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Contractor	EXXON BIOMEDICAL SCIENCES INC		
Document Title	INITIAL SUBMISSION: LETTER FROM EXXON CHEMICAL CO TO USEPA RE REPRODUCTION TOXICITY STUDIES IN RATS WITH MRD-94-775, WITH ATTACHMENTS (FINAL REPORTS) AND DATED 10/16/1998		
Chemical Category	1,2-BENZENEDICARBOXYLIC ACID, DI-C9-11-BRANCHED ALKYL EST*		

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EXXON CHEMICAL COMPANY

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Safety and Environmental Affairs Department
David J. Johnson
MANAGER, SAFETY PROGRAMS

October 16, 1998



8EHQ-98-14300

Document Processing Center (7407)
Attn.: TSCA Section 8(e) Coordinator
Office of Pollution Prevention and Toxics
U. S. Environmental Protection Agency
401 M Street, S. W.
Washington, D. C. 20460-0001

Contains No CBI



8899000019

Re: Notification Under TSCA Section 8(e)

Dear Sir or Madam:

Under the provisions of Section 8(e) of the Toxic Substances Control Act, Exxon Chemical Company is submitting the following information describing the toxicity of a substance described as 1,2-Benzenedicarboxylic acid, di-C₉₋₁₁-branched alkyl esters, C₁₀-rich (CAS Registry Number 68515-49-1). This substance is currently being manufactured for commercial purposes as defined by TSCA.

The data presented in this submission are from a two-generation reproductive toxicity study in rats. The definitive two-generation study was preceded by a one-generation range-finding study. The results of this study are summarized in the attachment, with complete details in the enclosed reports.

In brief, the results of the study clearly demonstrate that the test substance is not a reproductive toxicant. However, the results of the definitive study also included small but statistically significant reductions in live birth and early survival indices among the offspring. These results were not observed in the range-finding study and were not consistent within the definitive study. As documented in the report for the definitive study, these findings were considered to have been the result of biological variation and not treatment related.

Since the time when the study was completed, we have continued to review these results in the context of a larger body of data. Although we continue to believe that the results observed at the lower two doses (160 and 320 mg/kg/day) are the result of biological variation, we have not been able to clearly rule out treatment related effects, in particular at the highest dose level tested (640 mg/kg/day).

It is our understanding from previous Section 8(e) guidance from EPA that reproductive effects at dose levels greater than 250 mg/kg/day are considered to be of low concern and should generally not be submitted under Section 8(e). It should be noted that estimated human exposures from commercial use of this substance are well below the dose levels tested in the study. Nevertheless, we are submitting these results at this time since additional research will be needed to confirm that the observed effects are not treatment related. We will provide the results of additional research as they become available.

In the context of this submission, we also note two companion FYI submissions that are relevant to a broader understanding of the findings of this Section 8(e) submission. The first of these is a report on a two-generation reproductive toxicity study on 1,2-Benzenedicarboxylic acid, di-C₈₋₁₁-branched alkyl esters, C₉-rich (CAS Registry

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Number 68515-48-0). This substance is very similar both in chemical structure and molecular weight to CAS Registry Number 68515-49-1. On average, the alkyl ester groups are one carbon smaller and the carbon number ranges overlap in comparison to the Section 8(e) substance. This study demonstrated that the lower molecular weight substance is also not a reproductive toxicant and no effects on live birth or survival indices were observed at any of the dose levels tested, up to 0.8%.

The second FYI submission is a report on a one-generation range-finding reproductive toxicity study on 1,2-Benzenedicarboxylic acid, di-2,4-dimethyl heptyl ester (a CAS Registry Number for this substance is not known). Although not a definitive study, the results indicate that this substance is also not a reproductive toxicant. Small, but statistically significant, decreases in live birth and early survival indices were observed only at the second highest dose tested (0.8%).

If you have any questions or need additional information, please feel free to contact me on (281) 870-6874.

Sincerely yours,



Steven G. Hentges

SGH/jad
Attachment/Enclosures

*Per Ed Gross, after talking with Exxon officials,
raw data consisting of tables and charts were not
included in these two studies.*

11/03/98

Summary of Reproductive Toxicity Results

1,2-Benzenedicarboxylic acid, di-C₉₋₁₁-branched alkyl esters, C₁₀-rich

(CAS Registry Number 68515-49-1)

A definitive two-generation reproductive toxicity study was conducted with 1,2-Benzenedicarboxylic acid, di-C₉₋₁₁-branched alkyl esters, C₁₀-rich (CAS Registry Number 68515-49-1). The definitive study was preceded by a range-finding study.

In the first generation, adult male and female CD rats (P1 animals, 30 per dose group) were given dietary preparations containing 0, 0.2, 0.4 or 0.8% of the test substance beginning 10 weeks prior to mating, during the mating phase, and throughout gestation and lactation in the females. The dose levels were based on the results of the range-finding study. The dietary percentages corresponded to daily dosages of approximately 0, 160, 320 and 640 mg/kg/day, which increased during the lactation period. The offspring from this mating (the first generation or F1 animals) were exposed to the test substance throughout their entire life cycle; from the mothers during gestation and lactation, and from the diet from weaning until they had mated and produced a second generation of offspring. The second generation offspring (F2 animals) also received the test substance from their mothers during gestation and lactation and then were terminated at weaning (postnatal day 21).

No changes in reproductive parameters and no pathological changes in any of the reproductive organs were observed in this study. Based on these results, it can be concluded that the test substance is not a reproductive toxicant.

In the range-finding study, there were no statistically significant differences in live birth or offspring survival indices between treated and control animals at any of the dose levels tested, up to 1.0%. However, lower body weights were observed in the offspring. Similar body weight effects have been previously noted for certain similar test substances⁽¹⁾ and may have been related to maternal stress, changes in the quality and quantity of milk, decreased milk consumption or possibly direct toxic action of the test substance.

In the definitive study, treatment-related decreases in live birth and day 4 survival indices were observed in the F1 offspring. In the high-dose group (0.8%), these decreases were statistically significantly different from the control and outside the historical control range of the laboratory. After postnatal day 4, offspring survival in all groups exposed to the test substance was equal to or greater than that in the control group. Also in the high dose group, there were statistically significant effects on parental body weight and reductions in pup weight. A literature search revealed that decreased offspring survival has also been observed when high doses (1000-2500 mg/kg/day) of a similar test substance were administered to lactating rat dams or pups in studies of similar but not identical design.⁽¹⁾⁽²⁾

In the second generation, there were decreases in postnatal day 1 and day 4 survival indices in the F2 animals that were statistically significant and outside of the historical control range at all dose levels. After postnatal day 4, offspring survival at the 0.2% and 0.4% dose levels was equivalent to that in the control group. In the 0.8% dose level, offspring survival was also significantly reduced on postnatal day 7 and during the lactation period as a whole (lactation index). The results at 0.8% were similar to those in the first generation in that there were also significant effects on parental weight and pup weights. However, there were no consistent weight effects in either the parents or offspring in the two lower dose groups. Thus, in contrast to the 0.8% group, there is no other evidence in the 0.2% and 0.4% groups to suggest that a toxic process is occurring. Further, there is a lack of consistency between the first and second generations in the results of the 0.2% and 0.4% groups.

