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

FILE COPY

October 24, 1991

OFFICE OF
PESTICIDES AND TOXIC
SUBSTANCES

MEMORANDUM

SUBJECT: Carcinogenicity Peer Review Meeting on **ALIETTE**

FROM: Esther Rinde, Ph.D. E.R.
Manager, Carcinogenicity Peer Review
Health Effects Division (H7509c)

TO: Addressees

Attached for your review is a package on **ALIETTE** prepared by **Dr. Whang Phang.** ALIETTE was first evaluated by the Peer Review Committee (PRC) on March 5, 1986. At that time the PRC concluded that ALIETTE should be classified as a Group C Carcinogen.

A meeting to re-consider the carcinogenicity classification of **ALIETTE** is scheduled for **Wednesday Nov. 6, 1991, at 10:00 am in Room 821, CM2.**

Addressees

P. Fenner-Crisp
W. Burnam
R. Engler
R. Hill
R. Beliles
K. Baetcke
L. Brennecke
M. Van Gemert
M. Copley
K. Dearfield
J. Parker
H. Pettigrew
W. Sette
G. Ghali
B. Fisher
J. Du
Y. Woo
G. Burin
J. Quest
E. Saito (for microfiche-with one-liner)
W. Phang
J. Rowe

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

FILE COPY

4 SEP 1991

OFFICE OF
PESTICIDES AND TOXIC
SUBSTANCES

MEMORANDUM

SUBJECT: Carcinogenicity Peer Review Meeting on **ALIETTE (2nd)**

FROM: Esther Rinde, Ph.D. E.R.
Manager, Carcinogenicity Peer Review
Health Effects Division (H7509c)

TO: Addressees

Attached for your review is a package on **ALIETTE** prepared by **Dr. Whang Phang.** Aliette was first evaluated by the Peer Review Committee (PRC) on March 5, 1986. At that time the PRC concluded that ALIETTE should be classified as a Group C carcinogen.

A meeting to re-consider the carcinogenicity classification of **Aliette** is scheduled for **Wednesday Sept. 25, 1991, at 10:00 am in Room 821, CM2.**

CANCELLED
Rescheduled Nov 6, 1991

Addressees

P. Fenner-Crisp
W. Burnam
R. Engler
R. Hill
R. Beliles
K. Baetcke
L. Brennecke
M. Van Gemert
M. Copley
K. Dearfield
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UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

FILE COPY

OFFICE OF
PESTICIDES AND TOXIC
SUBSTANCES

MEMORANDUM

SUBJECT: Foestyl-Al (Aliette): Request for Re-evaluation of the Carcinogenic Potential of Foestyl-Al

TO: Esther Rinde, Ph.D.
Manager, Peer Review Committee for Carcinogenicity
SACB/HED (H7509C)

FROM: Whang Phang, Ph.D. 1/9/91
Pharmacologist
Tox. Branch II / HED (H7509C)

THROUGH: James Rowe, Ph.D. 1/9/91
Section Head
and
Marcia van Gemert, Ph.D. 1/9/91
Branch Chief
Tox. Branch II / HED (H7509C)

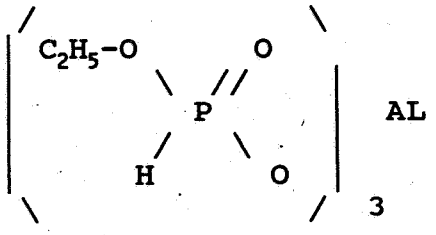
In 1986, the Peer Review Committee had evaluated the existing toxicology data of Aliette. Based upon the finding of an increase in the incidence of the urinary bladder tumor in high dose male rats (30,000 ppm), the chemical was classified as a Category C carcinogen, and no risk assessment was recommended. Currently, the registrant has submitted additional data and contends that this chemical should not be classified as a Category C carcinogen. This reviewer kindly requests that the Peer Review Committee evaluate the current data in conjunction with other relevant information and determine whether these data provide sufficient evidence for re-classifying the carcinogenic potential of Aliette.

rwd 1/9/92

Fosetyl-Al (Aliette): Request for Re-evaluation of the Carcinogenic Poetential of Fosetyl-Al

Prepared by Whang Phang, Ph.D.
Pharmacologist
Tox. Branch II / HED

Introduction: Fosetyl-Al is used as a fungicide on vines, vegetable crops, ornamentals, hops, pineapple, avocado, rubber, cacao, and citrus. The chemical structure of fosetyl-Al, aluminum tris (O-ethyl phosphonate), is shown below:



Aluminum tris (O-ethyl phosphonate)

