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CASWELL FILE

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

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JUN 19 1991

OFFICE OF
PESTICIDES AND TOXIC
SUBSTANCES

MEMORANDUM

SUBJECT: EPA Id. No. 100-524. Diazinon: Review of reproduction studies in rats submitted June, 1989.

TOX CHEM No.: 342
TOX PROJECT No.: 9-1765 and 1-0786
Record No.: 247888 and 242158

FROM: John Doherty, Ph.D., D.A.B.T. 6/13/91
Section IV, Toxicology Branch I
Health Effects Division (H7509C)

TO: George LaRocca
Product Manager #15
Registration Division (H7505C)

and

Jay Ellenberger
Product Mamager #50
SRRD (7508C)

THROUGH: Marion Copley, DVM, Section Head 9/14/91
Section IV, Toxicology Branch I
Health Effects Division (H7509C)

CONCLUSION

The rat multi generation reproduction studies submitted were reviewed and the definitive study (MRID # 411581-01, Study # 852218) was determined to be CORE-GUIDELINE. The NOEL for this study was set at 10 ppm (estimated 0.67 and 0.77 mg/kg/day for males and females) and the LEL at 100 ppm (estimated 6.69 and 7.63 mg/kg/day for males and females). At the LEL, diazinon was demonstrated to have effects on pup mortality and development (body weight gain) and parental (males) body weight gain was decreased. Tremors were evident and mating behavior and gestation length were affected at 500 ppm (estimated 35.15 and 41.43 mg/kg/day for males and females, refer to DER attached for details).

No additional data are required for rat multi-generation reproduction studies (series 83-4) at this time.

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Background/Action Requested

The Ciba-Geigy Co. has submitted two rat reproduction studies (series 83-4) with technical grade diazinon in response to the data gaps indicated in the Registration Standard for this chemical. The first study was terminated due to toxicity and the second study was completed and is considered the definitive study. DERs for both studies have been prepared by Toxicology Branch - I (TB-I) and are attached.

Toxicology Branch Comments

1. The one generation (two breedings) study was determined to be SUPPLEMENTARY. The study demonstrated that at 1000 ppm pup deaths and adverse effects on reproductive parameters resulted (refer to DER attached). The study also suggested that litter size and number of litters may be reduced at 10 and 100 ppm.

2. The definitive 2-generation reproduction study was reviewed and determined to be GUIDELINE (refer to DER attached).

This study demonstrated that diazinon affects the reproductive performance of males and/or females (NOEL = 100 ppm, LEL = 500 ppm) as indicated by decreases in male and female mating and fertility indexes. Gestation length was also consistently increased (500 ppm) Pup mortality and decreased pup weight gain (NOEL = 10 ppm and LEL = 100 ppm) as well as decreased litter size (LEL = 500 ppm) also indicated that diazinon affected pup development. No effects were recognized at the low dose of 10 ppm. Refer to DER for additional effects and details.

3. Submission of these studies satisfies the Agency's requirement to provide a multi-generation reproduction study (83-4). No additional reproductive toxicity studies are required at this time.

4. Studies reviewed.

Series	Study Identification	Results	CORE
83-4	One generation study. Ciba-Geigy Study # MIN 842294, January 25, 1989 MRID # 411581-02 (4 volumes).	Possible effects on litter size and number of litters (all dose levels). Ovary weight, female weight gain, and pup survival (100 and 1000 ppm. Pup weight and decreased fertility and mating indexes (1000 ppm). Dose levels tested 0, 10, 100 and 1000 ppm.	Supplementary.
83-4	Multi generation reproduction - rats Ciba-Geigy Study # MIN 852218, Feb 9, 1989. MRID # 411581-01 (7 volumes).	NOEL (parental toxicity) = 10 ppm. LEL = 100 ppm: Decreased weight gain (5-6% persistent for males but transitory for females in the second parental group. At 500 ppm: Tremors in females. Decreased body weight gain, decreases in male and female mating and fertility indexes (second parental group), and increased gestation length; two incidents of death/dystocia were an equivocal finding. NOEL (pup and development): = 10 ppm. LEL = 100 ppm: Pup mortality and decreased weight gain during lactation. At 500 ppm: decreased litter size and viable pups. Dose levels tested: 0, 10, 100 and 500 ppm or 0, 0.67, 6.69 and 35.15 mg/kg/day for males and 0, 0.77, 7.63 and 41.43 mg/kg/day for females.	GUIDELINE

Reviewed by: John Doherty 6/11/91
Section IV, Toxicology Branch I (H7509C)
Secondary reviewer: Marion Copley, DVM 6/14/91
Section IV, Toxicology Branch I (H7509C)

DATA EVALUATION REPORT

STUDY TYPE: 83-4. Multigeneration reproduction (one generation, two litters, abbreviated study).

MRID NO.: 411581-02 (4 volumes) TOX. CHEM. NO.: 342

TEST MATERIAL: Diazinon Technical, Obtained from Ciba-Geigy batch #FL 841650, 94.9% purity (as described by Ciba-Geigy).

TEST ANIMALS: Charles River albino rats (Crl:COBS CD(SD)Br) obtained from the Charles River Laboratories, Kingston, New York.

STUDY NUMBER(S): MIN 842294

SPONSOR: Ciba-Geigy

TESTING FACILITY: Research Department. Pharmaceuticals Division CIba-Geigy Corporation, Summit, New Jersey

TITLE OF REPORT: "Supplemental to the two-generation reproduction study a one-generation (two litter) reproduction study in rats"

AUTHOR: D. Raab, M. Youreneff, M.L.A. Giknis and E.T. Yau

REPORT ISSUED: January 25, 1989

CONCLUSIONS:

The study indicates that there are several parameters for which additional data will have to be generated because the present study implied a possible effect at either all dose levels or at the higher dose levels tested. These are:

i. effects on litter size (pups/liter). All dose levels.

ii. effects on number of litters. All dose levels.

iii. relative ovary weights. 100 and 1000 ppm.

iv. female weight gain during gestationn and initial (early phases of study) weight gain in males and females. 100 and 1000 ppm.

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v. reproductive indexes of the parents (decreased male and female fertility and mating indexes, increased gestation length). 1000 ppm.

vi. Pup weight at birth. 1000 ppm.

vii. Pup survival during lactation. 100 and 1000 ppm.

Dose levels tested: 0, 10, 100 and 1000 ppm.

Classification: SUPPLEMENTARY (one generation only). This study does not satisfy the requirement for a 2-generation reproduction study (83-4).

Special Review Criteria (40 CFR 154.7): N/A

Quality Assurance Statement: A statement signed by Lynn R. Miko attested that 16 inspections/audits were conducted over the span including Feb 20, 1985 to Jan 6, 1989. No discrepancies in the study report were indicated in the QAS report.