⁽¹⁾ Dostal et al, *Tox. Appl. Pharm.* 87:81-90, 1987

⁽²⁾ Cimini et al, *Cell Mol. Biol.* 40:1063-1076, 1994

Maternal and Offspring Effects Observed in Reproductive Toxicity Studies

Table 1. Range-finding Study

Dose (% diet)	Maternal Effects				Offspring Effects			
	Body weight PPD 21 (% decrease)	Food cons. PPD 0-21 (% decrease)	Relative Liver weight (% increase)	Live Birth Index ^a (%)	Day 1 Survival Index ^b (%)	Day 4 Survival Index ^c (%)	Lactation index ^d (%)	Birth weight (% decrease)
0	-	-	ND	97.8	96.3	97.7	100.0	-
0.25	-	12	ND	100	94.2	95.1	100.0	3
0.5	1	18**	ND	99.1	99.1	98.2	100.0	8
0.75	12**	24**	ND	98.7	98.7	100.0	100.0	7
1.0	21**	40**	ND	99.1	98.2	96.4	98.4	14*

PPD - Postpartum day

ND - Not determined

* Percentage of pups born alive

^b Percentage of pups born alive surviving to postnatal day 1^c Percentage of pups born alive surviving to postnatal day 4^d Percentage of live pups on postnatal day 4 surviving to postnatal day 21

* p < 0.05

** p < 0.01

Table 2. Definitive Study

F1 Generation

Dose (% diet)	Maternal Effects			Offspring Effects				
	Body weight PPD 21 (% decrease)	Food cons. PPD 0-21 (% decrease)	Relative Liver weight (% increase)	Live Birth Index ^a (%)	Day 1 Survival Index ^b (%)	Day 4 Survival Index ^c (%)	Lactation Index ^d (%)	Birth weight (% decrease)
0	-	-	-	98.7	95.5	93.9	93.4	-
0.2	-	5	4	97.6	95.8	93.0	100.0**	-
0.4	1	3	20**	96.8	94.2	91.5	98.9*	1
0.8	6**	12**	28**	94.2**	92.2	88.8*	96.4	5*

F2 Generation

Dose (% diet)	Maternal Effects			Offspring Effects				
	Body weight PPD 21 (% decrease)	Food cons. PPD 0-21 (% decrease)	Relative Liver weight (% increase)	Live Birth Index ^a (%)	Day 1 Survival Index ^b (%)	Day 4 Survival Index ^c (%)	Lactation Index ^d (%)	Birth weight (% decrease)
0	-	-	-	98.5	96.6	94.0	98.7	-
0.2	-	-	11*	94.7*	92.1*	85.8**	100.0	2
0.4	-	2	13**	98.2	89.6**	86.7**	97.8	5
0.8	6	17**	28**	96.8	85.2**	77.6**	92.9*	8*

PPD - Postpartum day

^a Percentage of pups born alive

^b Percentage of pups born alive surviving to postnatal day 1

^c Percentage of pups born alive surviving to postnatal day 4

^d Percentage of live pups on postnatal day 4 surviving to postnatal day 21

* p < 0.05

** p < 0.01

A 08

1,2-benzene dicarboxylic acid, di-C9 to C11 branched alkyl esters, C10 rich

CAS # 68515-49-1

FINAL REPORT

PROJECT NUMBER: 177533A

TEST MATERIAL: MRD-94-775

REPRODUCTION TOXICITY
STUDY IN RATS WITH MRD-94-775

PERFORMED FOR:

EXXON CHEMICAL COMPANY
13501 Katy Freeway
Houston, Texas 77079
and
EXXON CHEMICAL INTERNATIONAL INC.
Boulevard Du Souverain 280
B-1160 Auderghem, Belgium

PERFORMED AT:

EXXON BIOMEDICAL SCIENCES, INC.
Toxicology Laboratory
Mettlers Road
CN 2350
East Millstone, New Jersey 08875-2350

COMPLETION DATE: TBD

Revised October 13, 1998

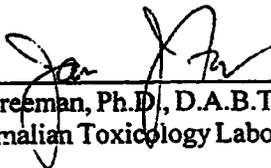
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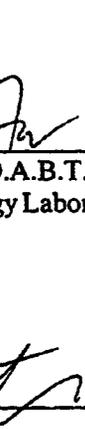
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APPROVAL SIGNATURES



J. J. Freeman, Ph.D., D.A.B.T.
Mammalian Toxicology Laboratory Director

13 Oct 98
Date



D. J. Letinski, M.S.
Analytical Chemistry Supervisor

13 Oct 98
Date

This study was conducted in compliance with the OECD Principles of Good Laboratory Practice and the E.C. Council Decision on Principles of Good Laboratory Practice.

This study was conducted in accordance with the EPA Good Laboratory Practice Standards set forth in 40 CFR Part 792 with the following exception:

- i. The stability, identity, strength, purity, and composition or other characteristics which appropriately identify the test material were determined prior to dose initiation. However, it is unknown if these analyses were performed in a GLP compliant manner. The test material was subsequently re-analyzed in a GLP compliant manner by the testing laboratory after dose initiation. The EPA Good Laboratory Practice Standards set forth in 40 CFR Part 792 require analyses of the test material prior to its use in a study.



G. W. Trimmer, B.A.
Study Director

13 Oct 98
Date

TWO GENERATION REPRODUCTION TOXICITY RANGE FINDING STUDY IN RATS
WITH MRD-94-775: 177533A

Revised October 13, 1998

QUALITY ASSURANCE STATEMENT

QUALITY ASSURANCE STATEMENT

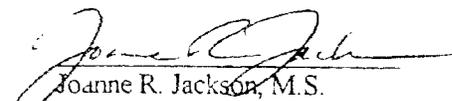
STUDY NUMBER: 177533A

TEST SUBSTANCE/ARTICLE: MRD-94-775

STUDY SPONSOR: Exxon Chemical Company, Exxon Chemical Europe Inc.

Listed below are the dates that this study was inspected by the Quality Assurance Unit of Exxon Biomedical Sciences, Inc. and the dates findings were reported to the Study Director and Management.

