group 5-7% and only a modest 3% in the 25 mg/kg group. The weight reductions (as mild as they were) were the only toxic effects seen in this experiment. The number of corpora lutea number of implantations, pups/litter, number of interuterine deaths, pup weights, and sex were all normal and the same among the dose and sex groups. There were no remarkable congenital defects observed. The NOEL in rats is set at 100 mg/kg (Teratogenesis).

In a comparible rabbit range-finding teratogenesis study at 0, 25, and 100 mg/kg, major congenital birth defects were seen at 25 mg/kg. Thus, a NOEL was not demonstrated in this experiment and is expected to be <25 mg/kg in the rabbit. At the 100 mg/kg, pronounced maternal toxicity was observed with 3 of the 5 total rabbits being humanely sacrified early (all three with dead fetuses), one not conceiving and the last doe delivering seven bunnies with no defects but were smaller than normal (46% reduction in bunny weight and 18% reduction in crown-to-rump distance).

Summarizing the Subchronic Studies:

Animal	Tox. Effect	(mg/kg) NOEL	LEL
Wistar S.P.F.	General Toxicity	5	25
Beagle Dog	General Toxicity	N.D.	8
Rat	Teratogenesis	100	N.D.
Rabbit	Teratogenesis	N.D.	25

N.D. = not determined

Chronic Studies

Rats were dosed (day 6 to day 15 of pregnancy) with 0, 10, 30, and 100 mg/kg of 2,4 DP acids in a 19 day teratology study.

Weight gains in the 0, 10, 30, and 100 mg/kg groups were normal as were food consumptions. The dosing was done via the feed and not by gavage to simulate human eating patterns (3X/day). This is judged to be a deficiency for dosing via feed would provide a constant intake throughout the day (instead of 3X/day) because rodents ingest feed continuously throughout the day.

Teratogenic observation showed the parturition, fetal viability, and frequency of resorption were normal among the dose groups. The abnormalities were qualitatively what might be expected in Sprague Dawley rats. The frequency of occurrence was low in all the groups and did not show any dose related effects with 2,4 DP acid.

A three-generation study was performed on CrL: COBS CD (SD) Br rats from Charles River Suppliers. Doses of technical 2,4 DP acid was administered via feed at 0, 12.5, 50 and 200 mg/kg (the highest dose was reduced to 100 mg/kg for the F_{1B} generation subsequent to F_{2A} birthing because of overt signs of general toxicity).

All parental rats survived in all three generations. The 200 mg/kg dose showed general toxicity and 5-10% weight loss; 200 mg/kg exceeded the MTD.

The pregnant rats (number of female rats conceiving/number inseminated) and the gestation periods were unaffected by 2,4 DP acid. Interestingly, the "average litter sizes" were not effected by dose but the number of litters with 8 or less pups were affected at 200 mg/kg2,4, DP acid. Fetal mortalities and neonatal mortilities were increased and pup weights were decreased at 200 mg/kg. When the high dose was reduced to 100 mg/kg these parameters were unaffected. Neonatal mortalities were increased only in F_{2A} litters at the 50 mg/kg dose only in F_{3B} litters at 12.5 mg/kg dose.

It is concluded that:

Rat Toxicity in Three Generation Study	(mg/kg) NOEL	LEL
Maternal toxicity (Smaller litters)	100	200
Fetal toxicity (increased litter mortility)	12.5	50
Neonatal toxicity (increased pys mortalities during laction)	100	200

An eighteen month oncology study was done in Swiss-Webster CD-1 mice at 0, 25, 100, and 300 mg/kg 2,4 DP acid. Survivals food consumption, and hemotology were normal among the dose and sex groups.

Stress toxicity was manifest at 25 and 100 mg/kg doses with increasing hematopoiesis, myelopoiesis, and granulopoiesis which is a typical stress response in aging mice. These increased synetheses were accompanied by some anisonormocytosis.

At the high dose (300 mg/kg) bile retention, increased liver weights, areas of degeneration and areas of regeneration were observed in the liver. Thus, for general toxicity in this 18-month mouse study: NOEL = 100 mg/kg and LEL = 300 mg/kg.

No tumor kinds (benign or malignant) or types (cell - or tissue-specific) were dose related to the feeding of 2,4 DP acid. Noteable is the increased heptomas at the high dose group (18% vs. 7.8% in controls). This response is viewed as weak tumor promotion due to obvious trauma to the liver by 2,4 DP acid at 300 mg/kg. It is concluded that this singular increase in tumors is not a dose-related response of 2,4 DP acid in mice at 300 mg/kg.

A two-year Sprague Dawley rat oncology study was done at 0, 50, 100, and 150 mg/kg in feed. At the high dose pronounced toxicity was observed in liver, kidney, and lymph nodes in both sexes. Males were affected by lung congestion, chronic prostatitis, and testicular atrophy, edema, and hyperplasia. These toxic effects were also observed at the mid-dose level 100 mg/kg but to a much lesser degree and were absent at 50 mg/kg.

For general toxicity, then, the NOEL = 50 mg/kg and the LEL = 100 mg/kg.

Analysis of the tumor response to Sprague Dawley rats by 2,4 DP acid showed the following:

1. Females had 34 to 66% more tumors of all kinds and tissue types than males. Females, however, did not show dose-related response in benign or malignant tumors (possably because of the high background in controls).

   Males showed an increase in malignant tumors of all kinds ($p>.006$) with a corresponding decrease in benign tumors (linear correlation coefficient = 0.999).

2. The malignant tumor load (no. malignant tumors per rat) in males rats increased with dose, 4-fold at the low dose and 8-fold at the mid dose ($p<.005$). Increased tumor tumor load (with dose) indicates increased malignancy in males.

3. A significant increase in male pituitary carcinomas ($p=.005$) and in male thyroid medullary carcinomas ($p<.005$) was observed with increased doses of 2,4 DP acid ($\geq$ 50 mg/kg). Occurrence of these tumor types in the controls agree well with occurrence in historical controls.

4. Life-times of rats with pituitary carcinomas were only moderately decreased (-8.5%) while rats with thyroid carcinomas were not significantly decreased (-2.4%).

5. There was a shift with dose from the malignant tumor pattern of the controls (pituitary + thyroid = 37% of total malignant tumors) to the treated groups (pituitary + thyroid = 85-86% of total malignant tumors). This shift to mostly pituitary and thyroid carcinomas indicates organ specificity of 2,4 DP oncogenic action.

6. Brain tumors, which are rare in the rat and in man, were observed in Group 2 (M and F) to be in excess of brain tumors in the controls (M and F). The degree of certainty is $p<.025$. The average life time of rats diagnosed with brain tumors was reduced 22% from term, i.e. brain tumors are significantly life-threatening.

It is concluded from this study that 2,4 DP acid is a carcinogen in male Sprague Dawley rats because of the following carcinogenic criteria were met.

1. Increased incidence and frequency in males with dose of all malignant tumor types compared to controls.

2. Increased incidence in males and frequency of three specific tumors types: pituitary, thyroid, and brain carcinomas.

3. A decrease in life-span in male rats with piruitary and brain tumors.

4. A shift with dose in the malignant tumor pattern in male controls to the malignant tumor pattern in the male treated groups. The treated groups had 85-86% of pituitary and thyroid malignant tumors whereas the controls had 37% of these two tumor types.

5. Increased tumor load (number of tumors/rat) in male rats with dose.

6. Occurrence in both sexes of a rare tumor type such as brain tumors.

Genetic toxicity testes showed positive effects in gene conversions and reverse mutations in Sacchromyces Cervisiae with unactivated (no. S-9) 2,4 DP. In repair of primary DNA damage 2,4 DP was positive but only with S-9 activation and only at the highest dose tested (40 ug/plate). These three positive genetic toxicity test lend support to the assertion that 2,4 DP is carcinogenic to male Sprague Dawley rats. Conversely, the Ames Salmonella mutagenic test was negative (up to 1000 ug/plate) on activated or unactivated 2,4 DP acid as was the mitotic crossing-over test in Sacch. Cervisial. The mouse micronucleus test was improperly done and no conclusions could be made.

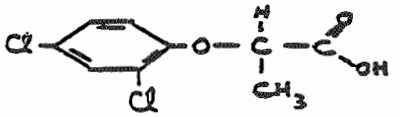
A preliminary rat metabolism study was presented. In this study it was shown that 2,4 DP acid (^{14}C-ring) is rapidly adsorbed (within 1.5 hours). Most of the radioactivity cleared in the urine 74-82% ($t1/2$ = 10-12 hours), 9-14% in feces, and none in expired air. The chemical identity of the metabolites was imprcperly done in urine and feces and was not done at all in the tissues. Kidney retained the highest amount of radioactivity with lesser amounts in liver, thyroid, and fat.

Radioactivity in fat was persistent (t1/2 = 48 hours) with an average of 1.0% of the administered dose (=117 mg/kg) in fat after 4 days.

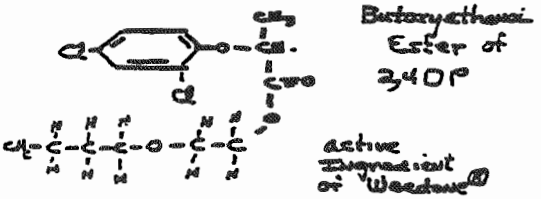
At least 50% of the radioactivity underwent enterohepatic eycling. In a repeated dose experiment (spanning 14 days), the radioactivity bioaccumulated in thryroid, liver, kidney, and fat (organs ordered on the basis of greater to lesser relative bioaccumulation of radioactivity).

INTRODUCTION

The herbicide 2-(2,4 dichlorophenoxy)-proprionic acid (esterified 2,4 DP occurs in Weedone® formulations in the U.S.). Other names for 2,4 DP are dichloroprop, Cornox-RD ™, RD-406 ™, and Fernoxone ™. The herbicide 2,4 DP was first introduced in 1961 and is generally used as the active ingredient* (often esterified with butoxyethanol) for weed control in the U. S., Austria Canada, Denmark, Finland, West Germany, Japan, and Sweden. 2,4 DP is often co-formulated with 2,4 DP or bromoxnil (only with 2,4 D in U.S.).



2-(2,4 dichlorophenoxy)- propionic Acid



Butoxyethanol Ester of 2,4 DP

active Ingredient of Weedone®

Current registrations in the U.S. granted to Union Carbide are 264-231, -222, -307, -308, -309, and -310. The 2,4 DP acid is esterified with butoxyethanol (2,4 DP/BOE) in Weedone® and occurs with the co-active ingredient 2,4 D in Weedone® 170, Weedone® super BK 32, Weedone® DP, Weedone® Weed and Feed 20, and Weedone HG herbicide formulations. There are no in-house petitions at this time for 2,4 DP acid (or its esters).

The summary of the toxicology studies precede this Introduction which summarize the toxicological effects of 2,4 DP acid to test animals in acute, subchronic and chronic studies. Weedone® was only tested in one acute dermal and one acute eye irritation study. Although Union Carbide did not submitt a rationale that 2,4 DP acid would be the residue of concern, it is assumed that 2,4 DP acid is expected to be the chemical form of 2,4 DP/BOE to which man may be exposed in application and use of Weedone®. If such is the case, the reviews in this report on 2,4 DP acid relate information which may be used to assess the relative safety of the pesticidal uses of Weedone® in the U.S.

A typical composition of the technical grade of 2,4 DP acid is:

	%(W/W)
2-(-dichlorophenosy)propionic acid	95.0
[redacted]	

*The positive optical isomer is twice as active as the negative isomer. Commercial preparations contain racemic 2,4 DP.

2,4 DP acid, the major component, is noted in the Registry of Toxic Effects of Chemical Substances as only slightly toxic (oral) and is Category III in skin toxicity. Dichlorophenol (0.50%) is only slightly toxic (oral) and is toxic to the skin, Category II, at 430 mg/kg. The dioxin content of 2,4 DP, or 2,4 DP/2,4 D formulations is not known at this time.

In review of the literature, no subchronic or chronic studies were found. Thus, the studies reviewed in this report are the first comprehensive reviews reviewed and compiled on 2,4 DP acid in the U.S.

**********ACUTE TOXICITY STUDIES ON WEEDONE® AND 2,4 DP **********

A. Acute Dermal and Acute Eye Irritation to the Rabbit from a Single Application of Weedone® or 2,4 DP Acid. (264-231, EPA No. 237875, Sections A, A-1, F & G of the Submission).

§ 1.0 Specific Conclusions on Acute Dermal and Eye Irritition Studies

1.1 Dermal irritation with Weedone ® was done adequately. Direct application of 0.5 mls of formulated Weedone® did not produce any dermal irritation.

1.2 Acute dermal application of 2g/kg with 2,4 DP acid showed only mild and temporary (<48 hours) erythemia with a PII = 0.48. The LD_{50} > 2g/kg placing 2,4 DP Acid in Dermal Toxic Category III.

1.3 Primary eye irritation with Weedone® showed no corneal or Iris involvement with some hyperemia and chemosis in 4/9 at 24 hours, 2/9 at 48 hours, and 1/9 at 72 hours; the PII at these respective times were 1.78, 0.89 and 0.44. Category III.

1.4 Weedone® [0.1 ml 2,4 DP (butoxyethanol ester] showed very mild redness in one-half the rabbits in < 5 hours which completely subsided in the rabbits by 24 hours. Weedone® is not considered an eye irritant and is placed in toxic Category III.

§2.0 Dermal Irritaiton of the Rabbit with Weedone® (Section A)

Six male New Zealand rabbits were shaved (AME Biological Research did study) and were abraded down to the dermis layer on one-half of the shaved area with a hypodermic needle discarding the stration corneum, epidermis and basal cells. The abraded half and the intact half were each treated with Weedone®. Both sides of rabbits were treated by placing a 1"X 1" gauze pad on the interscapular dorsal area which contained 0.5 ml of Weedone ®.

The test areas were scored for dermal irritation immediately following the 24 hour dermal exposure to Weedone® and again at 72 hours. The Draize Scoring system was utilized to measure irritation. No positive dermal irritant controls were used.

Both intact skin and abraded skin showed no postitives (PII = 0.0) at 24 or 72 hours. Delayed irritation at later times (7 or 14 days) was not monitored in this study. No other amount of Weedone®, other than 0.5 mls was tested. The actual composition of the applied Weedone® was not given.

Classification: Core Minimum

§3.0 Acute Dermal Irritation of the Rabbit with 2,4 DP Acid (Section G)

Rabbits (10) were shaved (CDC Research did study) and split into a group of 4 and a group of 6. The first group (4) was tested with intact shaved skin while the later group (6) had abraded skin.

The 2,4 DP acid was described as a brown liquid and was applied to a gauze pad at the rate of 2g/kg and placed on the rabbit dorsal test area by an "ace bandage" and adhesive tape for an exposure period of 24 hours. At the end of 24 hours the pad was removed, observations made, test area washed with water, dried and observations made once a day for 14 days.

The intact skin group showed only mild erythemia in two of four rabbits at 24 hours completely subsided by 48 hours. In the abraded group two of six showed only mild erythemia abated by 48 hours. The PII = 0.42 showing very low toxicity to the rabbit skin from 2,4 DP acid - Toxic Category III.

Classification: Core Guidelines

§4.0 Rabbit Eye Irritation with Weedone® Active Ingredient Technical 2,4 DP Butoxyethanol Ester of 2,4 DP Acid (Section F)

This study tested the Weedone® active ingredent 2,4 DP/BOE. All 10 New Zealand rabbits were treated by CDC Research with 0.1 ml of the technical material (not formulated) in the conjunctival sac. Six of ten were not washed after treatment while four of ten were washed with 20 ml of lukewarm tap water.

Nonwashed Group- Initial conjuctival irritation (redness) was noted in 3 of 6 rabbits for up to five hours. One of the three rabbits had occular discharge for 24 hours. All effects were subsided by 24 hours. No iris or cornea involvement. Blink reflex was normal in all six rabbits.

Washed Group- Initial conjunctival redness was observed for only 1 hour in one rabbit and up to 5 hours in another rabbit. The third rabbit was normal. No Iris or corneal involvement. Blink reflex was normal in all three rabbits.

It is concluded that technical 2,4 DP/BOE (a.i. of Weedone®) is not an eye irritant in the rabbit.

Classification of Study: Core Guideline

§5.0 Primary Eye Irritation of Weedone ® in Rabbits (Section A-1)

Affiliated Medical Enterprises (AME) Biological Research selected nine albino rabbits (New Zealand) and treated each by application of 0.1 ml of Weedone ® 2,4 DP into the conjunctival sac of the left eye; the right eye served as control. Irrigation was performed with lukewarm water: 3 rabbits 2 seconds after dosing, 3 rabbits 4 seconds after dosing, and 3 rabbits were not irrigated at all leaving the natural eye rinse to clear the eye contact with Weedone®. The Draize scoring method was employed.

No corneal or Iris involvement were noted in any of the three groups. Hyperemia and chemosis of the conjunctiva were noted at 24 hours (4/9), at 48 hours (2/9), and at 72 hours (1/9). All occurrences were in the non-irrigated or 2 sec. delay irrigation groups. The PIS were 1.78 (24 hours), 0.89 (48 hours), and 0.44 (72 hours). This is be considered only very mild irritation to the eye.

Classification: Core Guidelines

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§6.0 Study Classifications of Subpart A (four studies) On Dermal and Eye Toxicity

1. Acute Dermal irritation in the rabbit, Weedone®: Core Minimum

2. Acute Dermal irritation in the Rabbit, 2,4, DP Acid: Core Guideline

3. Eye Irritation in the Rabbit, Weedone®: Core Guideline

4. Eye Irritation in the Rabbit, 2,4 DP/BOE (technical): Core Guideline

B. Acute Inhalation by White Rats of Weedone ® 2,4 DP (Section C)

§ 1.0 Specific Recomendations on Inhalation Study

The inhalation of Weedone® should be repeated using suggested standard inhalation techniques, calculations, and presentation of data as outlined in §163.81-3.

§ 2.0 Specific Conclusions on Inhalation Study

2.1 The study of inhalation of Weedone® 2,4 DP is invalid because of the following deficiencies

Deficiency #1- The stain of test rat is unspecified.

Deficiency #2- Description of the % active ingredient, test material used for the inhalation experiment, should be completely specified.

Deficiency #3- Decription of the chamber, chamber concentrations of the a.i., dispersion of a.i., and actual dose concentrations (mg/l) for a given exposure peroid should be completely explained. It is impossible to calculate dose, nominal or otherwise, from the data or the discussion of the experiment done by Affilated Medical Research, Inc., Princeton, N.J.

Deficiency #4- The claim of a nominal concentration (for 1 hour) of 174 mg/liter is a non sequitor from the description of methods and the experiment as presented.

Deficiency #5- The claim that the LC_{50} is greater than 174 mg/l is not valid.

§3.0 Material Tested, Methods, and Animals Tested

Material: The sample used in this study was identified as Weedone ® 2,4-DP (no analysis or composition given) and was described a yellow clear solution. The sample was received and maintained in a one gallon can and stored at room temperature.

Methods: A dynamic chamber, containing a volume of 1000 liters, designed with a sliding tray and double sealing ports, was used in this study. Room air, drawn thrcugh the chamber at preselected rates, was monitored by means of a differential pressure flow meter and critical orifice, previously calibrated. The Weedone 2,4-DP was diluted in water to make

a 50% w/v suspension prior to each run. The suspension was maintained by constant agitation. An aerosol was generated in the chamber by passing the Weedone 2,4 DP suspension through a Devilbis atomizer, using compressed air at 40 psig and a flow of 4.0 l/min. The concentration of 2,4-DP in the in-put flow was not specified.

Particle size of the droplets was not measured; uniformity of the fog inside the chamber was monitored by observation of refracted light.