REVIEW

[Note: This study was originally designed to be a multi generation reproduction study but due to reproductive effects that resulted in an insufficient number of pups in the high dose group, only a single generation with two litters was produced.]

The experimental design for this study consisted of four groups of 30 male and 30 female rats which were dosed with either 0, 10, 100 or 1000 ppm of diazinon in their diets. The females were approximately 44 days old and the males were approximately 45 days old at the start of dosing. After about 12 weeks of dosing the rats were mated 1:1 (F_0 - F_1a). Three weeks were allowed for mating and the rats were separated on the day that there was evidence of mating (sperm and/or copulatory plug). The females were allowed to deliver and raise their pups until weaning on lactation day 21. Three weeks were then allowed before a second mating was begun (F_0 - F_1b) following the same procedures of the first mating. All pups were sacrificed after weaning (21-days old). The F_0 males were sacrificed after the second mating, the F_0 females were sacrificed after weaning the F_1b litter or 25 days in to the presumed gestation period if they had not delivered a litter.

Results

A. Parental animals.

1. Clinical signs and reactions and mortality.

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Few and indefinitely related to treatment signs of reaction were noted. One female in the high dose group had ataxia, tremors was 'unthrifty" and hypertensive. These symptoms are consistent with expected reactions to diazinon. There were five deaths or moribund sacrifices: i. one control female (mammary adenocarcinoma); ii. one low dose female (hindlimb paralysis), iii. one high dose male (apparent respiratory virus); and iv. a high dose female (severe nephritis unrelated to treatment). Only the death of one female which exhibited dystocia in the high dose group was described as being possibly related to diazinon treatment.

NOEL (Clinical signs) = 100 ppm. The single female rat showing signs at 1000 ppm is sufficient although marginally to assign this level as the LEL.

2. Body weight gain and food consumption. (Measured weekly).

Body weights for the dosed groups were comparable to the controls throughout the study (Table 6.4.1-4). Body weight gains for given weekly intervals (Tables 6.5.1 and 2) occasionally showed differences from the controls for the high dose group. For example, during the first week there was a 17% decrease ($p < 0.05$) that may have been related to the change in the palatability of the diet. Between days 119-126 there was a 65% increase ($p < 0.05$) which was probably incidental.

There were several apparent effects of treatment in the high dose female groups which were usually decreased in body weight (i.e. up to -9.5%, Table 6.4.5-10) and/or body weight gain over specific periods (Tables 6.5.3-9) such as gestation. Since body weight gain data is more meaningful than body weight, selected body weight gain data for females are discussed and/or illustrated below.

a. First Premating Interval. There was a 38% decrease ($p < 0.05$) in the first week in the high dose group that was probably related to the change in the palatability of the diet since weight gain was similar for the other dose levels and for the high dose group at later intervals. At days 35-42, all three dosed groups were 39 to 56% ($p < 0.5$) higher than the control without a dose response indicating that the control group may have been low for that period.

b. First gestation and lactation periods. Table 1 below illustrates the effects for these periods.

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Table 1. Parental Body Weight Gain Grams

Period	Dose Level: Control	10	100	1000
Gestation				
days 0-20	114.22 ± 8.11	113.86 ± 19.16	111.46 ± 22.12	92.82 ± 18.25*
F1a	(23)	(22)	(26)	(17)
F1b	114.10 ± 14.14	106.00 ± 17.85	107.15 ± 24.55	68.33 ± 17.12*
	(20)	(15)	(13)	(6)
Lactation				
days 0-21	9.24 ± 19.60	6.04 ± 16.90	5.42 ± 19.12	8.13 ± 24.32
F1a	(25)	(24)	(24)	(15)
F1b	-5.90 ± 27.53	0.47 ± 20.11	-3.38 ± 11.85	-7.33 ± 8.50
	(21)	(15)	(13)	(3)

*P < 0.05, study report statistics.
Data are mean ± the standard deviation. The number in () is the number N used in determining the mean.

The table shows that there was a decease in weight gain for the high dose group during gestation but not during lactation. In the F1a generation the high dose group was decreased (-22%, p < 0.05) during days 16-20. Smaller decreases (possibly not real as indicated by the large standard deviations of to 39%) were noted at other intervals. For the F1b generation, the gestation interval days 0-7 was 48% decreased and the interval days 16-20 was 50% decreased (both p <0.05).

Although there were some slight (about 5% for males, about 7% for females) decreases in food consumption during the first weeks of dosing, this was ascribed to unpalatability and there were no consistent effects in the latter weeks. Test article consumption was calculated from the body weight and food consumption data and these data were expressed as mg diazinon/kg/day for several phases of the study. In general younger rats consumed more test article and males consumed 0.49-0.99, 4.88-9.75 and 49.64-93.92 mg/kg/day and females consumed 0.60-0.96, 6.14-9.60 and 65.87-89.48 mg/kg/day for the low, mid and high dose test groups respectively.

NOEL (body weight gain) = NOEL = 100 ppm. LEL = 1000 ppm. Decreases in body weight gain during gestation.

Note: Although body weight gains may have been decreased for

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some intervals, final body weights for both sexes were not statistically different from the controls.

3. Organ weights. [Only the testis and ovaries were weighted.]

The testis weights for all dosed groups were comparable to the controls.

The Table 2 illustrates the ovary weight data and shows that the two highest test dose groups were decreased in absolute or relative weight.

Table 2. Ovary weight data.

	N	Body Weight	Paired Ovary Weight	
			Absolute Weight (g)	Relative Weight
Control	29	348 ± 31	0.15 ± 0.03	0.043 ± 0.010
10 ppm	29	365 ± 43	0.14 ± 0.03(-7%)^{ns}	0.038 ± 0.011(-12%)^{ns}
100 ppm	30	368 ± 46	0.13 ± 0.03(-13%)^{ns}	0.037 ± 0.009*(-14%)
1000 ppm	28	363 + 47	0.12 + 0.04*(-20%)	0.032 + 0.011*(-26%)

* $P \leq 0.05$ (Study report statistics, ANOVA)

NOEL (Organ Weights) = 10 ppm. LEL = 100 ppm. Relative ovary weight decreased. 1000 ppm absolute and relative ovary weight decreased.

Note: The study report recognizes a possible effect only for the high dose test group. The relaitve weight increase was said to be associated with increased body weight in the mid dose group.

4. Pathology (gross and histopathology).

Both the performing laboratory's Pathology and Reproductive and Experimental Toxicology Subdivisions examined specimens of the parental rats from this study. The Pathology subdivision sacrificed and necropsied 9 males from the control, 10 and 100 ppm groups and 11 males from the 1000 ppm group; and 16 control and 1000 ppm group females and 15 females from the 10 and 100 ppm groups. All remaining parental animals were necropsied by the Reproductive and Experimental Toxicology Subdivision. The necropsy consisted of inspection and counting of the uterine implantation sites, obvious gross lesions, and special inspections of the vagina, cervix, uterus, ovaries, testis, epididymides, seminal vesicles, prostate, pituitary, mammary glands (females only) and coagulation gland (males).