<u>Date(s) of Inspection</u>	<u>Reported to Study Director</u>	<u>Reported to Management</u>
25 May 94	25 May 94	03,09 Jun 94
09 Sep 94	12 Sep 94	15,22 Sep 94
15,16 Sep 94	19 Sep 94	22,26 Sep 94
10,18 Oct 94	18 Oct 94	25,30 Oct 94
27 Feb - 02 Apr 96	02 Apr 96	26 Jun 97
9,10,16 Apr 96	16 Apr 96	29 Apr,02 May 96
19,20,23 Jun 97	23 Jun 97	30 Jun 97


Joanne R. Jackson, M.S.
Quality Assurance Supervisor

13 Oct 98
Date

PERSONNEL

Study Director:	G. W. Trimmer, B.A.
Sponsor:	Exxon Chemical Company 13501 Katy Freeway Houston, Texas 77079 and Exxon Chemical International Inc. Boulevard Du Souverain 280 B-1160 Auderghem, Belgium
Sponsor Representative:	A. I. Nikiforov, Ph.D.
Mammalian Toxicology Laboratory Director:	J. J. Freeman, Ph.D., D.A.B.T.
Toxicology and Animal Care Supervisor:	R. C. Forgash, B.S.
Report Preparation Supervisor:	E. R. Frank, B.A.
Compound Preparation/Necropsy Supervisor:	M. A. Elliott, B.S.
Analytical Chemistry Supervisor:	D. J. Letinski, M.S.
Quality Assurance/Archives Supervisor:	J. R. Jackson, B.S.
Statistician:	M. J. Nicolich, Ph.D.
Maintenance Supervisor:	J. L. McGrath, A.S.
Veterinarian:	R. L. Harris, D.V.M.
Veterinary Pathologist:	C. F. Morris, D.V.M., D.A.C.V.P.

SUMMARY

This study was designed to provide general information in order to select dose levels for a subsequent definitive multigeneration study with MRD-94-775 in the rat.

Undiluted MRD-94-775 was blended in Certified Rodent Chow at a fixed concentration and was mixed thoroughly to assure homogeneity. The test material-diet admixtures were administered ad libitum to 10 rats/sex/group at 4 dosage levels. Group 1 served as a control and received carrier (Purina Certified Rodent Chow) only. Groups 2, 3, 4, and 5 received 0.25%, 0.5%, 0.75% and 1.0% of MRD-94-775 in feed, respectively. P1 males and females received test material daily for at least ten weeks prior to mating and during the mating period. Additionally, P1 female animals received test material during the gestation and postpartum periods, until weaning of the F1 offspring on Postpartum Day (PPD) 21.

Clinical inlife observations, body weight, and food consumption were recorded for all P1 animals at least weekly during the premating and mating periods (food consumption was not measured during mating due to cohabitation), and for females on Gestation Days (GD) 0, 7, 14, and 21 and on Postpartum Days (PPD) 0, 4, 7, 10, 14, and 21. Following birth, the offspring were counted and examined externally daily from Postnatal Day (PND) 0 to 21 and on PND 28. Offspring were sexed and weighed on PND 0, 1, 4, 7, 14, 21 and 28. P1 males were euthanized at the end of mating (Test Day 94), while females were euthanized following weaning of their litters on PPD 21 (Test Day 121 or 133). A gross necropsy was performed on all adult animals and on all animals which died during the study. A full macroscopic examination was performed on these animals.

All parental animals survived to scheduled terminal sacrifice and there were no clinical signs which were judged to be directly related to treatment with MRD-94-775. The majority of animals in all groups had no adverse clinical signs during the premating/mating, postmating, gestation, and/or postpartum periods.

Statistically or biologically significant lower mean body weight and corresponding suppression in body weight was observed in the 0.75% and 1.0% males compared with controls during the majority of the premating period. In the females, statistically significantly lower mean body weights compared with controls were observed in the 1.0% treated females during the gestation and postpartum periods and in the 0.75% treated females during the postpartum period.

SUMMARY (CONT'D)

Slight decreases in mean food consumption were observed in the 1.0% treated males and females during the majority of the pre-mating period. Additionally, statistically significant decreases in mean food consumption were observed in the 1.0% treated females compared with controls during gestation. During the postpartum interval, consistent decreases in mean food consumption were observed in the 0.5%, 0.75%, and 1.0% treated groups compared with controls. It is important to note that during the postpartum period test material consumption is substantially increased due to increased food consumption in the lactating dams. Thus, the animals in the treated groups are actually receiving higher doses than the dose rate at the end of the pre-mating period. This complicates interpretation of slight effects observed in lower dose groups during the postpartum period.

In general, the mean measured dose rate for the male and female animals during the pre-mating period decreased over time, as expected. This trend is characteristic of fixed concentration dietary studies since food consumption remains relatively constant, while body weight continues to increase over the course of the study. The measured dose rate for each group during pre-mating in mg/kg/day was as follows:

	Males	Females
0.25%:	264-132	257-165
0.5%:	521-262	484-335
0.75%:	776-414	709-503
1.0%:	1014-542	919-660

There were no gross postmortem findings judged to be related to treatment with MRD-94-775, and the majority of animals in all groups were free of observable abnormalities at postmortem examination.

There were no statistically significant differences in mean Male Mating, Male Fertility, Female Fertility, Female Fecundity, or Gestational Indices, or percentage of live/dead offspring, between treated and control animals.

SUMMARY (CONT'D)

There were no statistically significant differences in mean offspring survival indices between treated and control animals, no treatment-related clinical findings observed in the offspring of any group, and no gross postmortem findings in the F1 offspring judged to be related to treatment with MRD-94-775. However, lower body weights were observed in offspring. The mean body weight of the 1.0% treated male and female offspring was statistically significantly lower than controls during the majority of the postnatal weighing intervals and all values were lower than the laboratory historical control range. In the 0.75% dose males and females, there were statistically significantly lower mean body weight during the postnatal (PND) 14, 21, and 28 intervals, and the mean body weight was lower than the laboratory historical control range on PND 0 and 4. Similar effects have been previously noted for certain phthalates (Dostal et al., 1987) and may have been related to maternal stress, changes in the quality and quantity of milk, decreased milk consumption, and or possibly direct toxic actions of MRD-94-775.

Stability data indicated the test material was stable in feed at room temperature for at least 14 days. Characterization data demonstrated that the neat test material also was stable over the dosing period. Excellent uniformity of the test material in feed was demonstrated and the relative standard deviation ranged from 1.86% to 2.72%. Concentration verification analysis indicated that all dose samples were within 13% of the nominal values.

