UNITED STATES ENVIRONMENTAL PROTECTION AGENCY

DATE: January 16, 1979

SUBJECT: EL-222; Experimental Use Permits 1471 EUP-50 (EL-222 50W on Turf) 1471-EUP-51 (EL-222 EC on Rose) 1471-EUP-52 (EL-222 EC on Turf) 1471-EUP-54 (EL-222 EC on Apples) 1471-EUP-55 (EL-222 EC on Grapes) Caswell No. 207AA

FROM: William Dykstra, Ph.D
Toxicology Branch/HED

002610

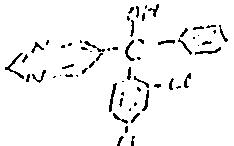
TO: Henry Jacoby (21)
RD,(TS-767)

THRU: Acting Chief, Toxicology Branch
HED (TS-769)

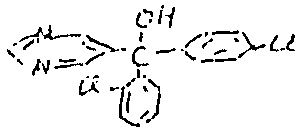
Registrant: Elanco Products Company

Recommendations:

1. The Experimental Use Permits are not toxicologically supported. Previous cancellation of the EUPs (memo 10/22/76, R. Coberly) was based on the structural similarity of EL-222 to EL-273 as shown below:



EL-273



EL-222

and the fact that EL-273 in a 2 year oral feeding study in rats at levels of 50, 100, 500 and 2500 ppm was judged to be a liver carcinogen in the rat at 2500 ppm and possibly at 500 ppm (memo of 3/15/72, C.H. Williams). The maximum tolerated dose (MTD) of 2500 ppm in the EL-273 2 year rat study is 7.1 X greater than the MTD of 350 ppm in the 2 year rat studies (R-405 and R-415) with EL-222. A comparison of acute and subchronic data between EL-273 and EL-222 does not reflect a difference in toxicity of a factor of 7.1. The MTD of EL-222 is significantly below the MTD of EL-273 which demonstrated the hepatocarcinogenic response. The registrant is requested to address the discrepancy in the MTD for EL-273 and the MTD for EL-222.

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AM 1320-6 (REV. 3-76)

2. The teratology study as reported is incomplete. The results of the visceral examination of fetuses were not submitted for review. These results are required to be submitted.

3. In the combined 2 year rat feeding studies (R-405 and R-415), there was a low, but statistically significant, increase in the incidences of hepatic adenomas (3.2% in high-dose group vs 0% in controls) and hyperplastic nodules (4.4% in high-dose group vs 0% in controls) in rats. The registrant is requested to submit data from their laboratory on the incidence of hepatic adenomas and hyperplastic nodules in the historical control rats of the type used in the studies for comparison to the incidences in the high-dose rats.

4. The toxicological studies submitted are summarized and classified below:

Book 1 - Toxicology Studies

a. Multi-generation reproduction study with EL-222 in the rat; NOEL is 50 ppm for reproductive parameters.

At dietary levels of 130 and 350 ppm EL-222 has a significantly, apparently irreversible, anti-fertility effect.

Classification: Core-Minimum Data

b. In a second multi-generation reproduction study with EL-222 in the rat; the NOEL is 12.5 ppm. Fertility was unaffected at 25 ppm but mean liveborn litter sizes for the 25 and 50 ppm groups were decreased in the 2nd mating of the F1 parents. There was an increase in hydronephrosis in the F3a progeny in the 25 and 50 ppm groups.

Classification: Core-Minimum Data

c. A one-year toxicity study with EL-222 in the rat; NOEL for systemic toxicity is 130 ppm. Relative liver and kidney weights were increased in females at 350 ppm.

Classification: Core-Minimum Data

d. 18-month chronic oral toxicity of EL-222 in rats; NOEL for systemic toxicity is 50 ppm. Atrophy of thymus occurred at 130 ppm. No increase in incidence of benign or malignant neoplasms.

Classification: Core-Minimum Data

e. Pilot reproduction study with EL-222 in the mouse; NOEL is 50 ppm. Reduced fertility and mean liveborn litter size at 170 ppm and above.

Classification: Supplementary Data

(1) Pilot Study

f. A multi-generation study with EL-222 in the mouse; NOEL for reproductive performance and progeny parameters is 140 ppm for 3 generations of mice. This was the highest dose treated.

Classification: Core-Minimum Data

Book 2 - Toxicology Studies

a. Twelve-month chronic oral toxicity of EL-222 in mice; The NOEL is 170 ppm in diet for systemic toxicity. Effects at 600 ppm were fatty metamorphosis in the liver in both sexes. Increased liver and spleen weights in males and liver weight in females.

Classification: Core-Minimum Data

b. Twenty-four month chronic oral toxicity of EL-222 in mice; No increase in benign or malignant neoplasms. NOEL for systemic toxicity is 170 ppm. 600 ppm dose showed fatty metamorphosis of liver in study m-9135 in both sexes.

Classification : Core-Minimum Data

Book 3 - Toxicology Studies

a. The effect of EL-222 on bacterial systems known to detect mutagenic events; EL-222 is non-mutagenic in S. typhimurium (8 strains) and E. coli (2 strains) with and without metabolic activation.

Classification: Core-Minimum Data

b. A dominant lethal study of EL-222 in the rat; Negative at a single dose level of 350 mg/kg.

Classification: Core-Minimum Data

c. A teratology study in EL-222 in the rabbit; Results of Visceral examination of fetuses were not submitted for review. No external or skeletal terata up to 35 mg/kg during days 6-18 of gestation.

Classification: Supplementary Data

(a) Results of visceral examination of fetuses were not submitted for review.

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d. Twenty-four month chronic oral toxicity of EL-222 in Rats; There was an increased incidence of fatty change in the liver of both sexes at the lowest dose (50 ppm). A low but statistically significant increase in hepatic adenomas (3.2%) and hyperplastic nodules (4.4%) was observed in the 350 ppm group of rats. The number of animals with other benign or malignant neoplasms was not increased by treatment.

