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

008748

OFFICE OF
PESTICIDES AND TOXIC
SUBSTANCES

MEMORANDUM

SUBJECT: Azinphos-methyl
One-Generation Rat Reproduction Study (83-4)
Section 6(a)(2)

Project No.: 1-2180
Tox Chem. No. 374

FROM; Ray Landolt 10/24/91
Review Section I
Toxicology Branch II
Health Effects Division (H7509C)

TO: Dennis Edwards, PM 19
Insecticide-Rodenticide Branch
Registration Division (H7505C)

THRU: Mike Ioannou, Section Head J.M. Ioannou 10/24/91
Review Section I
Toxicology Branch II
Health Effects Division (H7509C)
and
Marcia van Gemert, Ph.D., Branch Chief
Toxicology Branch II
Health Effects Division (H7509C) Marcia van Gemert 10/24/91

Registrant: Mobay Corporation; letter of June 12, 1991
EPA Reg. No. 3125 -108

Action Requested: Mobay has submitted a one-generation rat reproduction study (MRID 419168-01) to supplement a previously submitted two-generation rat reproduction study (MRID-403326-01). FIFRA Section 6(a)(2) was cited because of the adverse effects on reproduction reported at a dose of azinphos-methyl in the two-generation rat reproduction study that did not produce overt signs of parental toxicity.

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Conclusion: Classification of Data: Supplementary to a two-generation study reviewed (DER006533) December 1987.

Maternal NOEL < 5 ppm with significant decrease in plasma and erythrocyte cholinesterase activity during day five of lactation in parental females at the 5 ppm level.

Reproductive NOEL - 5 ppm with significant reduction in viability index and observed decreased pup body weight during days 14 and 21 of gestation were reported at the 15 ppm level.

Background Information:

A two-generation rat reproduction study (83-4) was reviewed (DER006533) by D. Ritter, December 1987 with the conclusion that the Maternal NOEL is 15 ppm, the Reproductive NOEL is 5 ppm and the data classified Core Minimum. This two-generation rat reproduction study (MRID 403326-01) and the attached 1-generation rat reproduction study (MRID 419168-01) were both conducted at dietary levels of 0, 5, 15 or 45 ppm. The purity of the technical material fed in the two-generation study was 87.2% as compared to a purity of 92% fed in the attached one-generation rat reproduction study.

Cholinesterase activity was not determined in the two-generation rat reproduction study. The registrant sought to "correlate the findings in the two-generation reproduction study with the cholinesterase data from a 28-day dietary feeding study and a chronic toxicity/oncogenicity study." Toxicology reviewer (DER 006533) was of the opinion that "these studies are not acceptable for fulfilling data requirements under EPA guidelines because no detailed analytical data are included and only the briefest outline of a protocol is provided. In addition there are no data for young and very young animals." These studies were not designed to evaluate reproductive performance to cholinergic stress in the parental animals, that was demonstrated in the attached one-generation rat reproduction study.

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Reviewed By: Ray Landolt 10/24/91
Section I, Toxicology Branch II - (H-7509C)
Secondary Reviewer: Mike Ioannou 10/24/91
Section I, Toxicology Branch II - (H7509C)

DATA EVALUATION REPORT

Study Type: One Generation Rat Reproduction Study (83-4)

Tox Chem No. 374
MRID No. 419168-01
Project No. 1-2180

Test Material: 0,0-Dimethyl S-[(4-oxo-1,,2,3 benzotriazin-3(4H)-yl)methyl]phosphorodithioate

Classification: Insecticide-Cholinesterase Inhibitor

Common Name: Azinphos-methyl

Study No.: 100646

Date of Study: October 8, 1990

Sponsor: Mobay Corporation

Testing Facility: Bayer AG, Department of Toxicology
West Germany

Title of Report: Investigation of Inhibition of Cholinestarase Activity in Plasma, Erythrocytes and Brain in a One-Generation Study

Author: Dr. B. Holzum

Quality Assurance: [illegible]n

Conclusions: Classifi[illegible]ata: Supplementary to a two-generation study reviewed (DER006533) December 1987.

Maternal NOEL < 5 ppm with significant decrease in plasma and erythrocyte cholinestease activity during day 5 of lactation in parental females at the 5 ppm level.

Reproductive NOEL 5 ppm with significant reduction in viability index and observed decreased pup body weight during days 14 and 21 of lactation were reported at the 15 ppm level.