In conclusion, signs of toxicity were apparent at dose levels of 0.75% and 1.0%, and were observed in both the parental animals and offspring. Signs of toxicity in the parental animals included decreased body weight, suppression in body weight gain, and decreased food consumption. In the 0.5% dose group, adverse findings were limited primarily to decreases in food consumption compared to controls in the females during the postpartum period. Similarly, body weight reductions were observed in offspring of the 0.75% and 1.0% dose groups of both sexes. There also was slight evidence of growth retardation in the 0.5% offspring. The PND 0 and 4 weights were outside historical control range for this laboratory (although not statistically significantly less than controls) indicating possible growth retardation. Based on these results, 0.8% was selected as the high dose for the definitive two-generation reproduction toxicity study in rats with MRD-94-775. This dose was anticipated to produce signs of toxicity, primarily lower body weights, in the parental males but also in the females during gestation and lactation. Additionally, this dose was considered low enough to allow for sufficient survivorship in the F1 generation. A low dose of 0.2% was selected because it was expected to be a level without effect, particularly in the F2 generation. Finally, 0.4% was selected as the mid dose.

SUMMARY (CONT'D)

There were no statistically significant differences in mean offspring survival indices between treated and control animals, no treatment-related clinical findings observed in the offspring of any group, and no gross postmortem findings in the F1 offspring judged to be related to treatment with MRD-94-775. However, lower body weights were observed in offspring. The mean body weight of the 1.0% treated male and female offspring was statistically significantly lower than controls during the majority of the postnatal weighing intervals and all values were lower than the laboratory historical control range. In the 0.75% dose males and females, there were statistically significantly lower mean body weight during the postnatal (PND) 14, 21, and 28 intervals, and the mean body weight was lower than the laboratory historical control range on PND 0 and 4. Similar effects have been previously noted for certain phthalates (Dostal et al., 1987) and may have been related to maternal stress, changes in the quality and quantity of milk, decreased milk consumption, and or possibly direct toxic actions of MRD-94-775.

Stability data indicated the test material was stable in feed at room temperature for at least 14 days. Characterization data demonstrated that the neat test material also was stable over the dosing period. Excellent uniformity of the test material in feed was demonstrated and the relative standard deviation ranged from 1.86% to 2.72%. Concentration verification analysis indicated that all dose samples were within 13% of the nominal values.

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INTRODUCTION

This study was designed to provide general information in order to select dose levels for a subsequent definitive multigeneration study with MRD-94-775 in the rat.

This study was conducted for Exxon Chemical Company, 13501 Katy Freeway, Houston, Texas 77079 and Exxon Chemical International Inc., Boulevard Du Souverain 280, B-1160 Auderghem, Belgium (subsequently referred to as the Sponsor).

The study was conducted by Exxon Biomedical Sciences, Inc. (EBSI) Toxicology Laboratory (an American Association for Accreditation of Laboratory Animal Care accredited facility and a Japanese Ministry of Agriculture, Forestry, and Fisheries certified facility), Mettlers Road, CN 2350, East Millstone, New Jersey 08875-2350.

Study Initiation (Protocol Signature Date)

June 8, 1994

Inlife Test Period

June 13, 1994 to October 12, 1994

Justification for Selection of Test System

The rat is among the species of choice for reproduction and fertility testing according to the E.C. Dangerous Substances Directive (67/548/EEC), Annex V part B "Two-Generation Reproduction Toxicity Test", and the U.S. EPA test guidelines for reproduction and fertility effects (40 CFR, Part 798).

Justification of Dosing Route

The dietary route is an accepted route of administration according to E.C. Dangerous Substances Directive Annex V, and U.S. EPA TSCA regulations, and represents a likely route of human exposure.

MATERIALS AND METHODS

TEST MATERIAL

Material Identification

EBSI Identification:	MRD-94-775
Sponsor Identification:	
Supplier:	Exxon Chemical, Holland, BV
Date Received:	April 8, 1994
Expiration Date:	April 1999
Description:	Colorless liquid
Storage Condition:	Room temperature

The test material was assumed 100% pure for the purpose of dosing.

Characterization of the Test Material

Analyses for the stability, identity, strength, purity, and composition or other characteristics which will appropriately identify the test material were performed by the testing laboratory. This documentation is maintained at Exxon Biomedical Sciences, Inc. (EBSI) Toxicology Laboratory, Mettlers Road, CN 2350, East Millstone, New Jersey 08875-2350.

The methods of synthesis, fabrication, and/or derivation of the test material are the responsibility of the Sponsor. Documentation is maintained at Exxon Chemical International Inc., Machelen Chemical Technology Centre, Hermeslaan 2, B-1831 Machelen, Belgium.

TEST MATERIAL (CONT'D)

Analysis of Mixtures

The stability and homogeneity of the test material in feed was determined by the testing laboratory prior to and/or concurrent with dose initiation. Homogeneity was evaluated at two concentrations representing the lowest and highest levels expected during the study. Triplicate samples were collected from the top, middle and bottom of each preparation (nine samples in total). The concentration was the mean of all nine samples.

Stability was assessed by measuring the concentrations of selected samples from each of the homogeneity preparations after room temperature and refrigerated storage.

Concentrations of test material-diet blends were checked by the testing laboratory at least once a month in order to assure continuing accuracy in mixing diets.

Solubility

Not applicable to this study.

Sample Retention

One archival sample of the undiluted test material was collected by the Compound Preparation Department and stored at room temperature.

Carrier

Furina Certified Rodent Chow (5002 Meal)
Manufacturer: PMI Feeds, Inc.
Richmond, Indiana

TEST SYSTEM

Test Animal

Species: Rat
Strain/Stock: CrI:CD¹BR - VAF/Plus⁷
Supplier: Charles River Breeding Laboratories, Inc.
Kingston facility, Stony Ridge, New York.
Area: Males - K64; Females - K97

Animal Receipt Information:

Receipt Date: May 31, 1994
Purchase Order Number: 4GM38177R

Quarantine and Acclimation Period

13 days; animals were checked for viability at least once daily.

Number and Sex

P1 Males: 50 virgin
P1 Females: 50 virgin

Age at Initiation of Test Material Administration

P1 Males: Approximately 7 weeks
Approximate Date of Birth: April 26, 1994
P1 Females: Approximately 7 weeks
Approximate Date of Birth: April 26, 1994

Body Weight at Initiation of Test Material Administration

P1 Males: 214.9 to 248.9 grams
P1 Females: 158.6 to 191.9 grams

TEST SYSTEM (CONT'D)

Animal Identification

P1 Generation: Ear tags and corresponding cage identification.

Selection

More animals than were required for the conduct of this study were purchased and acclimated. The P1 population was selected by exclusion of animals from the quarantine population (determined by the attending veterinarian, Study Director, or his designee) because of poor health, outlying body weights, or abnormalities. The selected P1 population were allocated randomly to groups by a computer-generated randomization procedure to most nearly equalize initial group mean body weight.

Husbandry

Housing

Room Number: 521
Housing: Individually housed during the test period, except during the mating and postpartum periods.
Caging: Suspended stainless steel and wire mesh with absorbent paper below cages.