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No effects of treatment were evident at either gross necropsy or by microscopic analysis. In particular, the decrease in ovary weights were not supported by pathological changes based on the evidence provided. The pathology report (p. 942, volume 4) indicates that only 16 rats in the control and high dose groups and only 2 rats in the low and none in the mid dose group were examined.

NOEL (gross and histopathology): > 1000 ppm.

5. Reproductive parameters.

a. Precoital interval. All groups for both matings had comparative numbers of matings in the first 4 days (84.2 to 96.3%). No effect of diazinon was evident.

b. Male and female mating and fertility indexes. Table 3 3 illustrates the male and female mating and fertility indexes.

Table 3. Male and FEmale Fertility Indexes.

Parameter		Test Group Control	Low	Mid	High
Male mating index	F1a	27/30(90)	24/30(80)	29/30(97)	29/30(97)
	F1b	27/29(93)	23/30(77)	25/30(83)	19/29(66)*
Female mating index	F1a	27/30(90)	24/30(80)	29/30(97)	29/30(97)
	F1b	27/29(93)	23/30(77)	25/30(83)	19/29(66)*
Male fertility index	F1a	25/27(93)	24/24	26/29(90)	17/29(59)*
	F1b	21/27(78)	15/23(65)	14/25(56)	6/19(32)*
Female fertility index or pregnancy index	F1a	25/27(93)	24/24	26/29(90)	17/29(59)*
	F1b	21/27(78)	15/23(65)	14/25(56)	6/19(32)*
Number of litters	F1a	25	24	24	17
	F1b	21	15	14	5

*p < 0.05. Study report statistics, Mantel's trend test.
Numerators are number giving evidence for mating/fertility; denominator is number mated or fertile; the number is () is the percent see also below.

The data from the above table are discussed as follows:

Female mating index. [Total females with positive evidence of mating/total females cohoused X 100].

In the first breeding, there was no indication of an effect. The mid and high dose groups had percentages (96.7%) or were greater than the control (90.0). In the second breeding, the high dose group (65.5%) was statistically significantly ($p < 0.05$) less than the control (93.1%). An effect of diazinon treatment is implied for the second breeding.

Male Mating Index. [No. of males with positive evidence of mating/No. of males cohoused X 100].

No difference was evident in the first breeding. In the second breeding, the high dose group (65.5%) was statistically significantly ($p < 0.05$) less than the control (93.1%). An effect of diazinon treatment is implied.

Note: There is no numerical and/or procedural difference between male and female mating indexes.

Male Fertility Index. [No. of fertile males/No of males with positive evidence of mating X 100].

In the first breeding, the high dose group (58.6%) was statistically significantly ($p < 0.05$) less than the control (92.6%). In the second breeding, the high dose group (31.6%) was statistically significantly ($p < 0.05$) less than the control (77.8%). An effect of diazinon treatment is implied for both breedings.

Pregnancy Index. [No. of litters delivered/No. of females with positive evidence of mating X 100).

In the first breeding, the high dose group (58.6%) was statistically significantly ($p < 0.05$) less than the control (92.6%). In the second breeding, the high dose group (31.6%) was statistically significantly ($p < 0.05$) less than the control (77.8%). An effect of diazinon treatment is implied for both breedings.

Number of litters.

For the first breeding, there were 25, 24, 24 and 17 (15 live) litters in the control, low, mid and high dose groups respectively. For the second breeding, there were 21, 15, 14, and 5 (3 live) litters in the control, low, mid and high dose test groups respectively. An effect of diazinon treatment is implied. The high dose test group and possibly the low and mid dose test groups have reduced numbers of litters.

c. Duration of Gestation. In the first breeding the length of gestation was slightly higher for all dosed groups when compared with the control. For example the lengths were 23.09, 23.18, 23.25 and 23.94 days for the control, low, mid and high dose test groups respectively. Only the high dose group was statistically significantly ($p < 0.05$) greater. In the second breeding, the length of gestation for the high dose group (24.60 days) was again statistically significantly longer than the control (23.25 days). An effect of diazinon treatment is implied.

CONCLUSION (Parental effects). NOEL = 100 ppm. At 1000 ppm, there were decreases in the male and female fertility indexes, male mating indies, pregnancy index, and number of litters per group; the duration of gestation was increased. TB-I considers the data showing a possible effect on the number of litters in the low and mid dose groups to be equivocal. Additional data will have to be generated to resolve this issue.

B. Pup Data

a. Litter size, viable pups and pup weight. Table 4 illustrates these data for day 0 or birth date.

Table 4. Litter size. viable pups and pup weight.

	First breeding			Second breeding		
	Litter size	Viable pups	Pup weight[1]	Litter size	Viable pups	Pup weight
Control	13.6	341	6.33/5.94	12.3	259	6.55/6.24
10 ppm	11.8*	282	6.42/5.94	9.2	138	6.72/6.33
100 ppm	12.4*	298	6.43/5.95	11.8	165	6.61/6.19
1000 ppm	9.3*	140	5.93*/5.50*	2.3*	7	7.00/6.84

[1]males/females.
* statistically significant $p < 0.05$

The above table demonstrates that litter size, the number of viable pups for both breedings, and for the first breeding the pup weights are affected in the high dose test group. The litter size for the low and mid dose groups for the first breeding, although lower than the control, was said to be within the historical control for this strain from this laboratory. No differences in the sex ratios were noted,

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b. Pup Survival and weight gain during lactation.

Pup weight gain during lactation is illustrated in Table 5.

Tbale 5. Pup weight gain during lactaion.

Interval		Dose group[1] Control	10	100	1000
Birth	F1a	6.33/5.94	6.42/5.94	6.43/5.95	5.83*/5.50*
	F1b	6.55/6.24	6.72/6.33	6.61/6.19	7.00/6.84
4 post	F1a	8.98/8.60	9.60/8.82	9.12/8.61	7.16*/6.44*
	F1b	9.20/8.80	10.20/9.95	8.98/8.51	No pups
7	F1a	14.15/13.57	14.60/13.47	13.83/13.07	9.43*/9.81*
	F1b	14.00/13.28	15.34/15.08	13.05/12.50	No pups
14	F1a	28.31/27.31	28.93/27.24	26.40/24.94*	17.36*/20.00*
	F1b	29.79/28.63	32.00/31.14	27.45/26.46	No pups
	F1a	42.69/41/80	43.73/41.62	39.82/38.10*	25.22*/29.83*
	F1b	47.83/45.78	50.63/49.28	45.73/42.75	No pups

*p < 0.05. Study report statistics.
Data are mean pup weight in grams. Numerators are male data and denominators are female pup data. The standard deviations were about 3-4% of the means except or the high dose group at day 21 when they approached 10%.