Classification: Core-Minimum Data

Book 4 - Toxicology Studies

Additional data from 2 year rat studies.

Review:

1. Toxicology Studies Book 1 (Accession#235175)

A. A Multi-Generation Reproduction Study with EL-222 in the Rat (. conducted by D.G. Hoffman, E.R. Adams, J.K. Markham, N.V. Owen, W.R. Gibson, and D.M. Morton; Toxicology Division, Lilly Research Laboratories, Nov. 1977.

Test Material: 56722 (EL-222, fenarimol) α-(2-chlorophenyl)-α-(4-chlorophenyl)-5-pyrimidinemethanol. Lot No. B30-C69-220; 97.9% EL-222.

Wistar-derived rats were used for control (30 per sex) and treatment groups (20 per sex) at dietary levels of 0, 50, 130 and 350 ppm test material. The study numbers were: R-715, F0 generation (May 7, 1975 - Jan. 16, 1976); R-1345, F1 generation (Nov. 12, 1975 - July 20, 1976); R-956, F1 generation males (Aug. 16, 1976 - Sept. 28, 1976); R-966, F1 generation females (Aug. 16, 1976 - Oct. 14, 1976). A similar number of untreated males and females were mated with the F1 generation animals (R-956, R-966). Growth and reproduction was examined for a duration of two generations. The parameters studied were survival, food consumption, body weight gain, physical condition, fertility, gestation survival, live born litter size; 1, 7, 14 and 21 day progeny survival; progeny weight gain and sex distribution, progeny condition. Gross necropsies of representative offspring and microscopic examination of reproductive organs of F1 generation adults.

The breeding scheme and protocol for the multi-generation reproduction study is shown below:

The results were evaluated by appropriate statistical methods.

Breeding Scheme and Protocol for a Multigeneration Reproduction Study is shown below:

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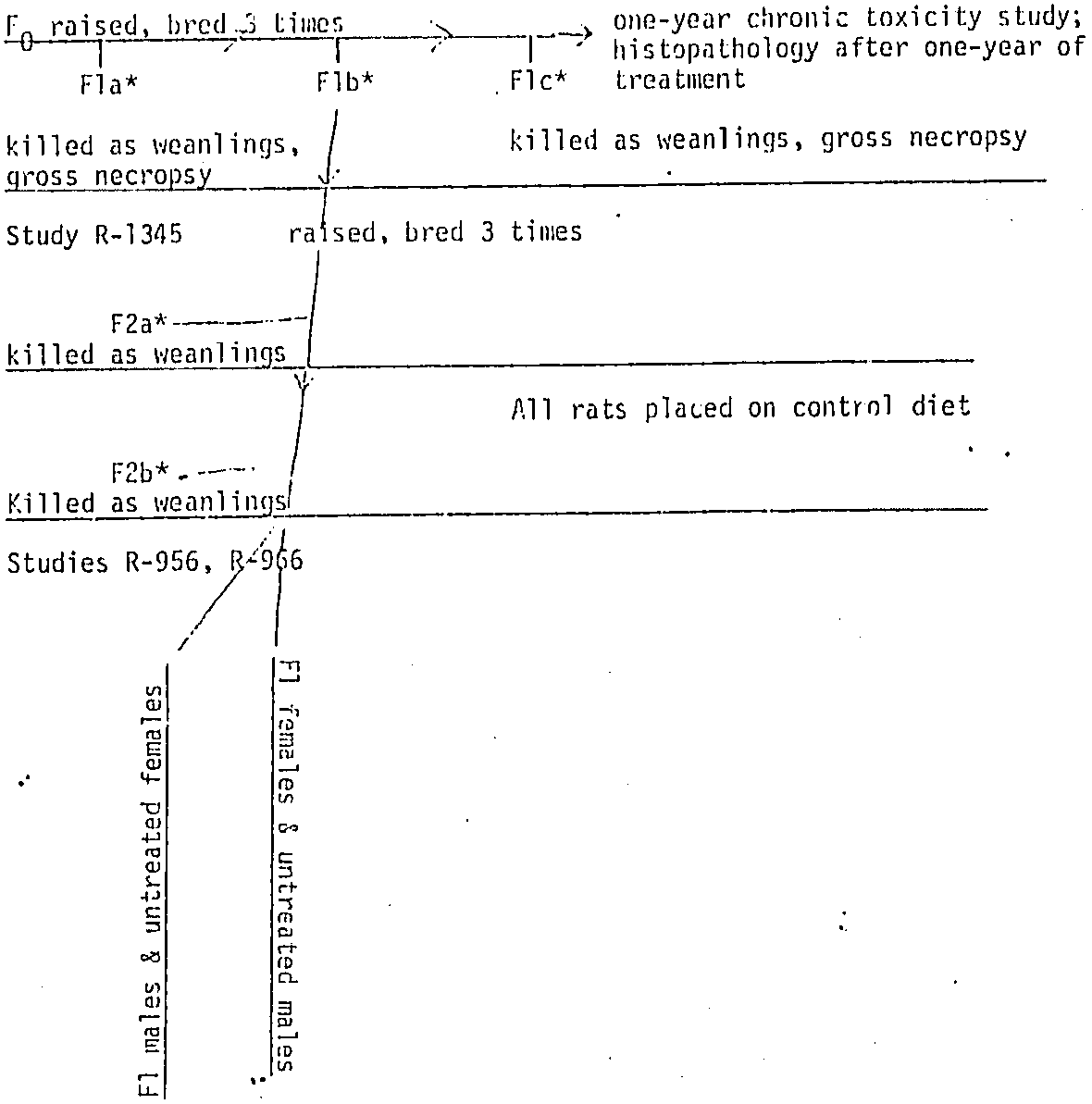
Study R-715



Offspring killed day 1 postpartum; histopathology on adult reproductive organs.