The flow of the Weedone 2,4-DP suspension through the atomizer was monitored, using an inline flow meter. No flow readings given.

Concentrations of Weedone 2,4-DP were calculated from the compound flow and chamber air flow data, and were considered nominal values (unconfirmed by chamber sampling and chemical analysis).

Animals: Young adult male and female albino rats, weighing from 150 to 300 g, were used in these studies. The rats were acclimated to the laboratory, individually housed, and provided food and water ad libitum. Groups of 10 rats were exposed at a time. Exposure time was 1.0 hour. The animals were observed during and following the exposure; mortalities were recorded, cumulatively, for the subsequent 14 days. At termination, the animals were submitted to autopsy [No results given]. Decedents were examined grossly except where autolysis was extensive [No results given].

§4.0 Discussion of Results:

At a nominal concentration of the aerosolized Weedone 2,4-DP at 174 mg/l (according to Union Carbide), slight hyperexcitability was observed during and immediately after the one-hour exposure. Within a few hours post-exposure, the rats appeared normal and remained normal over the 14-day observation period.

§5.0 Discusion of Results

Due to the fact the dosing was not adequately explained, it is not possible to specify the dose of the unspecified strain of rats received in this experiment. No necropy results were presented.

§6.0 Classification of Study

This study, because of the deficiencies given in §3.0, is Invalid.

C. Acute Rat Oral Lethality of Weedone and 2,4 DP (Sections B and D).

LD_{50} Calculations in the Rat.

§1.0 Specific Conclusions on Oral LD_{50} in the Rat

1.1 Weedone® LD_{50} = 2200 ± 350 mg/kg body weight. Clinical signs included early (< 4 hrs) depression, excessive salivation, reduced motor activity, and ataxia. Rats surviving the first few days recovery completely. Necropsy results were not presented.

1.2 Dichlorprop (or 2,4 DP acid) LD_{50} = 532 mg/kg with 95% confidence range of 446-633 mg/kg. LD_{10} = 344 mg/kg (95% range, 247-479 mg/kg) and LD_{90} = 823 mg/kg (95% range, 616-1099 mg/kg).

Clinical signs show early (2 hrs) hypersensitivity, decreased motor activity, abnormal gait, decreased grip and ptosis. At higher doses ($\geq$ 600 mg/kg) labored breathing, ataxia, loss of righting, loss of body tone all which lead to a loss of consciousness and finally death. Rats surviving the first 3 days recovered completely.

Necropsy of rats dying showed intestinal distention filled with fluid and gastrointestinal irritation.

Signs are progessive with dose in the number of rats affected and intensity becoming prominent at 400 mg/kg, medium to intense at 600 mg/kg, and intense at 800 mg/kg.

1.3 The dose/effect relationship, as measured by the slopes of the lethality curve, are similar for Weedone® and DP acid. These parallel curves suggest similar mechanisms of toxicity are operative for both compounds.

1.4 The relative toxicity of 2,4 DP acid to Weedone is 4.1:1.

1.5 Oral Toxicity Category Classifications are:

Weedone®	Class III
2,4 DP acid	Class III

1.6 For comparison to the above oral toxicity in rats, the following data were abstracted from the Registry of Toxic Effects of Chemical Substances (1976):

Compound	LD_{50} (mg/kg)
Dichlorprop	800
2,4 D	375
2,4,5 T	300
2,4 Dichlorophenol	580

§2.0 Results of Oral Toxicity of Weedone® in Rats

Weedone was fed to an unspecified strain of white male albino rats. The concentration of Weedone® formulation was not confirmed nor were the volumes administered to the rats specified. Rats weighed initially 135-155 grams and were fasted 24 hours before dosing. Three dosing levels (6 rats in each level) were chosen at 1000 mg/kg, 2500 mg/kg, and 5000 mg/kg body weight. The eighteen rats were dosed once, observed the first day, and then each day thereafter for 7 days.

All rats in each dose group showed early signs of intoxication in the first 4 hrs. of observation. Observed signs were depression, excessive salivation, reduced motor activity, and ataxia.

In Group 3 (5000 mg/kg) one of the rats died in the first 4 hours, one more died in the 4-8 hr. period, and four more died on the second day. The cumulative mortality for Group 3 male rats was 6/6.

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In Group 2 (2500 mg/kg) no rats died in the first 4 hours, one rat died in the 4-8 hr. period and 2 more rats died on the second day. The cumulative mortality for Group 2 males was 3/6. The three remaining rats recovered from the above clinical signs by the third day and remained normal for the duration of the 7-day experiment.

In Group 1 (1000 mg/kg) no rats died in the 7 days (0/6). As in the other groups clinical signs were onset in the first four hours. Group 1 recovered by the second day and were normal for the duration of the experiment.

The registrant reported an LD_{50} = 2,200 mg/kg $\pm$ 350 mg/kg for Weedone®.

Classification of Study: Core-Minimum

§ 4.0 Oral Toxicity of 2,4 DP Acid in Sprague Dawley Rats (Section D of Submission)

Dichlorprop (or 2,4 DP acid) was fed to Sprague Dawley rats (5 groups, 5M and 5F per group) at dose levels of 200, 400, 600, 800, and 1000 mg 2,4 DP acid/kg body weight. Rats were initially 133-185 grams in weight and 198-260 grams at the end of the 14 day experiment following a single dosing. Dosing was given in 0.25% methyl cellulose solution 5 ml/kg body weight. Rats were fasted 24 hours before dosing, then dosed and then allowed food and water ad libitum for the course of the 14 day acute experiment.

At the high dose (1000 mg/kg) all Sprague Dawley rats died the first day (10/10). At the 800 mg/kg dose, 4F and 1M died the first day, 3M and 1F died on the second day and 1M on third day for a cumulative mortality of 10/10.

At the dose of 600 mg/kg, 1M and 1F died on the first day and 2F died on the second day and remainer lived for a cumulative mortality of 4/10. At 400 mg/kg, 1F died the first day, 1F the second day and 1M the third day for a cumulative mortality of 3/10. At the 200 mg/kg dose no rat died during the 14 days.

The clinical signs exhibited in the 5 groups are given in Table I (next page). The signs were progressive with increased dose and effected more rats, but seemed to be acute in nature with those surviving the initial few days recovering completely. At 800 and 1000 mg/kg, the effects were intense with all rats showing shock, lack of motor coordination, decreased breathing, comatose, and death. Fluid filled intestines and gastrointestinal irritations were the primary findings upon necropsy of the rats that died during the course of the 14 days. Rats surviving the 14 days were normal upon necropsy. Clinical signs became prominant at 400 mg/kg and medium to intense at 600 mg/kg.

The registrant calculated an LD_{50} = 555 mg/kg (95% confidence range 396-777 mg/kg). A Finney probit analysis (program written by J. Worthington, RCB) was performed on the lethality data presented:

Lower 95% Limit	Parameter	Upper 95% Limit
446	LD_{50} = 532 mg/kg	633
247	LD_{10} = 344 mg/kg	479
616	LD_{90} = 823 mg/kg	1099

These calculations show the registrant calculations are essentially correct.

Additionally, these calculations show that 10% of the Sprague Dawley rat population will likely die by 344 mg/kg and 90% will likely die by 823 mg/kg.

Classification of Study: Core-Guideline

TABLE I

Dose Group (mg/kg)	Clinical Observations of Rats Fed 2,4 DP
200	All rats showed decreased motor activity in 2 hrs. up to 4 hrs., but all were normal by 24 hrs.
400	Rats showed hypersensitivity, decreased motor activity, abnormal gait, and decreased grip 2 hrs after dosing. Females showed piloerection. At 4 hrs decreased respiratory rate was noted. At 24 hrs rat #25 showed tremors and died the third day. Rats #26 and #29 were comatose at 24 hrs with #29 dying that day and #26 dying the next. Necropsy of these rats showed gastro-intestinal distention adn discoloration and fluid-filled intestines. All other rats were normal for 14 days and were normal upon necropsy.
600	All rats exhibited initial ptosis, piloerection, ataxia and labored respiration at 4 hrs. Rats #43 and #47 were comatose at 24 hrs and died that day. Rats #50 and #46 showed very labored breathing at 48 hrs and died. All other rats (6) became asymtomatic at 48 hrs. and were thereafter normal. Necropsy showed same as 400 mg/kg.
800	These rats exhibited hypersensitivity, loss of righting, decreased motor activity, abnormal gait, and loss of body tone within 2 hrs after dosing. Five of the rats became comatose at 24 hrs and died that day. Four more rats followed the next day and 1M survived til the third day and then he too became comatose and died. Necropsy showed all rats same as 400 mg/kg with dicoloration of the lungs in addition.
1000	All rats exhibited in less than 4 hrs all the clinical symptoms as at 800 mg/kg with one rat becoming catatonic. At four hrs, all rats showed depression and slowed respiration. By twenty hrs, all rats were comatose and subsequently died that day. Primary findings upon necropsy were gastrointestinal irritation and fluid-filled intestines in all rats.

§5.0 Discussion of Results of Both Oral Toxicity Studies (Sections B & D of Submission) on Weedone® and 2,4 DP

The LD_{50} for Weedone® is estimated to be approximately 2200 mg/kg. The LD_{50} for 2,4 DP acid (not esterified as in Weedone®) is 532 mg/kg with a 95% confidence range of 446-633 mg/kg.

It is notable that the lethality curves when plotted for 2,4 DP acid and Weedone are parallel in the rat with the mid-point for 2,4 DP acid being 1668 mg/kg lower than the Weedone® mid-point. This suggests the mechanisms of toxicity are the same for the two compounds. Further, since 2,4 DP acid is four times more toxic to Sprague-Dawley rats then Weedone®, the toxicity of Weedone® likely resides in the 2,4 DP acid released from Weedone® by murine esterases.

The LD_{10} for 2,4 DP in rats is 344 mg/kg and the LD_{90} is 823 mg/kg.

§6.0 Classification of Oral Toxicity Studies in the Rat

The Weedone® study (Section B of Submission) is classified as Core Minimum as the rats were not specified as to strain and the high dose produced a 100% kill and the low dose had zero kill making analysis of LD_{50} depend on the middle dose alone.

The 2,4 DP acid acute oral study (Section D of Submission) in rats is classified as Core Guideline.

D. Acute Oral Lethality of 2,4 DP Acid (Dichlorprop) in the Mouse (Section E of Union Carbide Submission 264-231, EPA No. 237875)

§1.0 Specific Conclusions on Oral Toxicity in the Mouse

1.1 The LD_{50} = 650 mg/kg (95% range, 484-872) for Blue Spruce CF Mice.

1.2 The LD_{10} = 247 mg/kg (95% range, 144-423).

1.3 The LD_{90} = 1709 mg/kg (95% range, 887-3293).

1.4 The LD_{50} for mice reported here is similar to the LD_{50} for rats (532 mg/kg).

1.5 The slope of the lethality course for 2,4 DP acid in mice is 0.4 of the lethality slope for rats. This indicates less sensitivity to 2,4 DP acid in mice as compared to rats.

1.6 The clinical effects in mice are acute gastrointestinal irritation which is essentially reversable at the lower doses ($\leq$ 400 mg/kg) but becomes intense at higher doses ($\geq$ 800 mg/kg) killing all the mice. All mice, even at lower doses, show reversable decreased motor activity, abnormal gait, straub tail, depression, ptosis, and hypersensitivity to touch and sound. This effects are onset at 2-4 hrs and are abated (in those rats that survive) by 48 hrs.

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§2.0 Methods

Pharmakon Laboratories did the following study for the Amchem Division of Union Carbide in 1977. Six groups (5M and 5F per group) of mice were chosen for a single oral dosing at 100, 200, 400, 600, 800, and 1000 mg 2,4 DP acid/kg body weight. Blue Spruce CF mice were selected for the experiment at an initial body weight of 18-25 grams. Mice were fasted 4 hrs. prior to dosing, then dosed, and then allowed water and Wayne Lab-Blox ad libitum.

§3.0 Results

At the high dose (1000 mg/kg) all 5M and 3F died in the first day (8/10). As early as 30 min. post dosing mice exhibited signs of writhing, depression, and ataxia. At 2 hrs loss of righting was observed. Five of the mice showed no remarkable findings upon necropsy whereas three mice showed distention of the bladder, stomach, and intestine. Two mice survived and were normal at 14 days.

At the 800 mg/kg dose, all 5F died the first day and all 5M lived (5/10). The same clinical signs seen at 1000 mg/kg were seen at 800 mg/kg. Four of the five females showed distention of the intestine. All other mice were normal upon necropsy.

At 600 mg/kg, five of ten died (5/10) on day 1, 2, 4, 5, and 6. Although the cumulative mortality was the same as 800 mg/kg, the mice that died lived longer (up to day 6). The same clinical signs were seen with straub tail in addition. Of those five mice that died distened, discolored, air and fluid-filled intestines were observed. The other five surviving mice were normal throughout the 14 days.

At 400 mg/kg, 2M died with rest surviving normally (2/10). Symptmatology was lesser compared to the higher 600 mg/kg dose. Ataxia and depression were seen in all mice with hypersensitivity to sound and to touch at 1 hr post-dosing. Abnormal gait was seen at 24 hrs. All clinical signs were abated at 48 hrs.

At 200 mg/kg, 1M died with rest normal (1/10). The same clinical signs as at 400 mg/kg were seen. Two surviving F mice showed slightly distended uteri. The one male which died showed ascites, discoloration of lungs and liver, and distended stomach and intestine.

At the 100 mg/kg dose, no mice died (0/10). Six mice were normal at necropsy with 4F having slightly distended uteri. The same clinical signs as before were seen which were abated completely by 48 hrs.

Union Carbide calculated the LD_{50} to be 620 mg/kg (95% limits, 453 - 849 mg/kg). A Finney Probit Analysis (program by J. Worthington, RCB) was performed for this report. The results were:

Lower 95% Limit	Parameter	Upper 95% Limit
484	LD_{50} = 650	872
144	LD_{10} = 247	423
887	LD_{90} = 1709	3293

These calculations show the registrant calculations are essentially correct.

Additionally, these calculations show that 10% of the Blue Spruce CF mice will likely die by 247 mg/kg and 90% will likely die by 1709 mg/kg.

§4.0 Discussion of Results

The 100% kill level was not reached in this experiment, but 80% were killed at 1000 mg/kg. The LD_{50} = 650 mg/kg for mice is somewhat less toxic than for rats (532 mg/kg, memo J. Holder to R. Mountfort, 11/3/81).

The mouse lethality curve slope (3.05) is lower than for the rat (7.38) and therefore in the mouse there is less sensitivity for the mouse to 2,4 DP acid even though the LD_{50} values are similar.*

Acute clinical signs were similar between the mouse and the rat. That is, primary irritation of the stomach and intestines with overt distention because of fluid build-up. These rodents tended to become ataxia with depressed motor activity in the first few hrs. and at 24-48 hrs became comatose leading to subsequent death. However, those rodents surviving the first few days were completely normal at the end of 14 days. It is concluded that acute gastrointestinal action of 2,4 DP acid could lead to death which became prominant at doses $\geq$ 400 mg/kg and became quite intense at doses $\geq$ 800 mg/kg effecting almost or all of the rodents.

§5.0 Classification of Study: Core Guideline

********** END OF ACUTE STUDIES ON WEEDONE AND 2,4 DP **********

* * * * * SUBCRONIC STUDIES ON 2,4 DP * * * * *

E. Subchronic Feeding of 2,4DP Range-Finding Studies in the Rat and the Dog (two studies) [264-222,231, 233; 237984, 5]

1.0 Subchronic 90-Day Oral Toxicity Study with 2,4DP Fed to Wistar SPF-Albino Rat

1.1 Conclusions of Rat Study

1.11 Survivals were good and the same for all doses and sex groups.

1.12 Body weights, food consumption, hemoglobin, packed cell volumes, number of erythrocytes, alkaline phosphatase, SGOT, SGPT, blood sodium, urine sodium and potassium, kidney and liver weights were all affected at the high-dose (2500 ppm) level. This level in the rat clearly exceeds the MTD.

1.13 Hemoglobin, packed cell volumes, blood sodium, and kidney weights were all affected at the mid-dose (500 ppm) group, although to a lesser degree than at the high-dose group. Accordingly, 500 ppm is assigned the LEL.

1.14 At 100 ppm, none of the effects summarized above were manifest. Thus, the rat NOEL in this feeding study is set equal to 100 ppm.

1.2 Design of Rat Subchronic Study

Wistar specific pathogen-free rats (40-60 g, 4 wks. old) were randomly divided into four groups (30 per dose group, 15 of each sex) and fed in a complete diet of 0, 100, 500, and 2500 ppm technical grade 2,4DP acid, respectively. GLP was followed by the Central Institute for Nutrition and Food Research on behalf of A. H. Marks (Yorkshire, England). This 13-week experiment was started January 17, 1977.

Signs of toxicity, behavior, body weight, food consumption, hematology urinalysis, organ weights, and histopathology of all relevant tissues were performed on each Wistar rat. The pathology procedures were adequate and complete for a subchronic investigation.

1.3 Results of Rat Subchronic Study

Survivals were good and the same among the dose and sex groups. Behaviors were normal except for alopecia and some muscular weakness noted in the high-dose group (2500 ppm).

The high-dose group exhibited, compared to controls, a lowered food consumption: 14% depression in males and 9% in females the first week. Both males and females continued to consume less food in the high-dose group throughout the 13 weeks, showing depressions as much as 21% for males and 19% for females. Since there were food consumption depressions the first week, it is assumed that at least 9-14% of the depression of food consumption was due to palatability.

Body weights also were depressed in the high-dose group compared to controls. All rats started at the same weight (approx. 64 g) but showed in the high-dose group (only) an ever-increasing depression of body weight of approximately 6% depression (M and F) starting at day 7 to 18% in M and 21% in F at day 91. Food efficiency was slightly depressed in females in the high-dose group.

Hemaglobin (Hb), packed cell volume (PCV), and number of erythrocytes were also depressed in the high-dose groups. HB and PCV were slightly depressed in the mid-dose group (500 ppm). Alkaline phosphatase (AP) was increased (M and F) in the high-dose group throughout the experiment. SGOT and SGPT enzymatic activities were elevated in the high-dose group. Plasma protein and serum albumin were both decreased in the high-dose group (M and F). The total protein levels were decreased at week 13 in the 500 ppm dose group. Na^+ was decreased in blood at 500 and 2500 ppm in females and at 2500 ppm in males.

Urinalysis results at week 13 showed decreased levels of Na^+ and K^+ in the top dose group (M and F).

The relative weights of liver and kidney were increased in weight at 2500 ppm. The kidney was increased at 500 ppm. Gross examinations showed a greenish discoloration of the top dose group livers.

1.4 Classification of Study: Not Classified

2.0 <u>Subchronic (4-week) Feeding Study of 2,4DP to Beagle Dogs</u>
[264-222, -231, -233; EPA No. 237987]

2.1 Conclusion of Beagle Dog Range-Finding Study

2.11 No change was seen among the groups in body weight, food consumption, behavior, appearance, blood electrolytes, sugar, and bilirubin. No changes were seen in globulins, albumin, or total serum proteins. SGOT and SAP were not significantly changed.

2.12 Hb, hematocrit, and RBC's were lowered somewhat at 32 mg/kg (high dose). SGPT was lowered in a dose/response manner. The thymus weight was reduced with dose (approximately 1/2 at 32 mg/kg as control thymus weights).

2.13 The symtomology demonstrated in this study does not permit the establishment of a NOEL, LEL, or MTD. This is not classified.