Feed

Purina Certified Rodent Chow (5002 meal), ad libitum
Manufacturer: PMI Feeds Inc.
Richmond, Indiana
Analysis: Performed by PMI Feeds Inc. Copies of the feed analyses are maintained in the EBSI Toxicology Laboratory.
Contaminants: There were no known contaminants in the feed believed to have been present at levels that may have interfered with this study.

The availability of feed was checked daily for all animals.

TEST SYSTEM (CONT'D)

Water

Automatic watering system, ad libitum

Supplier: Elizabethtown Water Company
Bound Brook, New Jersey.

Analysis: As provided by Elizabethtown Water Company. Copies of the water analyses are maintained in the EBSI Toxicology Laboratory.

Contaminants: There were no known contaminants in the water believed to have been present at levels that may have interfered with this study.

The availability of water was checked daily for all animals.

Bedding (Direct)

CELLU-Dri

Manufacturer: Shepherd Specialty Papers, Inc.

Analysis: Provided by the Manufacture. Copies of the analyses are maintained in the EBSI Toxicology Laboratory.

Contaminants: There were no known contaminants in the bedding believed to have been present at levels that may have interfered with this study.

Near parturition (Day 20 of gestation) and during the postpartum period, mated females were provided with clean bedding as necessary, usually every two days.

Environmental Conditions

Temperature range: 68 to 76 degrees Fahrenheit

Humidity range: 40 to 70 percent relative humidity

Lighting: Approximately 12 hours light (0700-1900 hours) and 12 hours dark (1900-0700 hours) by automatic timer.

Monitored at least once daily.

B 09

**TWO GENERATION REPRODUCTION TOXICITY RANGE FINDING STUDY IN RATS
WITH MRD-94-775: 177533A**

Revised October 13, 1998

EXPERIMENTAL DESIGN

Preparation of Animals

No special preparation of the animals was required prior to dose initiation.

Preparation of Test Material

Mixing of feed: The basal diet consisted of Certified Rodent Chow (5002 Meal). The test material was incorporated into the feed and mixed thoroughly to assure homogeneity. The test-material diet admixtures were prepared as fixed concentrations of test material.

Fresh diets were prepared weekly. Prepared diets were covered and stored at room temperature following dispensing.

Diets were prepared at the following target concentrations, not to exceed stability data on the test material-dietary admixtures.

Experimental Groups

Group Number	TARGET CONCENTRATION (in percent)	Number of P1 Females	Number of P1 Males
1 (Control)	0	10	10
2	0.25	10	10
3	0.50	10	10
4	0.75	10	10
5	1.0	10	10

B 10

TWO GENERATION REPRODUCTION TOXICITY RANGE FINDING STUDY IN RATS
WITH MRD-94-775: 177533A

Revised October 13, 1998

EXPERIMENTAL DESIGN (CONT'D)

Administration of Test Material

The homogeneous blend of the test material, prepared as a mixture in Certified Rodent Chow (5002 meal), was offered ad libitum to the treated rats of Groups 2, 3, 4 and 5. Control rats (Group 1) received Certified Rodent Chow (5002 meal) ad libitum only. Feed jars containing diet were replaced at least once each week. Animals had access to the test or control feeders until the day of scheduled euthanasia.

The dosing regimen for all groups proceeded as follows: P1 males were dosed for at least 10 weeks prior to mating and through the mating period for F1 litters. P1 females were dosed for at least 10 weeks prior to mating, during the mating and gestation periods, and until they were euthanized following weaning of the F1 animals on PPD 21.

Experimental Evaluation

Inlife Procedures:

All animals were examined for viability at least once a day.

Male body weight was measured prior to P1 selection, on the first day of dosing (Day 0), and at least weekly thereafter until euthanized. Female body weight was measured prior to P1 selection, on the first day of dosing and at least weekly thereafter until confirmation of mating, then on Gestation Days (GD) 0, 7, 14, and 21 and on Postpartum Days (PPD) 0, 4, 7, 10, 14, and 21, and/or at least weekly until euthanized.

Food consumption was measured concurrently with body weight during the test period, except that food consumption was not measured during mating or following weaning of the F1 litters.

A clinical examination was given to each male prior to P1 selection, on the first day of dosing, and at least weekly thereafter until euthanized. Females received a clinical examination prior to P1 selection, on the first day of dosing, and at least weekly thereafter until confirmation of mating, then on GD 0, 7, 14, and 21, and on PPD 0, 4, 7, 10, 14, and 21.

EXPERIMENTAL DESIGN (CONT'D)**Mating:**

The P1 mating period began after at least 10 weeks of P1 dosing and ended when all females were confirmed mated or approximately 3 weeks had elapsed. Each P1 male was assigned randomly (using animal reference numbers and a random numbers table) to be paired continuously with one P1 female of the same dose group to produce the F1 generation.

Mating was confirmed the morning following overnight pairing by observation of a copulatory plug (vaginal) and/or by the presence of sperm in a vaginal rinse. The day on which mating was confirmed was the female's Day 0 of gestation (GD 0). After confirmation of mating, each animal was returned to its own cage.

On GD 20, mated females were placed in clean cages fitted with a stainless steel litter pan and provided with fresh bedding material. Beginning on GD 21, mated females were examined at least twice daily for signs of parturition.

If a female was not confirmed mated, a litter pan was provided after examination on the last scheduled day of mating. Non-confirmed mated females were examined at least twice daily for signs of parturition after insertion of litter pans in their cages. One unconfirmed mated female was noted with red vaginal material approximately 2 weeks after overnight pairing. For husbandry purposes, this female was assumed to be at GD 14 and observations, body weight, and food consumption measurements were performed accordingly. These data are presented in the individual tables, however they are not included in gestation incidence or mean tables.

Postnatal Experimental Evaluation

Dams were allowed to give birth. The duration of gestation was calculated and any difficulties occurring at parturition were noted. The date of parturition was recorded as the dam's Postpartum Day 0 (PPD 0).

Each morning and afternoon during the postnatal period, the litters were checked for dead offspring and unusual conditions, and the dams were examined for viability, nesting, and nursing behavior.

Dead pups were removed from the litter immediately after their discovery. If intact, dead pups were examined externally and internally for anomalies. Dead pups discovered on PND 0 also were examined internally to determine whether they were stillborn.

EXPERIMENTAL DESIGN (CONT'D)

Postnatal Experimental Evaluation (cont'd)

On PND 0, 1, 4, 7, 14, 21, and 28 the offspring were counted, sexed, and each live pup was weighed. Pups were counted and examined externally on a daily basis during the postnatal period.

On PND 4, after counting, weighing, and examining the pups, the size of each litter was adjusted by eliminating extra pups by random selection to yield, as nearly as possible, 4 males and 4 females per litter. Partial adjustment (e.g., 5 males and 3 females) was permitted whenever there were not enough pups to obtain 4 per sex per litter. Litters of eight pups or less were not adjusted.