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A. Experimental Design

1. **Animals:** SPF-bred 6 to 8-week old Wistar rats, of the Bor:WISW strain, weighing between 106g and 142g for males and 92g and 146g for females were used in this study.

2. **Test material:** Technical material (92%), a yellow solid (flakes) of batch number 233 796 036 was mixed into Altromin 1321 feed with 1.0% peanut oil added to the food mix. Animals were fed treated diet throughout the duration of the study.

3. Dosing Schedule: Groups 1 to 4 are to determine whether "the slight effect on fertility (fertility index, number of pups born) observed at and above a dosage of 15 ppm in the previous 2-generation study could be confirmed."

Group	Male		Female	
	Dose (ppm)	No.	Dose (ppm)	No.
1	0	18	0	46
2	5	18	5	46
3	15	18	15	46
4	45	18	45	46

Groups 5 to 7 are to determine "whether the effect on fertility was attributable to treatment of the male or female animals."

Group	Male		Female	
	Dose (ppm)	No.	Dose (ppm)	No.
5	5	10	0	20
6	15	10	0	20
7	45	10	0	20

4. **Environmental control:** animals were housed individually, except during the mating phase, with temperature and humidity controlled to provide a uniform environment. A 12-hour light-dark cycle was provided.

5. **Procedure:** The 6 to 8 week old F0 animals used in this study were kept in individual cages for 14 weeks and then mated. During the 16-day mating period each male animal was mated a maximum of 12 times with 2 female animals from the corresponding group. Female F0 animals in which insemination was not established and female F0 animals which exhibited no weight gain suggestive of pregnancy during the 16 day mating period were mated up to 4 times during the following week with a male animal in the same group which had inseminated both females with which it was mated, with at least one of these females exhibiting weight gain suggestive of pregnancy. Five days after birth the F1 litters were reduced, where necessary, to 8 pups. Rearing of the F1 pups in groups 1 to 4 ended on day 28 p.p. and that of those in groups 5 to 7 on day 5 p.p. Animals were mated overnight, one male to one female rat. Vaginal smears were taken the morning after each mating. Day 0 in calculating the gestation period was the day positive sperm or the existence of a vaginal plug was observed.

6. Statistical significance was determined for body weight of parent, pup, litter, food consumption and food efficiency using the U-test by Mann, HB and Whitney, D.R. or by Wilcoxon, F. Fisher's exact test at significant levels of $p<0.05$ and $p<0.01$ were used for insemination, fertility, gestation, live birth, viability and lactation indices.

   Statistical significance of cholinesterase activity for plasma, erythrocytes, brain and brain weight were determined by F-test and T-test or T-test by Welch.

B. Methods and Results

1. Parental Observations (FO)

a. Mortality and signs of toxicity were observed for females, but not for males fed the 45 ppm level (group 4). At the 45 ppm level five females died during weeks 3 to 6. No signs of toxicity were observed for these five female rats. Emaciation, ataxia, decreased activity, ruffled fur and bloody nose were reported for the two female rats (at the 45 ppm level) sacrificed moribund during weeks 3 and 10 of the study. No treatment related deaths or signs of toxicity were recorded at the 45 ppm level during the pretreatment phase in the previous study. The author of this study associated these findings "with inhomogeneous distribution of the test compound in the food during the weeks these animals died." However, mortality and signs of toxicity were reported for females at the 45 ppm during the lactation phase in the previous study.

b. Body Weight and Dietary Intake. All FO animals were weighed at the start of the study. Thereafter, male body weights were recorded weekly until post mortem and food consumption recorded weekly to the end of the pretreatment phase. For females, body weight and food consumption were recorded weekly until insemination was established then on days 0, 7, 14 and 20 p.c., then on days 0, 5, 7, 14, 21 and 28 after birth of the pups. The food efficiency for the pretreatment phase was calculated as the rate of body weight gain(g)/food consumption(g) x 100 = % food efficiency.

A significant ($p<0.05$) decrease in body weight gain (3-4%) was reported for females fed the 45 ppm level (group 4) during weeks 2, 4 and 8 accompanied by a significantly ($p<0.01$) reduced food efficiency for the 45 ppm (group 4) level female rats during weeks 1, 2 and 8 of the pretreatment phase.

Male body weight gain was significantly ($p<0.05$) decreased (5-7%) at the 45 ppm level (group 7 only) during weeks 8 to 10 accompanied by a significant ($p<0.05$) reduced food efficiency for the 45 ppm (group 7) males during weeks 1, 3, 7 and 10 of the pretreatment phase.