For the first breeding, the decease in pup weight noted at birth for the high dose group persisted throughout the 21 day lactation period such that at termination the final weights of the pups in the high dose group were 41% for males and 29% for females less than the controls. The pups weight for the mid dose groups were also significantly less than the controls for female pups (-9.4% at week 14 and -8.9% at week 21) for the F1a generation. The low dose group was equivalent to the controls. For the second breeding, the weights of the low and mid dose groups pups were equivalent to the control or for males occasionally higher. There were no pups surviving after day 4 in the high dose group.

Table 6. Pup survival during lactation.

		Pup Survival (Percent of Cntrol)			
Group	Interval	Control	10 ppm	100 ppm	1000 ppm
F1a	0-4	97.3/95.1	94.9/93.0	98.6/96.5	28.5*/31.9*
F1b	0-4	99.2/97.9	100/100	98.4/100	all dead
F1a	4-21	100/100	98.9/99.2	95.8*/92.7*	60.0*/54.2*
F1b	4-21	100/97.5	96.4/100	93.2*/96.2	------

*p < 0.05. Study report statistics.

The above table 6 shows that pup survival was decreased in the high dose group and For the first breeding, only 28.5% of the male pups in the high dose group survived to day 4 (precull) and of these survivors only 60% survived until day 21. In the mid dose group, pup survival for days 4-21 was statistically significantly lower ($p < 0.05$) both males and females.

In the second breeding, none of the high dose group pups survived until day 4. Male pup survival for the mid dose group was also significnatly less than the controls but female pups survival was equivalent to the controls.

c. Clinical and Gross Pathology.

No dose related gross abnormalities were reported. Four pups in the high dose group (first breeding) were reported as being "lethargic" and this was considered by the laboratory to be a secondary effect to treatment with diazinon.

CONCLUSION (Pup Data): The number of viable pups was decreased for all dose levels for both breedings. The NOEL was otherwise 100 ppm. At 1000 ppm the litter size, number of viable fetuses and pup weight at birth, pup survival through lactation were decreased. Litter size was reduced for the low and mid dose groups for the first breeding, but since the litter size was within the historical control it is inconclusive if the apparent reduction was caused by diazinon.

CONCLUSION (Study): This study is classified as SUPPLEMENTARY. Only a single generation (with two litters) was bred. The study indicates that there are several parameters for which additional data will have to be generated because the present study implied a possible effect at either all dose levels or at the higher dose

levels tested. These are:

i. effects on litter size (pups/liter). All dose levels.

ii. effects on number of litters. All dose levels.

iii. relative ovary weights. 100 and 1000 ppm.

iv. female weight gain during gestationn and initial (early phases of study) weight gain in males and females. 1000 ppm.

v. reproductive indexes of the parents (decreased male and female fertility and mating indexes, increased gestation length). 1000 ppm.

vi. Pup weight at birth. 1000 ppm.

vii. Pup survival during lactation. 1000 ppm.

Dose levels tested: 0, 10, 100 and 1000 ppm.

Diazinon
Ciba Geigy Labs (Hanmit, New Jersey)
Study # HIN 842294
1/25/89

SUPPLEMENTARY

83-4 Reproduction

ACCEPTANCE CRITERIA

Does your study meet the following acceptance criteria?:

1. ✓ Technical form of the active ingredient tested.
2. ✓ At least 20 males and sufficient females to yield 20 pregnant /dose group
3. ✓ At least 3 dose groups and a control.
4. ✓ At the high dose, parental toxicity is observed (or a limit dose is given, 1,000 mg/kg/day).
5.* A At the low dose, no reproductive effects are observed.
6.* ___ Analysis for test material stability, homogeneity and concentration in dosing medium
7. ✓ P_1 animals 8 weeks old at the start of the study.
8. ✓ Dosing is continuous starting with the P_1 animals until an individual animal is sacrificed.
9. ✓ Mating is 1 male to 1 female.
10. ✓ The mating period is not more than 3 weeks.
11. B At least two generations are bred.
12. ✓ Individual daily observations.
13. ✓ Individual body weights.
14. ✓ Individual food consumption.
15. ✓ Individual litter observations.
16. ✓ Individual litter weights (pup weights) at birth and on days 4, 7 (optional), 14 and 21 .
17.* ✓ Sacrifice schedule, all mating males immediately after last mating, all breeding females immediately after weaning last litter, all animals not used for breeding immediately after weaning.
18.* ✓ Necropsy on all animals
19.* ✓ Histopathology of reproductive organs from all animals on the high dose and control P_1 and F_1 animals selected for mating. Animals from all other dosing groups if histological effects are observed at the high dose.
20.* ___ Histopathology of all organs with gross lesions.

A. Possible effects noted

B. 1 generation only

C. They were approx. 6 weeks old.

Criteria marked with a * are supplemental and may not be required for every study.

Reviewed by: John Doherty 6/13/91
Section IV, Toxicology Branch I (H7509C)
Secondary reviewer: Marion Copley, DVM
Section IV, Toxicology Branch I (H7509C)

DATA EVALUATION REPORT

STUDY TYPE: 83-4. Two-generation reproduction (rats)

MRID NO.: 411581-01 (7 volumes) TOX. CHEM. NO.: 342

TEST MATERIAL: Diazinon technical. Obtained from Ciba-Geigy batch #FL 841650, 94.9% purity.

TEST ANIMALS: Sprague-Dawley rats (CRCD, Crl:COBS CD(SD)BR) obtained from the Charles River Breeding Labs Kingston, New York. They were fed Purina #5002 Certified Rodent Chow and caged individually except during mating.

STUDY NUMBER: #852218

SPONSOR: Ciba-Geigy

TESTING FACILITY: Research Department, Pharmaceutical Division Ciba-Geigy Corporation Summit, New Jersey.

TITLE OF REPORT: "A two generation reproduction study in albino rats".

AUTHOR: D.M. Raab, M.A. Youreneff, M.L.A. Giknis and E.T. Yau

REPORT ISSUED: February 9, 1989

CONCLUSIONS:

NOEL (parental toxicity) = 10 ppm. LEL = 100 ppm: Decreased parental weight gain (5-6% persistent for males in the second parental group and transitory for females). 500 ppm: Tremors in females. Decreased parental body weight gain, decreases in male and female mating and fertility indexes (second parental group), and increased gestation length. Dystocia and death (equivocal) in first parental group.

NOEL (pup and developmental) = 10 ppm. LEL = 100 ppm: Pup mortality and decreased weight gain during lactation. 500 ppm: decreased litter size and viable pups.