2.2 Design of Beagle Dog Study

This four-week study was started on May 10, 1977. 2,4DP was mixed into complete dog chow at concentrations of 0, 8, 20, and 32 mg/kg body weight. Two M/F pairs were assigned per dose group, making a total of 16 dogs for the test. Adequate animal husbandry was followed.

Dogs (pure-bred beagles) were fed 50 g/dog/day containing the correct dose of 2,4DP and given water ad libitum. The test substance was tested and proved to be stable throughout the 4-week test.

Signs for toxicity were observed, such as general appearance, condition, and behavior patterns. Ophthalmoscopic examinations were made at day zero and on days 25-27; daily attention was given to the eyelids and buccal mucosa.

Body weight change and food consumption was monitored daily. Complete hematology was done: Hb, PCV, RBC's, Leuk., Diff. Leuk., platelets, and blood clotting times. Blood chemistries done were: SGPT, SGOT, SAP, serum protein, serum electrophoresis, BUN, glucose, bilirubin, electrolytes, and ornithine transcarbamylase. Urines were measured for electrolytes and specific gravity. Feces was examined for occult blood. Livers, kidneys, spleen, thymus, heart, brain, lung, thyroid, adrenals, pituitary, testicles, ovaries, and prostate weights were measured upon sacrifice. A complete set of histopathological slides were made from the following organs: liver, kidneys, stomach, buccal mucosa, bone marrow, spleen, testes, and thymus.

2.3 Results of Beagle Dog Range-Finding Study

In each dose group, one M/F was fed 2,4DP in the feed and the other M/F pair was given the same dose in a gelatin capsule. No differences were seen between the two methods of dosing; thus the pairs in a dose group will be discussed collectively below.

No mortality occurred in this study, and the general health, appearance, and behavior were all normal among the dose and sex groups. Ophthalmoscopic examinations revealed no abnormalities of the eye, eyelid, or duct.

There was no noticible body weight changes in any dose group nor change in food consumption.

Hb, hematocrit, and RBC were lowered somewhat in the high-dose (32 mg/kg) groups. Sugar, Na^+, K^+, and bilirubins were the same among the groups. α-, β- and γ-globulins, total serum protein, and albumin were unaffected among the groups. Liver enzyme SGOT, SAP, and ornithine transcarbamyl transferase were uneffected among the groups. SGPT was lowered in dose-response manner. The later observation did not show a large depression and is probably devoid of toxicological meaning insofar as SGPT (and the other hepatic enzymes, too) are elevated in the case of liver trauma (not lowered). No occult blood was found in feces.

Although it appears that liver organ weights were increasing (267, 264, 286, and 306), it was found not to be statistically significant; the same applies to the thyroid, testes, and kidney. Heart, spleen, brain, ovaries, pituitary, adrenals, lung, and prostate clearly did not show any dose-related weight changes.

Thymus, on the other hand, showed clearly an inverse dose/response relationship, i.e., dose increased, thymus weight decreased ($r = -0.91$). This relacionship is seen whether absolute or relative thymus weights are considered:

Dose	Absolute Thymus Wt. (g)	Relative Thymus Wt.
0	21.96	0.29
8	15.14	0.21
20	12.15	0.17
32	10.98	0.15

The effect of 2,4DP on the thymus was the only dose-related effect in the dog study.* No histopathological findings were noted that were dose related.

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*It should be kept in mind that there were only 4 dogs/dose group. Such a small number/group makes statistical inferences less reliable unless the effect is quite large in magnitude.

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F. Oral Teratogenic Range-Finding Studies in the Rat and in the Rabbit

(two studies, 264-222,-231,-233; EPA Accession No. 237981 & 237983)

§1.0 Conclusions of Sprague Dawley Rat Teratogenesis Study (0 - 20 day postmating)

1.1 Weights in the high dose (100 mg/kg) group were depressed 5 - 7% starting at day 6 till term (21 days). The low dose (25 mg/kg) group showed a modest 3% loss in weight in the last three days of dosing. The weight losses were the only toxic effect observed in this experiment.

1.2 The following parameters were not effected by 2,4 DP administration: no. of corpora lutea, no. of implantations, average no. of newborn pups, no. of interuterine deaths, newborn pup weights, or sex.

1.3 There were no increases in pups of congenital defects of any kind with 2,4 DP administration to gravid females bearing these pups.

1.4 A NOEL for the teratogenic effects can not be assessed from the present study. It is concluded that at 100 mg/kg 2,4 DP is not a teratogen.

§2.0 Methods of the Sprague Dawley Rat Teratology Study

Sprague Dawley (CD strain) were mated (4 females to one male) under good ainimal husbindry conditions. The test material was not identified other than "2,4 DP code T.P.H. (9/11/76)". Control rats received regular chow while two treatment groups received 25 mg/kg and 100 mg/kg body weight, respectively. Treatment started on inseminated females at the first sign of sperm in vaginal smears (day zero). Treatment was continued for 20 days of the 21-22 day gestation period. Dosing was performed by gavage using 10 mls of a 1% methyl cellulose solution as the vehicle.

Rats were observed daily for appearance, general health condition and body weights.

At termination on day 21 rat ovaries and uteri were examined for: No. of corpora lutea, no. of and positions of live and dead fetuses, fetal weights, fetal crown-to-rump lengths, and sex of fetuses. The percentage of pre-implantation loss was calculated as:

$$\%\ \text{pre-implantation loss} = \frac{\text{No. of corpora lutea} - \text{No. of Implantations}}{\text{No. of corpora lutea}} \times 100$$

§3.0 Results of Range-Finding Teratogenesis Study

All rats (at 0, 25, 100 mg/kg) survived the test. Dam weights were depressed at the 25 mg/kg level only in the last three days of the dosing period approx. 3%. In the high dose (100 mg/kg), weights were depressed by day 6 approx. 5% and was

depressed 7% at term (21 days).

The mean number of corpora lutea (13.0 - 13.6) and mean numbers of implantations (11.7 - 12.6) per dam were not effected by 2,4 DP administration. The pre-impantation losses (14.6 % in controls, 3.1% in 25 mg/kg, and 9.8% in 100 mg/kg) were also uneffected by 2,4 DP administration. The mean number of fetuses per dam were 11.7, 12.0, and 12.3 with the total interuterine deaths 0%, 4.8%, and 0%, respectively, control, low, and high dose groups. Live fetus weights were 68.6, 67.4, and 65.2 (not a significant decrease). Sex was uneffected.

There were no significant increases with 2,4 DP dose of any minor or major congenital defects. Those which did occur did so at a low frequency level and occurred randomly amoung the groups.

§4.0 Calssification of Study: Not Classified

§5.0 Dutch Belted Rabbit Range-Finding Teratology Study

5.1 Conclusions of the Rabbit Teratology Study

5.11 The control rabbits exhibited normal nest sizes with normal newborn weights and newborn sizes.

5.12 At 25 mg/kg, the NOEL is exceeded with two major birth defects - congenitally ruptured umbilical cord and 13 ribs on one side - and some fetuses born dead all to one doe (2 died early and 4 just before or during parturition). So, NOEL < 25 mg/kg, but the specific level is undetermined by this experiment.

5.13 At 100 mg/kg, one doe did not conceive, 3 died early bearing dead fetuses, and one bore 7 smaller (in size) newborn bunnies. Definite signs of maternal toxicity were observed at this higher dose.

§6.0 Methods of Rabbit Teratology Study

Rabbits were dosed at 0, 25, and 100 mg/kg just as in the previous rat study. The protocol was virtually the same between the two studies except: (1) rabbits were artificially inseminated and (2) dosing was done by gavage from one day after artificial insemination to day 27 of the 28 gestation period. Examinations followed the protocol (which was almost the same as the rat study) and the rabbit study was conducted using GLP.

§7.0 Results of the Rabbit Teratology

The high dose (100 mg/kg) group showed definite signs of maternal toxicity in the does. All high dose rabbits showed unsteadiness in gait, reduced food intake,

and some loss in weight gains leading to weight loss. Two rabbits in the high dose group were sacrificied on day 22 because of poor health conditions. One more rabbit was sacrificed on day 23 for the same reason. All these animals that were sacrificed were gravid, but were bearing dead fetuses. Of the two remaining rabbits only one rabbit produced a litter of normal size (7 bunnies) but with 46% reduction in fetal weight and 18% reduction in crown-to-rump distance.

The mid-dose (25 mg/kg) group showed 4 of 5 pregnant does with one of the four prematurely delivering on day 25 with normal weight newborn, but all newborn were dead (2 early in pregnancy and 4 late or during pregnancy). Only the mid-dose group showed major birth defects (two). The first bunny with major birth defects had omphalocele (plus displaced kidney), and the second with omphalocele, displaced kidney, and 13 ribs on one side.

The study does not demonstrate a NOEL in so far as teratogenic effects were observed in the low dose group (25 mg/kg). At 100 mg/kg pronounced maternal toxicity was observed leading to moribundity. At the high dose group, the MTD is exceeded for 2,4 DP in rabbits.

§8.0 Classification of Study: Not Classified

G. Teratology Study in Sprague Dawley Rats with the Administration of 2-(2,4 Dichlorophenoxy)-Propionic Acid Ad Libitum.

§1.0 Conclusions on Teratology Study

1.1 Weight gains and food consumption were normal in Groups I, II, & III (0, 150, & 450 ppm in standard rat chow) with Grp. IV (1500 ppm) showing a 12.5% weight loss. The 1500ppm dose exceeds the maternal fetal, and neonatal NOELs reported in the following section, Section H of this report, "The Three Generation Feeding Study of 2,4 DP Acid".

1.2 To simulate the human dosing pattern (normally 3X/day), rats should have have been gavaged. As it was in this experiment, the 2,4 DP was compounded with the feed and thus rats had access to this feed all the time. Rats are known to eat often thereby receiving constant dosage. Constant blood levels should be expected in contast to the human blood levels which peak and may likely force more of the test compound accross the placenta. This point is a deficiency of the present teratology study.

1.3 The parturition index and fetal viability were normal among all four treatment groups and showed no 2,4 DP acid dose effects.

1.4 The frequency of resorption was low and showed no dose related effects with 2,4 DP among the four treatment groups.

1.5 The abnormalities observed were qualitatively what might be expected in Spague Dawley rats. The frequency of abnormalities was low and showed no dose related 2,4 DP acid effects.

1.6 This study is classified as Core-Minimum due to the deficiency outlined in § 1.2.

§2.0 Introduction to Teratology Study in Spague Dawley Rats by 2,4 DP

Four dose groups were selected among gravid female Sprague Dawley rats: 0, 150, 450, and 1500 ppm 2,4 DP acid (94% pure)*. There were 21 rats/group and they were administered 2,4 DP acid in a commercial rodent diet from day 6 to day 15 of pregnancy (day zero = appearance of copulatory mucus plug).

The Dams were sacrificed on day 19 and the liver and uterus were removed and weighed (results not presented). The uterus was then opened and the number and location of live and dead fetuses were noted as well as readsorption sites. Each fetus was dissected free of placenta and surrounding membranes, weighed, and examined for gross abnormalities.

One third of each fetus group was examined for visceral abnormalieies by Wilson's Techniques. The remaining two-thirds was examined with Alizarin Red S stain for skeletal anomalies.

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*This corresponds to 0, 10, 30, and 100 mg/kg/day. The highest dose exceeds the MTD=50 mg/kg/day from chronic feeding studies.

§3.0 Results of Teratology Study

The mean maternal weight gain from day 6 to 15 was 112, 110, 116, and 98 grams for groups 1 to 4. Group 4 (98g) was not significantly lowered in weight gain. The mean food consumption per day was 19, 20, 21, 19 grams/day. As with the weight gains, no significant difference was seen in food consumption among the four groups.

In Table I, it seen the incidence of parturition was very good among the four groups and did not show any 2,4 DP effects.

The number of implantations are considered adequate with the mean number implantations per litter showing no 2,4 DP effects. The number of resorption sites was normal for Spague Dawley rats (0.48-3.3%) of the total number of implantation sites). The low level resorption sites showed no 2,4 DP effects.

The number of live fetuses per liter was good (10.1-10.7) and the fetal viability was very good showing no 2,4 DP effects (Table I).

The examination of the fetuses showed no skeletal or visceral abnormalities that were unusual to this strain of rat. The abnormalities observed included the following:

Control: One litter showed one fetus with a partial cleft palate and two other fetuses with separation of the thoracic ossification centers and the inter-parietal and occipital bones into two pieces as well as underdevelopment of frontal and parietal bones. A second litter showed one animal with a probable hydrocephalus and a second with an encephalocele. A third litter showed one individual with dilatation of the pelvis of both kidneys. (This last is of questionable importance).

Low Dosage: One litter showed four fetuses with parietal bones misshapen and underdeveloped.

Medium Dosage: One litter included a single fetus which showed deformation of the lumbar-dorsal arches, the vertebral column ending abruptly with the tail unusually short and small in diameter. Both hind paws appeared normal in ossification but were rotated inward. The third lumbar centrum was split, the occipital and inter-parietal bones of the skull were out of place to the left. The other 11 fetuses in this litter appeared normal.

High Dosage: One litter showed one fetus with substantial under-development of parietal bones, distortion of the inter-parietals and wavy ribs. A second fetus in this same litter showed a borderline wavy deformation of the ribs.

The frequency of abnormality occurrence is low as is the number of litters affected. Thus, 2,4 DP causes no increase in abnormal fetuses (Table I).

§4.0 Classification of Study: Core-Minimum

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RAT

Table I - Teratology

SUMMARY OF RESULTS

	GROUP I CONTROL	GROUP II 10 MG/KG	GROUP III 30 MG/KG	GROUP IV 100 MG/KG
No. of Gravid Dams	21	21	21	21
No. of Litters	16	21	19	19
Parturition Index*	0.76	1.00	0.90	0.90
No. of Implantation Sites	168	221	206	212
Per Litter	10.5	10.5	10.8	11.1
No. Resorption Sites	6	3	1	7
No. Live Fetuses	162	218	205	204
Fetal Viability*	0.964	0.986	0.995	0.962
Mean Live Fetuses Per Litter	10.1	10.3	10.7	10.7
Mean Fetal Mortality	0.0	0.0	0.0	0.5
No Fetuses Examined:	162	218	205	204
Visceral Exam.	49	67	64	55
Skeletal Exam.	113	151	141	141
No. Abnormal Fetuses	6	4	1	2
No. Abnormal Litters	3	1	1	1

*Parturition Index= No of paruritions/No of pregancies

Fetal Viability= No. of live fetuses/No of implantations

H. Three Generation Study on 2,4, DP Technical Acid in Rats. (264-231; 237875, Section I of Submission)

§ 1.0 Conclusions of Three Generation Study in Rats

1.1 Parental rats all survived in all dose groups and in all generations. Intercurrent deseases observed were low and not dose related. Appearance, behavior, and gait were normal in all groups.

1.2 Parental food consumption and weight gains were normal in Groups I, II and III, but were reduced 5-10% in the high dose group (Grp. IV). The same doses were given in the 2-year rat oncology study (section J) and 2000 ppm was toxic in that study indicating that Group IV weight loses were due to general toxicity. When the high dose was reduced to 1000 ppm, weights and food consumption were normal. The toxicity is not certain in so far as 2000 ppm equals a food concentration for 2,4 DP acid of 0.2% which is likely high enough to effect palatability.

1.3 The pregnancy rates (# female rats conceiving/# inseminated) and gestation periods were unaffected amoung the dose groups in any generation.

1.4 The "averages" of litter size were not significantly different among the dose groups, but smaller litters were seen ($n \leq 8$) in F_{1A} and F_{2A} litters at 2000 ppm.

1.5 Fetal mortality was significantly increased at 2000 ppm in F_{1B} and F_{2A} litters. These motality increases were abated when the high dose was reduced to 1000 ppm. Group III (500 ppm) fetal mortalities was slightly increased in F_{2A}, F_{2B}, F_{3A} and F_{3B} litters. There was no increase in fetal mortality at the low dose (125 ppm).

1.6 Mean pups weights at birth and at the end of lactation were affected at the high dose of 2000 ppm, but were unaffected at 1000 ppm. Sex of pups was unaffected.

1.7 Neonatal mortality was increased at the high dose of 2000 ppm. The 500 ppm dose (Grp. III) increased neonatal mortality only in F_{2A} litters and the low dose (125 ppm) only in F_{3B} litters. Although these lower doses had effect in these litters, the high dose (when reduced to 1000 ppm) was the same as controls.

1.8 It is concluded that maternal toxicity has a NOEL = 1000 ppm and LEL = 2000 ppm (reduced weights and increased number of smaller litters, i.e. where the number of pups/litter was 8 or less).

The fetal toxicity LEL = 500 ppm (increased litter mortality), and fetal NOEL = 125 ppm.

The neonatal toxicity NOEL = 1000 ppm and LEL = 2000 ppm (increased pup mortalities during lactation period).

§ 2.0 Recommendations

2.1 A complete chemical analyses should be submitted to the Agency of the technical grade 2,4 DP used to dose the rats in this three generation study.

2.2 A palatability study of the test material should be done and the results reported to the Agency.

§3.0 Introduction

Weedone® contains the active ingredient butoxyethanol ester of 2,4 DP acid. The acid moeity was fed in this study to Sprague Dawley rats at doses in rat chow: I (0 ppm), II (125 ppm) III (500 ppm), and IV (2000 ppm). The study was performed by the Huntingdon Research Center, 216 Congers Rd., NY. NY. 10956 (the center is now closed) for the Amchem Division of Union Carbide in order that the potential to affect the reproduction performance in rats may be assessed. Effects on the parental, fetal, and neonatal survivability and weight gains were also observed in order to assess the oral toxicity of 2,4 DP acid.

The three generation study was started on April 12, 1977 and completed on October 2, 1978. The composition of the technical grade 2,4 DP fed to the rats was not submitted by Union Carbide.

§4.0 Methods:

4.1 One hundred twenty (40 male, 80 female) Crl:COBS CD (SD) BR weaning rats were obtained from Charles River Breeding laboratories, Inc., 251 Ballardvale Street, Wilmington, Massachusetts 01887. Animals were housed five (5) per sex per cage in suspended wire meshed stainless steel cages. Light cycle was 0700 through 1900 hours. Urine and feces dropped onto DACB papter (Upjohn). DACB papter changed at least three (3) times per week. GLP was followed in these experiments.

During the mating phase one male and two females were housed per suspended wire meshed stainless steel England breeding box. After twenty days, and when sperm was observed microscopically in the vagina of the female, the males were housed five/cage. Females were individually housed in plastic breeding boxes containing approximately three inches of Sani-Chips (Saint Regis Paper Co.) for bedding. Presumably, these Sani-Chips were acceptable to the dams for nesting.

After a seven (7) day acclimation period to laboratory conditions the animals were assigned to the following groups and received the indicated diet: Group I, 0 ppm; Group II, 125 ppm; Group III, 500 ppm; Group IV, 2000 ppm into F_{1B} premating phase and 1000 ppm thereafter. In each dose group there were 20 males and 20 females. Basic diet given was Micro Mix Rat Diet.

§5.2 F_0 Generation:

Animals in the F_0 generation were maintained on their respective diets for sixty (60 days). One male and one female were then placed into mating cages for twenty (20) days. Upon the identification of sperm in the vagina of the female, the female was placed into a breeding box and allowed to give birth to the F1A generation. The F1A generation was raised until weanling and then sacrificed. Approximately one-third

(1/3) of the F1A generation was subjected to necropsy examination at twenty-one (21) days of age.

After a seven day rest period, the FC animals were remated, using different male/female pairings for no longer then twenty days. This mating producted the F1B generation. When the F1B generation was twenty-one days old, twenty (20) male and twenty female rats were selected from the litters of each group to become the parents of the next generation. Remaining weanling animals and the animals in the F0 generation were sacrificed. Approximately one-third (1/3) of the weanling animals from the F1B generation were subjected to a gross necropsy examination.