Food consumption was significantly ($p<0.01$) decreased during week 3 at the 45 ppm level for males (11%) and females (14%). The decrease in food intake was attributed to "inhomogeneous distribution of the test material in the 45 ppm mix during the third week of the study."

During weeks 5 to 14 of lactation female food consumption decreased significantly ($p<0.05$) at the 45 ppm level by 22% to 27% accompanied by an 8 to 14% decreased food consumption observed for females of the 15 ppm level also for the 5 to 14 day period of lactation.

No significant treatment-related effects on body weight gain, food consumption or food efficiency were reported for the parental males or females fed the 5 and 15 ppm levels.

Dietary Intake of Azinphos-Methyl
(mg/kg/day) During Pretreatment

Dietary Level	5ppm	15ppm	45ppm	5ppm	15ppm	45ppm
Group	2	3	4	5	6	7
Male	0.43	1.30	3.73	0.44	1.32	3.83
Female	0.55	1.54	4.87	0	0	0

Summary of Dietary Analyses for Azinphos-methyl

	Nominal Concentration (ppm)		
	5	15	45
Mean	5.1	15	42
Standard Deviation (%)	8.5	9.6	18.0
Mean (%) of Nominal	102	100	93

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c. Cholinesterase Activity (ChE) was determined in groups 1 to 4 from blood taken from the retro-orbital venous plexus and in brain from the left hemisphere of the brain of parental animals. ChE activity was determined in plasma and brain by a modified method of Ellmann* and erythocytes (RBC) by a modified method of Sagesaka**.

Cholinesterase Activity of Parental (FO) Animals

Interval	Sex	No.	Dose (ppm)	Percent Inhibition Plasma	RBC	Brain
End of mating	male	10	5	2.3	18.8††	0.6
		10	15	13.6†††	68.8†††	-
		10	45	43.2†††	93.8†††	18.6
End of pre-treatment	female	10	5	-	-	9.5†
		10	15	24.5†	45.8†††	3.9
		8	45	62.0†††	70.8†††	55.3†††
Day 11 Gestation	female	8	5	-	-	
	10	15	18.1	52.4†††	21.3††	
		8	45	60.4†††	81.0†††	69.0†††
Day 5 Lactation	female	10	5	26.1†	25.4††	-
		10	15	46.3†††	74.6†††	38.4††
		8	45	66.4†††	91.1†††	65.9†††
Day 28 of lactation		10	5	5.0	46.8†††	11.8
		10	15	38.8††	83.9†††	48.0†††
		7	45	62.5†††	88.7†††	67.7†††

† significant $p<0.05$
†† significant $p<0.01$
††† significant $p<0.001$

* Ellmann, G.L., Biochem. Pharmacol 7, 88-95(1961)
** Clin Chim Acta 80, 87-94 (1977)

In the previous table calculations for the female parental rats were based on animals which were pregnant or had live pups. Parental male ChE activity was significantly ($p<0.001$) inhibited at the 15 ppm level in two parameters, in plasma (14%) and RBC (25%) during day 5 of lactation. At the 15 ppm level, parental female ChE activity was significantly ($p<0.05$) inhibited in RBC (46-84%) at all intervals and in two or more parameters with either plasma (25-46%) or brain (21-48%) or both plasma and brain. Female ChE activity at the 45 ppm level was significantly inhibited in plasma, RBC and brain at all intervals.

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d. Terminal Observations

i. Necropsy finding

All animals which died, sacrificed moribund or by exanguination were subjected to gross necropsy. Males were anesthesized and sacrificed by exanguination at the end of the mating period with the same consideration given to females at the end of the lactation period.

Of the seven parental female rats which died or were sacrificed moribund at the 45 ppm level the lungs were dark red to brown in color (4/7), the spleen pale (1/7) and clear demarcation of the liver lobe (2/7) was noted.

No gross pathological findings were reported in the parental male and female rats sacrificed during the termination of the study relative to the dietary levels fed.

ii. Histopathological findings

At the 45 ppm level, the lungs of females which died or were sacrificed moribund were described as moderate to marked hyperemic with slight edema. These findings were reported to be "suggestive of terminally induced congestive disorders." No other histopathological findings were reported in the parental male or female rats sacrificed during the terminatiion of the study relative to the dietary level fed.