Dose levels tested: 0, 10, 100 and 500 ppm. Equivalent to 0, 0.67, 6.69 and 35.15 mg/kg/day in males and 0, 0.77, 7.63 and 41.43 mg/kg/day in females.

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Classification: CORE-GUIDELINE. This study satisfies the guideline requirement for a 2-generation reproduction study (83-4).

Special Review Criteria (40 CFR 154.7): N/A

Quality Assurance Statement: A statement signed by Lynn R. Miko attested that 14 inspections were made over the period from November 27, 1985 to January 30, 1989. No discrepancies were reported in the QAS.

REVIEW

[Note: A preliminary multi-generation reproduction study (MRID #411581-02, Study # MIN 842294, dated January 25, 1989) was run and was stopped after one generation because the high dose group (1000 ppm) displayed evidence of adverse reproductive effects and there were insufficient high dose group pups to continue the study. The findings of this study are summarized as follows:

The study indicated that there are two parameters for which additional data will have to be generated because the present study implied a possible effect at the low and mid dose levels. These are:

i. effects on litter size (pups/liter) at 10 and 100 ppm.

ii. effects on number of litters at 10 and 100 ppm.

The following summarizes other aspects of the results of this study:

NOEL = 10 ppm. LEL = 100 ppm: relative ovary weight decreases. At 1000 ppm: reproductive function of the parents (decreased mating, fertility and pregnancy indexes; both male and female parental groups were affected, gestation length was increased. The litter size and pup survivability were decreased.

The 2-generation definitive study should be carefully assessed as to whether the above findings are reproducible.]

The basic experimental design for the definitive study consisted for four groups of 30 male and 30 female Charles River albino rats (Crl.:COBS CD(SD)BR) which were dosed with either 0, 10, 100 or 500 ppm of technical diazinon. The F_0 or P_1 rats were 51 (females) or 52 (males) days old at the start of dosing. After 10 weeks of dosing, the rats were mated 1:1 to produce the F_1 litter. Pups from the F_1 litter were raised to become the parental rats for the second generation (P_2). The male F_0 parental rats were sacrificed on days 110-119 (after mating to produce the first litter). The F_0 parental females were

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sacrificed about three weeks after weaning the F_1 litter.

The P_2 rats were placed on the diazinon containing diets following weaning and after 11 weeks were mated 1:1 to produce the F_2 litters. Three pairs of siblings were reportedly inadvertently mated in the 100 ppm dose group but this is not sufficient to compromise the study. The P_2 males were sacrificed after 134 days on the study and the P_2 females were sacrificed after 146 days on the study. The F_2 pups were sacrificed after weaning.

Overall there are four sets of experimental groups (each with four dose levels): Parental groups P_1 and P_2 and litters F_1 and F_2. Parental group P_2 was derived from litter F_1.

An analytical chemistry report (p 121) described studies conducted to test the stability and homogeneity and confirmation of concentration of diazinon in the test diets. These studies revealed that diazinon was stable in the test diet for 35 days and that homogeneity had a maximum standard deviation of 2.7%. Most of the samples were reported as being 91-102% of the target dose level. One sample of the 10 ppm diet was reported as having 77% of the expected value. Such deviations are not considered sufficient to compromise the study.

A copy of the statistical methods used is attached.

Results

A. Parental animals.

1. Clinical signs and reactions and mortality. (Reported as being observed daily or twice daily).

First parental group: There were two deaths in the high dose female group and each was preceded by dystocia (difficulty in delivering pups). In addition, three rats in the high dose female group had tremors. One female in the mid dose group also died and death may have been associated with dystocia.

Second parental group: No compound related deaths or dystocia were noted although two females died (one each in the low and mid dose groups). Four high dose group females had tremors.

CONCLUSIONS (Clinical signs and mortality) NOEL = 100 ppm. LEL = 500. Tremors and deaths/dystocia (equivocal).

Tremors are an expected symptom of organophosphate intoxication.

TB-I does regard the deaths and dystocia in the first parental group as an equivocal observation. There were no compound

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related deaths or dystocia in the second parental group. This position is partially supported by the data from the preliminary one generation study which demonstrated only a single incidence of dystocia/death at 1000 ppm in two breedings. See Appendix I

2. Body weight gain. [Absolute body weight data were also presented in the report but body weight gain is discussed here since it is considered a more meaningful index. Note: See also pup weight at birth discussion below.] Body weight data were reportedly recorded weekly for males during premating, mating and sacrifice. Body weight data for females were reportedly recorded weekly during premating period, on days 0, 7, 14, and 20 of gestation and on days 0, 4, 7, 14 and 21 of lactation.

Male Data.

a. Premating (data presented for weekly measurements). First Parental Group (Table 6.5.1 in the report). An initial decrease in body weight gain for the first week was evident for both the mid (-8%) and high (-14%) dose groups. At termination, however, the high dose group was slightly higher (+4.3%) in net body weight gain.

Second Parental Group. Table 1 (adapted from Table 6.13.1 of the study report) illustrates the body weight gain data for males at selected intervals during the life of this group.

Table 1. Male Body Weight Gain[1], second generation (P2).

Interval	Control	Dose Group 10	100	500
0-7	60.27(17.09)	62.37(7.98) +3.5%	59.67(5.29) -1.0%	53.53(5.39)* -11.2%
21-28	40.33(6.78)	39.23(6.11) -2.7%	35.87(4.80)* -11.1%	34.93(5.49)* -13.4%
35-42	28.13(8.28)	30.90(6.84) +9.8%	26.31(4.65) -6.5%	21.87(4.40)* -23.3%
56-63	16.53(12.54)	17.67(6.32) +6.7%	15.70(7.29) -5.0%	15.27(4.55) -7.6%
70-77	21.80(6.41)	21.13(5.52) -3.1%	15.93(6.38)* -26.9%	12.53(4.58)* -42.5%
0-77	362.00(30.56)	355.73(46.67) -2%	338.90(27.97)* -6%	328.13(38.00)* -9%

[1] Data are body weigh gain in grams (standard deviation) and percentage different from the control. There were 30 males per dose level and group

except for occasional missed weighings when there were 29 per group. < 0.05. Study report statistics. ANOVA and Dunnett's Test.

Nearly all intervals for the high dose group were statistically significantly lower. The mid dose group was consistently lower in body weight gain but not all intervals reached statistical significance. The low dose group was often both higher and lower than the control. Overall net body weight gain was -2%, -6% and -9% lower for the low, mid and high dose test groups when compared to the control. Body weight gain in the mid and high dose groups appear to be minimally affected for males.

Female Data.

a. Premating (data presented as weekly measurements, large standard deviations were noted). First Parental Group (Table 6.5.2 in the study report). No statistical differences were reported.