On March 5, 1986, the Peer Review Committee met to evaluate the data base on fosetyl-Al (Attachment 1, Memorandum from J. Quest to H. Jacoby). The discussion focused on the carcinogenic potential of this chemical in a 2-year feeding/oncogenicity study in Charles River CD rats. Relative to the controls, increased incidences of urinary bladder tumor and pheochromocytoma were reported in high dose males. After considering all available toxicology data on this chemical, the Committee concluded that Aliette did not produce pheochromocytoma in high dose male rats. With respect to the urinary bladder tumors, the registrant had provided published articles to show that urinary bladder tumors were caused by "irritation and subsequent proliferation on the bladder epithelium due to the formation of urinary stones". However, the Committe decided that since "neither mineralization nor urolithiasis were observed in the bladder of high dose male rats upon histopathological examination", the increase in the incidence of bladder tumor (adenomas and carcinomas combined) in high dose male rats was considered to be compound-related. The chemical was classified as a Category C oncogen. The Committee also recommended that "the registrant pursue further studies to evaluate a possible urinary tract irritant effect of treatment resulting from either the urinary excretion of Aliette per se, calcium, the aluminum portion of the Aliette molecule, or the ethanol metabolite of Aliette".

Toxicological issues: The registrant has followed the Committee's recommendation and has specifically designed and conducted a 90-day feeding study in rats to investigate the theory that bladder tumors seen in the chronic study were the results of

a possible urinary tract irritant effect. The results of this 90-day study showed that large doses of fosetyl-Al (30,000 and 50,000 ppm) produced urinary calcium imbalance, diuresis, sharp drop in urine pH, formation of urinary calculi, and transitional cell hyperplasia in kidney tubules, ureter, and urinary bladder. These changes occurred after two weeks of treatment and were predominately in males. In a letter to the Product Manager for Aliette, Susan Lewis, the registrant argued that, based upon all the available toxicology data on this chemical, it should not classified as a Category C Carcinogen (Attachment 2). This reviewer requests that the Committee evaluate the current data in conjunction with other relevant information and determine whether these data provide sufficient evidence for re-classifying the carcinogenic potential of Aliette.

Summary of Relevant Toxicological Data

Much of the following summary is excerpted from the Peer Review Committee's document on Aliette (Memorandum of J. Quest to H. Jacoby, June 12, 1986).

1. Two-Year Feeding Oncogenicity Study in Rats

Groups of Charles River CD rats (80/sex/dose) received Aliette at dietary concentrations of 0, 2,000, 8,000, and 40,000/30,000 ppm for 2 years. Following observations of red urine and staining of the abdominal fur at 40,000 ppm, this dose level was reduced to 30,000 ppm after 2 weeks. Subsequent to reducing the dosage, the clinical signs were not observed. Based upon the reported data, Aliette did not produce compound-related effects on the survival rate, organ weights, and hematological and biochemical parameters. Urinalysis showed an increase in the amount of protein, and this increase was progressive relative to the dose level and the time on study. The initial 1-2 weeks administration of 40,000 ppm Aliette produced a decrease in body weights in males (-12%) and females (-9%) relative to the controls.

The original histopathologic examination showed an increase in the incidence of urinary bladder tumors and pheochromocytomas (Table 1). The slides of this study were read by several pathologists (as indicated in Table 1). For the incidence of pheochromocytomas, the diagnosis of the original pathologist indicated a significant increase in the incidence of adenomas and carcinomas combined in mid (8,000 ppm) and high dose (40,000/30,000) male rats. The increased pheochromocytoma incidence was primarlly due to an increase in the adenomas. However, this original diagnosis was not confirmed by two other consulting pathologists which had evaluated the same slides (Table 1). In 1986, the Peer Review Committee had evaluated the results and considered the differences in pathological

diagnosis of pheochromocytomas among the three pathologists was due to the fact that there is a high degree of variability in the interpretation of adrenal medullary hyperplasia versus adrenal medullary neoplasia. The Committee then concluded that the available data did not provide sufficient evidence to indicate that Aliette produced pheochromocytomas in male rats (Attachment 1, page 7).

The incidence of urinary bladder tumors was evaluated by two pathologists (pathologists 1 and 3) as indicated in Table 1. The evaluation of the original pathologist (pathologist 1) indicated a statistically significant increase in the combined incidence of adenomas and carcinomas in high dose male rats which were sacrificed at the termination of the study. A seconding reading of the same slides by another pathologist (pathologist 3) confirmed the findings of the diagnosis of the original pathologist. The second reading also showed a high ratio of carcinomas to adenomas and the presence of urinary bladder hyperplasia in high dose male rats (Table 1). Based on the generally similar histopathological findings provided by two independent pathologists, the Peer Review Committee concluded that the increase in the urinary bladder tumor incidence in male rats was produced by Aliette (Attachment 1, page 5).

The issue of maximum tolerated dose (MTD) was considered by the Committee, and it was concluded that an MTD level was reached in males at 30,000 ppm and 40,000 ppm in females (Attachment 1, page 12).

