1-3-85 PP6-316 TXR-4179

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

C04178

JAN 3 1985

DEC 7 1984

MEMORANDUM

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

Casewell # 652C

SUBJECT: EPA Petition No. 3F2941 (fenoxycarb): Status of Data Review for Use on Non-agricultural Turf (including residential lawns), and for Use on Pasture and Range Grass. Review of Studies Submitted.

FROM: David G. Van Ormer
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7 Dec 84

TO: Adam Heyward, Team No. 17
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Hazard Evaluation Division (TS-769)

1/2/85

1/3/85

For the uses of fenoxycarb on turf (including residential sites) not intended for grazing, Toxicology Branch requires data as follows:

Acute oral toxicity, technical (T) and formulation (F)
Acute dermal toxicity (T and F)
Dermal irritation (F)
Acute inhalation (T and F)
Dermal sensitization (F)
21-Day dermal toxicity (T)
Teratogenicity (two species)
Mutagenicity battery
90-Day feeding (rodent and non-rodent)
Metabolism

Of the above studies required for the subject petition, all have been submitted (and are acceptable) except for the following:

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1. 21-Day dermal study on technical material.
2. Teratogenicity study in a non-rat species.
3. 90-Day feeding study in a non-rodent.
4. 90-Day rat feeding study showing no-observable effects in all parameters.

The 90-day rat feeding study submitted (Hoffmann-LaRoche study No. B-104 779, 9/5/83; see attached reviews and addenda) shows significant increases in liver weight at low dose.

Acute Dermal - Rat; Hoffmann-LaRoche (Basle) Report No. B-97 341, May 5, 1982.

The test material was technical fenoxycarb, 10.3% (w/w) in [redacted] (formulation ACR 5023). The test animals were Wistar rats (outbred, SPF) from Kleintierfarm Madderin AG. The males weighed 207-238 g and the females weighed 169-202 g. The animals were randomized into dose groups and caged individually under standard ambient conditions. Pelleted rat died and tap water were available ad libitum.

Dosage groups of five males and five females received dermal application of test material at 1000, 3000, and 5000 mg/kg, respectively. An application area (20 cm^2) had previously been shaved with electric clippers. Exposure was for 24 hours under occlusive bandage. The skin was subsequently cleaned with lukewarm water prior to examination.

The animals were observed daily for 14 days, and their behavior was checked against a list of 57 components of symptomatology, according to the report. Body weights were recorded on days 1, 7, and 14. Animals killed were subjected to gross necropsy.

Results:

Animals in all dose groups exhibited dyspnea, curved body position, and ruffled fur. The high-dose animals (5000 mg/kg) also presented sedation and diarrhea. There were no deaths.

The Report tabulates no organ changes related to treatment. Body weight changes are not remarkable.

LD50 (dermal, rats) = > 5000 mg/kg

Tox Category: III (Guideline Data)

Reclassification of Inhalation Studies

The acute inhalation studies (rat) on the technical fenoxycarb as a dust and an aerosol (Huntington Research Centre) and on the formulation ACR 5023 [redacted] Research and Consulting Company, 3/16/82) have both been classified as Supplementary Data on the basis that the rats were not exposed to a sufficiently high concentration of dispersed material. For the studies on the technical material the concentrations obtained were reportedly the highest concentrations obtainable with the equipment used. The average chamber concentrations reported for the technical material were 0.26 mg/l (dust) and 0.48 mg/l (aerosol).

Inhalation toxicology experience at one laboratory (Dr. Charles Ulrich, International Research and Development Corp.) has led to the estimate that only about one-third of materials tested at that laboratory can be dispersed at as high a concentration as 5.0 mg/l. For comparison, a "nuisance particulate" concentration is often about 0.01 mg/l (ACGIH).

In consideration of the above opinion and of the low oral toxicity of the a.i., Toxicology Branch will reclassify the subject inhalation studies as limiting studies, providing Minimum Data for assigning Toxicity Categories to fenoxycarb technical and to the formulation ACR 5023.

Data Quality Reclassification:

		Tox Cat	Grade
Acute inhalation - rat (Huntington Research Center)	Technical	II	Minimum
Acute inhalation - rat	Formulation ACR 5023 [redacted]	III	Minimum

Primary Skin Irritation and Dermal Sensitization - Guinea Pig. Fenoxycarb Formulation ACR 5023 [redacted] Hoffmann-LaRoche No. 2330a; March 18, 1982.

The test animals consisted of two groups of 10-to-20 guinea pigs (experimental and control) and 4 animals for primary irritation test.

The protocol was the split adjuvant technique (Maguire). On Day 0, the animals are fixed with a window-dressing to cover the shaved areas (2x2 cm) of the shoulder girdle. After treating the sites with dry ice and 0.1 ml of test material, and covering with filter paper, the area is occluded until Day 2, at which time the window is opened and test material reapplied. On Day 4 the window is opened again and 0.1 ml of Freund's Complete Adjuvant (FCA) is injected i.d. twice into the sensitizing site before epicutaneous application of the test material and closing of the window. The procedure for Day 7 is the same as on Day 2. On Day 9 the wrappings are removed.

The Challenge phase began on Day 21, when both experimental and control groups received 0.1 ml of test material (a non-irritating quantity) applied under a patch to a closely-shaved area (2x2 cm) on the dorsal back. On Days 22 and 23 the area is shaved (if necessary) for reading of skin reactions. Rechallenge was performed on Day 35, with readings recorded on Days 36 and 37.

The control group received FCA injections and were challenged in the same manner and at the same time as the experimental group was rechallenged.

Tabulation shows that the experimental and control groups did not significantly differ in the frequency or intensity of skin reactions.

Not sensitizing at 0.1 ml in guinea pigs.

Guideline Data.

Addendum to Contractor Review of "Dermal Irritation and Sensitization - Guinea Pig". Fenoxycarb Formulation ACR 5023 [redacted] Hoffmann-LaRoche No. 2330; Jan. 4, 1983. Accession No. 671850.

The study was performed with an adequate number of animals and with the undiluted formulation. Testing utilized a standard protocol for the guinea pig open epicutaneous test (Marzulli and Maibach, p. 321).

The contractor review states imputed deficiencies in the study as follows:

1. Technical material was not used.
2. Test material was not applied intradermally.
3. The protocol did not specify use of "adult white" guinea pigs.
4. A positive control was not used.

Toxicology Branch notes that imputed deficiency (1) is not valid; "the end-use product shall be tested ..." (Guidelines, Nov. 1982, p. 60). Imputed deficiencies (2) and (4) are not valid according to standard protocol (Marzulli and Maibach). In regard to item (3), Toxicology Branch will accept the choice of test animal as proper.

Since deficiencies listed by the Contractor Review are not valid exceptions, Toxicology Branch elevates the data classification from Supplementary to Guideline.

Micronucleus Test-Mouse (Muta. Cat. 2): Hoffmann-LaRoche (Basle) Report No. B-96 679, July 20, 1982.

In response to TB request (J. Holder, Jan. 31, 1983) additional data have been submitted showing that oral administration of the positive control produces the same percentage of PCE with micronuclei as does i.p. administration. The study protocol had utilized oral exposure for the test material and the i.p. route for the positive control.

Separately, Toxicology Branch will also accept 5000 mg/kg (the top dose of the test material) as sufficiently high to provide valid data. The oral LD50 of fenoxycarb in mice is stated in the Report as > 5000 mg/kg. The Report does not provide data on the ratio PCE/NCE. A decrease of this ratio at top dose is a frequently accepted criterion for attainment of maximum tolerated dose.

Summary:

MAAG study No. 041/3106 (H-LR Report No. B-96 679) is reclassified as Acceptable; micronuclei not produced in mice at dosages up to 5000 mg/kg (HDT).

Addendum to Contractor Review of 90-Day Feeding Study in Rats. Hoffmann-LaRoche No. B-104 779; Sept. 5, 1983.

Body weight NOEL = 80 mg/kg
LEL = 250 mg/kg (decrease in male (B.W.)

Clinical chemistry NOEL = 80 mg/kg
LEL = 250 mg/kg (females show decreased ChE and slightly elevated cholesterol).

Alterations in liver enzyme values listed as significant show either inadequate dose response or fall within the normal range presented in the study. Calcium and phosphorus parameters were not examined in the study.

Hematology NOEL = 80 mg/kg
LEL = 250 mg/kg (decreased RBC, Hb, and PCV in females).

Organ weights (and O/B ratios) show very significant increases for the liver and thyroid. These changes are dose related and persist even at low dose in the liver for both sexes.

NOEL (increased liver wt.): not observed in either sex.

Histopathology NOEL = 80 mg/kg
LEL = 250 mg/kg (increased follicular activity in thyroid; hepatocyte hypertrophy and decreased glycogen in the liver).

OPP:HED:TOX:VAN ORMER:sb 12-6-84 X73713 #8-D21

J. Holder (Info copy)

Acute Oral Studies

Technical (97-98% a.i.) R_O 13-5223 was tested by gavage for oral acute toxicity (one dose), and it was found in one study the LD $_{50}$ > 10,000 mg/kg (rat) and in another study LD $_{50}$ > 16,800 mg/kg (rat). Still another study in the mouse showed the LD $_{50}$ >8000 mg/kg.

A formulation of R_O 13-5223 [redacted] showed LD $_{50}$ > 10,000 mg/kg. The formulation likely to be marketed [redacted] was not tested for acute oral toxicity.

To further underscore the low degree of toxicity of R_O 13-5223, the i.p. dosage LD $_{50}$ = 9220 mg/kg. Another study using a single dose (up to 10,000 mg/kg by gavage) and monitoring for 14 days showed two (2/5) females rats died showing gastric distress with crystals (presumed to be test substance) in the gastrointestinal tract. The rest of the rats lived and were sacrified at day 14 and necropsied. Histopathology showed splenic hemopoiesis in most of the treated animals.

Study Classification: Core Guideline (All acute oral studies).

Acute Inhalation Studies - Technical

Two types of 4-hour exposures in two separate experiments were performed: (1) R_O 13-5223 dusting with an average chamber concentration of 0.26g/m^3 (97% technical) of which 32.5% was respirable (<5 um a.d.), and (2) R_O 13-5223 aersol exposure with an average aersol concentration of 0.48g/m^3 of which 94.5% was respirable. The dust groups (10 of each sex) was compared to an air control and the aerosol group (10 of each sex) was compared to a vehicle control. The concentrations obtained were stated by Dr. Clark of Huntington Research Centre (performing contractor) to be the highest concentrations obtainable with the equipment used. Animals were observed for 14 days and then sacrificed for pathology.

Results show no deaths in dust or aerosol groups (or in controls). The dust group showed licking and excessive

salivation in the first 30 minutes of exposures with eye closure and lacrimation at 1 1/2 hours exposure. The aerosol group also showed licking of the mouth and excessive salivation during the first 1 1/2 hours, and then, they were apparently normal thereafter. During the 14 day period the dust group showed brown straining of the fur for 2-10 days and in the aerosol group brown straining of fur and a dark nasal discharge 1 day after exposure which cleared by day 5.