F1B Generation

Due to the reduced numbers of young and low body weights of parents the high dose level was reduced to 1000 ppm of the test material on October 19, 1977. Animals in the F1B generation were maintained on their respective diets for sixty days. The animals in the F1B generation were mated as described for the F0 generation and produced the F2A and F2B generations.

When the F2B generation was twenty-one days old, twenty male and twenty female rats were selected from litters of each group to become parents of the next generation. Remaining weanling animals and the animals in the F1B generation were sacrificed. Approximately one-third of the weanling animals from the F1B generation were subjected to a gross necropsy examination.

F2B Generation

Animals in the F2B generation were maintained on their respective diets for sixty days. The animals in the F2B generation were mated as described for the F0 generation and produced the F3A and F3B generations.

When the F3B generation was twenty-one days old, animals in the F3B generation and F2B generation were sacrificed. Animals in the F3B generation were subjected to a gross necropsy examination.

§5.3 Observations

5.31 Parental Animals:

a. Daily Observations: All animals were observed each morning and afternoon for mortality and were observed at approximately the same time each day for appearance, behavior, gait, and signs of toxic effect.

b. Food Consumption: Food consumption was measured weekly during the sixty day premating phase of each generation.

c. Body Weight: Body weights were recorded for each parental rat in all generations on a weekly basis during the sixty day premating phase. Maternal animals were weighed on days 0, 7, 14 and 20 of pregnancy. Maternal animals producing the F1B, F2B and F3B generations were weighed on days 0, 7, 14 and 21 post partum. New born animals were weighed on days 0, 4, 12 and 21.

d. Pregnancy Rate: The pregnancy rate was calculated for each generation as the ratio of the numbers litters born to the number of females paired.

e. Mating Performance: During the mating periods, vaginal smears were prepared daily and examined microscopically for the presence of sperm. The day that sperm were seen was termed the day of mating and day zero of pregnancy. Rats were observed to determine if there were any effects upon the estrous cycle.

f. Gestation Period: The length of gestation was recorded for all generations and data for test and control animals compared.

g. Litter Data: As soon as possible after birth, pups were counted, litters weighed, mortality determined where possible, and pups examined for external abnormalities. The pups were again weighed on days 4, 12, and 21 post partum. Pups were sexed at day 21.

§ 6.0 Results

6.1 Parental Animal Data

Survival - All rats survived (except one killed by a falling cage) in all close groups in all three generations. Thus, 2,4 DP did not effect survival of either male or female rats. Only a few rats were affected were affected by intermittant disease and was random in the dose groups and considered normal for this strain of rat. Appearance, behavior, gait were all normal.

Food consumption - Male and female rats were affected (less consumed) at the high dose of 2000 ppm in the F0 generation. Males in the Group III F1B and F2B generations ate less than controls. All other food consumption was the same among controls and treated rats. In those groups affected, food consumption was lowered 5-10%.

Body Weights - Commensurate with less food sonsumption, body weights in the high dose group were decreased 5-10% compared to controls. The low and mid dose group weights were the same as controls.

Pregnancy Rate - The rates of pregnancies is each group and generation were normal (90 - 100%) and no differences occur due to 2,4 DP treatments.

Gestation period - The gestation periods were unaffected by 2,4 DP acid and were 21 or 22 days.

6.2 Litter Data

Litter size - The litter sizes are given in Table I. There appears to be no significant differences in "averages" amoung the groups or amoung the generations, therefore 2,4 DP does not seem to affect litter number of pups. However, if the number of litters which have 8 or less pups are scored (Table I), it is seen that 2000 ppm in the F1A and F2A litters produce these smaller litters.

Fetal Mortality - At day zero (at parturition) the percent of dead fetuses show definite 2,4 DP affects at 2000 ppm in F0 --> F1B and F1B ---> F2A (Table I). This increase is dead detuses is not seen in successive generations where the high dose was reduced from 2000 to 1000 ppm. It should be noted that a slight fetal effect* at 500 ppm (Group III) is seen after the F1B ---> F2A generation which is missing altogether in the F0 whelpings.

Neonatal Mortality - Groups III (500 ppm) and IV (2000 ppm) show significant neonatal mortality responses to 2,4 DP in the F1B---> F2A generation whereas only the 2000 ppm shows neonatal responses in the F0 generations.

After the high dose was reduced from 2000 to 1000 ppm the mortalities are same in the high dose as controls. The mortality from 500 ppm treatments tend to show slight increases* (compared to controls). The low dose (125 ppm) produces increased neonatal mortality only in the F3B generation.

Mean Pup Weight - The mean pup weights are recorded in Table II pups at birth and at the end of the lactation period (at 21 days).

At birth (day zero) - a slight reduction (Group IV) in mean pup weight is seen in the F0 --->F1B generation. Mean pup weights are the same among the treatment groups in all other generations.

At the End of Lactation - A definite reduction was seen in Group IV weights in F1A and F1B pups. After the high dose reduction no decrease in pup weights were seen compared to controls in any other generation.

Sex - There was no change in the proportion of males and females in each litter with treatment of 2,4 DP in any of the generations.

§ 7.0 Classification of Study: Core Minimum (because of the deficiencies indicated in § 2.0).

*Not statistically significant from controls when compared by a standard 2x2 contingency test.

TABLE I
Three Generation Study
Litter Mortality Incidence
(Average Cumulative Percent)

	Average No. of Pups/Litter	(Parturition) At Birth	At 4 Days	At 12 Days	At 21 Days	No. of Litters with 8 or less Pups/No. of Litter in Group
		Litter Mortality Incidence % (Cumulative)				
F_0-F_{1A}						
I	13.8 ± 2.7	1.21	3.10	3.42	3.42 ± 4.45	1/19
II	13.0 ± 1.8	0.39	2.05	2.05	2.05 ± 4.19	0/18
III	13.6 ± 2.3	0.00	2.78	2.78	3.47 ± 6.98	1/19
IV	11.2 ± 3.4	0.83	4.32	5.00	5.00 ± 6.49	5/18
F_0-F_{1B}						
I	13.2 ± 4.1	0.00	1.33	6.39	8.38 ± 12.07	3/18
II	11.9 ± 3.6	0.79	0.79	2.32	3.5 ± 6.05	3/19
III	14.7 ± 1.8	0.39	2.50	5.06	5.66 ± 5.32	0/18
IV	12.3 ± 3.5	18.15	18.90	33.85	37.95 ± 37.31	3/20
F_{1B}-F_{2A}						
I	11.94 ± 3.8	0.00	1.06	5.12	6.88 ± 12.04	3/17
II	14.1 ± 2.0	0.00	2.21	4.32	7.95 ± 11.20	0/19
III	11.9 ± 3.9	3.53	17.11	26.05	26.88 ± 35.44	4/17
IV	11.3 ± 3.9	11.15	13.10	19.80	26.00 ± 34.81	6/20
[Following F_{2A} Birthing ... High Dose Group Lowered 2000 ppm to 1000 ppm]						
F_{1B}-F_{2B}						
I	13.6 3.1	1.50	2.06.	5.12	5.12 ± 7.59	1/16
II	15.0 ± 3.0	0.32	3.63	9.63	11.44 ± 13.93	1/19
III	14.1 ± 2.5	3.52	9.32	11.42	12.77 ± 22.79	2/19
IV	13.1 ± 2.3	1.37	4.52	8.32	9.32 ± 12.50	1/19
F_{2B}-F_{3A}						
I	13.8 ± 2.0	1.50	1.95	3.35	4.26 ± 7.21	0/20
II	13.2 ± 1.7	0.39	1.61	1.72	2.66 ± 5.61	0/18
III	12.2 ± 2.5	4.65	5.00	5.30	5.30 ± 5.11	1/20
IV	12.2 ± 3.5	.70	0.70	0.70	1.65 ± 3.54	2/20
F_{2B}-F_{3B}						
I	14.6 ± 2.5	0.65	1.80	2.45	3.55 ± 7.53	1/20
II	13.6 ± 2.6	1.80	5.80	10.10	10.40 ± 19.04	1/20
III	14.3 ± 2.2	3.95	4.30	9.20	9.50 ± 15.04	0/20
IV	14.8 ± 1.1	0.70	1.30	1.90	1.90 ± 3.65	0/20

TABLE II.

Mean Pup Body Weight (Grams) ± Standard Deviation (%)

Generation / Treatment	Group I (0 ppm)		Group II (125 ppm)		Group III (500 ppm)		Group IV (2000/1000 ppm)	
$F_0 \rightarrow F_{1A}$	(g)	(%)	(g)	(%)	(g)	(%)	(g)	(%)
At Birth (zero days)	6.18 ±	6.8	6.18 ±	15.7	6.22 ±	12.9	6.27 ±	14.2
End of Lact. (21 days)	43.1 ±	17.2	45.3 ±	17.8	43.0 ±	16.6	34.5 ±	27.2
$F_0 \rightarrow F_{1B}$								
At Birth (Zero days)	6.51 ±	11.1	6.34 ±	10.4	6.26 ±	8.9	6.00 ±	14.5
End of lact. (21 days)	46.4 ±	20.4	43.8 ±	17.7	40.1 ±	14.3	33.5 ±	31.1
$F_{1B} \rightarrow F_{2A}$								
At Birth (Zero days)	6.72 ±	13.7	6.34 ±	7.25	6.49 ±	12.4	6.70 ±	15.3
End of Lact. (21 days)	36.9 ±	24.4	33.19 ±	12.8	34.6 ±	[illegible]	34.4 ±	30.5
$F_{1B} \rightarrow F_{2B}$								
At Birth (Zero days)	6.56 ±	9.1	6.31 ±	7.3	6.46 ±	14.4	6.34 ±	10.4
End of Lact. (21 days)	39.8 ±	21.3	38.3 ±	16.9	39.6 ±	21.3	37.1 ±	20.8
$F_{2B} \rightarrow F_{3A}$								
At Birth (Zero days)	6.13 ±	17.9	5.99 ±	9.7	6.28 ±	8.9	6.18 ±	12.0
End of Lact. (21 days)	38.8 ±	16	39.0 ±	13.7	42.5 ±	27.0	39.7 ±	28.2
$F_{2B} \rightarrow F_{3B}$	6.73 ±	15.9	6.65 ±	9.3	6.75 ±	13.2	6.38 ±	7.1
End of Lact (21 days)	39.7 ±	17.5	38.3 ±	18.0	39.6 ±	20.0	33.9 ±	11.8

Note: Each value is given in grams weight ± the % variation in each group.

001995

I. Eighteen Month Oncological Feeding Study with 2-[2,4-dichlorophenoxy] propionic Acid to Swiss-Wester CD-1 Mice at Doses of 0, 25, 100, and 300 mg/kg Body Weight.

§1.0 Conclusions: [264-231; EPA No. 242035, 6,7,8]

1.1 Survivals, food consumption, and hematology were normal among the dose and sex groups.

1.2 Stress toxicity was manifest at 25 and 100 mg/kg by increasing hematopoiesis, myelopoiesis, and granuloporesis which is a typical response of ageing mice. These syntheses were accompanied by some anisonormocytosis.

1.3 Bile retention (with some bile duct duplication) was observed at the 300 mg/kg dose as well as areas of degeneration and other liver areas of regeneration. The LEL for general toxicity is set at 300 mg/kg and NOEL toxicity = 100 mg/kg.

1.4 No tumor kinds (benign or malignant) or types (cell- or tissue-specific) were dose related to the feeding of 2,4 DP. It should be noted that excessive (18.8%) hepatomas were ween at the high dose (300 mg/kg) males compared to control males (7.8%). This response is viewed as an effect not related to the dose of 2,4 DP.

§2.0 Introduction

Union Carbide is submitting for review a 1.5 year (18 Month) oncological Swiss-Webster CD-1 mouse study on 2,4 DP. The 2,4 DP in this study was administered in the feed at doses of 0, 25, 100 and 300 mg/kg body weight. This is equivalent to concentrations of 0, 175, 700, and 2100 ppm of 2,4 DP in the feed. The technical grade of 2,4 DP (EPA EST. No. 15440 EN-1) was used. The study was started by Chemcials, Drugs, and Cosemetic Research (CDC) on 6/17/77 and was terminated on 1/25/79.

§3.0 Methods

3.1 General Design and Conduct of Test

This 1.5 year feeding study to CD-1 mice was conducted in a manner similar to the rat study reviewed in Subpart J. § 3.1 and 3.2. Only experimental matters unique to the mouse study will be described here.

Mice weighed 10-12 grams upon arrival at CDC Laborities and were acclimated six weeks before starting feeding exposure to 2,4, DP.

GLP was followed in animal husbandry. Mice were observed daily for viability and Monday thru Friday for pharmacologic or toxicologic effects and behavioral changes. Food consumption and body weights were measured weekly (0-13 weeks) and then monthy thereafter. Organs weighed upon necropsy were brain, heart, liver, kidney, adrenal, and testes.

Tissues examined histopathologically in mice were the same as those listed in Subpart J. § 3.2 (rat study). All mice were necropsied whether sacfriced because of moribundity or died before term or sacrificed at term (81 weeks = 75% of CD-1 lifetime). All tissues in each necropsied mouse were examined with few exceptions.

Hematologic examinations of heart blood was odne on 10 male and 10 females. Measurements were made on blood hematocrit, hemoglobin, erythrocyte count, MCV, and differential leukocyte counts, and platlets.

3.2 Methods of Oncologic Toximetric Analysis

The same methods and criteria employed in the previous rat study (Subpart J, § 3.3) were used to analyzed the present CD-1 mouse oncologic response.

§4.0 Toxicology Observed in Swiss-Webster CD-1 Mice

4.1 Survival - Survivals were the same among the treatment groups and sexes. Thus, 2,4, DP as high as 300 mg/kg showed no life-shorting effects in CD-1 mice.

4.2 Clinical Signs - Signs of old age were manifest in term mice and were distributed randomly among the dose groups and sexes.

4.3 Body Weights - Mice were an average of 10-12 grams upon arrival, 22-23 g at start of test (average among randomized groups), and 41 grams at erm. There were no differences in body weights among the dose groups and sexes.

4.4 Food Consumption - At the start of the test all groups consumed Wayne Blox at an average of 10g/day. At the end of test consumption was 5-6g/day among the four groups. Thus, 2,4, DP did not effect food consumption.

4.5 Organ Weights - Only liver weight changed with dose, and then, only at the high dose of 300mg/kg. Males showed a 59% increase in relative liver weight (55% increase in absolute weight) and females showed a 25% increase in relative liver weight (21% in absolute weight).

4.6 Hematology - The values HCT, HGB, RBC, WBC, and MCV did not significantly change among the dose groups and sexes. Although WBC's were normal some anisonormocytosis was observed (see 4.8 below).

4.7 Hematopoiesis - There was a definite ($p=.01$) increase in male and females in hematopoiesis in marrow, spleen, and liver in the first three dose groups (0, 25, and 100 mg/kg). The high dose group did not show increased erythroid cell production.

001995

4.8 Myelopoiesis and Granulopoiesis - Males show an increase in low dose group compared to controls (=.02). Females showed a dose/response increase (p=.03) at all four dose levels.

4.9 Neural Involvement - Sciatic nerve, spinal cord, and brain were each reviewed for any responses to 2,4 DP. There were no dose related neural effects in these tissues caused by 2,4, DP.

4.10 Lymphoreticular System (LRS) Involvement-lymph nodes, spleen, thymus and myeloid tissues were each reviewed for arteritis, thrombosis, hemorrhage, infartion, lymphangiectasis as well as cellular hyperplasa, hypoplasia, and hypertrophy. There were no 2,4 DP - induced effects except some lymphoid depletion in the spleen in the high dose group.

4.11 Liver - Males and females showed bile retention at 300 mg/kg, only. Liver cells also became irregular in shape (anisocytosis). Considerable ($p<.001$) hepatic regeneration was observed in the high dose males along with areas of degeneration in both males and females.

4.12 Kidney - there was an effect in the high dose group of interstitial nephritis and intratubular pigment.

4.13 Gastro intestinal Tract - Some gastric erosions were seen in treated groups (II = 3, III = 2, IV = 2) with none observed in the control mice.

§ 5.0 Oncological Response of Swiss - Webster CD-1 Mice to 2,4 DP.

Review of over-all tumor incidence data showed: (1) no increase in tumors (male and female) of all kinds with dose, (2) no increase in begign or malignant tumors with dose, and (3) no increase in tumor load (# of tumors/mouse) with dose.

The most frequent observed tumors in this experiment occurred in the lung as benign alveologenic adenomas:

Dose:	0		25		100		300	
(mg/kg)	M	F	M	F	M	F	M	F
Lung freq.	20/90	12/90	9/50	6/50	8/50	5/50	10/50	1/50

It can be seen there are no 2,4 DP dose related increase of lung tumors.

The second most frequent tumor occurence was benign liver tumors (hepatomas):

Dose:	0		25		100		300	
(mg/kg)	M	F	M	F	M	F	M	F
Liver freq.	7/90	0/90	3/50	0/50	1/50	1/50	9/50	1/50

The hepatomas (M+F) are dose related according to the positive trend analysis of Peto (0.01). However, the trend is driven solely by the response in the high dose group. Thus, the response is viewed as occurring at 300 mg/kg possibly due to liver toxic stress (see section 4.11 for corroborating evidence).

The lymphoreticular system (LRS) showed collectively the largest malignant tumor response:

LRS Tumors (Malignant)

Dose: (mg/kg)	0		25		100		300	
Cell Type	M (90)	F (90)	M (50)	F (50)	M (50)	F (50)	M (50)	F (50)
Reticulum Cell Sarcoma	6	5	4	6	2	2	1	4
Thymic Lymphoma	1	1	1	0	1	2	0	1
Nonthymic lymphoma	2	0	0	0	0	1	1	0
Myeloid leukemia	0	2	1	1	1	1	1	1
Erythrocytic leukemia	0	0	0	1	1	1	0	0
Lymphocytic leukemia	0	0	0	0	0	0	1	0
Leukemia unspecified	0	0	0	0	0	1	0	0
Total:	9	8	6	8	6	8	4	6

These data show that males show no dose response of any specific LRS tumor or collected total LRS tumors. Females show only marginal effects in reticulum cell sarcomas and total LRS tumors ($p = .01$) and then only at the low dose.

Of mice dying before term 57 of 87 had LRS tumors, but these were randomly distributed among the dose and sex groups. Of the number of malignant tumors discovered at term 16 of 32 were LRS tumors, but these tumors were also randomly distributed among the dose and sex groups. Mice are known to exhibit a high level of LRS tumors especially in old age. The percentage mice before term (66%) with LRS tumors is high in so far as LRS tumors when they do occur are life-threatning thereby enriching the frequency of discovery of LRS tumors before term because the mice sucumbing to these tumors are likely to die early.

These data taken together suggest that CD-1 mice normally get some LRS tumors (control =9.4%, historical control average = 8%). 2,4 DP does not increase total LRS tumors nor any specific tumor type in the LRS.

§6.0 Discussion of Results

Survivals, foods consumption and hematology were normal in the 0-300 mg/kg feeding dose range. Stress toxicity was seen by increased hematopoiesis, granulopoiesis, and myelopoiesis in dose groups 0, 25, and 100 mg/kg. This is expected in old mice. Liver weights were increased by bile retention at the 300 mg/kg dose leading to areas of degeneration and other liver areas of regeneration.

It is conclused that the MTD (CD-1 Mice) is 100 mg/kg and LEL is 300 mg/kg for long term feeding toxic effects. No tumor kind or type was shown to be related to the feeding of 2,4 DP to CD-1 male or female mice for 1.5 years.