Absolute brain weights of male and female parental (FO) animals sacrificed for determining ChE activity were comparable between the control and the three dietary levels fed with no statistical difference between groups.

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2. Litter Data

a. Reproductive Indices

In calculating the insemination index, females were considered inseminated if they were inseminated during the 16 day matinig period or the 4 day subsequent mating period. Only those females inseminated during the 16 day mating period were considered in the calculations. Considered pregnant were those animals which gave birth to pups or which were found to have implantations in the uterus at post mortem. Female animals sacrificed on day 11 p.c. were not included in calculation of the gestation index.

$$\text{Insemination index} = \frac{\text{number of inseminated females}}{\text{number of mated females}} \times 100$$

$$\text{Fertility index} = \frac{\text{number of pregnant females}}{\text{number of inseminated females}} \times 100$$

$$\text{Gestation index} = \frac{\text{number of females with live pups}}{\text{number of pregnant females}} \times 100$$

The following table summarizes the reproductive indices from mating one FO male from each group with one FO female from the corresponding group.

Group	1	2	3	4	5	6	7
Dose (ppm)	0	5	15	45	5/0	15/0	45/0
Insemination Index (%)	100	100	100	100	100	100	100
Without additional mating	97.2	91.7	97.2	96.8	95.0	90.0	95.0
Fertility Index (%)	97.2	94.4	100	96.8	100	95.0	100
Gestation Index (%)	88.5	100	100	95.5	100	100	95.0
Gestation Period (days	22.8	22.4	22.6	22.7	22.6	22.7	22.5
Mated Females (n)	36	36	36	31	20	20	20

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b. Maternal Performance - The following indices were evaluated for maternal performance.

$$\text{Live Birth Index} = \frac{\text{number of live pups at birth}}{\text{number of pups born}} \times 100$$

$$\text{Viability Index} = \frac{\text{number of live pups after 5 days (before reduction)}}{\text{number of live pups at birth}} \times 100$$

$$\text{Lactation Index} = \frac{\text{number of live pups after 4 weeks}}{\text{number of live pups after 5 days (after reduction)}} \times 100$$

The rearing success of the FO female rats through day 5 for the viability index and through day 28 for the lactation index are summarized in the following table. Number of FO females available for evaluation were in group 1(11), group 2 (16), group 3 (14) and group 4(8). In groups 5 to 7 only male rats were fed the test material.

A significant decrease in the viability index was reported in groups 15 ppm ($p<0.05$) and 45 ppm ($p<0.01$). No treatment related effect on viability was reported for FO parental females fed the 5 ppm or on those fed control diet in groups 5, 6 and 7. No treatment related effect was reported for the lactation index. No treatment related effect on the live birth index was reported for the dietary levels fed as compared to the control values. Historical control data for 14 reproduction studies conducted between 1978-1986 is attached.

Group Dose (ppm)	1 0	2 5	3 15	4 45	5 5/0	6 15/0	7 45/0
Viability Index (%)	93.4	92.4	86.0*	48.3**	98.1*	90.8	95.7
Lactation Index (%)	62.1	74.2	69.8	57.7	-	-	-
Live Birth Index (%)	94.6	98.6*	98.9**	98.6*	99.1*	99.5**	98.6*
Total Pups Born	240	293	275	214	216	196	211
Number Dead	13	4	3	3	2	1	3
Litter Size at Birth	9.9	11.1	10.5	10.0	10.7	10.3	10.9
Percent Males	45.0	48.8	48.7	49.5	52.3	48.5	56.4
Percent Females	55.0	51.2	51.3	50.5	47.7	51.5	43.6

*Statistically significant $p<0.05$
**Statistically significant $p<0.01$

c. Pup Observations

No treatment related malformations were observed. No gross signs of toxicity were observed among F1 pups during the 28-day period relative to the dietary levels fed. Groups 5 to 7 were sacrificed on day 5 post parturition (p.p.)