There were no body weight changes in the dust or aerosol groups. The lung weight to body weight ratio was invariant. No macroscopic organ abnormalities were observed as there was no compound-related microscopic pathology in either exposure group.

The study was well conducted in all respects except: no dose response was established and the dose utilized was not high enough to rule out inhalation effects. The highest dose should be > 5mg/liter. This inhalation experiment should be repeated with a number of treatment groups and high dose > 5mg/liter.

Study Classification: Supplementary

Acute Inhalation Studies - Formulation [redacted]

This study on 10.3% a.i. (w/w) started on 3/16/82 at Research and Consulting Co. Ldt., Itingen, Switzerland and the sponsor was Hollmann-LaRoche. Two groups of 2221 and 3052 mg/m^3 (gravimelrically determined) were aersol tested and compared to vehicle control Wistar Rats. Exposure was for four hours in 100 l. chambers at a flow rate of 600 l. air/hour and a pressure of 3 atm. Observation was for 14 days post-treatment.

No deaths, body weight changes, organ weights or gross morphology or microscopic or histologic alterations were noted with 10.3% a.i. [redacted] aerosol exposures.

It should be noted as in the previous inhalation experiment, that high enough a.i. concentrations were not achieved in the experiment in order to assess inhalation effects. The highest concentration 3052 mg/m^3 = 3052 mg/1000 L= 3.05 mg/L X .103 (% a.i.) = 0.31 mg/L which could not ever rule out Category II toxicity.

Looks like the formulation could have >3.05 mg/L as the LC_{50}.

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Study Classification: Supplemental

Acute Dermal Irritation Studies

In this study (11/15/79) on 10 males and 10 females, test compound (from a 40% a.i. solution in corn oil on shaved dorsal area) at 2000 mg/kg was applied and patch occluded for 24 hours. Following exposure the R_0 13-5223 was rinsed off and rats were observed for 14 days.

No dermal irritation was observed in males or females during the 14 days. There were no significant gross, behavioral, or histopathologic dose related changes noted.

Formulated (10.3% a.i.) $R_0$13-5223 showed no dose-related effects with applications of 1000, 3000, and 5000 mg/kg.

These data indication a Category 3/4 dermal toxicity.

Study Classification: Core-Minimum

A primary skin sensitization test was run on Guinea Pigs. A induction dose (single) of .025 ml of 100%, 30%, 10% and 3% a.i. was applied to a 2 cm^2 area of flank skin (6-8 pigs per group). The applications were repeated daily for three weeks. On days 21 and 35 the colateral flank was treated with a challenge dose of R_0 13-5223. R_0 3-5223 was well tolerated and no allergic reactions reactions occured at 24 hours or 48 hours after challenge.

Study Classification: Core-Guidelines

Acute Eye Irritation

Rabbits were tested with 0.1 ml of 10% and 30% a.i. technical solutions in two eye irritation tests. Some mild redness was seen at first which subsided quickly by 24 hours. There were no corneal opacity or ulcerations, no iris involvement, nor any chemosis present.

J. Holder (duplicate)
Jan 31, 1983 (Info copy)

6. The Ames reverse mutation assay, the S. Cervisiae D-7 crossing over, mitotic gene mutation in Chinese Hamster V 79 lung cell line at the HGRPT Locus assay were performed. All three tests performed were negative for mutagencity.

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7. The new test submited by Maag, i.e., mouse micronucleus test, was found to be not acceptable because the positive control (procarbazine) and test compound (R_o 13-5223) were given by two different routes of administration. To supplement the present data R_o 13-5223 should be given I. P. and procarbazine should be given orally in peanut oil, or justification should be made as to why the positive control had to be given I.P. while R_o 13-5223 had to be given orally.

Mouse Micronucleus Test (Report No. 041/3106)
on Technical R_o 13-5223

The insect growth regulator was in vivo tested for chromosomal breaking activity and the ability to cause mitotic nondisjunctions. Fullinsdorf Albino Mice were treated orally (in peanut oil) with 1250, 2500, or 5000 mg a.i./kg. b.w. Treatment was done twice: 30 hours and 6 hours before sacrifice. Bone marrow cells were collected, washed, and smears were made. Stained PCE cells were counted, usually 2000 cells per mouse, and the number of cells containing micronuclei were scored.

Control mice showed the natural occurrence of micronuclei @ 5-12/2000, which is 0.25 to 0.60%. No differences were seen between the sexes.

Treatment with R_o 13-5223 orally in peanut oil did not cause any statistically significant increase in polychromatic erythroblast cells (PCE) which contained micronuclei. A positive control of procarbazine (50 mg/kg) was given I.P. (not orally) which caused 3-6% occurrence of PCE's which had micronuclei. Although this positive control may offer test standardization, it was not given by the same route of administration as the compound under test, R_o 13-5223.

To logically complete the test, procarbazine and R_o 13-5223 should be given by the same route. The data lacking which would make the present mouse micronuclei data acceptable would be from mice given R_o 13-5223 I.P. and from mice given procarbazine orally in peanut oil. Alternatively, the registrant can submit justification as why the positive control had

to be administered I.P. and R_o 13-5223 had to be administered orally. The present data is supplemental and not acceptable until the above two types of dosing are done and combined with the present data, or an acceptable justification for the present protocol is made. The registrant is refered to NTIS for mutgencity testing protocols.

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Study Classification: Not acceptable

J.W. Holder 1/21/83
1/21/83

James W. Holder, Ph.D.
Toxicology Branch
Hazard Evlauation Division (TS-769)

cc: Dave Severn, EFB

* CONFIDENTIAL BUSINESS INFORMATION *

MRID: Not reported

Study Type: 90 Day Feeding Study in Rats

Accession Number: Not reported

MRID Number: Not reported

Sponsor: F. Hoffman - La Roche and Co. Ltd., Basle, Switzerland

Contracting Lab: F. Hoffman - La Roche

Date: September 5, 1983

Test Material: Ro 13-5223/000
Ethyl (2-p-phenoxyphenoxy ethyl) carbamate

Protocol:

The following description of the materials and methods used for this study was abstracted and paraphrased from the original report.

1. Test substance and purity: The substance tested was ethyl (2-p-phenoxyphenoxy ethyl) carbamate, 98% pure.
2. Species of animals: Albino SPF rats weighing 82-90 g were randomly assigned to test groups. Each group contained 20 males or 20 females, except for the high dose groups, which contained 30 animals of each sex.
3. Dosing schedule: The rats were fed the test material at the following dietary dose levels: 0, 80, 250, or 800 mg/kg/day for 91 days. At the end of the period all survivors were sacrificed except the extra 10 males and 10 females from the 800 mg/kg/day dose groups. These 10 rats were given a standard diet, free of test material, for an

additional 29 days in order to observe the reversibility or late occurrence of any effects from the test material. There were no extra control rats for the reversibility test group.

4. Parameters to be examined: All animals were examined for morbidity and mortality. Clinical signs, body weights, and food consumption were recorded weekly. All control and high-dose animals were given opthalmoscopic examination in the 12th week. Blood samples for clinical chemisty measurement were taken from the first ten animals in each group of males and females during weeks 6 and 14. Blood samples for hematology examinations were collected during weeks 0, 6, and 14. Urine was also collected during weeks 5 and 11 from the same animals that were sampled for clinical chemistry. Samples of food remaining in the hoppers after weeks 2 and 12 were analyzed for the content of the test material. At termination, the survivors were sacrificed by means of CO_2, followed by exsanguination. The following organs were removed and weighed: thyroids, heart, liver, spleen, lungs, kidneys, adrenals, testes,

prostate, ovaries, uterus, brain and pituitary. Sections were prepared for histology.

5. Statistics Used: The authors used mean values and their standard deviations as well as mean ranks. MITRE has used students "t" test and Chi Square.

Results:

1. Chemical analyses of samples of the feed provided in weeks 2 and 12 and records of food intake showed that the levels of test material were as intended and there were no statistically significant deviations.

2. Mortality during this study was limited to two females receiving the high dose and assigned to the post-exposure recovery observation group. Their deaths were not related to exposure to the test material, but rather were due to blood sampling during week six.

3. Clinical observations during this study showed apparent dose-related effects of alopecia, diuresis, and soiled tail, as shown in Table 1. The effects disappeared during the recovery period.

4. Body weight gains (Table 2) were reduced in a time and dose-related manner throughout 91 days of feedings. The weights of the high-dose males and females at 91 days were 86.8% and 91.1%, respectively, of the weights of the corresponding control rats. These body weight differences were statistically significant.

* CONFIDENTIAL BUSINESS INFORMATION *

TABLE 1

INCIDENCES OF ALOPECIA, SOILED TAIL AND DIURESIS
AMONG RATS RECEIVING DIETARY FENOXYCARB

Observations	Incidences at Dietary Dose Level (mg/kg/day)							
	Males				Females			
	0	80	250	800	0	80	250	800
Alopecia	0	12/3	3/2	17/7	3/1	1/1	19/4	65/14
Soiled Tail	0	0	0	54/7	0	0	0	77/14
Diuresis	0	0	0	6/3	0	0	0	52/11

* CONFIDENTIAL BUSINESS INFORMATION *

TABLE 2

ABSOLUTE AND RELATIVE BODY WEIGHTS (%)
OF RATS RECEIVING DIETARY FENOXYCARB

Dose (mg/kg)	Week of Measurement 0	4	8	13	17
Males					
0	154.0 (100)	303.7 (100)	373.6 (100)	414.6 (100)	- -
80	155.5 (101.0)	302.8 (99.7)	365.8 (98.2)	406.8 (98.1)	- -
250	153.4 (96.6)	291.7 (96.0)	346.6 (93.0)	385.0 (92.9)	- -
800	153.7 (99.8)	271.3 (89.3)	325.6 (87.4)**	359.8 (86.8)**	378.6 -
Females					
0	131.6 (100)	185.4 (100)	212.7 (100)	232.7 (100)	- -
80	131.6 (100.2)	185.4 (97.3)	212.7 (97.8)	232.7 (98.3)	- -
250	131.1 (99.8)	176.0 (96.4)	199.0 (96.1)	212.0 (96.0)	- -
800	133.1 (101.3)	176.0 (92.4)	199.0 (91.5)	212.0 (91.1)*	213.9 -

* = Statistically significantly different from control at $p \leq 0.05$ by Chi square analysis.
** = Statistically significantly different from control at $p \leq 0.01$ by Chi square analysis.

5. There were no statistically significant differences in the food consumption of any of the dose groups.

6. The mean values for clinical chemistry determinations are shown in Table 3. The test material caused the following statistically significant changes compared to controls:

a) Glutamic-pyruvic transaminase levels were increased in both sexes by the high dose of 800 mg/kg/day. The LEL is 800 mg/kg/day and the NOEL is 250 mg/kg/day.

b) Glutamic dehydrogenase levels were increased in the males by the two higher doses of 250 and 800 mg/kg/day and in the females by the dose of 250 mg/kg/day. The LEL is 250 mg/kg/day and the NOEL is 80 mg/kg/day.

c) Alkaline phosphatase levels were increased in both sexes by all dose levels. The LEL was 80 mg/kg/day and the NOEL was not established.

d) Bilirubin was increased in the males by the high dose of 800 mg/kg/day. The LEL is 800 mg/kg/day and the NOEL is 250 mg/kg/day.

e) Cholinesterase levels were decreased in the females by the two higher doses of 250 and 800 mg/kg/day. There were no differences among the males. The LEL is 250 mg/kg/day and the NOEL is 80 mg/kg/day.