*growth and survival monitored through 21 days postpartum.

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Results

The compound was well-tolerated without significant mortality or overt signs of toxicity other than a depression of growth in F1 generation male parents at 130 and 350 ppm. During the 3 mating trials conducted with the F0 generation, the fertility rates deteriorated from a slight reduction in fertility at the 350 ppm level, to a significant decrease in fertility at that level, and finally to a significant reduction at both 130 and 350 ppm in the third mating trial. During the mating of the F1 generation rats, which were the f1b offspring of EL-222 treated parents, no pregnancies occurred at the 350 ppm level and only 4 of 20 females were pregnant at 130 ppm. The fertility performance of rats given 50 ppm EL-222 was comparable to controls.

Rats were placed on control diets for 2 months and then remated to assess the reversibility of the effect of EL-222 on fertility. The anti-fertility effect was not reversible.

Subsequent studies conducted in which the F1 rats previously given EL-222 were mated with untreated rats, in addition to reinforcing irreversibility, demonstrated that the anti-fertility effect although unquestionably occurring in previously treated males, was also apparent with previously treated females; however, the female data were not as conclusive due to the decreased fertility of the controls.

A second major effect of EL-222 on the reproductive process was an apparent lengthening of gestation and/or a delay in the onset and normal progression of parturition. Although an effect on parturition is somewhat difficult to define when it occurs at a low incidence, the following data primarily of the F0 generation rats are suggestive of such an effect: 1) reduction in live born litter size due to an increase in stillborn at 350 ppm; (2) a larger proportion of gestation lengths greater than 22 days in all groups given EL-222; (3) 1-day old progeny of the 350 ppm test group generally larger than controls; and (4) deaths of treated females during late gestation or parturition with no apparent anatomical problems other than large fetuses *in utero*.

Values of the reproduction parameters of progeny survival, weight, and sex distribution were not consistently affected, not affected in a dose-related manner, or as was the case in several mating trials, too few offspring were available for a meaningful evaluation. No abnormalities attributable to EL-222 treatment were apparent in adults or offspring with one possible exception. A high incidence of hydronephrosis occurred in weanling offspring of the 350 ppm level submitted for gross necropsy after the third mating of the F0 generation (f1c). The difference in incidence at 350 ppm from that of the control group was not statistically significant but this lack of statistical significance may be a function of the small sample size at 350 ppm.

Conclusions: EL-222 at dietary levels of 130 and 350 ppm had a significant, apparently irreversible, anti-fertility effect. Fertility rates of rats given 50 ppm EL-222 were comparable to controls. EL-222 also appeared to interfere with the normal initiation of parturition, although the evidence suggestive of such an effect is difficult to quantify from the data generated in this series of studies.

Classification: Core-Minimum Data

B. A Second Multi-Generation Reproduction Study with EL-222 in the Rat (Conducted by J.K. Markham, D.G. Hoffman, R.V. Owen and D.M. Morton; Toxicology Division, Lilly Research Laboratories, July, 1978)

Test Material: 56722 (EL-222, fenanimol) Lot No. B30-C69-220; 97.9% EL-222.

Wistar derived rats were used for the control group (30 per sex) and treatment groups (20 per sex) at dietary levels of 0, 12.5, 25 or 50 ppm of test material. A total of 180 parent animals were used per generation. Growth and reproduction was examined through three generations as follows: Study R-636, F0 generation (June 24, 1976 - Nov. 10, 1976), Study R-1076, F1 generation (Oct. 25, 1976 - May 6, 1977), Study R-217, F2 generation (March 10, 1977 - July 14, 1977). The parameters studied were survival, food consumption, body weight gain, physical condition, fertility, gestation, and survival, in the parent animals and live born litter size, gestation survival; 1, 7, 14 and 21-day survival; condition, weight gain, and sex distribution in the progeny. Gross necropsies were performed on parent animals that died. For the offspring gross necropsies were done as follows:

F0 generation - one pup per sex per litter in the control and all EL-222 treatment groups.