§7.0 Classification of Study: Core Guideline

001995

J. Two Year Oncological Feeding Study with 2-[2,4, Dichlorophenoxy] Propionic Acid in Sprague Dawley Rats at Doses of 0, 25, 50, 200 mg/kg.

[264-231, -222, -307, -308, -309; EPA Accession No. 244-476,-478, -479, -480, -481]

§ 1.0 Conclusions of Two Year Oncology Study in Sprague Dawley Rats

1.1 Dioxin content of 2,4 DP formulations (including those formulated with 2,4D) should be assessed. TOX defers to RCB as to dioxin content of these formulations.

1.2 The toxicity of the manufacturing impurities of 2,4DP Acid (listed in § 4.0) are unknown at this time.

1.3 The high dose (200 mg/kg for 60 weeks, 150 mg/kg 44 weeks) produced pronounced toxicity to Sprague Dawley rat liver, kidney, and lymph nodes (histiocytosis) in both sexes. Males were also particulary effected by lung congestion, chronic prostatitis, and testicular atrophy with hyperplasia and edema. Females had remarkable adrenal cortical degeneration and increases in pituitary pigmentation.

1.4 The MTD for the chronic feeding of 2,4DP acid is set at 50 mg/kg for Sprague Dawley rats.

1.5 Females had 34 to 66% more tumors of all kinds and tissue types than males. Females, however, did not show dose-related responses in benign or malignant tumors (possably because of the high background in controls).

Males showed an increase in malignant tumors of all kinds ($p>.006$) with a corresponding decrease in benign tumors (linear correlation coefficient $=-0.999$).

1.6 The malignant tumor load in males rats increased with dose, 4-fold at the low dose and 8-fold at the mid dose ($p<.005$). Increased tumor load (with dose) indicates increased malignancy in males.

1.7 A significant increase in male pituitary carcinomas ($p<.005$) and in male thyroid medullary carcinomas ($p<.005$) was observed with increased dose of 2,4DP acid ($\leq$ 50 mg/kg). Occurrence of these tumor types in the controls agree with occurrence in historical controls.

1.8 Life-times for rats with pituitary carcinomas were only moderately decreased (-8.5%) while rats with thyroid carcinomas were not significantly decreased (-2.4%).

1.9 There was a shift with dose from the malignant tumor pattern of the controls (pituitary + thyroid = 37% of total malignant tumors) to the treated grcups (pituitary + thyroid = 85-86% of total malignant tumors). This shift to mostly pituitary and thyroid carcinomas indicates organ specificity of 2,4DP oncogenic action.

1.10 Brain tumors, which are rare in the rat and in man, were observed in Grp 2 (M and F) to be in excess of brain tumors in the controls (M and F). The degree of certainty is $p<.025$. The average life time of rats diagnosed with brain tumors was reduced 22% from term, i.e. brain tumors are significantly life-threatening.

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1.11 It is concluded that 2,4DP acid is a carcinogen in male Sprague Dawley rats because of the following criteria:

1. Increased incidence and frequency with dose of all malignant tumor types compared to controls.

2. Increased incidence and frequency of three specific tumors types: pituitary, thyroid, and brain carcinomas.

3. A decrease in life-span in rats with piruitary and brain tumors.

4. A shift with dose in the malignant tumor pattern in controls to the malignant tumor pattern in the treated groups. The treated groups had 85-86% of pituitary and thyroid malignant tumors whereas the controls had 37% of these two tumor types.

5. Increased tumor load (malignancy) in male rats with dose.

6. Occurrence in both sexes of a rare tumor type such as brain tumors.

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§ 2.0 INTRODUCTION

Union Carbide Chemical Corporation is sumbitting a 2-year Sprague Dawley rat oncology study in partial fulfillment of Section 3 guidelines on 2,4 dichlorophenoxy-2-propionic acid (2,4 DP acid, also called dichloprop). Notably Union Carbide is submitting oncology data on the unesterified 2,4 DP acid not on the formulated active ingredient 2,4 DP/BOE. Union Carbide did not submitt a rationale for feeding 2,4 DP acid, although it follows that Union Carbide considers 2,4 DP acid as the residue of concern. No animal or plant metabolism studies have been reviewed by the Agency. Such studies could delineate the residue of concern in the environment or when contacting applicators per os, percutaneously or by respiration.

The chemical composition of the technical grade 2,4 DP acid used in the feeding study was:

Composition of Technical Grade Dichlorprop

Chemical Constituent	% (W/W)
2-(2,4 dichlorophenoxy) propionic acid	95.0
[redacted]	

MANUFACTURING PROCESS INFORMATION IS NOT INCLUDED

2,4 DP acid (the major component) is noted in the Registry of Toxic Effects of Chemical Substances as only slightly toxic (oral) and is Category III in skin toxicity. [redacted] is only slightly toxic (oral) and is toxic to skin, Category II, at 430 mg/kg. No carcinogenic effects of 2,4 DP acid, or the contamination products, are known to the Agency. The dioxin content of 2,4 DP acid or 2,4 D in Weedone ® formulations is not known at this time.

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§ 3.0 Methods

3.1 General Design and Conduct of Test

The 2-year feeding experiment was performed by Chemicals, Drugs, and Cosmetics (CDC) Research for Amchem Products Division of Union Carbide. The study commenced on 5/18/77 and terminated on 5/25/79. Sprague Dawley rats (randomly bred and selected) were used to test 2,4 DP acid.

Group 1 (control rats, zero mg/kg) were fed Wayne lab-Blox containing no 2,4 DP acid whereas Groups 2, 3, and 4 were fed 2,4 DP acid mixed into the Lab-Blox at levels of 25, 50, and 200 mg/kg body weight. These doses correspond to 2,4, DP acid concentrations of 0, 500, 1000, and 4000 ppm in the feed. It was found necessary to lower dose group 4 from 200 to 150 mg/kg (4000 to 3000 ppm) at 60 weeks of the 104 week experiment due to poor weight gain and general signs of toxicity.

Rats 75-100g. body weight (Charles River) were commenced on test at 4-6 weeks of age. Rats were radomized and singly caged just before onset of the test. handling of rats followed Standard Procedures of Good Laboratory Practice.

3.2 Experimental Evaluations

Rats were observed daily for toxic effects and examined once/week for the appearance of any swelling or mass. Rats showing advanced signs were euthanized & necropsied. Food consumption was recorded weekly in early life (13 weeks), every two weeks for the next 12 weeks, and then monthly. Samples of feed were taken to verify dose but the results were <u>not</u> reported to the Agency by CDC Research.

Ophthalmoscopic examination was performed on 10 rats of each sex for each dose group. Hematologic examination was performed twice, once at week 67 and once at week 103 on 10 rats/sex/dose group. Blood from clipped tails was analyzed for hemaglobin, RBC, MCV, differential leucocyte counts, and platlets.

All tissues* were examined histologically (H&E stains) in all animals killed during the 2-year test and in all animals sacrificed at term (104 weeks) except the parathyroids and esophagi in the low and mid dose groups. Pathology Report Sheets were supplied for <u>each</u> of the 480 Sprague Dawley rats listing results and comments on <u>each</u> tissue. Before the rats were killed gross observations were made and and upon killing the brain, liver, kidneys, adrenals, heart, and testes were weighed.

3.3 Methods of Oncologic Toximetric Analysis

Tumors showing significant incidence (by X^2-contingency tests) are reported as the number of those tumors scored and compared to the expected number of those tumors that would occur if 2,4 DP acid had no oncogenic properties, i.e. to those that would occur by random chance. A positive trend in tumor response with dose was evaluated in each case.

* All grossly visible lesions, esophagus, nasal skull, stomach brain, small intestine, pituitary, large intestine, eyes with Harder's gland, salivary glands, tongue, skin, larynx, mammary gland, trachea, lymph nodes, lungs, testes, thymus (if visible), epididymi, heart, seminal vesicles, thyroids /parathyroids, prostate, liver, ovaries, pancreas, spinal cord spleen, sternebrae adrenals, sciatic nerve kidneys, muscle, urinary bladder, uterus.

Frequency intervals evaluated were 1 week using Peto's "death rate method" [Peto, R. et al. IARC Monograph-Long Term and Short Term Screening Assays for Carcinogens: A Critical Appaisal Supplement #2, Annex, P. 311, (1980)]. A further comparision was made of dose effects with the frequency of observed tumors (FOOT) by calcutating the number of rats in which a specific tumor was discovered at a certain time in the test divided by the total number of rats alive at that time in that group. For a specific time frame, e.g. "during" or "at term", the sum of the FOOT was made to get the total frequency of observed tumors in that time period (sum FOOT/time period).

Criteria examined in this review for oncogenicity were: (1) increased incidence and (2) frequency of tumors among the dosed groups, (3) decreased life-span for animals bearing a certain tumor type, (4) existence of rare tumors, (5) increased tumor load (No. of tumors/rat), and (6) increased malignancy (increased No. of malignant rumors compared to No. of rats with tumors) in dosed groups, and (6) changes in tumor pattern in treated groups in comparision to controls.

§ 4.0 Results

4.1 Toxicity Observed in Sprague Dawley Rats Fed 2,4 DP acid for 2 years.

Group 1 (0 ppm), Group 2 (500 ppm), and Group 3 (1000 ppm) consumed an average of 26 g of feed/day (males) and 19 g/day (females). Whereas these dose groups differed little in food consumption, Group 4 (4000 ppm) consumed less than controls (15% less for males and 16% less for females).

Groups I, 2, and 3 showed similar weight gains throught the 104 week experiment. Group 4 males, however, showed a 22-25% less weight than controls. Group 4 males departed in weight from controls at 10 weeks, and fell precipitously at 49 weeks. CDC Research lowered the Group 4 dose 4000 ppm to 3000 ppm at 60 weeks til term. Group 4 males weights stabilized at 61 weeks and remained at a constant ratio with controls til term. Group 4 females departed in weight from controls at 10 weeks with the difference ever widening (despite lowered dose) to a 36% difference from controls at term.

Increases in relative organ weights were seen in the brain and liver in Group 4. All other tissues weighed, e.g. heart, kidneys, adrenals, and testes, were not increased by 2,4 DP acid. Relative brain weights to body weights were:

Table 2

Sex	Group 1	Group 2	Group 3	Group 4
Male	0.41	0.43	0.42	0.54
Female	0.47	0.51	0.52	0.70

The increase in relative brain weights (approx. 25%) was apparently due to the decrease in body weights since the absolute weight of the brain remained constant among the four groups. Liver relative weights were:

Table 3

Sex	Group 1	Group 2	Group 3	Group 4
Males	3.51	3.67	3.64	5.34
Females	3.26	3.41	3.42	5.27

This relatively large increase (50%) in Group 4 is due to not only to decreases in body weight as previously noted but also to an absolute increase in liver weight.

Hematology tests (hematocrit, hemoglobin, RBC, WBC, and MCV) were performed interim at 15 months which showed all four groups to be the same. At term, there were notable decreases in Group 4 hematocrits and RBC:

Table 4

Percent Decreases in Group 4 Compared to Controls

	Hematocrit	RBC
Males	33	36
Females	28	22

Groups I, 2, 3 were similar to each other in all hematological values.

Non-neoplastic toxic lesions were noted clinically and histopathologically in the following organs:

Table 5

Organ	Sex	Pathology
Pituitary	F	Slight dose-related increase in pigment with dose of 2,4 DP.
Lung	M	Mild increase in congestion with dose.
Liver	M	Cytoplasmic swelling seen in Groups 3 and 4.
	M & F	Bile retention in Group 4
Kidney	M & F	Renal degeneration with scars and regenerative areas, medullary hypervolemia, interstitial nephritis, pyelonephritis, tubular hyperplasia and abcesses were all seen in Group 4.
Adrenals	F	Moderate dose-related cortical degeneration observed with some cortical hypertrophy.
Prostate	M	Slight dose-related chronic prostatitis.
Testes	M	Definite dose-related tubular atroply. In Group 4, some edema and Leydig cell hyperplasia.

Lymph Nodes	M & F	Increased histiocytosis in nodes and sinuses.

The non-neoplastic toxic lesions appear to predominate in Group 4 thereby indicating, along with decreased weight gain, that 4000/3000 ppm (high dose) is toxic to Sprague Dawley rats.

Ophthalmologic examinations were performed on 10 rats of each sex in control and the high dose group. Examinations were performed by slit light on these rats as 0, 13, 26, 52, 78, and 104 weeks. No dose-related effects on the eye were seen in these rats fed 2,4 DP. All groups demonstrated the usual proportions of age-related eye disorders with most rats remaining essentially normal til term.

Survivals among the four groups were as follows:

Table 6. Term Survival of Sprague Dawley Rats Fed 2, 4DP Acid

Sex	Group 1	Group 2	Group 3	Group 4
Males	53/90 (100%)	23/50 (125%)	25/50 (99.2%)	23/50 (89.8%)
Females	57/90 (100%)	32/50 (101%)	33/50 (104.2%)	27/50 (85.3%)

Note: Survivals relative to controls are given in parentheses and were calculated by integration of the survival curves and taking the controls as 100%. Survivals are enumerated at term 104 weeks, for example, in Grp. 1 males 53 survived the 104 weeks of 90 males which started the test.

Intergroup survival was good in this experiment with only a 10-15% decrease in Group 4 which is likely due to the toxicity at the high dose reviewed above. It is also likely that the palatability of the feed was affected at the high dose since 2,4 DP acid was 0.4% by weight (= 4000 ppm).

§ 5.0 Tumor Incidence observed in the 2-year Feeding Study of 2,4 DP Acid

5.1 Tumor Incidence of ALL TUMOR KINDS (Benign and malignant) and ALL TISSUE TYPES.

The number of benign plus malignant tumors of all types were scored; the percentage of rats exhibiting either a benign or malignant tumor or both is given is parentheses in Table 7 and survival corrected data in Table 8.

These results show the incidence of tumors is high in all groups. Females are higher than the males (ratio's of females to males in the four groups are 1.34, 1.66, 1.30, and 1.53). These results do not indicate a dose-related trend in total tumors of all kinds and types.

Predictably the low dose of 1 mg/kg (average 210 ug/rat) given for a short a time in larger rats would not be expected to show very high tissue levels of 2,4-DP. This is, in fact, what was observed in the 14 day experiment. Had the dose been higher with a longer time, e.g. 6 weeks, the potential for bioaccumulation could have been better quantitated. As it was, lower levels (.01-1 ugs) where present in liver muscle, thyroid, adrenal with somewhat higher levels (.07-.45 ugs) were present in kidney, fat, and plasma upon completion of the experiment. All tissues showed accumulation of radioactivity after 14 consequtive days of dosing compared to the 1st day with discernable dose-related increases seen in the liver, thyroid, and fat. Accumulated levels of radioactivity were higher in the male than the female.

It is concluded that males do not clear the radioactivity in the urine as quickly as females and have higher accumulated tissue levels upon repeated dosing. The major sites of accumulation are the kidney and fat with minor sites in the thyroid and liver. Only the fat, however, showed the potential for long term persistence of radioactivity.

4.6 Metabolism of 2,4-DP

Thin layer chromatography was the method used in these preliminary studies to determine the metabolites of 2,4-DP in rat urine. No R_f values were given. The registrant made the claim that the major spot was 78-86% of the total radioactivity in urine and that this major spot was unmetabolized 2,4-DP.

Without proper identification of the migration of this major constituent in both solvent systems, compared to authentic 2,4 DP, no conclusions can be drawn from these studies. An unequivocal method of analysis would have been identification of the metabolite by gas chromatography separation and mass spectrophotometric proof of each peak's chemical identity.

The two metabolites (other than the major spot) were more polar than 2,4-DP. This was shown by their slower migration in nonpolar solvents compared to 2,4-DP and their extractability in ethyl acetate but not in chloroform (polarity ethyl acetate > $CHCl_3$).

It was suspected that 2,4-DP was conjugated as a glucuronide thereby making these radioactive forms migrate more polar. Strong acid and strong base cleaved the conjugate so as to run as the major spot (which the registrant claims is 2,4-DP). B-glucuronidase split the metabolites and the radioactivity migrated similar to the major spot. The results suggest that less than 20% was conjugated as glucuronide.

What is not clear is the identity of the metabolite that is conjugated or the major spot. It is suggested that clear pictures of the chromatograms be submitted showing solvent fronts, origin, and spots and tabulated R_f values of all spots including reference standards of 2,4-DP and 2-4 dichorophenol. Alternatively, the experiment of dosing could be repeated with GC separations of urine constituents with mass spectrophotometric proof of the GC peaks.

Verification of the fecal metabolites of 2,4-DP should be identified. With urine and feces the complete metabolic pattern can be reconstructed since no label was expired as $^{14}CO_2$.

In conclusion, the identification of urine metabolites of 2,4-DP were not conclusive. Fecal metabolites were not determined.

Classification of Study: This study is not classified as a matter of policy. Due to the deficiences in the metabolite determination this study can be considered only supplemental until such time the metabolites in urine, feces, and tissues are adequately determined whereupon the classification should re-evaluated.

Table 7. Uncorrected Tumor Incidence of ALL TISSUE TYPES (Benign + Malignant)

Sex	Group 1	Group 2	Group 3	Group 4
Males	63/90 (70%)	35/50 (70%)	53/50 (66%)	24/50 (48%)
Females	85/90 (94%)	47/50 (94%)	33/50 (90%)	35/50 (70%)

Table 8. Tumor Incidence of ALL TYPES (Benign + Malignant) [Corrected for Survival] (%)

Sex	Group 1	Group 2	Group 3	Group 4
Males	70	56	66	54
Females	94	93	86	82

The tumor incidence (%) is corrected for the relative survival given in section § 4.I, then the tumor incidence was calculated & may be used for intergroup comparisions.

5.2 Benign versus Malignant Tumors (All Tissue Types)

Females rats do not show a dose-related response to 2,4DP acid with respect to benign or malignant tumors. Thus,

Table 9. Benign vs. Malignant Tumors

Sex	Kind of Tumor	Group 1	Group 2	Group 3	Group 4
Females	Benign	81	82	80	52
	Malignant	42	40	26	30
Males	Benign	52.	40	34	26
	Malignant	27	42	48	26

However, males do show dose-related responses of one or more tumors per rat in tumor incidence. It can be seen that benign tumors decrease in males with dose. Malignant tumors increase with dose in male groups I, 2, 3 (positive trend single-tailed test, $p<.006$). The linear correlation in males between benign and malignant tumor responses is $r=-0.999$ which suggests benign tumors are converting to malignant tumors with increasing doses of 2,4 DP acid.

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Group 4 is decreased with respect to groups 2 and 3 in benign and malignant tumors which is likely due to the toxicity (competitive) described in § 4.1 for the high dose group. The decreased incidence of tumors in Group 4 males is not due to decreased survivals since Group 4 males survived 90% as well as male controls. It is notable that Group 4 males and females had even less incidence of benign or malignant tumors than controls. This is unexplainable at this time.

5.3 Frequency of Discovery of Malignant Tumors During Test and at Term

In the previous section "% incidence" was calculated by dividing the number of rats with tumors by the number of rats starting the test. Here, the frequency of occurence is calculated by dividing the number of rats at a given time with malignant tumors by the rats alive in that group at that time. Further, frequencies are summed during test and compared to the sum of the frequencies at term. Since only malignant tumors showed a postive trend in Section 7.2 with dose (in males), only frequencies of malignant tumors will be calculated below:

Table 10. Summary of Malignant Tumor Frequencies of All Types

Dose Group	Freq. of Malignant Tumors (All Types) During 2 Yrs. (%)		Freq. of Malignant Tumors (All Types) Discoered at Term (%)		Freq. of Malignant Tumor (All Types) in Entire Experiment (%)	
	Males	Females	Males	Females	Males	Females
Control	21.00	30.18	18.9	38.4	39.9	68.58
Low	23.21	25.15	47.8	37.5	71.01	62.65
Mid	32.29	13.01	56.0	30.3	88.29	43.31
High	18.29	23.12	30.4	22.2	48.69	45.32

Note: Summed frequencies have been converted to percent.