Pup Mean Body Weights (g)

Group	1	2	3	4	5	6	7
Dose (ppm)	0	5	15	45	5/0	15/0	45/0
Day 0 Male	6.04	5.86	6.00	5.99	5.97	5.97	5.87
Female	5.86	5.54	5.65	5.71	5.67	5.78	5.55
Day 5 Male	9.24	8.90	9.26	8.08	9.09	9.24	8.87
Female	9.04	8.52	8.87	7.80	8.61	9.34	8.47
Day 5 Male	9.39	8.85	9.44	8.88 {after litters were			
Female	9.15	8.33	8.86	8.97 {culled to eight pups			
				{on day 5 p.p.}			
Day 7 Male	12.38	11.03	11.76	11.37			
Female	11.87	10.44	10.71	11.39			
Day 14 Male	24.20	22.50	21.92	20.97			
Female	24.81	22.38	22.39	19.51*			
Day 21 Male	37.00	35.20	32.68	29.81*			
Female	37.52	35.51	33.58	28.07*			
Day 28 Male	52.77	52.79	51.52	50.45			
Female	52.21	52.07	50.86	45.46			

* Significant ($p<0.05$)

During days 14 to 21 of the observation period a significant ($p<0.05$) decrease in female body weight gain of 21% to 25% was reported at the 45 ppm level accompanied by an observed decrease in female body weight gain of 10% to 11% at the 15 ppm level. A decrease in body weight gain of 13% to 19% was observed for males at the 45 ppm level during days 14 to 21.

d. Terminal Observations

i. Pups Brain Weight and Cholinesterase Activity
Determination of brain cholinesterase activity followed the modified method of Ellmann described for the FO generation. Plasma and erythrocyte cholinesterase activity were not determined for the F1 generation.

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The following table summarizes the mean brain weight and cholinesterase activity of males and females combined for the respective dietary levels of the F1 pups during days 5 and 28 post parturition.

	Day 5 p.p.				Day 28 p.p.			
Dose ppm	n	Activity U/g	Inhib. %	Absolute Brain Wt. (g)	n	Activity U/g	Inhib. %	Absolute Brain Wt. (g)
0	10	1.79		0.476	9	2.49		0.695
5	10	1.92	-	0.516	10	2.84	-	0.724
15	10	1.77	1.1	0.500	10	2.14	14.1	0.665
45	8	1.49*	1.68	0.386**	7	1.34***	46.2	0.691

* significant p≤0.05
** significant p≤0.01
*** significant p≤0.001

Brain cholinesterase activity of male and female values combined at the 45 ppm level were significantly reduced during day 5 post parturition ($p<0.05$) by 17% and during day 28 post parturition ($p<0.001$) by 46% as compared to the control values.

Pup absolute brain weights were significantly p(<0.01) lower at 45 ppm level by 19% as compared to the control values on day 5 post parturition, as the result of a corresponding 27% decrease in body weight during this interval.

Conclusions: The two-generation rat production study (MRID 403326-01) concluded that "azinphos-methyl produces maternal toxicity at 45 ppm but not at levels of 5 and 15 ppm. The reproductive NOEL is 5 ppm." The data was classified as Core Minimum.

This one-generation rat reproduction study (MRID-419168-01) is supplementary to the two-generation rat reproduction study with the additional cholinesterase data provided.

The cholinesterase NOEL is less than 5 ppm with a significant decrease in plasma and erythrocyte cholinesterase activity reported during lactation in parental females at the 5 ppm level. Cholinesterase activity was significantly inhibited in plasma erythrocyte and brain in parental animals fed the 15 and 45 ppm levels.

The reproductive NOEL is 5 ppm with a significant decrease in the viability index reported for the 15 ppm level accompanied by an 11% decrease in pup body weight observed during days 14 to 21 of lactation. There was no effect on reproductive indices as evident in parental performance at the 15 and 45 ppm levels.

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Historical Control Data
for the FO Generation of Fourteen Reproduction Studies
Conducted Between 1978-1986
Bayer AG, Department of Toxicology, West Germany
Report Number 19594

Parameter	Mean Values First Mating (FIA)	Second Mating (FIB)
Insemination Index (%)	97.7	98.5
Fertility Index (%)	89.7	92.5
Gestation Index (%)	99.0	98.1
Gestation Period (8 days)	22.3	22.1
Number of Dead Pups at Birth	3	5
Ratio of Male to Female Pups at Birth (%)	50.4/49.7	49.3/50.7
Mean Litter Size at Birth	10.4	9.5
Viability Index (%)	97.1	95.4
Lactation Index (%)	98.3	97.2
Mean Body Weight of Pups at Birth (g)	5.9	5.9
Mean Body Weight of Pups at One Week (g)	12.7	13.9
Mean Body Weight of Pups at Two Weeks (g)	22.8	25.5
Mean Body Weight of Pups at Three Weeks (g)	34.9	39.2
Mean Body Weight of Pups at Four Weeks	54.5	60.8

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