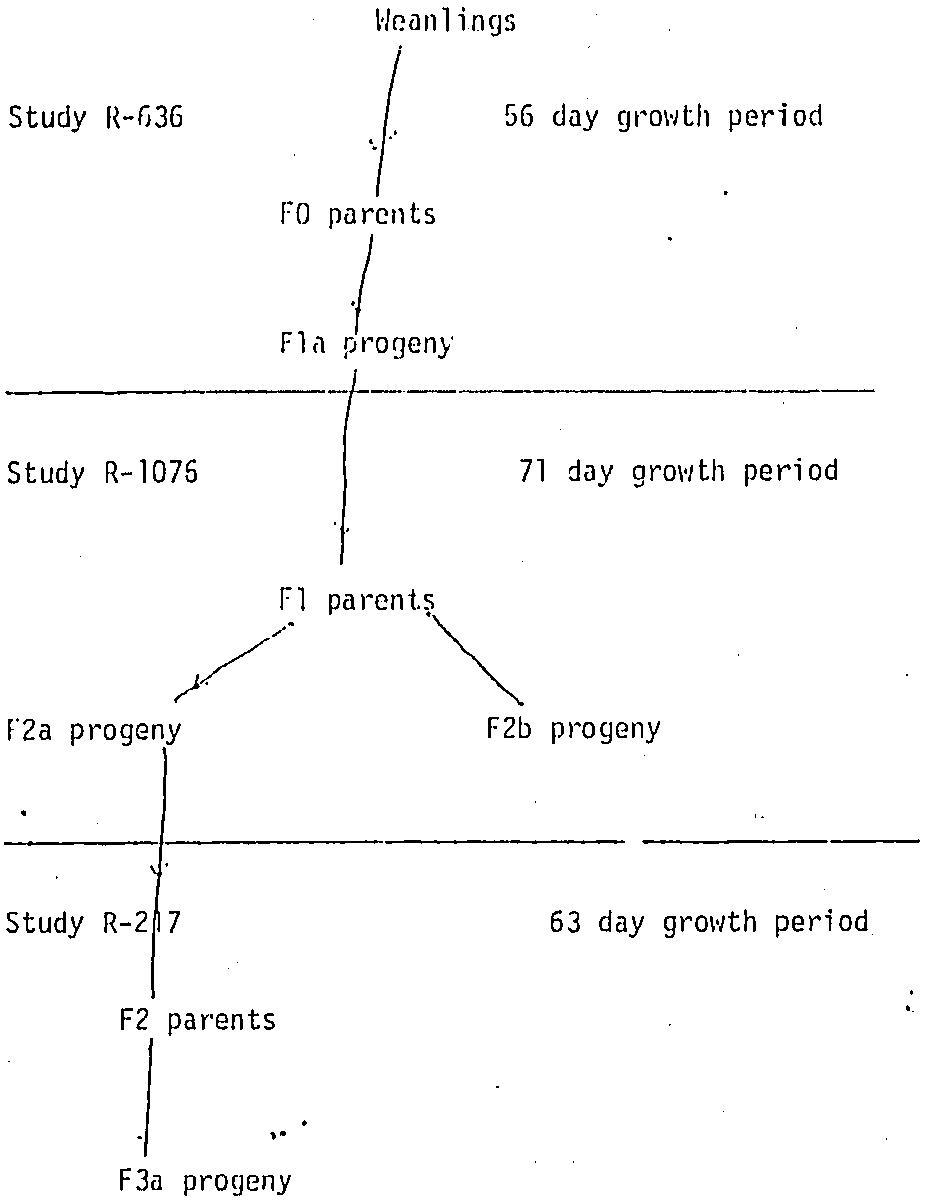
F1 generation - first mating trial - one pup per sex per litter in the control and in the 12.5 and 25 ppm EL-222 treatment groups .

F1 generation - second mating trial - gross pup necropsy examinations were not done.

F2 generation - on all offspring; in addition, tissues of one pup per sex per litter were examined microscopically.

Results were evaluated by the appropriate statistical methods. The Breeding scheme for the multi-generation study with EL-222 in rats is shown below:

Breeding Scheme for a Multi-Generation Study with EL-222 in Rats.



Results:

Growth was significantly depressed in the 50 ppm F1 male parents but not in other groups or generations. Parental fertility was not decreased in the F0 parents. In the 50 ppm dietary group, fertility was significantly depressed in the 2 mating trials of the F1 parents and in the F2 mating trial. Dietary levels of 12.5 and 25 ppm had no effect on fertility. One female in the 50 ppm group died during parturition on the calculated gestation day 23; an additional female of this group showed signs of hemorrhage during parturition.

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Previous studies demonstrated that EL-222 interferes with the reproductive process resulting in a delay in the onset and normal progression of parturition and lengthened gestation periods. Consequently, although of low incidence, these findings must be attributed to compound.

Mean liveborn litter sizes for the 25 and 50 ppm groups were decreased in the second mating trial of the F1 parents. Mean liveborn litter sizes for the EL-222 treated females were normal in all of the other mating trials. There was no evidence of a consistent, substantive effect on progeny survival, weight, or sex distribution. In the terminal generation (F3a progeny), the incidence of hydronephrosis was higher in weanling animals from the 25 and 50 ppm treatment groups than in the control weanlings. The increase in hydronephrosis was not statistically significant.

Conclusion: 50 ppm of EL-222 resulted in decreased fertility. Fertility was unaffected at 12.5 and 25 ppm. Mean liveborn litter sizes for the 25 and 50 ppm groups were decreased in the 2nd mating of the F1 parents. There was an increase in hydronephrosis on the F3a progeny in the 25 and 50 ppm groups.

Classification: Core-Minimum Data

C. A One-Year Toxicity Study with EL-222 in the Rat (Study R-715) (Conducted by D.G. Hoffman; E.R. Adams, P.N. Harris and D.M. Morton; Toxicology Division, Lilly Research Laboratories, June, 1978)

Test Material: 56722, EL-222; Lot No. B30-C69-220; purity 97.9%.

Wistar derived rats were used in the study. Control (30/sex) and EL-222 (20/sex/dose) test groups were administered 0, 50, 130 or 350 ppm of test material in the diet for one year. The phases of the test included growth (2 months), reproduction (7 months, 3 mating trails) and chronic feeding (3 months). Parameters studied included appearance and behavior mortality, growth, food consumption, terminal hematology, clinical chemistry, organ weights and gross and microscopic examination of tissues. Appropriate statistical analysis of the data was performed.

Results: No differences in the mortality rate resulted from dietary administration of EL-222. There were no apparent gross signs of toxicity. The weight gain of male rats given 350 ppm diets was depressed slightly but the terminal body weight was not statistically different from the control. Food consumption was not affected.

The WBC count was decreased and the RBC count increased in male rats given the diet containing 350 ppm. No other significant effect on hematological parameters was apparent. Mean blood glucose concentrations were increased slightly in the EL-222 test groups. The increases were statistically significant in low and high-dose males and middle and high-dose females. However, an unusually low control mean glucose concentration may account for the apparent increase in treated groups. There were no other toxicologically significant changes in blood chemistry parameters.

Relative liver and kidney weights were increased in females given the diet containing 350 ppm EL-222. There were no other toxicologically significant changes in organ weights. Slightly proliferation of small bile ducts occurred in all groups but more frequently in the treated animals. The incidence was not related to the dietary concentration of EL-222. Slight atrophy of the pancreas occurred more frequently in high-dose animals.