Culled pups were euthanized. Culled pups that appeared normal received only an external examination and tissues were not saved. Culled pups that appeared abnormal were subjected to a visceral examination.

At weaning (PND 21), the surviving neonates in each litter were group housed by sex until sacrificed on PND 28. On PND 28, after counting, weighing, and examining the neonates externally, all surviving F1 rats were sacrificed and discarded.

Study Termination

Euthanasia was by CO₂ asphyxiation and exsanguination.

P1 males selected for mating were euthanized at the end of the mating period (Test Day 94). P1 females were euthanized after weaning of the F1 litters on PPD 21 (Test Day 121 or 133). Confirmed mated P1 females which did not give birth by presumed GD 26 or those females which had not been confirmed mated and did not give birth by 26 days after completion of the mating period, were euthanized and received gross necropsies. Special attention was paid to the reproduction system.

F1 litters were euthanized on PND 28.

EXPERIMENTAL DESIGN (CONT'D)**Necropsy:**

Gross necropsies were performed on all adult animals. Body weight was recorded on the day of necropsy. The uterus of each female used for mating, but failing to deliver, was examined grossly for evidence of implantations and these data were recorded.

All F1 pups that died were subjected to a visceral examination. Abnormal tissues were not preserved as per the discretion of the Study Director.

Abnormal tissues were preserved in 10% neutral buffered formalin at the discretion of the Study Director.

Records

A copy of the protocol, final report, raw data, computer generated listings of raw data, supporting documentation, specimens, and one sample of the test material are maintained in the Archives of the EBSI Toxicology Laboratory.

EXPERIMENTAL DESIGN (CONT'D)

Statistical Analyses

The following statistical methods were employed, where appropriate.

I. Continuous data were tested for statistical significance as follows:

Bartlett's test of homogeneity of variance was used to determine if the groups have equivalent variances at the 1% level of significance (Snedecor and Cochran, 1989).

If the variances were equivalent, the hypothesis that there was no difference in response between the groups was tested using a standard one-way analysis of variance (Snedecor and Cochran, 1989). If the ANOVA was significant, Dunnett's test was performed to determine which treated groups differed from control (Dunnett, 1964). A linear regression to test for a dose response also was performed and tested for lack of fit (Snedecor and Cochran, 1989). All tests were reported at the 5% or 1% level of significance.

If the variances were not equivalent, then a Kruskal-Wallis (non-parametric) test was performed to determine if the treatment effects are equivalent (Hollander and Wolfe, 1973). If there was a difference, Dunn's Rank Sum comparison was used to determine which treatment groups differed from control (Hollander and Wolfe, 1973). Jonckheere's test for ordered response also was performed (Hollander and Wolfe, 1973). All tests were reported at the 5% or 1% level of significance.

II. Pup weight was analyzed by a standard nested analysis of covariance (Snedecor and Cochran, 1989) with pups nested within dams and with dams nested within doses, and litter size (both sexes combined) as the covariate. If differences in groups were identified, the Least Significant Difference (LSD) technique was used to determine which groups differed from the control group (Snedecor and Cochran, 1989). Male and female pups were tested separately (the covariate was combined sexes in each analysis). All tests were reported at the 5% or 1% level of significance.

EXPERIMENTAL DESIGN (CONT'D)**III. Parental reproductive and offspring survival incidence data were analyzed for statistical significance as follows:**

First, a standard chi-square analysis was performed to determine if the proportions of incidences differed between the groups tested (Snedecor and Cochran, 1989). In keeping with standard statistical practice, if any one cell had an expected value less than 5, this step was not reported. Next, each treatment group was compared to the control group using a 2 x 2 Fisher Exact test (Bradley, 1968). Thirdly, Armitage's test for linear trend in the dosage groups was performed (Snedecor and Cochran, 1989). All tests were reported at the 5% or 1% level of significance.

RESULTS

P1 GENERATION - PARENTAL FINDINGS

1. PARENTAL CLINICAL INLIFE OBSERVATIONS - P1

Incidence of Inlife Observations: Tables 1-3

Individual Inlife Observations: Appendices A-C

There were no clinical signs which were judged to be directly related to treatment with MRD-94-775. The majority of animals in all groups had no adverse clinical signs during the premating/mating, postmating, gestation, and/or postpartum periods.

There was a very low incidence of incidental findings observed in the male and/or female animals in one or more groups during the premating/mating, postmating, gestation, and/or postpartum periods. These findings included dental abnormalities, scabs/sores, soft stool, alopecia, and/or ocular discharge. These findings were considered incidental and unrelated to treatment with MRD-94-775.

2. PARENTAL SURVIVAL - P1

Summary of Survival: Table 4

Individual Survival Data: Appendix D

All parental animals in all groups survived to scheduled termination.

RESULTS (CONT'D)**3. PARENTAL BODY WEIGHT AND BODY WEIGHT CHANGE - P1**

Mean Body Weight and Body Weight Change: Tables 5-7

Individual Body Weight and/or Body Weight Change: Appendices E-G

There were statistically significant lower mean body weights compared with controls in the 0.75% dose males (6-11%) and 1.0% dose males (7-13%) from Day 21 through Day 70 during the pre-mating period. These lower body weights occurred in a dose-related manner. Similarly, there was statistically significant suppression in body weight gain in the 0.75% and 1.0% dose males during the Day 7/14, 14/21, and 28/35 intervals, and in the 1.0% dose males during the Day 42/49 interval compared with controls. Suppression in body weight gain also was observed during the remaining pre-mating intervals in the 0.75% and 1.0% dose males, however these differences were not statistically significant.

In the females, there were no biologically significant changes in mean body weight or body weight change between treated and control animals. A statistically significant increase in mean body weight was observed in the 0.25% dose females when compared with controls at the Day 21 pre-mating interval. This single occurrence was considered spurious and unrelated to treatment. There were several differences, both increases and decreases, in mean body weight gain between treated and control animals observed sporadically during pre-mating. In the absence of a consistent pattern of response, these differences were considered incidental and unrelated to treatment with MRD-94-775.

During gestation, the mean body weight of the 1.0% dose females was statistically significantly lower (10-11%) than controls at the GD 7, 14, and 21 intervals. Similarly, the mean body weight over the entire gestation period (GD 0-21) was lower than controls (14%), although this difference was not statistically significant. These lower body weights are due, at least in part, to the slightly lower body weights observed during the pre-mating period which were evident at the start of gestation. There were no statistically significant differences in mean gestation body weight change between treated and control animals at any interval.