TABLE 3

MEAN CLINICAL CHEMISTRY VALUES FOR RATS RECEIVING DIETARY FENOXYCARB

Dose mg/kg/day	GOT IU/L	GPT IU/L	GLDH IU/L	AP IU/L	ChE mg/DL	Urea mg/DL	Glue mg/DL	Bili mg/DL	Chol mg/DL
Males									
0	35.0	17.9	2.72	335	158	47.0	169	0.15	66.8
80	39.5	19.9	3.17	400*	177	43.9	152	0.14	57.4
250	43.8	21.1	26.33***	441***	247	45.7	173	0.17	56.1
800	31.5	24.9***	11.33*	448*	191	49.3	153	0.19**	99.7***
Post[a]	31.9	25.1	10.21	383	216	52.0	153	0.18	78.2
Females									
0	35.0	16.0	3.80	264	687	56.6	143	0.09	63.5
80	33.5	15.5	1.42	336**	716	57.9	149	0.11	62.8
250	29.2	15.8	16.46*	364*	555**	50.1	137	0.16	89.3**
800	28.8	18.9*	5.93	332*	428**	51.6	132	0.21	87.7**
Post[a]	27.8	17.1	7.08	346	415	61.6	138	0.11	85.9

* Statistically significantly different from control at $p \leq 0.05$.
** Statistically significantly different from control at $p \leq 0.01$.
*** Statistically significantly different from control at $p \leq 0.001$.
[a] These rats received the high dose throughout the test and were then placed on control diets for four weeks prior to sacrifice in order to obtain information of reversibility of effects.

f) Cholesterol levels were increased in the females by the two higher doses of 250 and 800 mg/kg/day and in the males by the high dose of 800 mg/kg/day. The LEL is 250 mg/kg/day and the NOEL is 80 mg/kg/day.

There are no clear trends in these biochemical parameters among the high dose animals that were given the four week post-exposure recovery period in comparison to those sacrificed at 13 weeks.

7. Representative mean values for hematologic determinations are shown in Table 4. The test material caused the following statistically significant changes compared to controls:

a) Red blood cell counts were decreased in the females by the two higher doses of 800 and 250 mg/kg/day. The LEL is 250 mg/kg/day and the NOEL is 80 mg/kg/day.

b) Hemoglobin was decreased in the females by the mid dose of 250 mg/kg. There were no other statistically significant changes in hemoglobin.

c) Packed cell volumes were decreased in the females by the two higher doses of 800 and 250 mg/kg/day. The LEL is 250 mg/kg/day and the NOEL is 80 mg/kg/day.

d) Prothrombin times were increased in the males and females by the high dose of 800 mg/kg/day. The LEL is 800 mg/kg/day and the NOEL is 250 mg/kg/day.

TABLE 4

MEAN HEMATOLOGIC VALUES FOR RATS RECEIVING DIETARY FENOXYCARB

Dose (mg/kg/day)	RBC ($10^6/\mu l$)	Hb (g/100 ml)	PCV (%)	Retic (%)	PT (Sec)
Males					
0	8.86	17.1	44.7	2.4	15.4
80	8.73	16.6	43.5	2.4	15.2
250	8.68	16.7	43.5	2.8	14.8
800	8.78	16.6	43.4	2.6	18.9*
Post	8.51	16.4	42.4	3.0	17.4
Females					
0	8.62	16.7	44.0	3.0	12.8
80	8.36	16.3	42.8	2.9	13.2
250	8.13*	15.7*	41.8*	2.9	13.2
800	8.20*	15.8	41.3*	3.1	14.2*
Post	7.94	15.3	40.6	3.6	12.5

* Statistically significant different from control at $p \leq 0.05$.

There appears to be a trend in reversing these effects in the high dose exposed group during the four week recovery period; however, statistics were not conducted on the recovery group due to a lack of control animals.

8. The urinalyses and the opthalmoscopic examinations gave no indications of any treatment related effects.

9. There were no treatment related findings from the gross pathology examinations.

10. The organ weights and organ/body weight ratios for the liver and thyroid glands were increased in a dose related manner as shown in Table 5. These changes appeared to be reversed in the high dose animals during the four-week post exposure recovery period, except for the absolute liver and thyroid weights of the high dose males. For the high dose males, the liver/body weight ratios did not appear to regress.

11. The major histopathological findings involved the liver and the thyroid gland. Both organs showed dose-related, reversible effects. The liver changes consisted of moderate hypertrophy of the centrilobular hepatocytes associated with decreased glycogen content. Effects on the thyroid glands were most noticeable in the high dose females and comprised a predominance of active follicles with large epithelial cells, some of which had vacuoles. The authors

TABLE 5

MEAN ORGAN WEIGHTS AND MEAN ORGAN/BODY WEIGHT RATIOS (x 100)
FOR LIVER AND THYROID GLANDS OF RATS RECEIVING DIETARY FENOXYCARB

Dietary Exposure (mg/kg/day)	Liver				Thyroid Gland			
	Males		Females		Males		Females	
	Org. Wt. (g)	O/BW x 100	Org. Wt. (g)	O/BW x 100	Org. Wt. (g)	O/BW x 100	Org. Wt. (g)	O/BW x 100
0	5.82	1.41	8.47	3.60	11.72	5.66	15.44	6.43
80	6.99**	1.78****	10.47**	4.51****	12.11	6.01	16.47	7.33
250	8.54****	2.23****	13.98****	6.07****	13.19	6.48*	19.10**	8.42***
800	10.26****	2.80****	16.55****	7.67****	16.38*	8.53***	21.68*	10.07***
Post-Exposure Period	15.06	3.96	9.64	4.43	21.47	5.65	18.13	8.34

*: Statistically significantly different from control at $p \leq 0.05$.
**: Statistically significantly different from control at $p \leq 0.01$.
***: Statistically significantly different from controls at $p \leq 0.0005$.
****: Statistically significantly different from controls at $p \leq 0.0001$.

Note: Statistical analyses are by Students "t" test.

interpreted this as a stress-related effect rather than a specific action of the test material. The LELs for increased liver weight and liver/body weight ratio in both sexes were 80 mg/kg/day and the NOEL was not established. The LEL for increased thyroid weight and/or increased thyroid/body weight ratios was 250 mg/kg/day and the NOEL was 80 mg/kg/day.

There were minor histopathological changes in the kidneys which were not dose-related.

Conclusions:

1. Male and female, albino, SPF rats were fed diets containing the test material at levels of 0, 80, 250, or 800 mg/kg/day for 91 days. Additional animals, 10 males and 10 females, received the dietary test material at 800 mg/kg/day for 91 days and then were placed on diets free of test material for 28 days.

2. The LEL for mortality was not established and the NOEL is 800 mg/kg/day.

3. Body weights were decreased at the high dose of 800 mg/kg/day in the males at weeks 8 and 13 and in the females at week 13. The LEL for decreased body weight gain was 800 mg/kg/day and the NOEL was 250 mg/kg/day. There were no differences in food consumption of any of the groups.

4. Glutamic-pyruvic transaminase levels were increased in both sexes by the 800 mg/kg/day level. The LEL for increased glutamic-pyruvic transminase is 800 mg/kg/day and the NOEL is 250 mg/kg/day.

5. Glutamic dehydrogenase levels were increased in the males by the 250 and 800 mg/kg/day levels and in the females by the 250 mg/kg/day levels. The LEL for decreased glutamic dehydrogenase is 250 mg/kg/day and the NOEL is 80 mg/kg/day.

6. Alkaline phosphatase levels were increased by all dose levels in both sexes. The LEL for increased alkaline phosphatase was 80 mg/kg/day and the NOEL was not established.

7. Bilirubin was increased in the males by the 800 mg/kg/day level. The LEL for increased bilirubin is 800 mg/kg/day and the NOEL is 250 mg/kg/day.

8. Cholinesterase levels were decreased by the 250 and 800 mg/kg/day levels in the females. The LEL for decreased cholinesterase is 250 mg/kg/day and the NOEL is 80 mg/kg/day.

9. Cholesterol levels were increased in the females by the 250 and 800 mg/kg/day levels and in the males by the 800 mg/kg/day level. The LEL for increased cholesterol is 250 mg/kg/day and the NOEL is 80 mg/kg/day.

10. Red blood cell counts were decreased in the females by the

250 and 800 mg/kg/day levels. The LEL for reduced red blood cell count is 250 mg/kg/day and the NOEL is 80 mg/kg/day.

11. Packed cell volumes were decreased in the females by the 250 and 800 mg/kg/day levels. The LEL for decreased packed cell volume is 250 mg/kg/day and the NOEL is 80 mg/kg/day.

12. Prothrombin times were increased in the males and females by the 800 mg/kg/day level. The LEL for increased PCV is 800 mg/kg/day and the NOEL is 250 mg/kg/day.

13. The LEL for organ weight and organ body weight ratio was 80 mg/kg/day for both sexes and the NOEL was not established. The effects at this feeding level and above were increased liver weights and liver/body weight ratios. There were also increases in the thyroid weights and thyroid/body weight ratios at the 250 and 800 mg/kg/day feeding levels. Most of these effects were apparently reversible, since the mean values for these effects decreased in the 800 mg/kg/day group during 28 days on a diet free of test material.

CORE Classification: Core Guideline.

MITRE CONTROL NO.
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* CONFIDENTIAL BUSINESS INFORMATION *

MRID: Not reported

Study Type: Subchronic Oral Toxicity Study in Mice

Guideline data
PVO 7 Dec 84

Accession Number: Not reported

MRID Number: Not reported

Sponsor: Dr. R. MAAG Ltd.

Contracting Lab: Roche

Date: May 31, 1983

Test Material: Ro13-5223/000

Protocol:

1. Test substance and purity: Ethyl [2-p(p-phenoxyphenoxy) ethyl]carbamate (98% pure).
2. Species of animals: Albino-SPF mice, Fullinsdorf strain, 7 weeks old, 16 males and 16 females per dose level.
3. Dosing schedule: Groups of mice received 0, 100, 300, or 900 mg/kg/day in the diets.
4. Parameters to be examined: Clinical health, clinical pathology, and gross and histopathology including organ weights.

5. Statistics used: Dunn Test of multiple comparision.

Results:

Dietary Level Study: Actual dietary consumption of Ro13-5223/000 was 0, 101, 302, and 921 mg/kg for males and 0, 103, 310, and 923 mg/kg for females based on nominal concentrations of Ro13-5223/000 in the feed. No analytical data verifying the concentration of Ro13-5223/000 in the feed were given; thus, the exact doses received were not determined.