Conclusions: 50 and 130 ppm EL-222 had no toxicologically significant effects on rats when administered in the diet for one-year. The NOEL for systemic toxicity is 130 ppm.

Classification: Core-Minimum Data

D. Eighteen-Month Chronic Oral Toxicity of EL-222 (56722) in Rats, Study R-435 (Conducted by D.G. Hoffman, W.R. Gibson, E.C. Pierce, and D.M. Morton; Toxicology Division Lilly Research Laboratories, May, 1978). Study Number R-435.

Test Material: EL-222 (56722) Lot No. B30-C69-220; purity 97.9%.

Wistar derived rats were used in the study. Control (30/sex) and EL-222 (20/sex/dose) test groups were administered 0, 50, 130 and 350 ppm of test material in the diet for 18 months.

Parameters studied included appearance and behavior, mortality, growth, food consumption, terminal hematology, blood chemistry, serum prolactin organ weights and gross and histological examination of tissues. Appropriate statistical analyses of the data were performed.

Results: Dietary administration of EL-222 had no adverse effect on survival of rats. Seventy percent of the control females and 63% of the control males survived the 18-month treatment period. At the end of the test a higher fraction of the animals were alive in the groups given 350 ppm than in those given control feed. There were no grossly detectable signs of toxicity that would be attributed to administration of EL-222. EL-222 produced a slight, dose-related decrease in the growth of rats. The effect was more pronounced in males than in females. However, at the termination of the test only the low dose females weighed significantly less than the control females. There were no compound-related effects in food consumption or food utilization. There were no effects on any of the measured hematology parameters except for a slight decrease in prothrombin time in females given 350 ppm and a decrease in white cells in males given 350 ppm. No compound related effects were noted in blood chemistry parameters. Administration of 350 ppm diet for 18-months resulted in an increase in absolute and relative ovary weights in females and a decrease in absolute spleen weight in males. The relative liver weight was increased slightly in all treated groups but the difference was statistically significant only in the 350 ppm group.

The incidence of fatty change of the liver was increased slightly in the treated male rats, but not in a dose-related manner. In the females, there was no change in incidence but in the 350 ppm group, the severity of the fatty change was increased.

Atrophy of the thymus was a common finding in all groups, but occurred more frequently in the 130 and 350 ppm treated groups. There was no evidence of benign or malignant neoplasms.

Conclusions: Dietary administration of EL-222 did not increase the incidence of benign or malignant neoplasms. The NOEL for systemic toxicity is 50 ppm.

Classification: Core-Minimum Data

E. Pilot Reproduction Studies with EL-222 in the Mouse; Studies M-9165 & M-9215 (Conducted by J.K. Markham, D.G. Hoffman, E.R. Adams, N.V. Owen and D.M. Morton; Toxicology Division, Lilly Research Laboratories, July, 1978)

Test Material: EL-222; Lot No. B30-C69-220, purity 97.9%.

The ICR mouse was used in these studies at 10 per sex per group. Study M-9165 had dietary levels of 0, 50, 170 and 600 ppm of test material administered to animals for one week prior to mating, during the mating and gestation periods, and during a 21-day lactation period. Study M-9215 had dietary levels of 0, 170, 350 and 600 ppm of test material administered to animals for two weeks prior to mating, during the mating and gestation periods, and during a 14-day lactation period. In the parent animals, the parameters studied were physical condition, fertility, reproductive performance and mortality. In the progeny, the parameters studied were live-born litter size, gestation and postpartum survival, weight gain and sex distribution. The results were evaluated by the appropriate statistical methods.

Results: No adverse effect on maternal body weights. In study M-9165, female 353 (600 ppm) died postpartum day 14. No necropsy data was available. In study M-9215, female 352 (600 ppm) was killed on gestation day 21. Necropsy revealed dystocia.. Four mice were killed in the 600 ppm group (3) and 170 ppm group (1) and appeared normal on gross necropsy examination. The reproduction findings are summarized below:

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Study	EL-222 Group (ppm)	Fertility	Mean Liveborn Litter Size
M-9215	0	100	11.4
M-9165	50	90	12.0
M-9165	170	70	8.9
M-9215	170	70	11.3
M-9215	350	40	8.0
M-9165	600	90	5.3
M-9215	600	50	6.5

Decreased fertility values and decreased liveborn litter sizes at dietary concentrations equal to or greater than 170 ppm were attributed to EL-222 treatment. At the 600 ppm dietary level, progeny survival values through postpartum day 14 were lower than control survival values. Postpartum progeny survival was normal in the 50, 170 and 350 ppm dietary groups.

Conclusion: Diet levels of 170, 350 and 600 ppm adversely affected fertility and mean liveborn litter size. The reproduction performance of mice maintained on 50 ppm of EL-222 was not affected.

Classification: Supplementary Data

(1) Pilot Study only.

F. A Multi-Generation Study with EL-222 in the Mouse; Study Nos. M-9086, M-9296, M-9326 (Conducted by J.K. Markham, D.G. Hoffman, W.D. Broodle, N.V. Owen, and D.M. Morton; Toxicology Division, Lilly Research Laboratories, July, 1978)

Test Material: EL-222; Lot No. B30-C69-220; purity 97.9%.

The ICR mouse was used in these studies at 30 per sex (control group) and 20 per sex (test groups) at dietary levels of 0, 35, 70 or 140 ppm.

Study M-9086, F0 generation (April, 1976 - August 12, 1976); Study M-9296 F1 generation (July 28, 1976 - Dec. 14, 1976); Study M-9326, F2 generation, (Nov. 11, 1976 - April 7, 1977). Growth and reproduction was studied through three generations according to the following scheme.