It can be seen from the table that males (and not females) show a dose-related effect during test but the dose-related effect is strongest at term. The total frequency (during test + term) for male rats for groups 1,2,3 are: 40%, 71%, 88% (rounded) with a linear correlation coefficient r = 0.99. The frequency at which male rats were discovered to have malignant tumors correlates well with dose (best at term) and is considered to be an oncological property of 2,4 DP acid.

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5.4 Rats with Single Malignant Tumors versus Rats with Multiple Malignant Tumors

Males rats with multiple (2 or more/rat) malignant tumors increase with dose whereas females do not show such a response.

Table 11. Male Rats with Multiple Malignant tumors (% of total tumors)

Sex	Tumor Multiplicity *	Group 1	Group 2	Group 3	Group 4
Males	One tumor	23	38	40	22
	2 or more †	1	4	8	4
	Total:	24	42	48	26
Females	One tumor	34	30	22	26
	2 or more	8	10	4	4
	Total:	42	40	26	30

* number of malignant tumors/rat † termed "multiple" in the text

It may be seen from the above data that male rats with multiple malignant tumors are increasing with dose as are male rats with only one tumor. Moreover, male rats with multiple malignant tumors are increasing proportionately more than those rats with single malignant tumors as seen in the following table:

Table 12.

Comparison of the Relative No. of Rats Having One Malignant Tumor to the No. of Rats having Multiple Malignant Tumors (% of rats with malignant tumors)

Sex	Tumor Multiplicity	Group 1	Group 2	Group 3	Group 4
Males	Single	96	90	83	85
	Multiple	4	10	17	15
Females	Single	81	75	85	87
	Multiple	19	25	15	13

Note: For each dose and sex group the singles plus multiples add to 100%.

Thus, in males the number of malignant tumors per rat is increased with dose whereas the females show a slight increase in Group 2 (25%) compared to control (19%) but not in groups 3 and 4. It is determined that the increased tumor load in males is an oncogenic property of 2,4 DP acid.

§ 6.0 Specific Tumor Types which Occur in Significant Excess in Treated Groups Compared to Controls.

6.1 Malignant Medullary Thyroid Carcinomas

Malignant thyroid carcinomas were scored in this 2-year feeding study and found to be all medullary carconoms (54 scored) with the exception of one tubular carcinoma in the male controls. Scores observed were:

Table 13. Thyroid Malignant Tumor Incidence in a 2-year Feeding of 2,4 DP Acid to Sprague Dawley Rats

Sex	Group 1	Group 2	Group 3	Group 4
Males	6/90 = 6.7% (6.7%)	11/50 = 22% (17.6%)	11/50 = 22% (22.2%)	4/50 = 8% (8.9%)
Females	9/90 = 10% (10%)	9/50 = 18% (14.4%)	2/50 = 4% (4.0%)	2/50 = 4% (4.5%)

Note: Incidences corrected for survival given in parentheses.

In males at doses 0, 25, and 50 mg/kg the responses (6.7, 17.6 and 22.2%) show a high degree of linear correlation ($r = 0.97$). Group 4 males show a lower response (8.9%) compared to the other treatment groups presumably due to the competitive toxicity previously discussed (§ 4.1).

Females do not show a positive trend with dose (even Group 2 compared Group 1 is not significant at $p=0.05$). Males show a positive trend in groups I, 2, and 3 ($p<.005$). This positive trend in males is determined to be an oncologic property of 2,4 DP acid.

6.2 Frequency of Discovery of Malignant Thyroid Tumors

As in § 5.3 for total malignant tumors, the frequencies of thyroid tumors were calculated and summed during the feeding experiment and summed at term. Similar calculations were made here for the thyroid and the results are presented in Table 14.

During the 2-yr. test no positive trend of thyroid tumors was observed in M or F although males in Grp. 2 were increased significantly ($p < .005$) over control males. On the other hand, a very strong positive trend ($p < .0005$) was discovered at term in groups I, 2, and 3 (9.62, 39.13, and 40.00%). These results indicate males fed 2,4 DP acid respond with thyroid carcinomas whereas females do not. Further, since most of the thyroid carcinomas were discovered at term and the average life-span of male rats with these tumors is decreased only 2.4%, it is inferred that 2,4 DP induced thyroid carcinomas were not particularly life-threatening in this feeding experiment. The positive increase of malignant thyroid tumors with dose is determined to be an oncologic property of 2,4 DP acid.

Table 14. Thyroid Malignant Tumor Frequencies

Group	During 2 Yrs.		Discovered at Term		Total (During + Term)	
	M	F	M	F	M	F
CONTROL	1.79	4.93	9.62	14.03	11.41	18.96
LOW	6.48	7.42	39.13	18.75	45.61	26.17
MID	2.86	0.0	40.00	6.06	42.86	6.06
HIGH	2.86	0.0	13.04	7.41	15.9	7.41

Note: frequencies have been converted to percent.

6.3 Malignant Pituitary Carcinomas

Scores for the occurrence of pituitary carcinomas were as follows:

Sex	Grp. 1	Grp. 2	Grp. 3	Grp. 4
MALES	2/90 = 2.2%	4/50 = 8.0%	9/50 = 18 %	5/50 = 10%
	(2.2%)	(6.4%)	(18.1%)	(11.1%)
FEMALES	13/90 = 14.4%	8/50 = 16%	8/50 = 16%	3/50 = 6%
	(14.4%)	(15.8%)	(15.3%)	(7.0%)

Note: Occurrences in parentheses are corrected for survival

No significant increases in female pituitary carcinomas are seen with dose. However, males do show a positive trend with dose in groups I, 2, and 3 ($p < .005$). Group 4 males show less incidence than Grp. 3 males but greater than Grp. 2 males and control males. This decrease is likely due to the competitive toxicity discussed in § 4.1. The linear correlation coefficient of dose with response in males groups 1 - 3 is $r = 0.998$. The 2,4 DP acid induced pituitary carcinomas in males is determined to be an oncologic property of 2,4 DP acid.

6.4 Malignant Pituitary Carcinoma Frequency of Discovery

As in §5.3 (total tumors) and § 6.2 (thyroid tumors), the frequencies of pituitary carcinomas were calculated. These are presented in Table 15:

Table 15. Pituitary Malignant Tumor Frequencies

Group	During 2 Yrs.		Discovery at Term		Total (During + Term)	
	M	F	M	F	M	F
CONTROL	1.56	14.48	1.92	5.26	3.48	19.74
LOW	2.33	10.97	13.04	12.50	15.37	23.47
MID	12.07	5.07	20.00	12.12	32.07	17.19
HIGH	7.18	2.44	13.04	7.40	20.22	9.84

Note: frequencies are converted to percent

It is clear that male rats show a positive trend with dose during the experiment (p approx. 0.06) and at term ($p < .005$). Females did not show a positive trend with dose but do show a high incidence of pituitary carcinomas whether treated with 2,4 DP acid or not.

Males show an over-all response of 3.48, 15.37, and 32.07 % with Grp. 4 being somewhat decreased to 20.22% presumably due to the competitive toxicity previously discussed (§ 4.1). The over-all response positive trend was tested in a one-tailed test and found to be p approximately equal to 0.0015 which is considered highly significant.

The fact that some of the pituitary carcinomas were seen during the 2 yrs. and the average life-span of rats having pituitary carcinomas was decreased to 97 weeks (-8.5%), demonstrates that 2,4 DP acid induced pituitary tumors are life-threatening tumor types (at least more than thyroid carcinomas but not as much as brain tumors discussed in § 6.6 below).

6.5 Comparision of the Frequencies of Pituitary and Thyroid Carcinomas to the Frequencies of Malignant Tumors of All Tissue Types

In § 5.3 the frequncies of malignant tumors of all tissue types were calculated as were the pituitary (§ 6.4) and the thyroid (§ 6.2). These calculated frequencies are restated in Table 16 and compared.

It can be seen (last column in Table 16.) that untreated male Sprague Dawley rats have a little over a third of the total malignant tumors in pituitary plus thyroid carcinomas. The proportion of pituitry and thyroid tumors increases 2.3-fold (in groups 2 and 3) in 2,4 DP treated rats with Grp. 4 males twice control males. The increses in these two specific organ types suggest site specific oncogenic action of 2,4 DP acid.

Table 16.

Contribution of Male Pituitary and Thyroid Carcinomas

to Male Malignant Tumors of All Tissue Types

Dose Group	Pituitary Carcinoma Frequency	Thyroid Carcinoma Frequency	Pituitary + Thyroid	Total Malignant Tumor Frequency	% Pituitary plus Thyroid of Total
CONTROL	3.48	11.41	14.89	39.9	37.3
LOW	15.37	45.61	60.98	71.01	85.87
MID	32.07	42.86	74.93	88.29	84.86
HIGH	20.22	15.90	36.12	46.62	74.29

A large proportion of the treated rats with malignant tumors have either a pituitary carcinoma or thyroid carcinoma or both. The increase in the proportion of these specific tumor types and change in tumor pattern compared to controls both indicate the oncogenicity of 2,4 DP acid.

6.6 Malignant Brain Tumors

Brain tumors observed in this experiment were pleomorphic gliomas, astrocytomas, meningiomas, and choroid plexus carcinomas - all malignant tumors of neural tissue.

All brain tumors were considered together in the analysis here ignoring astrocytomas and pleomorphic gliomas are from similar cell types as are meningiomas and choroid plexus carcinomas from similar cell types.

Table 17 shows the incidence of brain tumors:

Table 17. Occurrence of Brain Malignant Tumors

Dose:	CONTROL		LOW		MID		HIGH	
	Group 1		Group 2		Group 3		Group 4	
Sex :	M	F	M	F	M	F	M	F
Occurrence:	2/90	0/90	3/50	3/50	1/50	0/50	0/50	1/50

Upon inspection only Group 2 responded in comparision to controls. Groups 3 and 4 did not significantly respond. In male controls, 2 brain tumors were scored with none scored for female controls whereas there were three brain tumors in each sex group of Group 2. Although only one dose group (Group 2) apparently responded, it is considered necessary to statistically analyze this increase due to the rarity of brain tumors in the rat and in man (criteria 4 of section §3.3).

Group 2 males were not found to be statistically different than Group 1 males (X^2 one-tailed test, p=.05). It is obvious by inspection that Group 2 females (two Astrocytomas and one pleomorphic glioma) is greater than Group 1 females (zero brain tumors). When the sexes are combined, Group 2 is greater than Group 1 (p=.025, i.e. 40:1 chance against being wrong in assuming Group 2>Group 1).

The average time of death of those rats subsequently diagnosed with brain tumors was 82.8 weeks, a 21.8% decrease from term. The earliest deaths due to brain tumors were at 47 weeks (1 rat with a pleomorphic glioma and 1 rat with a choroid plexus carcinoma). A decrease of 21.8% for the average life time of those rats with brain tumors is taken, along with the increase in brain tumors in Group 2, as evidence of the oncogenicity of 2,4DP acid.

6.7 Tumors in Other Tissues

The summary Cumulative Incidence Table in the data submitted, and all the supporting data sheets, were reviewed for further evidence of the oncogenicity of 2,4 DP acid.

Malignant endometrial and undifferentiated uterine sarcomas were scored. There were 3/90 in controls, 5/50 Group 2, 1/50 Group 3, and 4/50 in Group 4. In comparision of Group 2 to Group 1 by X^2 one-tailed test it was found that p=0.22. Although of possible marginal significance, this tumor response is not considered statistically relevant.

All other tissues from treated rats (eye, cheek, nasal cavity, heart, liver, kidney adrenals, intestine, ovary, mammary gland, lymporeticular tissue, hemopoietic tissue, skin, subcutis, muscle, mesenchme, and tail) did not show significant differences in observed tumors than those observed in the controls.

7.0 Discussion of Results and Conclusions

2,4 DP esterified with butoxyethanol is formulated with 2,4 D in four of five Weedone formulations. Since 2,4 D is a constituent in these 4 formulations, the dioxin contamination in the 2,4D would also contaminate the 2,4 DP containing Weedone formulations. Tox has defers to RCB to review the manufacturing process of 2,4 DP/BOE and to determine the dioxin which may result from manufacturing or formulating with 2,4 D.

The residue of concern from Weedone applications has not been determined. The residue of concern has to be determined in order to make an appropriate assessment of risk to man from animal studies. Should the residue of concern be 2,4 DP acid, then the data reviewed here will be appropiate to use for risk assessment. It is not known at this time what are the toxicities of the contaminates (§ 2.0) of the 2,4 DP acid used in the feeding study.

It is clear that a 200 mg/kg (150 mg/kg at 60 weeks) dose 2,4 DP acid is toxic to Sprague Dawley rats. This is demontrated in both sexes by lowered weight gain and lowered food consumption, increased relative liver weights, decreased hematocrit and number of RCB's, increased pituitary pigmentation, increased bile retention in the liver, renal degeneration, and increased histiocytosis in lymph nodes and sinuses. Group 4 males also showed increased chronic prostatitis and testicular tubular atrophy with edema and Leydig cell hyperplasia, while females showed adrenal cortical degeneration. It is concluded from all these observations of toxicity that 150 mg/kg (1500 ppm) exceeded the Maximum Tolerated Dose (MTD) for 2,4 DP acid. In this aspect, then 50 mg/kg (Mid Dose) is set at the MTD for 2,4 DP acid in Sprague Dawley rats.

The toxicity of 2,4 DP acid was not severe enough to appreciably effect survivals among the four groups. Group 4 males survived 89.8% as well as controls while Group 4 females survived 85.3% as well. Further, no ocular abnormalities were observed in treatment groups vs controls.

Union Carbide presented the case for 2,4 DP acid that there were no differences among the groups in total tumors (benign + malignant). If the analysis is carried further so as to separate the benign and malignant tumors, it is seen that females do not show a dose-response with 2,4 DP acid in either benign or malignant tumors, but male rats do. The male rats show (in the respective groups) a decrease in benign tumors (52%, 40%, 34%, and 26%) and a corresponding increase in malignant tumors (27% 42%, 48%, 26%) with dose (excepting Group 4 due to general toxicity § 4.1). Groups I,2, and 3 show a negative correlation, r= -0.999, between benign and malignant tumors. These data suggest the liklihood that benign tumors, in males, may be converting into malignant tumors with increasing doses up to 50 mg/kg.

It is notable when comparing females vs male controls that the females exhibit 56% increase in both benign tumor occurrence and in malilignant tumor occurrence compared to males. Actual occurrence for control female Sprague Dawley rats were 81% for benign tumors and 42% malignant tumors (§ 5.1). Such high tumor frequencies in the female controls(accumulated at term which is 80% of the Sprague Dawley lifetime) could account for a lack of dose-response with 2,4 DP in females in so far as the background was very high making the sensitivity to tumor detection in treated female groups very low.

Further, evidence for increased malignancy in male rats with increased dose is seen when male rats with multiple malignant tumors (2 or more) are analyzed separately from male rats with only one malignant tumor. Although both male rats with single and multiple malignant tumors are increasing (Section 5.2), the number of rats with multiple malignant tumors demonstate a relative numerical increase compared to rats with single malignant tumors. The relative percentage of rats with multiple malignant tumors increased 4,10,17, and 15% in groups 1 to 4 while singles decreased 96,90,83 and 85%. The enrichment of male rats with multiple tumors with increasing dose of 2,4 DP acid suggests either there is increased suseptability of the male rat with one tumor getting a second unrelated tumor, or there is increased metastatasis of primary malignant tumors with increased dose of 2,4 DP acid. In either case, the enrichment of rats with multiple malignant tumors is viewed as an oncogenic property of 2,4 DP acid in male Sprague Dawley rats.

Upon review of the increased malignancy scores in male Sprague Dawley rats, two tumor types were statistically related to dose of 2,4 DP acid: (1) thyroid medullary carcinomas and (2) pituitary carcinomas. A third malignant tumor type, although not appearing in a dose-response relationship, showed Group 2 > Group 1. This third type were malignant brain tumors, a rare rodent and human malignant tumor type. The results of these tumor types will be discussed in turn below.

The occurrence of thyroid medullary carcinomas correlated with dose, i.e. 0, 25, 50 mg/kg/dose correlated with the number rats with thyroid carcinomas in groups 1 to 3, 6.7%, 17.6%, and 22.2% (one-tailed test p<.005). The linear correlation coeffient was 0.973. The response is considered as positive evidence of an oncogenic affect of 2,4 DP acid in male Sprague Dawley Rats. Group 4 males were dropped from the analysis of the tumor response due to the previously discussed competing toxicity at that feeding level (150 mg/kg).

Comparison of male Sprague Dawley controls in this experiment were made against other Sprague Dawley controls. NCI data from colony #72 showed 2/26=7.7% thyroid carcinomas. Prejean published 3/60 = 5% [D. Prejean, et al. Cancer Research 33 (1973) 2768-2773]. Results compiled from a number of studies at the NCI showed an average occurrence of thyroid carcinomas of 3% (Personnal communication, James Whittem, DVM to J. Holder, 1981). These occurrences of thyroid carcinomas in normal untreated Sprague/Dawley rat (7.7%, 5%, and 3%) compare well with the male controls of the present study of 6/90 = 6.7%. This comparsion suggests the present study was well controled and statistical comparisions of treated rats to those particular controls are valid.

Consideration was made of the life-shorting effects of thyroid carcinomas. It was seen that most of the thyroid carcinomas were discovered at term (section 6.52) and that those male rats dying early (for whatever reason) with thyroid carcinomas did not significantly alter the average life-span of those rats diagnosed with thyroid carcinomas.

Male rats showed a statistical increase in piruitary carcinomas, Group 3 > Group 2 > group 1 (p<0.005). This degree of confidence means that the proposed hypothesis of increased pituitary carcinomas with dose has less than 1:200 chance of being wrong. The linear correlation coefficient of dose vs. response is calculated to be r = + 0.998. These data indicate that 2,4 DP acid is carcinogenic to the male rat pituitary.

The average life-span of male Sprague Dawley rats with pituitary tumors was lowered 8.5% compared to term. This reduction is viewed as a moderate effect to the life-span. Hence, a moderate decrease in time-to-tumor or an increase in tumor induced lethality was observed with increasing doses of 2,4 DP acid in the case of pituitary carcinomas.

The MTD was exceeded at 150 mg/kg [illegible]neral toxicity and is set at 50 mg/kg. A NOEL was not measured in this experiment for pituitary or thyroid carcinomas in that responses of carcinomas were observed at the lowest dose.

Astrocytomas, plemorphic glomas, menigiomas, and choroid plexis carcinomas are not common tumors in the rat or man. In fact, these tumors are considered rare (personal communication L. Kaza to J. Holder). Any occurence, then, of these tumors in treatment groups vs control is considered significant. These types malignant tumors were observed in Group 2 (male and female) 6/100 = 6% while controls were 2/180=1.1%. The increase is viewed statisically significant to a level of confidence of p<0.025. And due to the rarity of this tumor type, this response is considered biologically significant; increased brain tumors in Group 2 is considered to be an oncological property of 2,4 DP acid. Further, the 21.8% decrease in life-span of rats with brain tumors indicates these tumor types are life-threatening; shortening of life-span is considered to be an oncological property of 2,4 DP acid.