Clinical Health: No adverse reactions to treatment with Ro13-5223/000 were noted among the mice. One control male died inadvertly during the ophthalmoscopic examinations. Body weight data are given in Table 1. The mean body weights for all test groups were not equal at week 0, ranging from 94 to 104 percent of control values. The increase or decrease of mean body weight for the treated groups did not vary more than 3% relative to the appropriate controls for either males or females over the course of the study. MITRE performed an analysis of variance for selected body weight data and found that there were no statistically significant differences among treated and control values. Thus, the NOEL for body weight data in this study is 900 mg/kg (highest dose tested) and the LEL was not established.

TABLE 1

SELECTED GROUP MEAN BODY WEIGHT FOR MICE

Treatment Level (mg/kg)	Mean Body Weight (g) at Week		
	0	7	13
Males			
0	36	56	61
100	36(100)[a]	56(100)	63(103)
300	34(94)	52(93)	57(93)
900	35(97)	53(95)	59(97)
Females			
0	23	40	43
100	29(104)	42(105)	46(107)
300	28(100)	40(100)	42(98)
900	28(100)	39(98)	42(98)

[a]Numbers in parentheses are the percent of control value.

Clinical Pathology: The authors reported that all hematologic changes were within normal limits; however, there were elevations in the total number of white blood cells and in the number (percent) of polymorphonuclear neutrophils in selected animals in all groups, but especially in the control male group. These elevations are indicative of an intercurrent infection. The number of white blood cells ranged as high as 22.3 x 10^3 per microliter (normal range: 5-12 x10^3 per microliter) and the percent polymorphonuclear neutrophils ranged as high as 63 (normal range: 2-22). The mean values for all groups were within normal limits except for the percent polymorphonuclear neutrophils in the male control group (24 percent) where the normal mean is 12 (2-22) x 10^3 per microliter. These changes did not appear to be treatment related. Thus, the NOEL for clinical pathology data in this study is 900 mg/kg (highest dose tested) and the LEL was not established.

Remarkable changes in clinical chemistry determinations included dose-related alterations of alkaline phosphatase activity and plasma protein concentrations as shown in Table 2. Based on these data, the NOEL for clinical chemistry was selected as 100 mg/kg and the LEL as 300 mg/kg.

No remarkable differences in the urinalysis results were noted among treated and control groups. Thus, the NOEL for urinalysis in

TABLE 2

SELECTED MEAN CLINICAL PATHOLOGY DATA

Treatment Level (mg/kg)	Clinical Parameter: Total White Cell Count ($x10^3/mm^3$)	Percent Polymorphonuclear Neutrophils (%)	Total Plasma Protein (g/L)	Percent Alubumin (%)	Alkaline Phosphatase (I.U./L)
Males					
0	9.6 (4.7-13.5)[a]	24 (9-42)	55 (53-60)	50 (47-54)	107 (86-147)
100	8.2 (2.4-22.3)	11 (1-25)	54 (50-59)	55 (49-60)*	115 (88-148)
300	6.6 (4.1-11.4)	10 (3-20)	51(48-57)*	51 (39-59)	127 (94-195)
900	5.5 (3.2-9.3)	12 (5-36)	52 (48-56)	56 (44-63)*	209 (106-435)*
Females					
0	6.8 (3.9-8.5)	8 (2-24)	50 (47-52)	56 (50-64)	172 (74-544)
100	7.8 (4.5-9.5)	7 (2-14)	51 (48-54)	61 (55-65)	146 (76-223)
300	7.0 (3.4-10.4)	12 (1-63)	49 (44-54)	64 (59-69)*	190 (139-409)
900	5.6 (3.6-9.7)	7 (3-19)	49 (44-52)	66 (62-68)*	157 (90-206)

*Statistically different from control values ($p \leq 0.05$)
[a]Numbers in parenthesis are the range of values.

this study is 900 mg/kg (highest dose tested) and the LEL was not established.

Organ Weight Data: As shown in Table 3, some very high and low values were recorded among the brain weights for individual control males. However, nothing was noted in the histopathology section concerning the brains with elevated weights. In addition, there were some computational errors in the relative organ weight data presented by the authors.

The absolute and relative organ weight data presented in Table 3 include the range of values to illustrate the variation in the data. The authors noted differences in the absolute and relative liver weights for both sexes receiving 900 mg/kg and in females receiving 300 mg/kg. Based on liver weight data, the NOEL for organ weights is 100 mg/kg and the LEL is 300 mg/kg.

Histopathology: The only organ with remarkable findings was the liver; the changes which reflect the organ weight data. Fatty changes noted in the control and 100 mg/kg group were predominately in the centrilobular region, while fatty changes in the 300 and 900 mg/kg groups were predominately in the periportal region. Glycogen depletion was noted in the 300 and 900 mg/kg groups. There was a dose-response in the incidence of multinucleated hepatocytes. The incidences in males were 2/15, 4/15, 5/15, and 7/15 and in females were 0/16, 2/15, 4/16, and 10/16 for the 0, 100, 300,

TABLE 3

SELECTED ABSOLUTE AND RELATIVE ORGAN WEIGHT DATA FOR MICE

Treatment Level (mg/kg)	Body Weight (g)	Absolute Brain Weight (g)	Relative Brain Weight (% Body Weight)	Absolute Liver Weight (g)	Relative Liver Weight (% Body Weight)
Males					
0	61(49-81)[a]	0.562(0.306-1.352)	0.918(0.510-2.253)	3.66(2.41-6.05)	5.92(4.16-7.47)
100	64(54-76)	0.518(0.459-0.588)	0.822(0.624-0.943)	4.14(2.98-5.53)	6.47(5.43-7.68)
300	57(46-69)	0.503(0.421-0.552)	0.893(0.761-1.117)	3.76(2.88-4.77)	6.63(5.37-8.22)
900	59(51-67)	0.510(0.439-0.555)	0.867(0.708-1.025)	4.23(3.41-5.40)*	7.24(5.78-8.48)*
Females					
0	43(35-58)	0.524(0.451-0.604)	1.220(1.041-1.451)	2.57(2.07-4.07)	5.95(4.80-9.25)
100	46(36-61)	0.521(0.464-0.595)	1.150(0.843-1.478)	3.03(2.14-5.11)	6.50(5.53-8.38)
300	43(26-50)	0.515(0.431-0.598)	1.242(1.005-1.759)	3.13(2.01-3.95)*	7.35(6.06-8.35)*
900	42(33-54)	0.527(0.466-0.623)	1.231(1.048-1.416)	3.49(2.51-5.20)*	8.22(7.12-9.63)*

[a]Numbers in parenthesis are the range of values.
*Significantly different from control values ($p \leq 0.05$).

and 900 mg/kg groups, respectively. The authors attempted to minimize the importance of these hepatocytes; however, MITRE contends that these changes are treatment-related. Based on these data, the NOEL for histopathology is 100 mg/kg and the LEL is 300 mg/kg.

Only one tumor, an alveologenic carcinoma in a control male, was noted in this study.

Conclusions:

Groups of 16 male and 16 female weanling Fullinsdorf mice were fed 0, 100, 300, or 900 mg Ro13-5223/000 per kg body weight for 13 weeks. Based on liver weight data and liver pathology, the NOEL for Ro13-5223/000 is 100 mg/kg and the LEL is 300 mg/kg. Effects were also noted in hematology and clinical chemistry data. Body weight and mortality were not affected.

CORE Classification: Core Guideline.

MRID: Not assigned

Study Type: Dermal Irritation and Sensitization

Accession Number: Not assigned

MRID Number: Not assigned

Sponsor: Hoffman-LaRoche

Contracting Lab: Hoffman-LaRoche

Date: January 4, 1983

Test Material: Ro 13-5223/029-ACR5023

Protocol:

1. Test substance and purity: Ro 13-5223/029 ACR5023 (10% fenoxycarb).

2. Species of animals: Guinea pigs (strain and age not specified).

3. Dosing schedule: Application of 0.1 ml on clipped flank skin 5 days per week for 3 weeks followed by a challenge dose on day 21 and day 35 at a contralateral site.

4. Parameters to be examined: Check for dermal irritation and hypersensitivity at 24, 48, and 72 hours following the application of the challenge doses.

5. Statistics used: None

Results:

A preliminary study (results not given) determined that a 10% fenoxycarb solution in soybean oil was the minimal irritating concentration. When applied topically, this concentration (10% a.i.)

did not produce any evidence of sensitization and is not considered to be allergenic.

Conclusions:

Guinea pigs were treated epicutaneously with 0.1 ml 5 days per week for 3 weeks followed by a challenge dose at day 21 and day 35 at a contralateral site. Under the conditions of this study, a 10% solution of fenoxycarb in soybean oil does not possess a sensitizing potential for guinea pigs.

CORE Classification:

Supplementary: 1) technical grade fenoxycarb was not used; 2) the test material was applied epicutaneously rather than intradermally [epicutaneous application is allowed only if 0.5 ml is applied in a patch application rather than the 0.1 ml (applied without a patch) used in this study]; 3) it was not possible to tell if adult white guinea pigs were utilized--only that guinea pigs were used; and 4) a positive control was not utilized.

* CONFIDENTIAL BUSINESS INFORMATION *

MRID: Not assigned

Study Type: Metabolism

Accession Number: Not assigned

MRID Number: Not assigned

Sponsor: Maag Agrochemical R & D, NLR Sciences, Inc.

Contracting Lab: Not specified

Date: June 21, 1983

Test Material: Fenoxycarb, Ethyl[2-(p-phenoxyphenoxy-1,4-^{14}C) ethyl] carbamate

Protocol:

1. Test substance and purity: The test substance was ^{14}C-labeled fenoxycarb (^{14}C-fenoxycarb) with a specific activity of 36.06 micro Ci/mg. The chemical purity was 99% (GC analysis) and the radiochemical purity was 98% (TLC/HPLC analysis).
2. Species of animals: Five male and five female rats were used. Body weights, ages and strains were not specified.
3. Dosing schedule: One rat from each sex was used as controls. The remaining four males and females received orally 48.9 mg/kg (9.65×10^7 dpm) and 48.7 mg/kg (8.51×10^7 dpm) of ^{14}C-fenoxycarb, respectively.
4. Parameters to be examined: a) Biotransformation - Fenoxycarb and its metabolites in the feces (6-48 hour samples) and urine (6-24 hour samples) of rats were

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isolated and identified. b) Excretion - Radioactivity levels in the feces and urine were determined.

5. Statistics used: Not reported

Results:

Biotransformation

The distribution of fenoxycarb and its metabolites in feces and urine is presented in Table 1. Based on the amounts of the fenoxycarb metabolites found, this study indicates that the metabolism of fenoxycarb in male and female rats is quantitatively and qualitatively similar. Structures for these metabolities were confirmed using gas chromatographic/mass spectrometric data of the isolated metabolites and synthesized standards. These metabolites are from the following metabolic reactions: ring mono-hydroxylation, aliphatic hydroxylation, aliphatic oxidation, ether cleavage, condensation, and conjugation (Figure 1). A total of 8 metabolites were found. Four metabolites have been identified. The remaining four metabolites are conjugates but the identities of the conjugating moieties are unknown.