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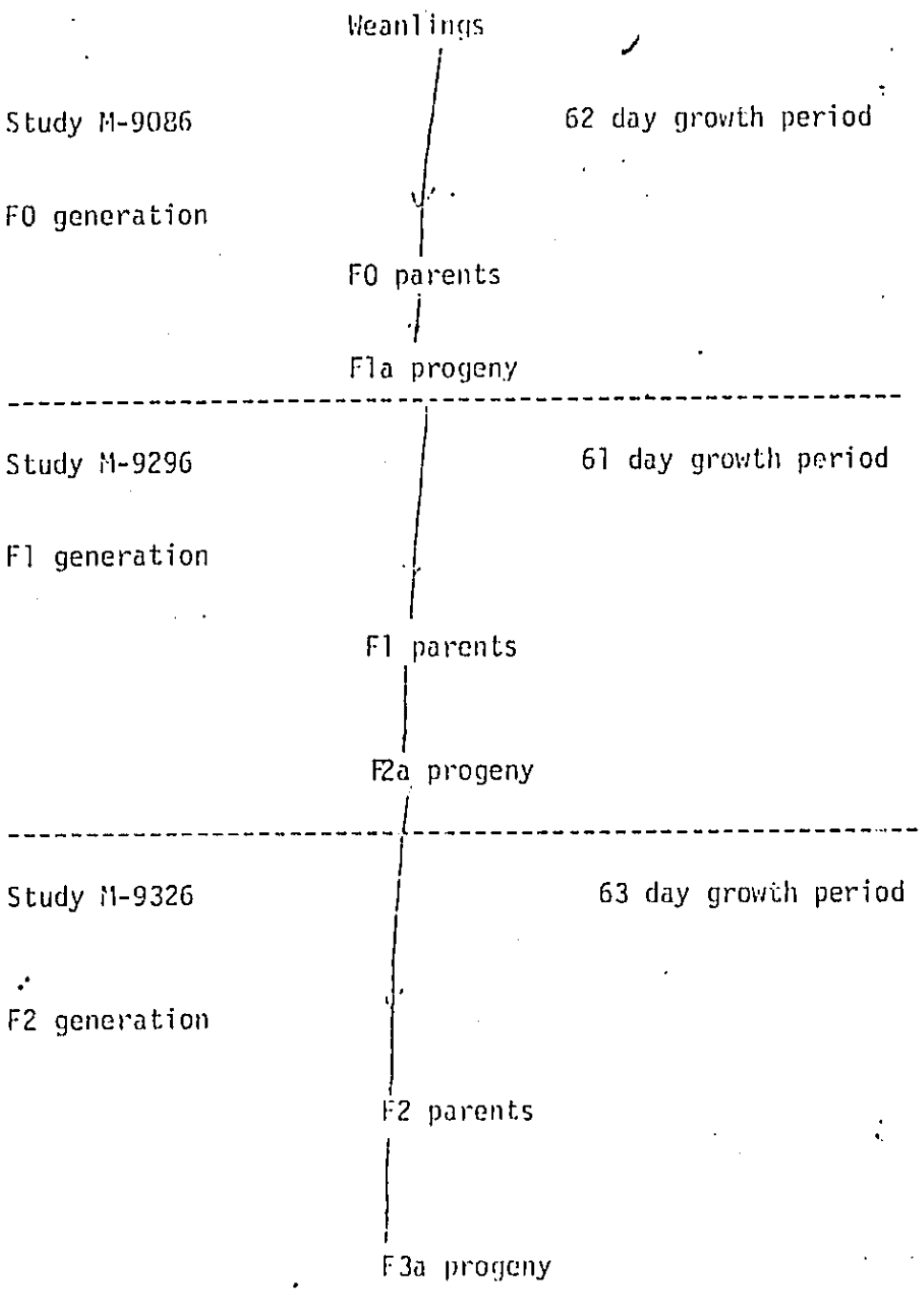

The parameters studied in parent animals were growth, physical condition, body weight gain, fertility, reproductive performance and mortality. The parameters studied in the progeny were liveborn litter size, gestation survival; 1, 7, 14 and 21 day survival; condition, weight gain, and sex distribution. Gross necropsies were performed on parent animals that die . For the offspring gross necropsies were done as follows:

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F0 generation - on all F1a progeny not assigned to the F1 generation.

F1 generation - one pup per sex per litter.

F2 generation - on all offspring (F3a); in addition tissues of one pup per sex per litter were examined microscopically. The results were evaluated by appropriate statistical methods.

Results: Nine parent mice died. These deaths were not related to EL-222 treatment. No adverse effects of the compound were seen in the growth phases of F_0, F1 & F2 was not altered by EL-222. In the F2 generation, 21 day survival and 21-day body weights were depressed in the control group as well as in the EL-222 dietary groups. Offspring pathology revealed no treatment-related findings. Chronic respiratory disease, which was diagnosed by histological examination of tissues from the F3a pups, was believed to be the probable cause of the increased offspring mortality in the F2 generation.

Conclusion: NOEL for reproductive performance and progeny parameters is 140 ppm (the highest dose level) for 3 generations of mice.

Classification: Core-Minimum Data

2. Toxicology Studies Book 2

A. Twelve-Month Chronic Oral Toxicity of EL-222 in Mice; Study M-9155 (Conducted by D.G. Hoffman, W.R. Gibson, E.C. Pierce, P.N. Harris, and D.M. Norton, Toxicology Division, Lilly Research Laboratories, April, 1978)

Test Material: EL-222, Lot No. B30-C69-220 97.9% purity.

The ICR mouse was used in these studies at 30/sex in the control group and 20/sex in treatment groups. The mice were fed diets containing 0, 50, 170 and 600 ppm of test material ad libitum for 12 months. The parameters studied included mortality, appearance and behavior, growth, terminal hematology and blood chemistry, organ weights and gross and histologic examination of the tissues. Statistical Analyses were performed.