Second Parental Group (Table 6.13.2 in the study report). The mid (-14%) and high (-13%) dose groups for the period days 0-7 were lower but after that time premating body weight gains were not statistically significantly different in a consistent pattern.

b. Gestation (measured for days 0-7, 7-14, 14-20 and 0-20). First Parental Group (Table 6.5.3 in the study report). There was an initial decrease ($p < 0.05$, study report statistics, Dunnetts's test) in the high dose group indicating a -17% lower body weight gain. For the succeeding intervals, body weight gain was also reduced (-11 and -5% and -10% for the gestation period) but statistical significance was not attained.

Second Parental Group (Table 6.13.3 in the study report). The high dose group again had a lower body weight gain (-26%, $p < 0.05$) for the interval 0-7 days. No difference was noted for the 7-14 day interval but the 14-20 day interval was 24% lower ($p < 0.05$). Net weight gain during gestation was 18% lower for the high dose group.

c. Lactation (Data reported for measurements made for days 0-4, 4-7, 7-14, 14-21 and 0-21). First parental group (Table 6.5.4 in the study report). Net weight gain (days 0-21) for the high dose group was 16% lower but for the mid dose group it was 27% higher. The standard deviations were 100-200% or more obscuring statistical evaluations.

Second Parental Group (Table 6.13.4 in the study report). Again the large standard deviations obscured the interpretation of the data. The net weight gain (days 0-21) was about 3 time higher for the high dose group than for the control.

2d

CONCLUSION (Body Weight Gain): NOEL = 10 ppm. LEL = 100 ppm. Transitory decreased male body weight gain and less definite transitory decreased female body weight gain in P2.

3. Food Consumption. (Reported for same interval as for body weight gain for females but for premating only for males).

First parental group: Food consumption was <u>increased</u> slightly in the high dose female group but was comparable to the controls in the other groups. Second parental group: Food consumption was reported to be <u>reduced</u> slightly in the mid and high dose group males during premating. No effects on female food consumption were reported.

Note: Food consumption data will not be included in the assignment of the NOEL/LEL since it is closely related to body weight and other endpoints.

4. Test Material Consumption. (Based on body weight gain and food consumption). In general, the dose level of diazinon in mg/kg/day consumed varied with the age of the rat with younger rats receiving a higher level. The approximate test material consumed is illustrated in the Table 2 (adapted from tables 6.3.1 and 2 and 6.11.1 and 2 in the study report).

Table 2. Compound consumption.

		Diazinon Consumed (mg/kg/day)[1]			
Group		Males	"Dose"	Females	"Dose"
Control		0/0	"0"	0/0	"0"
10	range	0.50-0.89/0.51-1.07		0.67-0.91/0.61-1.00	
	mean	0.65(0.12)/0.69(0.18)	"0.67"	0.77(0.08)/0.76(0.13	"0.77"
100	range	5.05-8.92/5.19-10.73		6.39-9.09/6.18-10.46	
	mean	6.41(1.26)/6.97(1.80)	"6.69"	7.48(0.87)/7.78(1.36)	"7.63"
500	range	26.08-44.24/27.25-59.73		33.10-46.86/31.97-61.12	
	mean	32.85(6.17)/37.45(10.59)	"35.15"	40.26(4.72)/42.60(9.39)	"41.43"

[1] Numerator = first generation, denominator = second generation.
[2] "Dose" is the mean of both the first and second generations.

5. <u>Organ weights</u>. (Only the ovaries and testis were weighted.)

First parental group. Both the testis and ovary weights were equivalent to the control with respect to absolute and relative weights.

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Second parental group. Ovary weights (absolute and relative) were comparable to the controls. Absolute testis weight for the high dose group was statistically significantly less than the controls (-9.9%, $p < 0.05$, study report statistics, ANOVA and Dunnett's test), but relative weight was equivalent to the controls.

CONCLUSIONS (Organ weights): NOEL > 500 ppm. [The decrease in testis weight was not accompanied by pathological changes and is regarded as being associated with the lower body weight in this group.]

Note: The decrease in ovary weights noted in the one generation study was not noted in this study for either generation.

6. Pathology (Gross and Histopathology)

Gross necropsy included inspection of the uterine contents to determine if the females were pregnant. All grossly abnormal tissues (any organ) and specimens of the vagina, cervix, uterus, ovaries, mammary tissue, testis, epididymides, seminal vesicles, prostate, pituitary and coagulating gland were collected and prepared for microscopy. Tissues from the control and high dose groups were examined.

No treatment related effects were reported for either parental group. In particular the testis was examined for 30 control and 30 high dose group rats. No pathological conditions were reported in the high dose group but two incidence of "focal atrophy" were reported in the controls. No pathological conditions were reported in the ovaries.

7. Reproductive parameters.

a. Precoital interval (assessed on days 1-4, 5-8, 13-16 and 17-21).

The precoital interval was not affected by diazinon treatment for either parental group. Most pairs (> 86.2%) mated during the first four days. Occasional matings were observed at later intervals. For example, the highest interval besides days 1-4 was days 13-16 when 10.3% (3 matings for 29 pairs) of the controls in the P_2 group mated. One hundred percent of the pairs in the high dose group, however, mated during days 1-4.

b. Male and Female mating indices. [No. of males with positive evidence of mating/No. of males cohoused and total females with positive evidence of mating/total females cohoused X 100].

The male and female mating indexes were slightly reduced for the high dose group as indicated in Table 3 (adapted from Tables 6.6.4 and 6.14.4 in the study report).

Table 3. Male and female mating indexes.

Dose Level	Males P_1	Males P_2	Females P_1	Females P_2
Control	100%	29/30 (96.7%)	100%	29/30 (96.7%)
10 ppm	100%	27/29 (93.1%)	100%	27/29 (93.1%)
100 ppm	100%	28/29 (96.6%)	100%	28/29 (96.6%)
500 ppm	96.7%	26/30 (86.7%)	96.7%	26/30 (86.7%)

P_1 data are from table 6.6.4; P_2 data are from table 6.14.4. Note there were 30 males and females for all groups for the P_1 generation.

c. Male and female fertility indices. [No. of fertile males/No. of males with positive evidence of mating X 100 and number of fertile females/number of females with positive evidence of mating].

The male and females fertility indexes were also slightly reduced in the high dose group as indicated in Table 4 (adapted from Tables 6.6.4 and 6.14.4).

Table 4. Fertility Indexes

Dose Level	Males P_1	Males P_2	Females P_1	Females P_2
Control	28/30 (93.3%)	26/29 (89.7%)	28/30 (93.3%)	26/29 (89.7%)
10 ppm	28/30 (93.3%)	22/27 (81.5%)	28/30 (93.3%)	22/27 (81.5%)
100 ppm	29/30 (96.7%)	23/28 (82.1%)	29/30 (96.7%)	23/28 (82.1%)
500 ppm	27/29 (93.1%)	19/26 (73.1%)	27/29 (93.1%)	19/26 (73.1%)

P_1 data are from table 6.6.4; P_2 data are from table 6.14.4.