Excretion

The cummulative percents of the administered radioactivity recovered in feces and urine after oral administration of

TABLE 1

DISTRIBUTION OF FENOXYCARB AND ITS METABOLITES IN FECES AND URINE OF RATS FOLLOWING ORAL ADMINISTRATION OF ^{14}C-FENOXYCARB

	Percent of Administered Radioactivity Recovered			
	Males		Females	
	Feces [a]	Urine [b]	Feces [a]	Urine [b]
Fenoxycarb (Ro 13-5223)	3.1	0	0.5	0
Ro 16-8797	4.9	1.1	9.6	2.8
Ro 17-3192	11.6	2.5	8.9	7.0
Ro 17-3193 + Ro 1-1374	24.9	3.3	22.6	2.1
Total	44.5 (85)[c]	6.9 (37)[d]	41.6 (77)[c]	11.9 (60)[d]

[a] Means of the 6-48 hour fecal samples from 2 rats.
[b] 6-24 hour urine sample from one rat.
[c] Percent of total fecal radioactivity.
[d] Percent of total urinary radioacitivity.

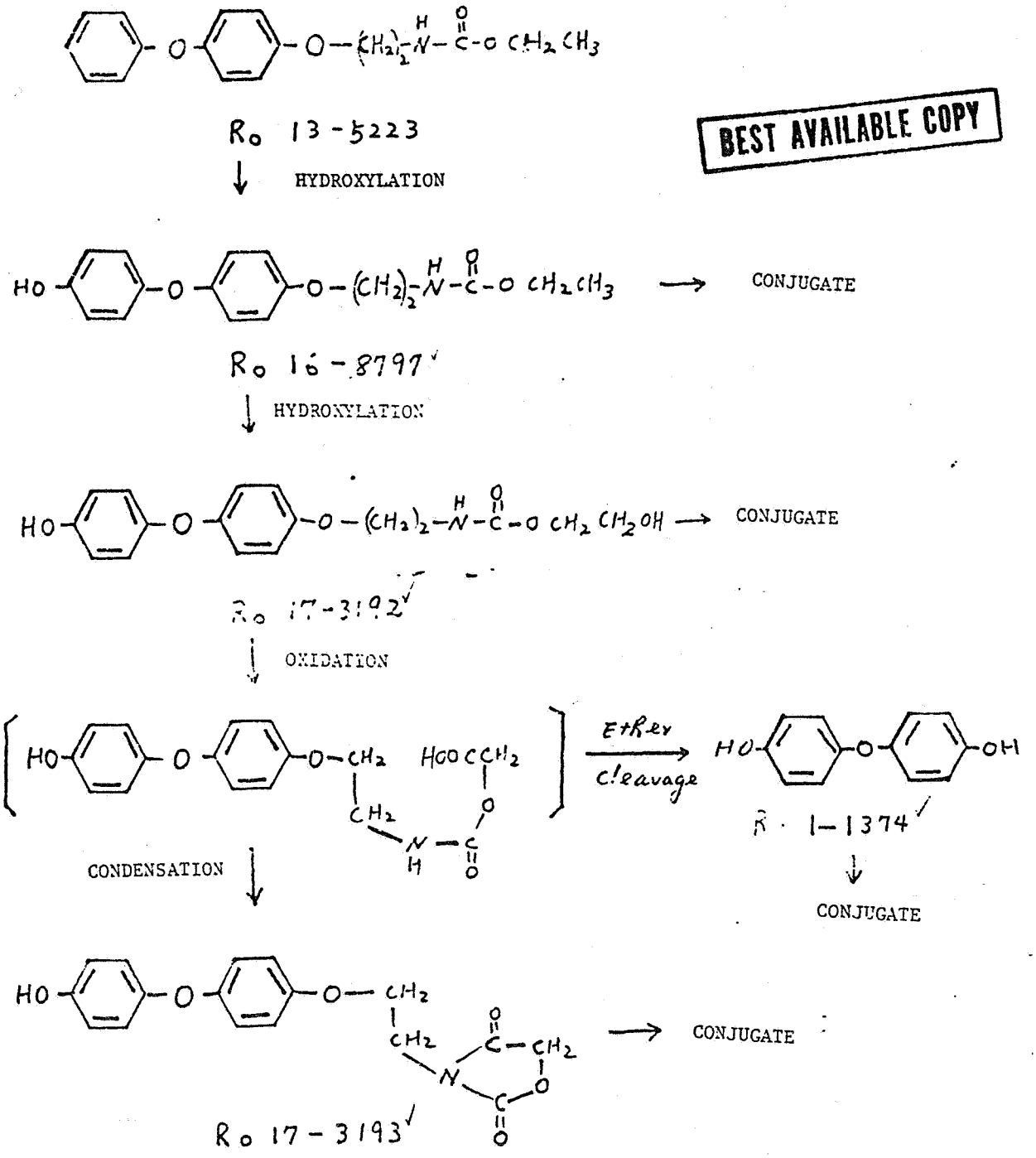

Figure 1. Metabolic Pathway of Fenoxycarb in the Rat.

^{14}C-fenoxycarb in rats are listed in Table 2. The excretion patterns of fenoxycarb in males and females were similar. Male rats excreted 52.14 and 18.65% of the dose in feces (6-48 hours) and urine (6-24 hours), respectively. Female rats excreted 54.01 and 19.77% of the dose in feces (6-48 hours) and urine (6-24 hours), respectively.

Conclusions:

- The metabolic pathway and excretion patterns of fenoxycarb in male and female rats were similar.
- Fenoxycarb is extensively metabolized in rats by a series of oxidations and conjugations followed by urinary excretion in free and conjugated forms.
- The total radioactivity recovered in feces (6-48 hours) and urine (6-24 hours) from male and female rats was 70.79 and 73.78% of the radioactivity administered, respectively.
- Minor deficiencies in this study included the use of only one dose and a duration which was too short for the excretion study.

CORE Classification: Not applicable. Guidelines for classification of metabolism studies are not available.

TABLE 2

PERCENT OF ADMINISTERED RADIOACTIVITY RECOVERED IN FECES AND URINE AFTER ADMINISTRATION OF FENOXYCARB TO RATS

Dose Level (mg/kg)	Percent of Administered Radioactivity Recovered					
	Male			Females		
	Feces [a]	Urine [b]	Total	Feces [a]	Urine [b]	Total
48.9	52.14	18.65	70.79	-	-	-
48.7	-	-	-	54.01	19.77	73.78

[a] Means of the 6-48 hour fecal samples from 2 rats.
[b] Total for the 6-24 hour urine sample from one rat.

Study Type: Metabolism

Accession Number: Not assigned

MRID Number: Not assigned

Sponsor: Maag Agrochemical R&D, NLR Sciences, Inc.

Contracting Lab: Not specified

Date: March 14, 1983

Test Material: Fenoxycarb, Ethyl [2-(p-phenoxyphenoxy)ethyl]carbamate

Protocol:

1. Test Substance and Purity: The test substance was fenoxycarb (Ro 13-5223). The purity of the test substance was not reported.
2. Species of Animals: Sixteen male beagle dogs were used. Ages and body weights were not specified.
3. Dosing Schedule: Four groups of four dogs were dosed orally with fenoxycarb at concentrations of 0, 50, 150, or 500 mg/kg/day for 26 consecutive weeks.
4. Parameters to be Examined: At day one and day 19 following the 26-week administration of fenoxycarb, 2 animals from each dose group were autopsied and three tissues (fat, plasma, liver) were removed and analyzed for fenoxycarb residues.
5. Statistics Used: Not reported.

Results and Discussion

Residual levels of fenoxycarb in the three tissues on day one and day 19 following the 26-week administration (0, 50, 150, or 500 mg/kg/day) of fenoxycarb to dogs are shown in Table 1. There was wide variation both within and between treatment groups. However, there was as much as a ten-fold difference of values within group, whereas differences between treatment groups did not exceed three-fold. Due to these wide variations in the data, the residual levels of fenoxycarb did not appear to be related to dosage.

The highest amount of fenoxycarb was found in fat (6.0 to 30.2 ppm) followed by plasma (0.18 to 2.04 ppm) and liver (0.11 to 0.5 ppm) on day one after the 26-week treatment period. The amount of fenoxycarb in fat (0.10 to 0.26 ppm) was equivalent to two- to five-fold above the 0.05 ppm limit of detection at day 19 after the 26-week treatment period. However, the residual levels of fenoxycarb in plasma (up to 0.08 ppm) and liver (up to 0.06 ppm) were slightly above the 0.05 ppm limit of detection.

Conclusion

In general, this study was inadequately reported for proper review. Additionally, more tissues should have been analyzed in addition to the three tissues examined because the possibility remains

TABLE 1

RESIDUE LEVELS IN TISSUES OF DOGS FOLLOWING 26-WEEK ADMINISTRATION OF FENOXYCARB

Tissues	Tissues Autopsied After Last Dose	Mean (Ranges) of Fenoxycarb Found[a] Daily Dosage Over 26-Week Treatment Period (mg/kg/day)			
		0	50	150	500
Fat	1 day	<0.05	9.7 (8.1-11.3)	6.0 (5.8-6.1)	30.2 (15.7-44.7)
	19 day	<0.05	0.26 (<0.05-0.52)	0.10 (0.06-0.15)	0.18 (0.12-0.24)
Plasma	1 day	<0.05	0.50 (0.3-0.7)	0.18 (0.14-0.21)	2.04 (0.57-3.52)
	19 day	<0.05	0.08 (<0.05-0.16)	<0.05	<0.05
Liver	1 day	<0.05	0.32 (0.14-0.51)	0.11 (0.10-0.12)	0.5 (0.25-0.76)
	19 day	<0.05	0.06 (<0.05-0.11)	<0.05	<0.05

[a]Values (in ppm) represent the mean of two animals.

that fenoxycarb may accumulate in other tissues. The amount of fenoxycarb in fat from dogs at day 19 after the final dosing with 50, 150, or 500 mg/kg/day of fenoxycarb was 0.1 to 0.26 ppm which is equivalent to two- to five-fold above the 0.05 ppm limit of detection. The amount of fenoxycarb in plasma and liver at day 19 after the treatment was slightly above the 0.05 ppm limit of detection.

Core Classification: Not applicable. Guidelines for classification of metabolism studies are not available.

D. D. Van Orner
14 Dec 84

Study Type: Teratogenicity in Rats

Accession Number: Not specified

MRID Number: Not assigned

Sponsor: Roche

Contracting Lab: F. Hoffmann-La Roche and Company Ltd.
Basle, Switzerland

Date: May 12, 1983

Test Material: Fenoxycarb (ethyl[2-p(p-phenoxyphenoxy)ethyl] carbamate:Ro 13-5223/000)

Protocol:

The following description of the materials and methods used for this study was abstracted and paraphrased from the original report. Fenoxycarb (Ro 13-5223/000: purity unspecified) was obtained from Roche. The physical state and complete chemical composition of the test material were not supplied by Roche although the compound was stated to be stable at 20° C in daylight.