Results: Dietary administration of test material has no adverse effect on survival. There was no overt evidence of toxicity that could be related to administration of compound. The rate of growth of mice given diets containing EL-222 was not adversely affected. The WBC count of male mice given the 600 ppm diet was increased significantly. No other hematological parameters were affected. In female mice, mean blood glucose and creatinine values were increased significantly in the 170 and 600 ppm groups. In males, the glucose and SGPT values were increased and bilirubin and Alkaline phosphatase values were decreased in the 600 ppm group. In the high dose male mice, the liver and spleen weights were increased significantly.

Uterus weight of female mice was slightly decreased and liver weight increased in the high-dose group. There were no apparent treatment-related abnormalities detected histologically except for slight fatty metamorphosis in the liver of male and female mice at 600 ppm.

Conclusion: The NOEL is 170 ppm for systemic toxicity for 12 months in mice. Marginal effects at 600 ppm were slight fatty metamorphosis in the liver in both sexes and increased liver and spleen weight in males and liver weight in females.

Classification: Core-Minimum Data

B. Twenty-four Month Chronic Oral Toxicity of EL-222 in Mice (Studies M-9135 and M-9145) (Conducted by D.G. Hoffman, W.R. Gibson, E.C. Pierce, P.M. Harris, and D.M. Morton, Toxicology Division, Lilly Research Laboratories, August, 1978)

Test Material: EL-222, Lot. No. B30-C69-220 purity; 97.9%.

The ICR mouse was used in two replicate studies, each containing a control group with 60/sex and dietary groups of 50, 170 and 600 ppm containing 40/sex/group. A total of 720 mice were used in studies M-9135 and M-9145. The test material was administered for 24 months and the parameters studied included mortality, appearance and behavior, growth, terminal hematology and blood chemistry, organ weights and gross and histologic examination of tissues. Appropriate statistical analyses were performed.

Results: Dietary adminstration of EL-222 to mice for 2 years had no significant effect on survival. Between 22 and 34% of each group survived the full two years. Nearly 50% of all the animals were alive for 21 months. There were no grossly detectable signs of toxicity that would be attributed to treatment. The growth of the high-dose males was slightly less than the controls or the two lower doses but the controls or the two lower doses but the difference was not significant. The RBC and hematocrit were decreased significantly in 170 ppm females in study M-9145 but not in any other treated groups. Creatinine was increased significantly in low- and middle-dose females of study M-9145. Glucose was increased in high-dose males and alkaline phosphatase decreased in middle-dose males of study M-9135. The changes in hematological and blood chemistry values do not appear to be of toxicological significance.

Dietary administration of EL-222 had no toxicologically significant effect on mouse organ weights.

A wide variety of lesions occurred in low incidence in the mice in both the control and treated groups. Alterations in liver histopathology were analyzed separately because of the known sensitivity of this organ to the effects of certain enzyme inducers. The table below shows the incidence of hyperplastic nodules, hepatic cell adenomas and hepatocellular carcinomas in both male and female mice of studies M-9135 and M-9145.

	Dietary EL-222 conc. (ppm)			
	0	50	170	600
No. mice	240	160	160	160
Hep. nodules	5	2	1	6
Hep. adenoma	4	1	0	1
Hep. carcinoma	1	1	3	3

None of the liver lesions in the treated groups were statistically significantly different from the controls. Slight fatty metamorphosis occurred to a greater extent in males and females of study M-9135 at 600 ppm. These liver effects were not observed in study M-9145 at 600 ppm.

Conclusion: No increase in benign or malignant neoplasms. The NOEL for systemic toxicity is 170 ppm. Slight fatty metamorphosis occurred to a greater extent in males and females of Study M-9135 at 600 ppm. These liver effects were not observed in Study M-9145 at 600 ppm.

3. Toxicology Studied Book 3

A. The effect of EL-222 on Bacterial Systems known to detect mutagenic events.

Test Material: EL-222, Lot No. B20-69-21

The test material was tested in ten bacterial systems; 8 Salmonella typhimurium: G46, TA1535, TA100, C3076, TA1537, D3502, TA1538 and TA98; and 2 E. Coli: WP2, WP2 UVrA⁻; at 100 µg of test compound impregnated into filter disks using either H_2O or DMSO as solvent. The system was run with and without metabolic activation using S-9 microsomal fraction from rat liver. The S-9 fraction was not obtained from PCB treated rats. Postive controls were streptozotocin which does not require microsomal activation and 2-acetylaminofluorene which does require microsomal activation.

Results: The positive controls gave postive results with the expected organisms: EL-222 was found to be negative when tested with and without metabolic activation against ten organisms designed to detect mutagenic events.

Conclusion: EL-222 is not mutagenic under the conditions of the assay.

Classification : Core-Minimum Data

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B. A Dominant Lethal Study of EL-222 in the Rat; Study No. R-346; (Conducted by D.G. Hoffman, J.K. Markhan, N.V. Owen, and D.M. Morton, Toxicology Division, Lilly Research Laboratories, Jan., 1977)

Test Material: EL-222, Lot No. B30-C69-220; 97.9% purity.

Twenty Harlan-Wistar - derived rats, proven males which ranged in weight from 404-490 gm, were used. Ten males were designated at random as vehicle controls and the remaining 10 were given a single oral dose of 350 mg/kg BW of EL-222. This dose is approximately 10X greater than the high dose used in the rat reproduction study (R-715) and is greater than 1/10 of the rat oral LD_{50}. The test material was prepared as a 7% (w/v) suspension in 5% (w/v) acacia solution and given in dosage volumes of 5 ml/kg body weight. Control males were given an equivalent volume of 5% (w/v) acacia solution.