During the postpartum period, there was a dose-related decrease in body weight. Statistically significantly lower body weights were observed in the 1.0% dose females (9-23%) at every body weight interval and in the 0.75% dose females (12-14%) at every body weight interval except for PPD 0 when compared with controls. Statistically significant body weight gain suppression and/or body weight loss was observed in all treated groups at the PPD 0-4 interval, in the 1.0% dose females at the PPD 4-7 interval, and in the 0.75% and 1.0% dose females over the entire postpartum interval (PPD 0-21).

RESULTS (CONT'D)**4. PARENTAL FOOD CONSUMPTION - P1**

Mean Food Consumption: Tables 8-10

Individual Food Consumption: Appendices H-J

During the pre-mating period, dose-related decreases in mean food consumption were observed in male animals during the majority (Weeks 2-10) of the pre-mating intervals and lower mean food consumption values compared with controls were observed in the 1.0% dose males during the majority of the study, although differences from controls were statistically significant only during Weeks 3 (8%), 5 (10%), and 9 (11%).

In the females, there were statistically significant lower food consumption in the 0.5% (9%), 0.75% (8.5%), and 1.0% (15%) dose groups during Week 7 and in 1.0% dose group (13%) during Week 9 compared with controls. Lower mean food consumption compared with controls was observed in 1.0% dose females during the remaining pre-mating intervals. However, these differences were not statistically significant.

During gestation, dose-related decreases in mean food consumption were observed in the female animals during the GD 0-7 and GD 7-14 intervals, as well as for the overall gestation period (GD 0-21). Statistically significant lower mean food consumption values when compared with controls were observed in the 1.0% dose females during the GD 0-7 (18%) and GD 7-14 (18%) intervals, as well as the overall gestation period (17%).

During the postpartum period, dose-related decreases in mean food consumption were observed in female animals at every interval, including the overall postpartum period (PPD 0-21). Statistically significant lower mean food consumption values when compared with controls were observed in the 0.5% dose females at the PPD 0-4, 7-10, 10-14 and 14-21 intervals (16-30%), in the 0.75% dose females at the PPD 4-7, 7-10, 10-14 and 14-21 intervals (21-25%), and in the 1.0% dose females at every postpartum interval (37-41%). For the overall postpartum interval (PPD 0-21), statistically significant lower mean food consumption when compared with controls was observed in 0.5% (18%), 0.75% (24%) and 1.0% (40%) dose groups.

In the 0.25% dose females, statistically significant lower mean food consumption values compared with controls also were observed. However, these significant decreases were limited to the PPD 0-4 and PPD 4-7 intervals (18-28%). Mean food consumption during the overall postpartum interval (PPD 0-21) was not statistically significant lower than controls. Thus, the decreases observed in the 0.25% group during the early postpartum period were not considered biologically significant.

RESULTS (CONT'D)

5. PARENTAL MEASURED DOSE RATE - P1

Mean Measured Dose Rate: Table 11

Individual Measured Dose Rate: Appendix K

In general, the mean measured dose rate for the male and female animals during the pre-mating period decreased over time, as expected. This trend is characteristic of fixed concentration dietary studies, since food consumption remains relatively constant, while body weight continues to increase over the course of the study.

The measured dose rate during pre-mating in MG/KG/DAY was as follows:

DOSE (%)	WEEK 1 (mg/kg/day)	WEEKS 2-9 (mg/kg/day)	WEEK 10 (mg/kg/day)
MALES: 0.25	264.4	227.9-135.3	131.7
MALES: 0.5	521.3	445.9-269.0	262.4
MALES: 0.75	776.1	668.3-421.3	413.9
MALES: 1.0	1013.8	875.7-546.9	542.0
FEMALES: 0.25	257.3	231.0-172.2	165.0
FEMALES: 0.5	484.0	434.8-342.3	335.4
FEMALES: 0.75	708.9	674.0-502.8	520.9
FEMALES: 1.0	908.4	918.7-659.5	688.1

Mean measured dose rate was not calculated for the female animals during gestation or lactation due to the great variation in number of animals gestating/lactating at any particular time. However, mean measured dose rate during gestation was most likely similar to Week 10 of the pre-mating period, and slightly decreased at the end of gestation since maternal body weights significantly increase (although food consumption remains relatively constant) during the latter part of gestation.

During the postpartum period, mean measured dose rate most likely exceeded the Week 10 pre-mating values. This results from the steady increase in food consumption during the postpartum period, while body weights remain relatively constant. Food consumption measurements during the final week of the postpartum period is confounded since the pups begin eating from the dams feeder during this time.

RESULTS (CONT'D)**6. PARENTAL GROSS POSTMORTEM OBSERVATIONS - P1**

Incidence of Gross Postmortem Observations: Table 12
Individual Gross Postmortem Observations: Appendix L

There were no gross postmortem findings judged to be related to treatment with MRD-94-775.

The majority of animals in all groups were free of observable abnormalities at postmortem examination. There were single or low occurrences of dilated renal pelvis, scabs, dental abnormalities, alopecia, distended uterus, and/or dry red material around the eye. In addition, three females had intestinal parasites presumed to be pinworms. The source of the parasites was unknown. These findings were considered incidental and unrelated to treatment.

7. REPRODUCTION INDICES - P1

Summary of Reproduction Data: Table 13
Individual Reproduction Data: Appendix M

There were no statistically significant differences in mean Male Fertility, Male Mating, Female Fertility, Female Fecundity, or Female Gestational Indices between treated and control animals. However, the mean Male Fertility (60%) and Female Fecundity (71.4%) indices of the 0.75% dose group was substantially lower than controls, lower than all other treated groups, and lower than the historical control range for this laboratory (Male Fertility 66.7-100%; Female Fecundity 72.2-100%). In the absence of a clear dose response or statistical significance, the biological importance of this finding is questionable.

Mean days of gestation of the treated and control groups were essentially equivalent.

There were no statistically significant differences in the mean percentage of live and dead offspring or in the sex ratio of the treated offspring compared with controls.

One control female, three 0.25% dose females, one 0.5% dose female, four 0.75% dose females, and two 1.0% dose females were not pregnant.

RESULTS (CONT'D)**F1 GENERATION - OFFSPRING FINDINGS****8. OFFSPRING SURVIVAL - F1**

Summary of Offspring Survival: Table 14

Individual Offspring Survival Data: Appendix N

There were no statistically significant differences between treated and control offspring in any of the offspring survival indices.

9. OFFSPRING CLINICAL INLIFE OBSERVATIONS - F1

Incidence of Offspring Inlife Observations: Table 15

Individual Offspring Inlife Observations: Appendix O

There were no treatment-related clinical findings observed in the offspring of any group. The majority of offspring in all groups were free of observable abnormalities from PND 0-21. Some offspring across most groups were observed without milk in their stomachs, during the first week of the postnatal period, with the highest incidence occurring on PND 0.

Single or low incidences of lacerations, scabs, alopecia, truncated tail, and/or missing limb were observed in one or more groups. These findings were considered incidental and unrelated to treatment.