In particular, the high dose P_2 parental group is 73.1% vs 89% for the control.

d. Pregnancy index or gestation index. [No. of litters delivered/No. of females with positive evidence of mating X 100].

The pregnancy or gestation index was not affected for either parental group. It was 92.6 to 100% for the P_1 group and 86.4 to 100% for the P_2 group. In the second generation the high dose group had 100%.

e. Duration of gestation.

The mean duration of gestation was said by the study author to be increased for the second parental group. The duration of gestation data are shown in table 5 (adapted from Tables 6.6.2 and 6.14.2 in the study report).

Table 5. Mean gestation length data.

Dose Group	Generation P_1	P_2
Control	23.22 (4/27, 15%)[1]	23.48 (10/25, 40%)
10 ppm	23.11 (3/28, 11%)	23.40 (7/20, 35%)
100 ppm	23.07 (2/26, 8%)	23.37 (7/19, 37%)
500 ppm	23.36 (9/25, 36%)	23.76 (13/17, 76%)

[1] Data are mean gestation length in days and in () the number of dams delivering on day 24 or later (numerator) and number of dams carry fetuses to term (23 days) and the percentage.

The above table shows that for both the P_1 and P_2 parental groups, the high dose group has a slightly longer gestation length and more dams (about twice the percentage) deliver their litters on day 24 or later. The duration of gestation was also noted to be increased in the one generation study for both sets of litters in the high dose group (see review). These data, however, did not reach statistical significance (study report statistics. Mandel's Trend Test).

f. Number of viable litters.

There were 26, 28, 27, and 25 viable litters for the first parental group and 24, 19, 21, and 19 viable litters for the second parental group for the control, low, mid and high dose test groups respectively. This does not provide an indication of a compound related decrease in the number of litters produced.

CONCLUSIONS (Reproductive parameters for parental groups] NOEL = 100 ppm. LEL = 500 ppm. Male and female mating indexes and fertility indexes decreases and increased gestation length.

B. Pup Data.

a. Litter size, viable pups, still births and pup weight. Table 6 illustrates these data for day 0 or birth date (adapted

from Tables 6.8.1 and 6.8.3.1 and 2; 6.16.1 and 6.16.3.1 and 2).

Table 6. Litter Data (litter size, viable pups, still births and pup weight).

	First litters			Second Litters		
	Viable Mean Litter size	Total Viable pups	Mean Pup weight[1]	Viable Mean Litter size	Total Viable pups	Mean Pup weight[1]
Control	13.5	351	6.26/5.97	13.5	324	6.45/6.06
10 ppm	13.6	380	6.26/5.89	13.9	265	6.28/5.99
100 ppm	15.1	409	6.16/5.87	13.4	289	6.47/6.13
500 ppm	12.1 (-10.4%)[2]	303 (-13.7%)	6.17/5.86	8.7* (-35.6%)	166 (-48.8%)	6.21/5.84

[1] male pup weight/female pup weight (standard deviations were about 2%.
[2] the number in () is the percent decrease.
* statistically significant $P < 0.05$.

As indicated in table 6 mean litter size (-10.4% and -35.6%) and number of viable pups (-13.7% and -48.8%) in the high dose groups are reduced. Mean pup weight for both males and females is also slightly reduced (1.4% to 3.7%) but statistical significance was not attained.

There was no evidence presented of a compound related effect on the number of or percentage of stillbirths. For example, the percent stillbirths were 6.46, 3.18, 2.22, 4.90 and 7.66, 8.04, 1.43 and 8.86 for the control, low, mid and high dose groups for the first and second litters respectively.

c. Sex ratio.

The sex ratio of the pups indicated slightly higher ($p < 0.05$) male pups in the high dose group (54.1%) than in the control (52.7%) for the first breeding. The sex ratio was not significantly different for the second breeding being 49.1% for the control and 50.6% for the high dose group.

b. Pup survival.

Pup survival data are illustrated Table 7 (adapted from Tables 6.8.1 and 6.16.1 in the study report).

Table 7. Pups surviving (%)

	F_1		F_2	
	0-4	4-21	0-4	4-21
Control	88.7	99.0	96.2	100
10 ppm	97.6	98.7	98.4	96.5
100 ppm	95.4	93.5*[2]	97.9	98.2
500 ppm	61.3*[1] 64.3*/60.5*[3]	61.7*[1] 59.5*/67.6*	44.0* 44.6*/42.9*	76.7* 78.0*/72.2*

* $p < 0.05$. Study report statistics.
[1] Separate analysis indicated both sexes were statistically significantly reduced.
[2] Statistical analysis failed to show a statistically significant decrease for either sex when male and female data were assessed separately. Both sexes had 93.5% survival.
[3] Data for males (numerator) and females (denominator) are shown for the high dose test group.

The above table shows that for days 0-4 (pre-cull) only 61.3% of the pups in the first breeding and 44.0 of the pups in the second breeding in the high dose group survived as compared to > 88.7% for all other groups. Thus, pup survival is affected in the high dose group during the precull (days 0-4 interval) when the pups are receiving nearly all of their diet from mothers milk.

For days 4-21 (post-cull) only 61.7% of the pups in the first breeding and 76.7% of the pups in the second breeding in the high dose group survived as compared to > 93.5% for all other groups. Survival of both sexes was about equally affected.

When the total male and female pups for the mid dose group in the first generation are considered there was 93.5% survival for days 4-21 vs 98.7 and 99.0 for the low dose and control groups. The mid dose group was statistically significantly less than the control. When the data for males and females are considered separately, neither sex was statistically significantly different from the control, although both sexes had 93.5% survival vs > 97.8% for all other groups. For the second generation, survival for days 4-21 was > 96.5% for all of the controls, low and mid dose groups.

CONCLUSION (pup survival) NOEL = 10 ppm. LEL = 100 ppm.

Note: The study report (p.35 and again on page 45) asserts that the decrease in pup survival for the mid dose group (100 ppm) for days 4-21 is treatment related. Pup survival for days 4-21 was also decreased for the 100 ppm dose group for the first breeding in the one generation study (se review).

c. Weight gain during lactation.

Pup body weight gain during selected subintervals during lactation are shown in Table 8 and 9 (one table each for males and females.