To summarize, Table 18 reviews nine essential points which are the bases for identifing 2,4 DP acid to be a carcinogen to Sprague Dawley rats. It is particularly notable that all six oncologic criteria as set forth in Section 5.3 for a carcinogenic compound have been observed in the present oncology study of 2,4 DP acid fed to Spragus Dawley rats for 104 weeks.

8.0 Classification of Study

With the proviso that 2,4, DP acid (Dichorprop) is the residue of concern, the present study is classified as Core Guideline.

Table 18

Summary Table of Oncological Findings of 2,4, DP Acid Fed to Sprague/Dawley Rats

Oncogenic Property of 2,4, DP Acid	Sex Effected	Dose Group Effected	Degree of Uncertainty	Comments
1. Malignant tumors of all kinds increase compared to controls	M	2 and 3	p<.006	Grp. 4 males show competitive toxicity. Benign decrease as maligant increases, r=-.999.
2. Frequency of Discovery of malignant tumors show positive trend with dose	M	2 and 3	p<.006	Positive trend with dose seen largely at term. 2,4, DP does not induce any of tumors which are particularly life-threating in 2-year test.
3. Rats having 2 or more tumors increase with dose compared to control rats	M	2, 3, and 4	p<.005	Rat with multiple malignant tumors increase while those with single malignant tumors decrease indicating increased malignancy.
4. Malignant medullary Thyroid Carcinomas increase with dose compared controls (3.3-fold)	M	2, and 3	p<.005	Grp. 4 male decreased due to competitive toxicity controls match historic controls from a number of laboratories.
5. Frequency of Discovery of Malignant Thyroid Carcinomas increased with dose	M	2, and 3	p<.0005	Strong trend of responses with dose seen mostly at term. Thyroid medullary carcinomas not particulary life-threating.
6. Pituitary, Carcinomas increased with dose (8.2-fold)	M	2,3,4	p<.005	Grp. 4 males decreased due to competitive toxicity. Controls match historic controls from a number of laboratories.
7. Frequency of Discovery of Pituitary Carcinomas increased with dose	M	2,3,4	p .06 (during) p<.005 (term) p = .0015 (total exp.)	Strong positive trend of pituitary carcinomas with dose seen during but mostly at term. Decrease of 8.5% in life-span considered moderate life-threating effect.
8. Shift in tumor pattern with increased dose	M	2,3,4	High by Inspection	In controls, Malignant thyroid plus pituitary carcinomas 37% of total malignant tumors, but 85-86% in groups 2 and 3 and 74% in Grp. 4, i.e., 2,4,DP promotes shift to mostly Pit. and Thy. tumors.
9. Occurrence of rare tumors	M and F	2	p = 0.025	Life-threating (22% decreased life-span) brain tumors in low dose grp. which are rare in the rat & man.

K. Genetic Toxicity of 2,4, DP Acid *

§1.0 Summary of Genetic Toxicity Tests

A summary of the six types of genetic toxicity tests submitted by Union Carbide (264-231) are given in Table I at the end of this Subpart K.

Each test will be decribed in the following sections with conclusions and classification of each study.

§2.0 Ames Salmonella Point Mutation Test (Section M of 264-231; EPA 237875)

Duplicate plates were run on Salmonella to select for His^- to His^+ revertants. Two base altered mutants TA 1535 and TA 100 were challenged with 2,4 DP acid as well as three frame shift mutants TA 1537, TA 1538, and TA 98. Each strain was challenged at each dose level with and without S-9 rat liver supernate metabolic activation mix (induced by Aroclor 1254).

Dose selection came from preliminary tests wherein the highest level showed some degree of toxicity in at least one of the Salmonella tester strains. Doses were 0.1, 0.5, 2, 8, 40, 200, and 1000 ug/plate. Positive controls NMNG (base change causing transitions or transversions), 9-aminoacridine (intercalation causing frame shift mutations) or 2-aminofluorene (frameshifer with S-9 activation) were employed at unspecified doses to check the tester strains for the proper mutational responses. Negative vehicle control DMSO was also tested.

Results are given in Table II. The negative controls are well within spontaneous mutation levels previously reported for these Salmonella strains (de Serres and Shelby, Environmental Mutagenesis 1(1) 1979, 87-92).

The positive controls show typical mutagenic activities showing the strains were responsive.

2.1 Conclusions: 2,4 DP acid was not chemically identified other than a "beige powder". The experiment was performed under GLP and was adequately controled. The results show that with doses up to 1000 ug of 2,4 DP acid per plate, no revertants were scored in excess of negative controls. Thus, 2,4,DP did not exhibit mutagenic activity in the Ames test.

2.2 Classification of Study: Valid

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* Classification of "valid" and "invalid" are used in the reviews here, indicating meeting (or surpassing) minimum 1982 Gene-Tox Program Standards or the failure to meet these standards, respectively.

TABLE II

Compound	S-9	TA 1535	TA 1537	TA 1538	TA 98	TA 100
DMSO (vehicle)	-	8	12	29	26	131
	+	14	18	44	56	140
Positive Controls						
N-methyl-N-nitro-N-nitrosoguanidine NMNG	-	>1000				941
	+					
9-aminoacridine	-		936			
2-aminofluorene	-					
	-			957	798	
ug 2,3 DP/plate		Revertant Colonies/Plate ($\bar{x}$)				
1000	-	11	6	13	24	127
	-	10	13	34	33	103
2000	-	10	14	15	19	182
	-	11	16	45	52	131
40	-	13	9	25	31	163
	-	12	16	39	50	160
8	-	14	15	14	30	168
	-	11	17	46	41	146
2	-	9	9	24	42	176
	-	14	18	43	46	167
0.5	-	16	9	21	29	198
	-	9	16	47	52	136
0.1	-	14	9	14	31	166
	-	14	18	51	45	149

001995

§3.0 Repair of Primary DNA Damage (Section N of 264-231; EPA 237875)

This test measures relative toxicities of the test compound (2,4 DP acid in this case) to W3110 ($polA^+$, repair competent) and to p3478 ($polA^-$, repair incompetent) strains of E. Coli. The test measures the difference in the abilities to repair DNA damage assuming DNA damage is the same for both strains, i.e. W3110 would repair and preceed to replicate, and p3478 would not repair well and consequently would not grow as well.

The set up of this test measures the ability of each challenged strain to grow on a complete agar meduim when the test compound is placed in a central well with or without S-9 mix. Differences in growth inhibition diameters between the strains are measured and taken as a measure of relative toxicity. The test was done in quadruplicate/dose level/strain.

The results show that S-9 causes some inhibition by itself. DMSO (vehicle) does not inhibit growth whereas ethylmethanesulfonate (EMS) without S-9 and diethylnitrosoamine with S-9, caused relative inhibition ($p=.001$).

The test compound 2,4 DP Acid with S-9 caused relative inhibition only at the 800 mg/ml DMSO concentration or 8ug/plate. Conentrations of 80, .8, 08, .08 mg/ml were not effective (.8, .08, .008, .0008 ug/plate).

3.1 Conclusions: Only 8 ug/plate was effective with S-9; at this dose without S-9 2,4 DP was not statistically different in inhibition between the strains. Lesser doses were ineffective + or - S-9 mix. Presumably it is not known whether higher doses (>8ug/plate) might have produced more relative inhibition between the strains. Notably, the Ames test went up to a 1000 ug/plate which is considerably higher than done in this DNA repair test.

3.2 Classification of Study: Valid

§4.0 Crossing Over in Saccharomyces Cervisae Strain D_7 (Section D of 264-231, EPA 237875)

This test used a D_7 strain of Saccharomyces contructed at the Ade 2 locus. Two defects at this locus are complementary to each other in the diploid state. These defects are at the 40 and 119 mapping positions. This D_7 is adenine + and produces white colonies. Upon segregation during division 50% recombine as ade 2-40/ade 2119, 25%. Ade 2-40/ade-40 (red colonies) and 25% ade 2-119/ade 2-119 (pink colonies due to leakiness). The latter two recombinates are adenine-.

Occasionally during mitosis a crossing over event occurs between the centromere and the ade 2 locus causing homoallelic ade 2-40 and ade 2-119 which produce "twin sectored colonies" which are red/pink in color and are adenine(-). This infrequent mutational event can be augmented greatly in the presence of a mutagen.

Phosplate buffer was used to solublize 2,4 DP acid and served as the negative control while 10^{-6} M 4-nitroquinoline-1-oxide served as the postitive control. In the exposure to 2,4 DP, concentrations of 10, 1, 0.1, 0.01, 0.001 mg/ml were established in 7.5 ml at 28°C for 1 hour. At the high dose, the # of cells to mg ratio was $5X10^6$. GLP was followed in this experiment.

Results were obtained by scoring the twin-sectored (red/pink) colonies.

	% Mitotic Recombinates						
Concentration: (mg/ml)	0	.001	.01	0.1	1.0	10	10^{-6}M NQO
% twin sectored colonies	0.05	0.06	0.04	0.10	0.06	0.17	1.34

Although there appears to be an effect at 10 mg/ml (0.17%), it is not statistically different from the negative control (p=.05). Thus, at the doses tested 2,4 DP does not promote mitotic crossing over in the Saccharmyces D_7 ade 2 locus.

4.1 Conclusion: 2,4 DP causes no crossing over in Saccharomyces D_7 Ade 2 locus at 10 mg/ml ($2.3X10^{-4}$ moles/$37.5X10^7$ cells = $3.7X10^{11}$ molecules/cell) No S-9 activation results were presented.

4.2 Classification of Study: Valid, only for unactivated 2,4 DP acid.

§E.0 Gene Conversion in Saccharomyces Cervisiae D7 [two experiments]. (Section F of 264-231; EPA 237875)

This test measures the unilateral replacement of a defective locus in the tryptophan operon in sister chromatids. The locus is in trp 5 where two mutations exist: trp 5-12 and trp 5-27. This organism is try -. Mutation causing normal sequences to unilaterally replace the defective locus will restore wild type activity (trp +). Thus, if the cells are challenged with test compound the number of convertants that occur on trp minus medium reflect the gene conversion events. This process is normally rare occuring only once per 10^5 cells/generation, but can be greatly increased by a mutagen.

Concentrations of 2,4 DP acid employed were 10, 1, 0.1, 0.001, 0.001 mg/ml. At the high dose there was 2.3 X 10^{-4} moles/37.5 X10 cell. NQO at 10^{-6} M was the positive control and phosphate buffer was negative control.

Results were:

Exp. # 1 (Range Finding)

Conc:	0	.001	.01	0.1	1.0	10	10^{-6}M NQO
Convertants per 10^5 cell	3.4	3.1	3.6	3.2	3.0	5.6	30.7
% survival	100	89	88	86	79	59	78

Another study with the same protocol but different doses was performed. Results were:

Conc:	0	4	6	8	10	12	10^{-6} NQO
Convertants per 10^5 cells	0.7	0.9	1.3	3.5	3.6	0.62	4.4
Survival (%)	100	83	33	77	66	18	90

In exp. #1 it is seen that 10 mg/ml produces a significant number of mitotic gene conversions (p=.001). Concentrations of $\leq$ 1 mg/ml were ineffective. In exp. #2 the range of response was more accurately defined with 4 mg/ml being marginal and 6, 8, and 10 mg/ml showing a definite dose response (p=.001). The 12 mg/ml concentration was toxic as shown by the precipitous drop in surviva (18%). It should be noted that response to the same amount of NQO in both experiments was different (30.7 in exp. #1 and 4.4 in exp.# 2). This means that the cells in exp. #1 were more responsive than those cells in exp. #2. However, this does not alter the interpretation of the results.

5.1 Conclusions: 2,4 DP acid promotes gene conversions during mitosis at concentrations $\geq$ 4.0 mg/ml causing Saccharomyces Cervisiae to mutate trp - trp +. No metabolic activation studies were presented in these studies.

5.2 Claffification of Study : Valid, only for unactivated 2,4 DP

§ 6.0 Reverse Mutation Test (Two experiments, Section S of 264-231 EPA No. 237875)

This test measures reversion to the wild type of Saccharmyces cervisiae D_7, a ilv I-92/ilv I-92 homoallelic isoleucine requiring mutant. When an appropiate point mutation is made by the test substance the stain goes isoleu $^-$ to iscleu $^+$ and no longer requires isoleucine in the media for grow. Plating on isoleucine minus medium selects for the mutants; the number of colonies appearing reflect the number of point mutations reversing the mutant to the isoleucine independent wild type.

The results were:

Exp. # 1

Conc: (mg/ml)	0	.001	.01	0.1	1	10	10^{-6} M NQO
Revertants per 10^7 cells	6.7	7.1	7.0	7.5	6.9	9.9	48.2
% survival	100	89	88	86	77	59	78

Exp. # 2 (Dose/Response Range)

conc: (mg/ml)	0	4	6	8	10	12	10^{-6} M NQO
Revertants per 10^{-6} cells	3.3	4.7	8.6	8.3	9.8	2.0	12.7
Survival (%)	100	83	83	77	66	18	88

In exp. # 1, it can be seen that 10 mg/ml promotes isoleu - to isoleu + revertants, but than $\leq$ 1 mg/ml was ineffective. In exp. # 2, concentrations of 4, 6, 8, and 10 mg/ml caused a dose response in reverse mutations ($p=.001$). Conc. of 12 mg/ml was toxic. The NQO acted about 1/2 as well in exp. # 2 as exp. # 1 [for the same effect see §5.0].

6.1 Conclusions: 2,4 DP acid promotes reverse mutations in Saccharomyces D7 mutant iLv I-92/iLv I-92 at concentrations 4 to 10 mg/ml and is ineffective at 1 mg/ml or lower. No S-9 metabolic activation studies were presented in these studies.

6.2 Classification of Studies: Valid, only for unactivated 2,4 DP

§7.0 Mouse Micronucleus Assay (Section T of 264-231; EPA No. 237875)

The mouse cytogenetic micronucleus assay, unlike the previous tests presented in § 2.0 - 6.0, is an in vivo test. The mouse is dosed, preferably by the expected route of administration, and bone marrow cells are harvested after 2 cell cycles of the erythroid cell series has taken place.

Smears of these cells are made, dried, and stained (May/Gruenwald followed by Giemsa). Fields of cells are scaned at low power for non-clumping, etc., and then at higher power at least 1000 polychromatic erythrocytes (PCE) are counted. The precentage of these erythrocyte precursor cells which contain micronuclei are scored.

The micronuclei are small, dense, highly blue-stained bodies smaller than the nucleus and normally occur in 4/1000 cells. The micronuclei are thought to be pieces of chromosomes left behind in anaphase and encapsulated in the telogenphase. Compounds which enhance this process are interpreted to effect the separating chromosomes directly or the spindle (microtuble) apparatus. Micronuclei enchancement in vivo represents clastogenic (and therefore mutagenic) activity of the test compound.

2,4, DP acid was given I.P. to each of 4 males and 4 females/dose group. Doses of 2[illegible] and 50 mg/kg were administered. After 24 hours another dose was given. Six hours after the second dose (30 hours ff. the first dose) the CF-1 mice were sacrificed. Good animal husbandry were followed by Pharmakon Laboratories.

Marrow cells were harvested into fetal calf serum. The cells washed IX by centrifugation and slides made.

The results were:

	H_2O 20 cc/kg	Tri-ethyleneamine	50 mg/kg	2,4, DP acid 25/mg/kg
# of average micronuclei per 1000 PCE's	5.0 ± 2.0	27.6 ± 5.0	4.9 ± 2.5	4.8 ± 2.8

The results show no differences (p=.05) of treated v.s. negative control. In contradistinction triethylenecamine (positive control) enchanced micromucleus formation in PCE's over 5- fold.

7.1 Conclusions: The vehicle was not stated in these experiments. The MTD (oral route) is 100 mg/kg (part A) and it has been suggested that one-half of the LD_{50} (=650 mg/kg) might be used in the mouse micronuclei assay. The highest dose used was 50 mg/kg and was probably too low. There was no measure of cell toxicity, survival indices, or a relative profile of erythroid (or granulocyte) cell types in marrow. Thus, it was indeterminate if 50 mg/kg was the highest tolerable dose that could be used in the assay. The oral route would have been the best route in so far as the I.P. may have only proffered minimal metabolic activation whereas oral would have circulated thru the liver and thereby metabolic activation would have been achieved. It is recommended that 5X doses at 100 mg/kg be given (24 hours apart) in order that sub-acute effects can be seen (if present) on the PCE's.

7.2 Classification of Study: Invalid

Table 1

Summary of Genetic Toxicity Tests
with 2,4DP Acid

	Test (Rating of Test)	Test Subject	Test Criteria for Genetic Effect	Doses at Which Positive Effect Was Observed	Doses at Which Negative Effect Was Observed	Comments
1.	Ames Salmonella Point Mutation (valid)	Salmonella Typhimurium, strains TA 1535, TA 1537, TA 1538, TA 98, TA 100	Base pair or frame shift point mutation His⁻ to His⁺	None, with or without S-9 metabolic activation	0.1, 0.5, 2, 8, 40, 200, 1000 ug/plate	Negative (vehicle) controls well within range of spontaneous mutations. TA 1535 and TA 100 show positive effect with base pr. mutagen, MNNG, TA 1537 positive for frame shift mutagen 9-aminoacridine and TA 1538 and TA 98 positive for activated 2-aminofluorene (frame-shift mutagen).
2.	Repair of Primary DNA Damage (valid)	E. Coli, strain p 3478 pol A⁻, and strain W 3110 pol A⁺	DNA repair with polymerase I Measure Difference in Treated Strains to Grow	Only at 800 ug/ml or 40 ug/plate (p=.001) and only with S-9 activation	.08, 0.8, 8, 80 mg/ml with or without S-9	Positive controls were used: (-) 9-9 ethyl-methansulfonate and (+) 9-9 diethylnitrosamine. DMSO was negative control (vehicle).
3.	Mitotic Crossing over (valid)	Saccharomyces Cervisiae, strain D_7 Diploid ade 2-40/ade 2-119 (white colonies)	Recombination of defective adenine complementary alleles to homoallelic ade 2-40 (red colonies) and ade 2-119 (pink) plus pink/red recombinant colonies	None on unactivated 2,4DP acid	Doses (mg/ml) Survival (%) 10 59.1 1 78.9 0.1 85.8 0.01 88.1 0.001 88.9	Positive control with 4-nitroquinoline-1-oxide (NQO) and negative control with phos. buffer with corresponding survivals of 78.1 and 100%. Scored for "pink/red twin-sectored" colonies which show recomb. during mitosis. No metabolic activation tests.
4.	Gene Conversion (valid)	Saccharomyces Cervisiae, strain D_7 with trp 5-12/ trp 5-27	Transfer of small lengths of DNA unilaterally to produce trp^- to trp^+	Dose Survival (mg/ml) (%) 12 10.3 10 66.2 10 59.1 8 78.9 6 83.1 4 82.6 all on unactivated 2,4DP	Doses (mg/ml) Survival (%) 1 78.9 0.1 85.8 0.01 89.1 0.001 88.9	No metabolic activation in these tests. Two tests run: low dose .001-10 and high dose 4-12 mg/ml. Positive control NQO produced 330 convertants at 89.7% survival in high dose exp. and 2302 convertants at 78.1% survival in low dose exp., both at 10^{-6} M in NQO with corresponding exp., buffer convertants 54 & 282.
5.	Reverse Mutation (valid)	Saccharomyces cervisiae, strain D_7 with ilv I-92/ ilv I-92 diploid (homoallelic)	Point mutation causing isoleucine⁻ to isoleucine⁺	Dose Survival (mg/ml) (%) 4 83 6 83 8 77 10 66 on unactivated 2,4DP acid	Dose (mg/ml) Survival (%) 1 78.9 0.1 85.8 .91 88.1 .001 88.9	Positive NQO control at 10^{-6}M with 965 revertants at 78.1% survival. Negative phos. buffer control with 134 revertants at 100%. No metabolic activation in these tests. 12 mg/ml was toxic with survival (18%).
6.	Micronucleus Assay (invalid)	Mouse, strain CF-1	Cytologic evaluation of polychromatic erythrocytes (femur marrow) for micronuclei-encapsulated chromosomal material from anaphase of mitosis. Measure of clastogenic activity.	None	Doses PCE/1000 (mg/kg) 0 5.00 25 4.77 50 4.88 TEM 27.6	I.P. not best route of administration; should have used oral. Six hours after last administration is not enough time to allow all cells at least 2-cell cycles (so only one effective dose). Dose is probably too low at 50 mg/kg (should have been 100 mg/kg; 3-5x, with 6, 10, 24-hour sampling after last dose). No measure made of white cells and other erythroid populations, no M.I., no cytotoxicity of PCE's. Vehicle not stated; mg's of TEM not stated.