10. OFFSPRING BODY WEIGHT - F1

Mean Offspring Body Weight: Table 16

Individual Offspring Body Weight: Appendix P

Statistically significantly lower mean body weights compared with controls were observed in the treated offspring of the 0.75% and 1.0% dose groups. In the male offspring, statistically significant lower mean body weight was observed in 0.75% dose group on PND 14 (19%), PND 21 (20%), and PND 28 (15%), and in the 1.0% dose group on PND 0 (15%), PND 4 (22%), PND 7 (28%), PND 14 (35%), PND 21 (39%), and PND 28 (35%). Similarly, in the female offspring, statistically significant lower mean body weight was observed in the 0.75% dose group on PND 14 (21%), PND 21 (21%) and PND 28 (17%), and in the 1.0% dose group on PND 0 (12%), PND 7 (27%), PND 14 (36%), PND 21 (39%) and PND 28 (32%). These lower mean body weights were considered treatment-related.

RESULTS (CONT'D)

10. Offspring body weights (cont'd)

There also were statistically significant lower mean body weights in the 0.5% dose males and females on PND 14 (14% and 12%, respectively) and 21 (14% and 12%, respectively). However, these values were essentially within the historical control range of this laboratory and thus were considered incidental. However, the PND 0 and 4 weights for both males and females were outside the historical control range for this laboratory, although not statistically significantly less than controls. This may be indicative of slight growth retardation.

11. OFFSPRING GROSS POSTMORTEM OBSERVATIONS - F1

Incidence of Offspring Gross Postmortem Observations: Table 17
Individual Offspring Gross Postmortem Observations: Appendix Q

The majority of animals which died prior to scheduled termination (PND 0-20) were free of observable abnormalities or were too autolyzed/cannibalized to be examined at the necropsy. There were single incidences of dilated renal pelves, scabs, emaciated, anogenital staining, and/or apparent anophthalmia/anencephaly/protruding tongue. Two pups in the control group were stillborn and two pups in the 1.0% group were emaciated. Due to the very low incidence of findings, all were considered incidental and unrelated to treatment with the test material.

12. ANALYTICAL CHEMISTRY

Analytical Chemistry Report: Appendix R

Excellent uniformity was observed. The relative standard deviation (RSD) ranged from 1.86 to 2.27%. Stability data indicated the test material was stable in feed at ambient temperature for at least 14 days. Concentration verification analysis indicated that all feed samples were within 13% of the nominal concentrations. Characterization data obtained prior to and after dosing was completed demonstrated the neat test material was stable.

DISCUSSION

Administration of MRD-94-775 via diet at graded doses to one generation of CrI:CD⁷BR rats was associated with signs of overt toxicity at dose levels of 0.75% and 1.0%. There was slight evidence of adverse effects at the 0.5% dose level. Both parental animals and offspring were affected.

Overt signs of toxicity in the parental animals included decreased body weight, body weight gain suppression, and decreased food consumption in both the male and female animals. Statistically significant decreases in body weight and/or food consumption from controls were generally limited to the 0.75% and 1.0% dose males and females, although significant decreases from controls also were observed sporadically during the test period in the 0.5% dose animals.

In the 0.25% dose animals, noted differences were limited to slight suppression in body weight gain during the first postpartum interval and decreased food consumption at the first two intervals during the postpartum period, compared with controls. However, the mean body weight change and mean food consumption of the 0.25% dose females during the overall postpartum interval (PPD 0-21) were not statistically different from controls. Thus, these small decreases in mean body weight gain and food consumption of the 0.25% dose females during postpartum were not considered biologically significant.

It is important to note that during the postpartum period test material consumption is substantially increased due to increased food consumption in the lactating dams. Thus, the animals in the treated groups are actually receiving higher doses than the dose rate at the end of the pre-mating period. This complicates interpretation of slight effects observed in lower dose groups during the postpartum period.

The mean Male Fertility (60%) and Female Fecundity (71.4%) indices of the 0.75% dose group was substantially lower than controls, lower than all other treated groups, and lower than the historical control range for this laboratory (Male Fertility 66.7-100%; Female Fecundity 72.2-100%). In the absence of a clear dose response or statistical significance, the biological importance of this finding is questionable. Additionally, the small number of animals (10/sex/group) makes interpretation of this finding difficult. Organs were not weighed and microscopic evaluations were not performed; therefore, it was not possible to determine if any functional or structural changes occurred in the reproductive organs. However, there was no macroscopic evidence at the postmortem examination which would indicate an effect. Therefore, this finding was considered incidental and unrelated to treatment with MRD-94-775.

DISCUSSION (CONTD)

In the offspring, adverse effects were limited to low body weights and suppression in body weight gain. Biologically or statistically significantly lower mean body weights were observed in the treated offspring of the 0.75% and 1.0% dose groups when compared with controls during most of the postnatal period. These lower mean body weights were considered treatment-related. Similar effects have been previously noted for certain phthalates (Dostal et al., 1987) and may have been related to maternal stress, changes in the quality and quantity of milk, decreased milk consumption, and or possibly direct toxic actions of MRD-94-775. There also was statistically significantly lower mean body weight in the 0.5% dose males and females on PND 14 and 21 and a biologically significant decrease in the PND 0 and 4 weights for both males and females. The PND 0 and 4 weights of the 0.5% dose offspring were outside the historical control range for this laboratory, although not statistically significantly less than controls. This may be indicative of slight growth retardation.

In conclusion, signs of toxicity were apparent at dose levels of 0.75% and 1.0%, and were observed in both the parental animals and offspring. Signs of toxicity in the parental animals included decreased body weight, suppression in body weight gain, and decreased food consumption. In the 0.5% dose group, adverse findings were limited primarily to decreases in food consumption compared to controls in the females during the postpartum period. Similarly, body weight reductions were observed in offspring of the 0.75% and 1.0% dose groups of both sexes. There also was slight evidence of growth retardation in the 0.5% offspring. The PND 0 and 4 weights were outside historical control range for this laboratory (although not statistically significantly less than controls) indicating possible growth retardation. Based on these results, 0.8% was selected as the high dose for the definitive two-generation reproduction toxicity study in rats with MRD-94-775. This dose was anticipated to produce signs of toxicity, primarily lower body weights, in the parental males but also in the females during gestation and lactation. Additionally, this dose was considered low enough to allow for sufficient survivorship in the F1 generation. A low dose of 0.2% was selected because it was expected to be a level without effect, particularly in the F2 generation. Finally, 0.4% was selected as the mid dose.

PROTOCOL EXCEPTIONS

DEAD OFFSPRING EXAMINATIONS: Both an external and internal examination was performed on offspring found dead after PND 0. The protocol only required an external examination.

No other circumstances occurred that would have affected the quality or integrity of the data.