A total of 144 female Fu-Albino rats were obtained from the Institute of Biological and Medical Research, Fullinsdorf, Switzerland for this study. The rats were allowed to acclimate for three weeks prior to the initiation of mating. The age and weights of the rats at the time of receipt were not given in the report, however, according to the protocol, the rats were to be two to three months of age at the initiation of study. During acclimation and mating the females rats were housed two per cage in suspended

wire-mesh cages. During gestation and lactation they were housed in macrolon cages with dust-free wood shavings. They were housed two per cage except from the day of gestation 21 through lactation when the females were housed individually with their own litters. The ambient room temperature was maintained at approximately 22° C and the relative humidity was maintained at approximately 50 percent. The light cycle was maintained at 12 hours of light to 12 hours of darkness. Throughout the study, they were given free access to tap water contained in drinking bottles and a diet which was Nafag 850 cubes.

The breeding males for this study were a stock of male rats maintained at Hoffmann La-Roche. The male rats were changed on a twice yearly basis. Following the acclimation period, females were randomly selected and placed together with a single male overnight. The females were examined for the presence of a vaginal plug on the following morning. The date at which the vaginal plug was observed was designated day 1 of gestation. Dams with evidence of insemination were randomly assigned to treatment groups on day of gestation 1. Identification was apparently effected by means of color coding the animals with a number. The color code on the fur was made by picric acid, malachite green, methylene blue or eosin.

The dams were observed on a daily basis for general condition, signs of pharmacological action or toxicity, and changes in behavior. All changes were recorded. The dams were weighed at the beginning of the experiment on gestational day 1; daily during the treatment period (which included days 7-16); on gestational day 17; and again on gestational day 21.

Fenoxycarb was administered via gastric intubation to female rats on gestational days 7-16 in nominal dosages of 50, 150, or 500 mg per kg body weight. All dosages were maintained at a constant volume of 10 ml per kg. Batches of dosage solutions were prepared fresh every 10 days. The dosage fluid was a suspension of Fenoxycarb in a "standard solvent vehicle". The solvent vehicle was comprised of 4 percent carboxymethylcellulose, 0.9% sodium chloride, 0.5% benzylalcohol, and 0.4% Tween 80 in distilled water. Each daily dosage of Fenoxycarb was determined by the body weight of the subject dam on that day of gestation. Control dams received 10 ml per kg of the vehicle only. All pregnant or presumed pregnant rats which died prior to the time of sacrifice were necropsied to determine the cause of death.

On presumed gestational day 21 the surviving dams of each group were separated into two subgroups. The dams in one group were to be necropsied for standard teratological analysis and the dams in the

second group were to give birth to their litters and to rear the pups until weaning (which apparently occurred on day 21 of lactation). The teratology subgroup was determined by randomly selecting dams and sacrificing them until 15 successful litters were obtained. This means that in some cases more than 15 dams were sacrificed on gestational day 21 (given some dams were not pregnant) in order to obtain 15 litters for teratological analysis per dose group. The remaining surviving dams from each dose group comprised the rearing subgroup.

Animals in the teratology group were sacrificed by carbon dioxide (CO_2) asphyxiation. The abdominal cavity of each dam was laparotomized, and the uterus was exteriorized to examine its contents. The uterus was scored for the presence, sites, and numbers of implantations, resorptions (both early and late), and living and dead fetuses. The ovaries were examined and scored for number of corpora lutea. Individual fetuses were removed from the uterus, examined macroscopically and weighed. Approximately one-half of the fetuses from each litter were subjected to evaluation of osseous skeletal elements by the alizarin red S technique. The remaining one-half of the pups were fixed (presumably in Bouin's fluid) and subsequently examined by a modified free-hand razor sectioning technique (Barrow and Taylor, 1969).

Uteri from females which did not bear litters were examined for sites of possible resorptions. If none were found, the uteri were subjected to the ammonium disulfide staining method of Saleweski (1964) in order to determine the possible presence of occult implantation sites.

Dams which were not sacrificed on gestational day 21 made up the rearing subgroup. These dams were allowed to give birth spontaneously to their litters and to rear their young to weaning which occurred on either lactation day 21 or day 23. (Both dates were given in the text of the report.) Since the usual date for termination of weaning is day 21, MITRE has assumed that postnatal day 21 was the true end of the lactation period. Both the dams and their individual pups were weighed on postnatal days 1, 4, 12, and 21. On those days the size of the litter was also recorded. On postnatal day 21 all pups were examined externally, sacrificed, and discarded. Any external malformations or anatomic alterations were described and/or photographed. The mothers of those weanlings were also sacrificed on postnatal day 21. Their abdominal cavitities were opened and the uteri were examined for possible resorption sites.

Females in the rearing subgroups which did not give birth within the one week after their expected end of gestation were sacrificed. Their uteri were examined for possible resorptions and/or

implantation sites; in the event none were found, the uteri were subjected to the staining technique of Salewski as mentioned above.

The statistical evaluations utilized by the authors were limited to the t-test for independent samples and the Chi-square test.

RESULTS AND DISCUSSION

Prior to discussing the results of this study some comments concerning its method of execution are in order. This study was well-designed, well-executed, and well-documented. Several minor discrepancies were noted; at least a portion of these may be due to a problem in translation of the original document from German into English. For instance, the ages of the rats were not found in the English translation of the document; however, they were given in the Protocol which was appended and was written in German. Several other discrepancies such as the actual age of the weanlings at sacrifice (either 21 or 23 days) were also noted. The number of litters examined for teratology was limited to 15 as opposed to the usual number of 20 litters. However, due to the lack of deleterious findings in this study and the rather consistent findings across all groups, this deficiency is considered to be minor and does not weaken the conclusions which can be drawn from this study.

This study reported few clinical signs. Those which were reported dealt primarily with "slightly nervous" activity following

treatment of animals in the mid (150 mg/kg) and high (500 mg/kg) dose Fenoxycarb treated groups. Nervousness were observed in the high dose treated group from gestatio al day 11 through the end of treatment (on gestational day 16) and in the mid dose group from gestational day 13 through the end of treatment. The incidences of clinical signs were not given in this report; thus, the reader is left with merely a qualitative statement by the authors that such signs occurred, with no objective statement of the frequency with which they occurred. This makes it difficult to judge the biological significance of these findings.

A total of 5 animals died on test: Four of these animals (one each from the low and mid dose Fenoxycarb groups and two from the high dose) were killed by intubation errors. The remaining mortality was a mid dose treated female which died on gestational day 10 from unknown causes.

The mean body weights for pregnant rats for treated and control groups are depicted in Figure 1. This figure is reproduced from the original submission by Roche. Inspection of that figure discloses that the treatment with Fenoxycarb had virtually no effect on the mean maternal weights of animals during the period of treatment (days 7 through 16) or throughout the entire period of gestation. Table 1 presents the mean change in body weights for surviving dams treated

FIGURE 1

MEAN BODY WEIGHT (g) DURING GESTATION OF PREGNANT RATS WHICH WERE INTUBATED WITH VARIOUS DOSES OF FENOXYCARB FROM GESTATIONAL DAYS 7-16
(Reproduced from original report)

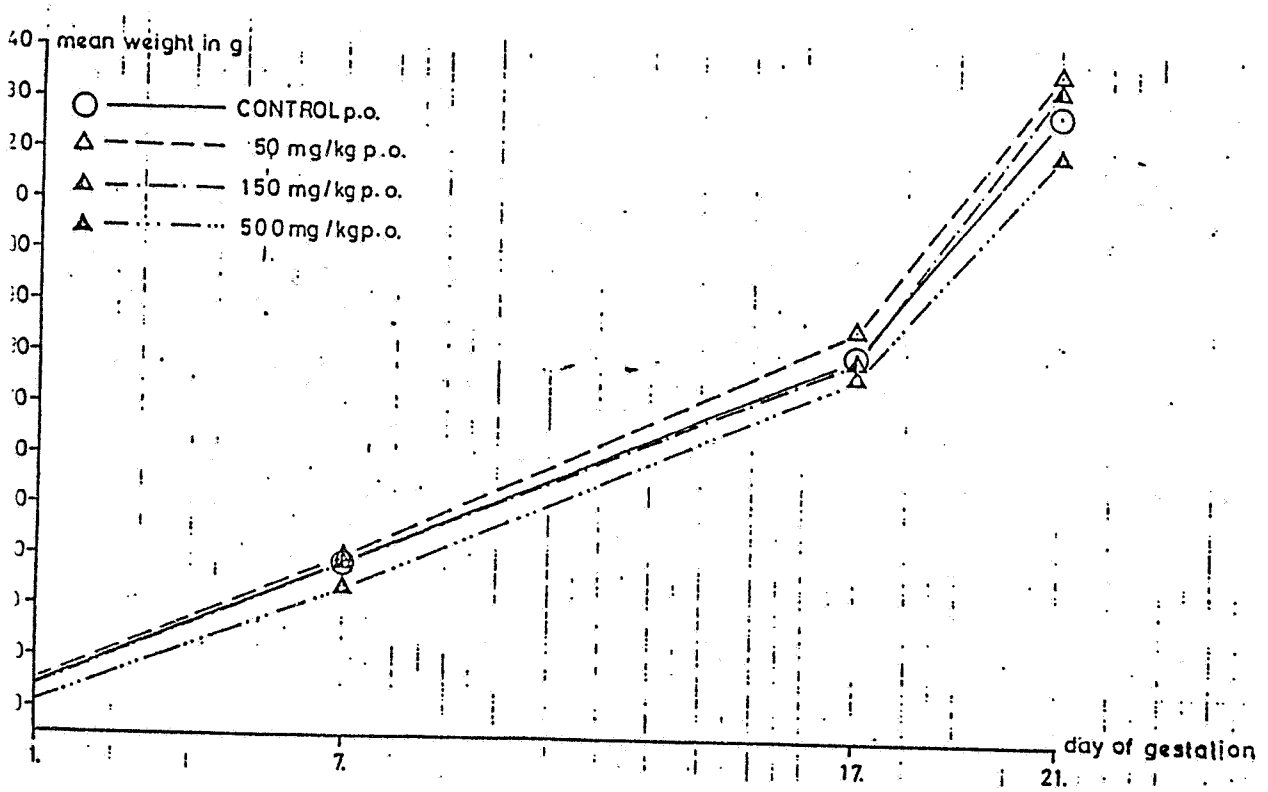

TABLE 1

MEAN INITIAL MATERNAL BODY WEIGHTS AND BODY WEIGHT CHANGES FOR PREGNANT RATS INTUBATED WITH VARIOUS DOSES OF FENOXYCARB DURING GESTATIONAL DAYS 7-16

Treatment	No. of Surviving Pregnant Dams	Mean Body Weight at day 1 (g)	Mean Change in Body Weight (g) for Surviving Pregnant Dams	
			Treatment (day 7-16)	Gestation (day 1-21)
Control	31	214	42	116
Fenoxycarb (mg/kg/day)				
50	31	215	47*	122
150	31	214	45	120
500	33	211	42	110

*Significantly different from control, $p \leq 0.05$ ('t'-test) (Authors' statistics.)