Table 8. Mean Pup Body Weight[1] for Males,

Body Weight in Grams

Interval		Control	Low	Mid	High
Birth	F1	6.26	6.26	6.16(-2)	6.17
	F2	6.45	6.28(-3)	6.47	6.21(-4)
Day 4 (pre)	F1	8.73	8.67	8.34(-4)	7.44(-15)*
	F2	9.57	9.26(-3)	9.33(-3)	7.40(-23)*
Day 4 (post)	F1	8.63	8.68	8.38(-3)	7.42(-14)*
	F2	9.69	9.29(-4)	9.39(-3)	7.40(-24)*
7	F1	13.50	13.49	12.57(-7)	11.11(-18)*
	F2	15.42	14.76(-4)	14.37(-7)	9.79(-37)*
Day 14	F1	29.04	28.07	26.79(-8)*	21.81(-25)*
	F2	30.84	30.20(-2)	29.06(-6)	18.63(-40)*
Day 21	F1	45.07	44.88	42.88(-5)	32.37(-28)*
	F2	48.81	47.60(-2)	47.30(-3)	29.41(-40)*

See next page for footnotes.

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le 9. Mean Pup Body Weight[1] for Females

Body weight in Grams

Interval		Control	Low	Mid	High
Birth	F1	5.97	5.89	5.87(-2)	5.86(-2)
	F2	6.06	5.99	6.13	5.84(-4)
Day 4	F1	8.16	8.23	7.95(-3)	7.39(-9)
(pre)	F2	9.14	8.88(-3)	8.91(-3)	7.26(-21)*
Day 4	F1	8.21	8.21	7.90(-4)	7.40(-10)*
(post)	F2	9.17	8.90(-3)	8.96(-2)	7.24(-21)*
Day 7	F1	13.81	12.82(-7)	11.88(-9)*	10.15(-27)*
	F2	14.52	14.18(-2)	13.80(-5)	9.91(-32)*
Day 14	F1	27.74	26.37(-5)	25.93(-7)	20.74(-25)*
	F2	29.38	29.80	28.12(-4)	18.60(-37)*
Day 21	F1	43.29	41.96(-3)	40.58(-6)	31.23(-28)*
	F2	45.92	47.09	45.06(-2)	27.70(-40)*

* $p < 0.05$.

Data for the F1 male pups is from Table 6.8.3.1 and for female pups is from le 6.8.3.2. Data for the F2 male pups is from Table 6.16.3.1 and for ale pups is from Table 6.16.3.2.

Day 4 data are for pre cull and post cull to 8 pups per litter. The F1 generation has 25-28 litters per group and the F2 generation has 19-24 litters per group.

[1] Data are mean pup body weight. The standard deviations are not presented here but were about 1-5% (but occasionally higher) of the mean. The percent decrease in pup weight is shown in ().

The high dose group pups gained less weight than the other groups such that the males -28% and -40% and females -28% and -40% for the first and second breedings respectively were less than the corresponding controls at day 21. For the first generation, the mid dose group also had at least one occasion of reduced weight gain during lactation ($p < 0.05$, -9% for females and -8% for males on day 14 post-culling) and on all other occasions this group was less (2-7%) than the control. Pup body weight gain for the mid dose group did not reach statistical significance for the second generation although some decreases in weight (up to 7%) were noted.

Pup weight gain for the low dose group did not reach statistical differences when compared to the control although some decreases were noted up to -7%.

CONCLUSION (pup weight gain during lactation): NOEL = 10 ppm. LEL = 100 ppm. The rather consistent decrease in both male and female weight although only occasionally statistically significant at 100 ppm justifies the assignment of this level as the LEL.

c. Clinical and Gross Pathology.

No definite treatment related findings were evident. In the F1 generation some of the pups in the high dose group in one litter appeared to be "unthrifty" (14 pups) and/or lethargic (6 pups) in appearance possibly secondary to maternal neglect. In addition, pups from 6 litters (total 90 pups) had "no milk". It is not possible to determine exactly what is meant by this observation. Necropsy revealed that 1 litter (4 pups) had "no milk in stomach"

In the F2 generation, there were no compound related observations and necropsy was unremarkable.

TB-I does not regard the conditions noted in the F1 generation as direct effects of treatment. If they are real, they will be covered by other aspects of the NOEL/LEL setting since they were reported in the high dose group only.

NOEL (Pup Data): NOEL = 10 ppm. LEL = 100 ppm: decreased pup survival and body weight gain during lactation. 500 ppm: Decreases in litter size.

DISCUSSION: The study report author assigns a NOEL of 10 ppm and a LEL of 100 ppm. The responses to treatment recognized by the study report author included: deaths (500 ppm), deceased food consumption in males (100 and 500 ppm), decreased body weight (100 and 500 ppm), increased gestation length (500 ppm), decreased number of pregnancies and viable newborns and adverse effects on mating and fertility indices (500 ppm), reduced litter size, decreases in pup survival and pup weight (100 and 500 ppm), necropsy findings ("no milk in stomach", 500 ppm).

TOXICOLOGY BRANCH CONCLUSION (Study): This study is classified as CORE GUIDELINE.

NOEL (parental toxicity) = 10 ppm. LEL = 100 ppm: Decreased parental weight gain (5-6% persistent for males in the second parental group and transitory for females). 500 ppm: Tremors in females. Decreased parental body weight gain, decreases in male and female mating and fertility indexes (second parental group),

and increased gestation length. Dystocia and death (equivocal) in first parental group.

NOEL (pup and developmental) = 10 ppm. LEL = 100 ppm: Pup mortality and decreased weight gain during lactation. 500 ppm: decreased litter size and viable pups.

[Note: Most of the observations noted in the preliminary study were also noted in the definitive study. No compound related effects, however, were noted on any parameter at 10 ppm in the definite study. In particular, diazinon was shown to affect the reproductive performance as indicated by mating indexes (NOEL = 100 ppm) and the pup survival and body weight (NOEL = 10 ppm).

A NOEL for litter size is established at 100 ppm and for number of viable litters at > 500 ppm (i.e no decrease in the number of viable litters was evident in the definitive study). No effects on the ovary weight were evident in this study.

Diazinon D

Appendix I. Comparision of the "one generation" and "two generation" reprduction studies with diazinon for incidence of deaths and dystocia among the dams.

Table A. Postmating maternal deaths related to dystocia in both the "one generation" and "two generation" reproduction studies with diazinon.

	"One Generation Study"		"Two Generation Study"	
Dose Level[1]	First Breeding	Second Breeding	First Generation	Second Generation
0	0/30[3]	0/30	0/30	0/30
10	0/30	0/30	0/30	0/29
100	0/30	0/30	1?/30[5]	0/29
500	--[2]	--	2/30	0/30
1000	0/30 (1)[4]	1/29	--	--

[1] Dose level in ppm.

-- Not tested at this dose level for this study.

[3] Data are numerator: number of rats with death or sacrifice and also showing dystocia; denominator: number of dams mated.

[4] The number in () is the number of dams reported as dying from causes other that being related to dystocia.

[5] This dam was found dead on day 24. It was uncertain if dystocia was associated with the death. It condition prior to death was described as unthrifty", having chromodacryorrhea and a nasal discharge. The cause of death was not reported.