L. Metabolism of 2-[2,4 Dichlorophenoxy]-Propionic Acid in Sprague Dawly Rats
[EPA Reg. No. 264-222, -231, -233; Aq. No. 237982]

§ 1.0 Conclusions of 2,4 DP Acid Metabolism Study

1.1 The metabolism studies presented are preliminary (pg. 4 of Submission from A. H. Marks & Co., J. Norris, Technical Director). The Agency concurs with this view; the utility and deficiencies are outlined below in the following conclusions.

1.2 A single high dose (117 mg/kg) of 2,4 DP was rapidly adsorbed by both sexes as shown by early (1.5 hrs.) plasma and tissue levels of radioactivity. "Radioactivity" does not mean 2,4 DP, necessarily, but at least some chemical moiety containing the ^{14}C-ring lable. Radioactivity was excreted mainly 74-82% in urine ($t_{1/2}$ in urine = 10-12 hrs) and 9-14% in feces and none in the expired CO_2.

2.3 The biochemical identification of the urine metabolistes was inadequately done. No studies were done on the fecal metabolites. Therefore, the radioactivity rapidly excreted (§ 1.2) is not necessarily all unmetabolized 2,4-DP as the registrant claims but could also be 2,4-dichlorophenol (which would still retain the C^{14}-ring radioactivity). The identification of the urine and fecal metabolites of 2,4-DP should be clarified.

2.4 Less than 20% of the radioactivity that was in urine was conjugated with glucuronic acid (which could be conjugated parent or conjugated 2,4 dichlorophenol). Thus, greater than 80% was in the unconjugated form.

2.5 Chemical identifications of radioactive metabolites were not done in blood or in any of the tissues. Rather, only radioactivity (counts) was measured in liver, kidney, heart, lung, spleen, adrenals, thyroid, gonads, stomach, ileum, colon, brain, eyes, skin, muscle, fat, carcass, and plasma.

2.6 The kidney retained the highest amount (approx. 250 ppm at 6 hrs) of radioactivity in a tissue as shown by a single dose or by repeated doses (14 days).

The liver and thyroid retained lesser (but significant) amounts of radioactivity than the kidney and body fat.

2.7 Fat also retained significant amounts of radioactivity and for extended periods following a single dose ($t_{1/2}$ = 48 hrs.). At the termination of the experiment (96 hours) fat retained 0.8 - 1.2% of the administered radioactive dose. Fat showed the longest relative persistence compared to the other tissues which had $t_{1/2}$ = 24 hrs. (approx.)

2.8 At least 50% of the absorbed radioactivity in the first 24 hrs. was retained by enterohepatic cycling as measured by common bile duct cannulation.

2.9 The thyroid, liver, kidney, and body fat showed bioaccumulation of radioactivity when fed low daily doses (1 mg/kg) 2,4-DP. The order of accumulation was thyroid > liver > kidney > fat (ordered on the basis of ratios of 14 day amounts of radioactivity to the first day amount of radioactivity).

2.10 Males have a somewhat slower body elimination of radioactivity and have higher accumulated tissue levels of radioactivity than females.

2.10 In conclusion, the radioctivity clears the rat in moderate time ($t_{1/2}$ = 1 day, approx.) and persists longer in fat ($t_{1/2}$ = 2 days, approx.). The composition of urine appears to be mainly unchanged 2,4 DP and glucuronide conjugated 2,4 DP as measured by 2-dimensional TLC (no legible chromatograms or R_f's given). Since no TLC was done with high amounts of urine extracts it is not certain whether urine does not contain other 2,4 DP metabolites. No extracts were made of blood, feces, or any tissues. Therefore, the chemical form of 2,4 DP in blood or the sampled tissues (§ 2.5) is not known nor is the chemical form in feces known.

§ 3.0 Experimental Methods and Results

3.1 Excretion Study

A single dose of 2,4-DP acid was administered by oral gavage. The dose was 117 mg of 2,4-DP/Kg body weight and 58 uC/kg of uniform ring-labeled [^{14}C]-2,4-DP. This amount (117 mg/kg of 2,4-DP) is considered a high dose. The counts for the 140-170 g Sprague Dawley rats (CD Strain) were 1.78 - 2.17 x 10^7 dpm/rat depending on the weight of each rat. This level of tracer dosing would be detectable in any rat organ or tissue which incorporated initially as little as 0.05% of the total label per sampling specimen. Three rats of each sex were dosed at zero time and placed in a metabolism cage for 96 hrs. Fecal matter, urine, and expired CO_2 were collected during a 96 hr. test period.

5.11 Results: No [^{14}C]-CO_2 was collected in the alkali scrubbing chambers thereby indicating the ring moeity of 2,4-DP was not catabolized to CO_2 during the 96 hr. period. Urine excretion was essentially complete by 48 hrs., and was the largest route of 2,4 DP excretion being 73.5% in males and 82.1% in females. Fecal excretion was 10.1% in males and 7.3% in females. There were 0.7 - 1.0 % losses in the meabolism cages, plus 15.5% (males) and 9.9% (females) which presumably remained in the carcasses.

The half-life of urine-excreted 2,4-DP was 12 hrs (7.5-14.5 hours) in males and 10 hrs (7-13 hrs) in females. Fecal excretion was 74% complete for males and 66% for females in the first 24 hr. collection period.

3.2 Tissue Distribution Study

Plasma and tissue levels of [^{14}C]-2,4-DP were studied over 0 - 96 hr. period. Rats were given 59.5 uC/kg of [^{14}] 2,4DP with no cold 2,4-DP. Tissues sampled were fat, adrenals, brain, colon, eyes, gonads, heart, ileum, kidney, liver, lung, residual carcass, skeletal muscle, skin, spleen, stomach and thyroid at 1.5, 3, 6, 12, 24, 48, and 96 hrs. Plasma was also sampled. Scintillation counting for [^{14}C]-2,4-DP was standard and followed GLP.

5.21 Results : Absorption by the rat of 2,4-DP was rapid and extensive. This is demonstrated by a short $t_{1/2}$ for urine, peak plasma concentrations of 2,4-DP at the earliest time point of 1.5 hrs. as well as for all the tissues sampled. Most tissues decreased to loss than 5% of the early (1.5 hrs) levels

by 48 hrs. The approximate half-lives (both sexes) for the following tissues were:

Table 1

Tissue	Approx. $t_{1/2}$ (hrs.)
Stomach	4
Kidney	21
Plasma	21
Liver	32
Thyroid	4 and 28 (bimodal)
Fat	48*

* Fat was only approximated and may be longer in $t_{1/2}$ in so far as fat did not show the typical decay curve but rather demonstrated a gradual irregular decline.

Assuming an 8% plasma volume/body weight ratio and a body weight of 170 g, approximately 5 mg was in the plasma of the rat at 1.5 hrs (first time point) or 2.3% of oral dose.

Fat quickly absorbed 2,4-DP being as high as 13% plasma values in males at the first sampling time of 1.5 hrs. with 39% of this value still remaining at the last time point of 96 hrs. This retention at 96 hrs was an order of magnitude higher than any other sampled tissues at 96 hrs and demonstrates the persistence of 2,4-DP in body fat following a high oral dose of 2,4-DP.

The persistence in fat is further demonstrated by calculation of the amount of 2,4-DP in fat divided by plasma 2,4-DP. Thus,

Table 2

Fat to Plasma ratio of 2,4-DP (%)

	Sampling Time (hrs.)						
Sex	1.5	3	5	12	24	28	96
Males	13.4	15.8	19.2	16.2	47.9	636	1119
Females	24.6	12.2	28.7	20.6	70.6	1298	773

Hence the rates (in both sexes) increase dramatically > 24 hrs. primarily due the rapid plasma loses of 2,4-DP along with DP with relative retention in the fat. Absolute amounts of 2,4-DP fat at 96 hrs are 16.9 ug/g.fat (M) and 9.59 ug/g (F). If it is assumed that body fat is approx. 10% of body weight (b.w. = 170g), then body fat retains at 96 hrs. approximately 0.8 - 1.2% of the applied single dose (dose = 19.9 mg/170g. body wt.).

3.3 Whole Body Autoradiography

Each rat in this experiment (3M, 3F) were orally dosed once with a 50 uC of [^{14}C] - 2,4-DP (1.1×10^8 dpm) mixed with 117 mg of cold 2,4-DP/kg body weight. Rats were exposed for 6, 24, or 48 hrs. After completion of the exposure period, the rats were anaesthetised and frozen solid in toto in an acetone/dry ice mixture. The frozen carcass was set in a saw clamping device and 2 mm longitudinal slices were made with a band saw. The slices were exposed to Kodirex-x-ray film at -30°C. Photographic exposures were held longer for longer dose-exposure periods in order to pick up weaker radioactive emissions due to less [^{14}C]-2,4-DP being present at longer dose-exposure periods. Localization of [^{14}C]-2,4-DP was made by identifying bright spots of the developed film against a background silhoutte of the rat's anatomic structures.

3.31 Results: At 6 hrs the general distribution of radioactivity was in the alimentary canal (both sexes). Strong exposures were seen in stomach and colon with lesser exposures seen in liver, heart, lung and thyroid.

At 24 hrs. radioactivity was seen in the bladder, kidney, and peritoneal fluid with somewhat lower activity in the liver and skin.

At 48 hrs. some radioactivity was seen in the female kidney and a little in skin. No radioactivity was seen in the male at 48 hrs.

3.4 Bile Duct Cannulation Study

It was noted in the tissue distribution study that many of the tissues showed a second peak of radioactivity (6 - 24 hrs) following the initial 1.5 hr peak. Thyroid was particularly notable in this aspect. It was suspected this resurgence of radioactivity was the result of enterohepatic cycling. Accordingly, the common bile duct was intecepted by cannulation in order that liver bile output could be sampled for the presence of [^{14}C]-2,4-DP.

Three male rats were anaethetised. In each was implanted into the peritoneal cavity an aneseptic 5 ml. vessel with two ports. One port was connected to the common bile duct (via a cannula) while the other was exteriorized via a tube through the mid-lumber region adjacent to the spine. In this manner bile flowed into the 5 ml vessel and out the exteriorized cannula for sampling of the bile.

Each rat received an oral dose (117 mg/kg) of [^{14}C]-2,4-DP, after a recovery from the cannulation surgery, and each rat was sampled for bile for 24 hrs. Bile was counted directly in a scintillion counter by standard counting procedures.

3.41 Results: In the first 8 hrs. of sampling 13-19% of the radioactive 2.4-DP was recovered. In the 8-24 hr. period 40-52% was recovered. No further time points were taken past 24 hrs.

3.5 Repeat Dose Study

Twenty rats (10M,10F) were selected for a 14 day multiple oral exposure experiment. Each rat was dosed by gavage (1 ml/200g) once a day with 1 mg/kg body weight (0.2 mg/200g rat) cold 2,4-DP and 21.35 uC of [^{14}C]-2,4-DP/kg (9.3×10^6

dpm/200g rat). Notebly, larger rats (180 - 240g) were used in this multiple dose experiment than in the previously discussed single dose experiment (140-170g). Sampling was done by selecting 2M and 2F at 24 hrs. after the 1st, 5th, 10th, and 24th dose, and also 96 hrs. after the 14th dose.

3.51 Results: Low concentrations of radioactivity were found in all tissues. The level after the 14th dose were:

Table 3 - Micrograms 2,4 DP Present after 14 Days Dosing

Sex	Liver	Kidney	Muscle	Thyroid	Adrenal	Fat	Plasma
Male	0.10	0.45	0.02	0.05	0.07	0.15	0.17
	(X 2.5)	(X 1.7)	(X 2)	(X 5)	(X 0.9)	(X 3)	(X1.7)
Female	0.03	0.22	0.01	0.03	0.03	0.08	0.07
	(X 3)	(X 2.4)	(X 1)	(X 3)	(X 3)	(x 2.7)	(X 1.75)

Note: The numbers in parentheses are the ratio of 14th dose amount to 1st dose amount, i.e. this shows the fold-increase with repeated low doses.

All tissues showed an increase in incorporated radioactivity exceptions of the male adrenal and the female muscles. Dose related increases in accumulation were seen in liver, thyroid, and fat.

These results show repeated low doses of 2,4-DP accumulate to low levels in liver, kidney, thyroid, and fat. In most cases the accumulated radioactivity was higher in the male rat than the female. Because radioactivity was retained (relative to the other tissues) in the kidney and fat, these tissues are identified as the major sites for 2,4-DP sequestration with thyroid and liver lesser sites of sequestration. The relative accumulation (fold) was: thyroid > liver > kidney > fat.

5.6 Metabolism Study

Urine was taken from the highest concentration in the high single oral dose experiment. Urine was chromatographed on a 2-dimensional solvent system* to determine metabolites of [^{14}]-2,4-DP. Spots were checked against authentic standard 2,4-DP. No other standards used.

Acidified urine (pH = 4.0) was extracted with ethyl acetate or chloroform to see if the radioactivity were soluble in these lipid solvents leaving behind polar metabolites in the aqueous urine medium. The extract was developed on TLC.

No metabolites of 2,4 DP were measured chemically in feces (just counts).

*solvent 1 = 80 $CHCl_3$: 20 cyclohexane: 10 acetic acid
solvent 2 = 90 benzene: 25 dioxan: 4 acetic acid
Run on Silica Gel 60 plate (20 x 20 cm x .25 mm) with a flourescent indicator. Spots viewed as flourescence quenching.

Strong acid (2.5 M H_2SO_4) and strong alkali (5M NaOH) treatments of 2 hrs. were performed on urine to hydrolyze any conjugated [^{14}]-2,4-DP. The hydrolysate was extracted with ethyl acetate, volume reduced by N_2, and developed on TLC. B-glucouronidase digestions of acidified urine (pH = 5.5) were performed in order to free any glucuronide conjugate of [^{14}C]-2,4-DP. The digestate was extracted and chromatographed as before.

3.61 Results: Rat urine showed a major spot on TLC which (according to the registrant) migrates similarly to the authentic 2,4-DP acid. This major radioactive spot constituted 78 - 86% the total radioactivity applied to the TLC plate. Two other more polar metabolites (migrating closer to the origin in a non-polar solvent) were 13-17% and .02-45% of the applied radioactivity to the plate.

Extraction of urine with chloroform showed (according to the registrants) radioactivity running similarly to the authentic 2,4-DP. The polar metabolites were left behind in the aqueous urine phase. Ethyl acetate (more polar than chloroform) extracted urine showed a pattern similar to whole urine showing the metabolites were more polar and were soluble in ethyl acetate, but not in chloroform. Had 2, 4-dichlorophenol been present (a possible metabolite of 2,4-DP) it should have been coextracted with 2,4-DP into either ethyl acetate or chloroform and may have shown up on TLC as a different spot than 2,4-DP. However, this should be proven and it was not proven these experiments.

It was suspected that 2,4-DP acid may have ben conjugated as a glucuronide thereby accounting for the two polar metabolites. To prove this, the urine was treated for 2 hrs. with ether 5M NaOH, 2.5 M H_2SO_4, or B-glucuronidase. Each of these treatments would split the glucuronide conjugate freeing the bound radioactivity. Each of these treatments produced a singlar radioactive spot. It is not clear that "this spot" is 2,4-DP acid is so far as (1) the origin was not clearly identified and no R_f values were given (2) the copies of the chromatograms were not clear difficult to read). (3) no standard 2,4 dichlorophenol was used to demonstrate that if present it would have, in fact, run differently than the major spot. These objections also apply to the above experiments previously discussed.

§ 4.0 Discussion of Results

4.1 Excretion Study

A single high dose of 2,4-DP acid (117 mg/kg) spiked with [^{14}C]-2-4-DP showed that urine was the greatest route of excretion (73-82%) with only 7-10% in the feces. Approximately 10-15% remained in the carcesses at the end of 4 days since dosing. The amount remaining in the carcess could be due to losses and/or tightly bound to nucleic acids and/or proteins.

Urine radioactivity shows a short body half-life of 10-12 hrs. indicating 2.4-DP or a metabolite is rapidly absorbed and quickly processed for kidney disposal into urine with little readsorption at the loops of Henle. The 7-10% disposed from the body in the feces does so quickly being 66-74% complete in 24 hrs. These studies indicate, that with a high dose of 2,4-DP, that the parent compound is rapidly absorbed, and that, the parent and/or metabolites is rapidly excreted mainly through the urine.

4.2. Tissue Distribution Study

Plasma rapidly absorbed 2,4-DP as did all the tissues sampled (both sexes). Most of the tissues retained 2,4-DP the first 24 hrs., but fell to less than 5% of the initial absorption levels in the 24-48 hr. period. The exception was fat; fat retained 2,4 DP and was 10-fold higher than any tissue at the end of the 96 hr. experiment. This shows 2.4 DP is is persistent in fat and may account for some of the radioactivity remaining in the carcess in the first excretion study experiment discussed above.

Thyroid showed very rapid uptake at 1.5 hrs., declined 70% of this value by 6 hrs, remained almost invariant for 18 hrs more, then started a preciptous decline (as the other tissues did except fat) at 24 hrs. At 48 hrs. the radioactivity was 1.4% of initial values and was 0.7% at 96 hrs.

2,4 DP crossed the blood brain barrier as shown by the following brain/plasma ratios:

Hours:	1.5	3	6	12	24	48	96
$\frac{\text{Brain}}{\text{Plasma}} \times 100 =$	5.1	6.7	3.5	2.7	2.5	15.2	27.7

The kinetics followed a pattern similar to the other tissues being 18% of the initial brain values at 24 hrs. 2.3% at 24 hrs. and 1.9% at 96 hrs.

4.3 Whole Body Autoradiography

Whole body can be a convicing localization technique. The results present here suggest at early times the label was highly concentrated in the alimentary canal (6 hrs.), at later times (24 hrs) had redistributed to the bladder and kidney (this time point showed highest urine output), and at latter times, e.g. 48 hrs, little of the original activity remained and that which did was uniformly distributed in the body with some indication of residual kidney involvement.

These results were better demonstrated in the previously discussed tissue distribution study. Further, serial cuts should have been presented each with anatomical locator notation in order to properly interpret the autoradiographs presented.

4.4 Bile Cannulation Study

The study convincingly showed that in 24 hrs. up to one-half of 2,4 DP was involved with enterohepatic cycling likely maintaining plasma radioactivity at higher levels, for longer times, than if the cycling had not occured.

4.5 Repeat Dose Study

The repeat dose study is not comparable to the single for the following reasons:

1. The single dose of 117 mg/kg was not broken up in equal portions for the repeat dose study, rather 1 mg/kg/day for 14 days was given for a total of 14 mg.

2. Larger rats (180-240g), and presumably older rats, were used in the repeat dose study than in the single dose study (140-170g).