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with Fenoxycarb. Fenoxycarb caused no effect on the mean body weight change over the entire gestational period. The only significant difference from control values was seen in the body weight gain of the low dose Fenoxycarb treated group over the period of treatment (i.e. gestational day 7-16) which was greater than that of the controls. Thus, based upon the lack of effects of Fenoxycarb on maternal body weight, body weight gains, and the relatively few clinical signs observed, the maternal systemic NOEL is estimated to be 500 mg/kg/day (highest dose tested) under the conditions of this study.

Pertinent gestational data for control and Fenoxycarb treated litters are summarized in Table 2. Fenoxycarb treatment had no adverse effects on the incidence of fertilized animals in any of the treated groups, on the mean numbers of corpora lutea per litter, nor on the mean numbers of implantations per litter. Similarly, the implantation rate was not affected by Fenoxycarb treatment. Although the incidence of litters which exhibited resorptions did increase with increasing Fenoxycarb treatment, that increase was not found to be statistically significant and is not considered to be important biologically.

The embryotoxicity data for Fenoxycarb treatment are summarized

TABLE 2

GESTATIONAL DATA FOR PREGNANT RATS INTUBATED WITH VARIOUS DOSES
DOSES OF FENOXYCARB DURING GESTATIONAL DAYS 7-16

Treatment	No. Fertilized No. Inseminated (%)	Cases of Maternal Mortality		No. Pregnant Survivors	No. Litters Evaluated	Corpora Lutea		Implantations			No. Exh. Res. Par.
		Iatrogenic	Idiopathic			Total	Mean/ Litter	Total	Mean/ Litter	Rate (%)	
Control	31/36 (86)	0	0	31	15	214	14.3	204	13.6	95	
Fenoxycarb (mg/kg/day)											
50	32/36 (89)	1	0	31	15	225	15.0	218	14.5	97	
150	32/36 (89)	1	1	31	15	227	15.1	214	14.3	94	1(
500	35/36 (97)	2	0	33	15	222	14.8	218	14.5	98	1:

[a]Implantation Rate = (Total Implantations/Total Corpora Lutea) x 100.

in Table 3. Fenoxycarb treatment was not associated with any statistically significance differences between control and treated values for the number of dead fetuses, the percent of dead or resorbed fetuses, the mean number of fetuses per litter, mean fetal weights, nor the male/female sex ratios. However, some changes were noted in the incidence of resorptions, which were not reported in the original report by Roche. In particular, the number of early resorptions was significantly greater for the high dose treated group than for controls ($p \leq 0.02$ by Fisher's exact test performed by MITRE.) However, due to the large number of late resorptions among the control group (five) compared to only one late resorption in all three treated groups, the total number of resorptions for control and treated animals were not statistically significantly different. The authors reported no statistically significant change among the total resorptions of the animals because they apparently analyzed only total resorptions. Since there was a significant increase in early resorptions in the high dose treated group, that is considered to be the embryotoxic LEL.

There were very few malformations detected in this study. A total of 4 malformed fetuses were found, including one malformed pup in the control group. That control fetus exhibited both anasarca (generalized body edema) and "ectopia of the viscera". It should be noted that the authors did not state exactly what was meant

TABLE 3

EMBRYOTOXICITY DATA FOR OFFSPRING OF PREGNANT RATS INTUBATED WITH VARIOUS DOSES OF FENOXYCARB DURING GESTATIONAL DAYS 7-16

Treatment	Total Implantations	Resorptions			Dead Fetuses	Percent or Resorbed	Live Fetuses		Mean Fetal Weight (g)	No. Malformed Fetuses	Male/Fem Ratio of Fetuses (% male)
		Early	Late	Total			Total	Mean/ Litter			
Control	204	13	5	18	0	9	186	12.4	3.4	1[a]	75/111
Fenoxycarb (mg/kg/day)											
50	218	17	0	17	0	8	201	13.4	3.4	1[b]	101/100
150	214	14	0	14	0	6	200	13.3	3.5	0	102/98 (
500	218	30**	1	31	0	14	187	12.5	3.4	2[c]	95/92 (

**Statistically different from control $p \leq 0.02$ (x^2., Fisher's Exact Test) (Calculations performed by MITRE.)
[a]One fetus exhibited anasarca and "ectopia of the viscera".
[b]One fetus exhibited anasarca, cleft palate, and multiple skeletal malformations.
[c]One fetus exhibited fusion of sternebrae 4 and 5; a second fetus exhibited partial fusion of thoracic vertebrae 11 and

by ectopia of the viscera. The treated groups displayed a total of 3 malformed pups: one in the low dose and two in the high dose group. The low dose Fenoxycarb fetus displayed both anasarca and multiple skeletal abnormalities including cleft palate, short and bent limbs, and wavy ribs. The two malformed pups in the high dose Fenoxycarb group exhibited relatively minor skeletal anomalies. These consisted of one fetus which displayed a fusion between sternebrae 4 and 5 and a second fetus which exhibited partial fusion of thoracic vertebrae 11 and 12. It should also be noted that the authors did not list the two high dose treated fetuses as malformed fetuses either in their summary tables or in the summary of the study. This is an error which has been corrected by this MITRE report. Although MITRE's reclassification of the malformations has resulted in additional numbers of malformed pups among the treated groups, the malformations are not considered to be life-threatening nor do they impinge on the quality of life of the animals. It is concluded that the malformations observed in this study were not related to Fenoxycarb treatment.

Similarly, the anatomical variations which were listed by the authors were similar among controls and treated groups. These consisted primarily of partial or faulty ossification of the axial

skeleton including the cranium, the sternum, and certain areas of the sternebrae. The changes were distributed evenly among all groups and can not be associated with Fenoxycarb treatment. Thus, it appears that treatment with Fenoxycarb was not associated with teratogenesis in fetal rats.

The authors' report was internally inconsistent in terms of what the low effect level was for Fenoxycarb. Thus it is not clear whether MITRE is taking issue with the authors or not. For instance, on page 11 of the Roche report it is stated that the low effect level for Fenoxycarb was 150 mg/kg per day. However, on page 13 the authors state in their conclusion section that "it can be concluded that under the conditions of the present study Ro 13-5223/000 when applied orally to pregnant rats up to a dose of 500 mg/kg per day is neither embryotoxic, teratogenic, nor impairs the postnatal development of the offsprings." The authors also stated on page 11 on their report that "a slight but statistical (sic) insignificant increase of the number of embryonic resorptions was found in the 500 mg/kg necropsy subgroup. This increase does not exceed the range of our historical control data and cannot be confirmed by the resorption rate found in the comparable rearing subgroups. Therefore, this finding is regarded to be of no biological significance."

MITRE takes issue with those statements because the possibility of finding resorptions among the rearing subgroups at the end of the lactation period is not easily done and because the number of animals in the teratology subgroup which were necropied (15) is below the minimum number usually used for teratology studies in this country (20). Consequently, MITRE suggests that the embryotoxic lowest observed effect level (LEL) is 500 mg/kg/day based upon increased early resorptions; the NOEL is 150 mg/kg/day.

In addition to the standard teratologic analyses which were employed by Roche, a second portion of the animals in each treatment group was designated the rearing subgroup. The rearing subgroups were those animals remaining after sufficient sacrifices to attain 15 litters for teratological evaluation. Thus, there were 16 animals each in the control, low and mid-dose treated rearing subgroup and 18 animals remaining in the high-dose Fenoxycarb subgroup. The dams were each placed in an individual cage with nesting material and were allowed to litter and to rear their young to weaning. The rearing subgroup is a different population of animals, and are in addition to the animals which have already been described.

Survival data were gathered for these animals and reproductive indices were calculated by MITRE. Although the reproductive indices are similar to those which are found in the reproduction toxicity

tests, it must be stressed that this test differs from a standard reproduction toxicity test in that the exposure of the fetuses occurred only in utero during the stage of major organogenesis (gestational days 7-16).

The reproductive and survival data for the offspring of the rearing subgroups are summarized in Table 4. MITRE calculated 4 indices. These were:

$$\text{Gestation Index} = \frac{\text{no. live litters}}{\text{no. pregnant dams}} \times 100$$

$$\text{Viability Index} = \frac{\text{no. pups alive on day 4}}{\text{no. live born pups}} \times 100$$

$$\text{Lactation Index} = \frac{\text{no. pups alive at weaning}}{\text{no. pups alive on day 4}} \times 100$$

$$\text{Birth to Weaning Survival} = \frac{\text{no. pups alive at weaning}}{\text{no. live born pups}} \times 100$$

The gestation indices for all treatment and control groups were 100%; that is, all of the dams which were not sacrificed for the teratology experiment were pregnant and gave birth to live pups. Similarly, the lactation and birth to weaning survival indices were similar for treatment and control groups. However, the viability index for the 150 mg/kg group of Fenoxycarb animals was significantly smaller than either the control or the other treated groups ($p \leq .01$ Fisher's exact test). The meaning of this difference is not clear

TABLE 4

REPRODUCTIVE DATA FOR REARING SUBGROUPS AND SURVIVAL DATA FOR OFFSPRING OF RATS EXPOSED IN UTERO TO VARIOUS DOSES OF FENOXYCARB DURING GESTATIONAL DAYS 7-16

Treatment	Gestation Index[a] Incidence	%	Viability Index[b] Incidence	%	Lactation Index[c] Incidence	%	Birth to Weaning Survival[d] Incidence	%	Male:Female Ratio at Weaning (% male)
Control	16/16	100	201/203	99	190/201	94	190/203	94	94/96 (49)
Fenoxycarb (mg/kg/day)									
50[e]	16/16	100	193/199	97	183/193	95	183/199	92	89/94 (48)
150[e]	16/16	100	171/183**	93	166/171	97	166/183	91	91/75 (55)
500[e]	18/18	100	200/205	98	197/200	99	197/205	96	104/93 (53)

[a]Gestation Index = $\frac{\text{no. live litters}}{\text{no. pregnant dams}} \times 100$

[b]Viability Index = $\frac{\text{no. pups alive on day 4}}{\text{no. live born pups}} \times 100$

[c]Lactation Index = $\frac{\text{no. pups alive at weaning}}{\text{no. pups alive on day}} \times 100$

[d]Birth to Weaning Survival = $\frac{\text{no. pups alive at weaning}}{\text{no. live born pups}} \times 100$

[e] Fenoxycarb was administered to pregnant dams; pups were exposed in utero only.

**Survival of pups was significantly less than controls ($p \leq 0.01$ Fisher's Exact Test; calculated by MITRE).

and is probably not important biologically, because the birth to weaning survival of pups in that group was not statistically significantly smaller than the controls and because the survival of control pups from the day of birth to day 4 was extremely high (99%). In addition, there was no dose response among the Fenoxycarb groups and, thus, there was no corroborative indication of toxicity caused by Fenoxycarb. Furthermore, the male/female ratios of pups at weaning were not found to be different among the treated or control groups. The exact length of the lactation period in this study is difficult to assess because the authors reported that weaning occurred on postnatal day 23 (as depicted on Figure 2) in the text. However, in their tables of data, the survival rates for animals (at weaning) are given as day 21. Although it is not clear how long this period was, the results concerning survival do not appear to be affected since apparently no pups died between days 21 and 23.