The male rats were dosed and then rested for 3 days. On test day 4, an adult virgin female (202-288 gm) was caged with each male and the animals were allowed to mate. The date of conception was estimated by the presence of an expelled copulatory plug (gestation day 0). After 7 days, the females were removed, caged individually, and replaced with a new group of females. This breeding schedule was maintained for 8 consecutive weeks in order to cover the duration of the spermatogenic cycle in the rat. Throughout the study, food and water were available ad libitum. The females were killed on gestation day 20 and evaluated for reproduction performance. Ovaries were examined for the number of corpora lutea, and the uterus for the number and location of fetuses and resorptions. Fetuses were examined for viability; resorptions were categorized as early or late to indicate the relative time of mortality. For each set of 10 females, the mean values for the reproduction parameters and the following reproduction indiced were obtained:

Gestation Survival Index - the proportion of fetuses that were alive.

Resorption Index - the proportion of implanted conceptuses that resulted in resorptions.

Implantation Index - the proportion of implantations to the number of corpora lutea.

Results: The animals were not clinically affected by treatment with EL-222. No treatment effect was apparent on fertility, implantation occurrence, fetal number or resorption incidence.

Conclusion: EL-222 administered to Harlan Wistar Rats at a single dose of 350 mg/kg prior to mating, did not produce a dominant lethal effect.

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C. A Teratology Study on EL-222 in the Rabbit (Study B-7125) (Conducted by D.G. Hoffman, J.K. Markham, E.R. Adams, N.V. Owen and D.M. Morton, Toxicology Division, Lilly Research Laboratories, Jan., 1977)

Test Material: EL-222, Lot No. B30-C69-220 97.9% purity.

Dutch Belted Virgin female rabbits (15 females/group) were administered orally by gavage doses of 0 (vehicle), 3, 10 or 35 mg/kg of test material during gestation days 6-18. The females were sacrificed on day 28 of gestation. Maternal parameters examined were appearance, food consumption, body weight, reproduction data on gestation day 28 including the number of corporea lutea, number and distribution of fetuses and resorptions. The fetal parameters examined were fetal viability, weight, sex and external, visceral and skeletal examinations. The results were statistically analyzed.

Results: Mean food consumption and body weight values of dams were not affected by treatment. Three control rabbits aborted and three rabbits in the 3 mg/kg group died during the study. One control rabbit, two rabbits in the 3 mg/kg group and one rabbit in the 35 mg/kg group were anorectic during the study. Mean litter size in the 10 and 35 mg/kg groups was slightly lower than the control litter size. External fetal defects were confined to 1 fetus in the 3 mg/kg group (omphalocele. Note, this condition has been seen in the control population previously) and 5 littermates in the 35 mg/kg group with edema and varus bones. These deviations have been observed previously in control offspring. Skeletal examination revealed no treatment related findings. Results of the visceral examination of fetuses examined were not presented in the report.

Conclusion: EL-222 is not teratogenic (for skeletal variances and external appearance) up to 35 mg/kg in rabbits during days 6-18 of gestation.

Classification: Supplementary Data

(1) Results of the visceral examination of fetuses, including the brain, were not submitted for review.

D. Twenty-four Month Chronic Oral Toxicity of EL-222 in Rats (Studies R-405 and R-415) (Conducted by D.G. Hoffman, W.R. Gibson, E.C. Pierce, and D.M. Morton, Toxicology Division, Lilly Research Laboratories, April, 1978)

Test Material: EL-222, Lot. No. B30-C69-220, 97.9% purity.

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Wistar derived rats were used in two replicate studies, each containing a control group (60/sex) and three treatment groups (40/sex/group) at dietary levels of 50, 130 and 350 ppm. A total of 720 animals were used in studies R-405 and R-415. The initial age of the rats was 4-5 weeks and the initial weight of females averaged 120 grams and of males averaged 140 grams. Parameters studied included mortality, appearance and behavior, growth, food consumption, efficiency of food utilization, terminal hematology (hematocrit, hemoglobin, RBC, total and differential WBC, and RBC morphology, prothrombin time), terminal clinical chemistry (glucose, urea (BUN), creatinine, bilirubin, Alkaline phosphatase, SGPT), terminal serum prolactin and luteinizing hormone levels (study R-405 only), organ weights and gross and histologic examination of tissues. Statistical analyses of the data was performed.

Results: Dietary administration of EL-222 resulted in a dose-related increase in survival. There were no grossly detectable signs of disease or toxicity that could be attributed to administration of EL-222. Dietary administration of EL-222 had no effect on the growth of female rats. Males of the high dose group weighed less than the controls for the first nine months of study R-415 and for the first 17 months of study R-405. The difference was not considered toxicologically significant although at some time points it was statistically significant. Food consumption was unaffected by treatment, as was efficiency of food utilization. There was a statistically significant decrease in the mean WBC values for the groups given 350 ppm in the females in studies R-405 and R-415. Blood glucose concentrations were statistically significantly increased at all dietary levels in the female rats of study R-405 and the high-dose males of study R-405 and R-415. There were no other statistically significant changes in any measured clinical chemistry parameters. Serum prolactin concentrations were decreased in female rats and increased in male rats given the diet containing 350 ppm EL-222. In addition, serum luteinizing hormone was increased in females of the same group. The significance of these hormone changes, which were investigated to aid in elucidating the mechanisms of the effects of EL-222 on reproduction, is unknown.

Dietary administration of EL-222 for 2 years had no apparent adverse effect on any of the measured organ weights, although absolute brain weights were decreased significantly in low and high dose females in study R-415. There was no change in any of the other groups of either test and relative brain weights were not affected. Pathological examination of the tissues showed that EL-222 administration for 2 years had no statistically significant effect on the total incidence of benign and malignant tumors in rats.

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