Figure 2 depicts the growth of pups from birth until weaning in a pictorial fashion. This figure was reproduced from the Roche report. As can be seen by inspection of that figure, the growth of pups in both the treated and control groups were virtually identical throughout the period of weaning. Consequently, it can be concluded that the treatment of mothers with Fenoxycarb had no effect on the growth of their young after birth.

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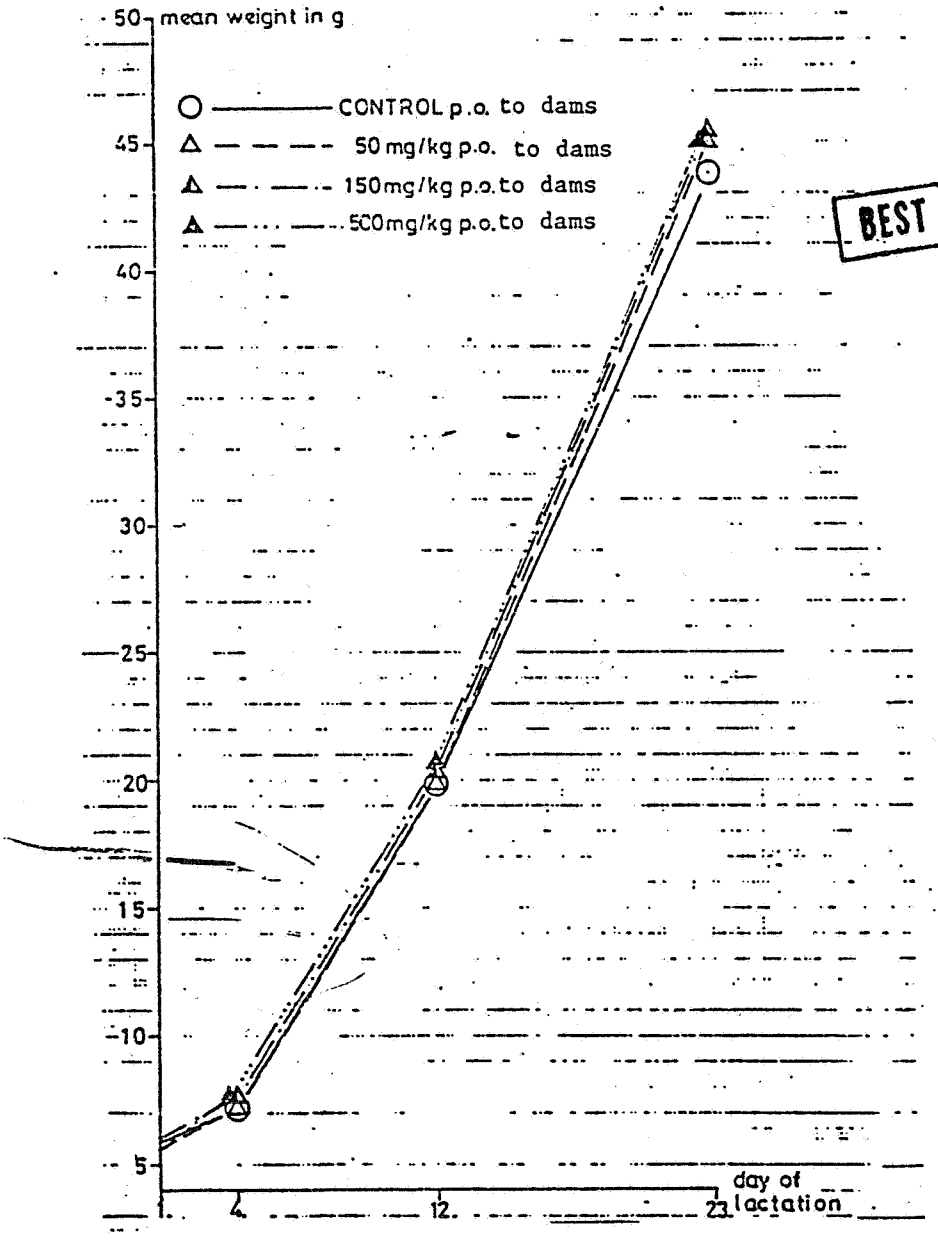
FIGURE 2

MEAN BODY WEIGHTS UNTIL WEANING (g) OF PUPS
EXPOSED IN UTERO TO VARIOUS DOSES OF FENOXYCARB
DURING GESTATIONAL DAYS 7-16
(Reproduced from the original report).



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CONCLUSIONS

In this teratology study, Fenoxycarb (ethyl[2-p (p-phenoxyphenoxy) ethyl] carbamate; Ro 13-5223/000; purity unspecified) was dissolved in a vehicle comprised of 4 percent carboxymethylcellulose, 0.9% sodium choloride, 0.5% benzylalcohol alcohol, 0.4% Tween 80 and distilled water and administered by gastic intubation to groups of 36 mated Fu-Albino rats at nominal dosages of 50, 150, 500 mg/kg per day on gestational days 7-16. Control dams received an equal volume (10 ml/kg) of the vehicle only. The animals' dosages were calculated daily based upon the body weight on each day of treatment. Few clinical signs were observed in any treatment group throughout gestation, and no statistically significant differences were observed among maternal body weights, body weight gains, numbers of corpora lutea, implantations, pregnancy rates, or implantation rates among all groups. Although five animals in treated groups died during the course of the treatment period, four of those deaths were due to intubation errors and only one (a dam from the mid-dose group) resulted from unknown causes. Based on these data and under the conditions of this study, the NOEL for maternal systemic toxicity for Fenoxycarb in pregnant rats is 500 mg/kg/day (highest dose tested).

Fenoxycarb treatment at 500 mg/kg per day was associated with statistically significant increases in early resorptions. No other

Fenoxycarb related adverse effects were seen in the pups in terms of reduction in mean fetal weights, increased incidences of malformations, or increased incidences of anatomical variations. The malformations observed in the Fenoxycarb-treated pups included one case of a fetus which had anascara cleft palate, and associated other skeletal defects, and two fetuses which exhibited minor skeletal malformations (one partially fused vertebrae; one case of fusion of the sternebrae). From these data the LEL for embryotoxicity caused by Fenoxycarb in rats under the conditions of this study was 500 mg/kg per day; the NOEL is 150 mg/kg per day. Fenoxycarb was not fetotoxic or teratogenic to rats under the conditions of this study.

CORE Classification: Conditional Core Minimum. It should also be noted that the purity of Fenoxycarb used in this study was not given. Consequently, an indication of any other ingredients or impurities/contaminants were not identified. That information should be requested from the registrant. The numbers of litters per group were less than the usually prescribed number of litters per teratology study (i.e. 15 versus 20). However, the overall scientific quality of the study was excellent, and the absence of serious adverse effects allows one to be comfortable with the conclusions.

* CONFIDENTIAL BUSINESS INFORMATION *

Study Type: Mutagenicity

Accession No.: Not assigned

MRID No.: Not assigned

Sponsor: Roche

Contracting Lab.: Roche

Date: 12 July 1983

Test Material: Fenoxycarb (Rol3-5223) Technical

No DER was prepared. The report consisted only of an internal memorandum justifying the different routes of application of Fenoxycarb and the positive control, Procarbazine in a micronucleus test in the mouse. Data for Procarbazine were presented. No data for Fenoxycarb were presented.

* CONFIDENTIAL BUSINESS INFORMATION *

CONFIDENTIAL BUSINESS INFORMATION

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Type of Study	:	Mutagenicity - Micronucleus Test Clarifying Data
Animal Tested	:	Mouse
Chemical Tested	:	Fenoxycarb (Ro 13-5223) Technical
Report Date	:	July 12, 1983
Report Number	:	Roche Internal Memo

CONFIDENTIAL BUSINESS INFORMATION

Name/Abt. Dr. A. Hugentobler PF/BP
Bau/Raum 61/436 Tel. 47 97

cc: Dr. Theiss
Dr. Hummler
Dr. Schüpbach

004

Betrifft Ro 13-5223/000

Dear Sirs,

Thank you for your letter of June 15th 1983 concerning the RCR 96 679 "In vivo mutagenicity evaluation of the IGR Ro 13-5223/000 with the micronucleus test in the mouse" (Dr. A. Hugentobler).

We do understand your doubts concerning the different routes of application of Ro 13-5223 and Procarbazine; however, for the following reasons we do not judge them to be a handicap for proving the sensitivity of the test system:

- Due to its high insolubility, Ro 13-5223 had to be applied orally to the test animals. Therefore we unfortunately cannot supplement the present data by giving Ro 13-5223 intraperitoneally to the mice. In addition we judge the oral application the appropriate one for an agrochemical test substance.

- Procarbazine, on the other hand, is a hydrophilic substance. For this reason it does not make sense to suspend it in an oily vehicle.

- From our point of view the positive control substance serves as a control of the test system, that is, it has to prove the sensitivity of the test animals. Its route of application is therefore of minor relevance as long as the values are within the range of previous data with the same positive control compound. In the test with Ro 13-5223 the results obtained with Procarbazine

TABLE 1: Füllinsdorf Albino Mice (SPF) given twofold application of Procarbazine 50 mg/kg dissolved in phosphate buffered saline.

Expt.	route of application	A: No of PCE with one or more micronuclei	mean value of A	Percentage of with one or mc micronuclei (mean value)
I(*)	intraperitoneal	64 55 107	75.3	3.8
	oral	101 89 78	89.3	4.5
II	oral	95 77 89 82	85.8	4.3
III	oral	96 94 64	85.0	4.3
IV	oral	61 83 77	73.7	3.7
V	oral	87 93 97	92.3	4.6

(*) in experiment I the results for the intraperitoneal and oral appli come from the same test

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clearly prove that the test animals are sensitive to substances inducing micronuclei in polychromatic erythrocytes.

- Neither in the OECD guidelines for testing of chemicals Draft 474, nor in the EEC Directive 79/831 of 18th September 1979 indications are made that the positive control substance has to be applied by the same route as the test substance.

- As a literature survey shows, Procarbazine has always been applied intraperitoneally (W.R. Bruce + J.A. Heddle, Can. J. Genet. Cytol. 21 (79) 319 / U. Kliesch et al., Mut. Res. 80 (81) 321 / D. Wild, Mut. Res. 56 (78) 319).

- In the meantime we have conducted studies to find out if Procarbazine is also active when it is applied orally. As Table 1 shows, there is no significant difference between the percentage of polychromatic erythrocytes with micronuclei resulting after intraperitoneal and oral application.

With these considerations in mind, we do not think that the different routes of application render our micronucleus test inadequate.

./.

A.L. Hugentobler

M. Schlyter

Basel 12.7.83