2. Rat Chronic Feeding/Oncogenicity Study on Mono-Sodium Phosphite (Metabolite of Aliette)

Groups of Charles River CD rats (60/sex/dose) received mono-sodium phosphite, the major urinary metabolite of Aliette in rats, at dietary concentrations of 0, 2,000, 8,000, and 32,000 ppm for 27 months (117 weeks). The results showed that the test article did not induce any clinical signs of toxicity, increased mortality, or hematological and biochemical changes. A compound-related increase in the incidence of either neoplastic or non-neoplastic changes was not found. The Peer Review Committee had previously considered the results of this study and concluded that no evidence of an oncogenic response in the urinary bladder, the adrenal medulla, or at any other sites was found. In addition, the MTD level was not a major issue because the study had tested an unusually high dose of 32,000 ppm (Attachment 1, pages 8 & 9).

3. 90-Day Feeding Study in Rats (Special Study)

The details of this study is presented in Attachment 3, Data Evaluation Report.

Groups of rats (70/sex/dose) received Fosetyl-Al at dietary concentrations of 0, 8,000, 30,000, and 50,000 ppm. Each dose group was further divided into 7 subgroups each of which received the test article for a different length of time (ranged from 2 weeks to 13 weeks), and some subgroups were then placed on the normal diet for different recovery periods (ranged from 8 weeks to 21 weeks). The results indicated that Fosetyl-Al at 30,000 and 50,000 ppm produced death and diuresis in males and some females. A decrease in body weight and food consumption was consistently seen in 50,000 ppm males and females and to a lesser extent in 30,000 ppm males. However, during the recovery period the body weights and food consumptions were comparable to those of the controls. An increase in water consumption was also seen in 30,000 and 50,000 ppm males and in 50,000 ppm females. Hematological changes were found in 50,000 ppm males and females, but the changes were related to diuresis.

Clinical chemistry data indicated increases in BUN nitrogen, phosphorous, and CO_2 in 50,000 ppm males and females. During the recovery periods, CO_2 and phosphorous levels were comparable to those of the controls, but the increase in BUN level persisted.

The urinalysis results indicated a decrease in the pH values in all treated animals after 2 weeks of treatment and an increase in urinary Ca++ levels in 30,000 and 50,000 males and females. The urinary phosphorous level was decreased in 30,000 and 50,000 ppm males and females; this decrease was related to diuresis. Urinary aluminum level was increased, and it was thought to be due to contamination of the urine samples since the blood level of aluminum was low.

Fecal analysis revealed that there was an overall decrease in the Ca++ level in 50,000 ppm males and females relative to that of the controls. The fecal aluminum level was consistently higher in the treated animals, and this increase was dose-related. The normal level of blood aluminum and the dose-related increase in the fecal aluminum level indicated that aluminum was not absorbed via the intestinal tracts.

Both gross pathology and histopathology data showed that calculi or uroliths were present in kidneys, ureters, and urinary bladder of 30,000 and 50,000 ppm males and of a few 50,000 ppm females. The chemical compositions of the urinary bladder calculi were approximately 23% phosphorous, 33% calcium, 0.2% magnesium, and less than 0.1% aluminum. In the kidneys, an increased incidence of urolithiasis, hydronephrosis, pyelitis, pyelonephritis, papillary necrosis, dilatation of the collecting tubules, and transitional hyperplasia of the pelvis was seen in 30,000 and 50,000 ppm males and 50,000 ppm females. In the ureters, an increase in

the incidence of urolithiasis, ureteritis, and dilatation was found in 30,000 and 50,000 ppm males and 50,000 ppm females. In the urinary bladder, an increase in the incidence of urolithiasis, submucosa edema, papillary hyperplasia, and cystitis was seen in 30,000 and 50,000 ppm males at different treatment durations, and some of these findings persisted to the recovery periods. The urinary bladder calculi were found to be in greater number and size than those found in kidneys and ureters and were in larger size and number at the 2 week sacrifice. After 13 weeks of treatment, the absolute kidney weights of 30,000 and 50,000 ppm males and 50,000 ppm females were significantly increased ($p < 0.05$).

Based upon the data presented in this report, the histopathological findings in the kidneys, ureters, and urinary bladder were related to the presence of uroliths in these organs because, in 8,000 ppm males and females and 30,000 ppm females, uroliths were virtually absence in their urinary systems, and accordingly the histopathological changes were not seen. Most importantly, an evaluation of the individual animal histopathology and the urinalysis data revealed that the presence of calculus was almost always associated with urinary hypercalciuria, acidic urine, transitional cell hyperplasia and/or papillary hyperplasia of the urinary bladder, ureter, and kidney tubules. The report did not attempt to offered any explanation for the substantially lower incidence of calculi formation in 50,000 ppm females relative to that of the males.

4. Mouse Oncogenicity Study

Groups of Charles River CD-1 mice (60/sex/dose) received Aliette at dietary dose levels of 0, 2,500, 10,000, and 20,000/30,000 ppm for 2 years. At treatment week 19, 20,000 ppm was increased to 30,000 ppm because of absence of any effect in the early part of the study. No evidence of an oncogenic response was found with Aliette, and no other toxicities were seen (Attachment 1, page 9).

Based upon the data, the Peer Review Committee believed that the highest dose tested in this study did not approximate a MTD level and the study was inadequate because the the increase from 20,000 ppm to 30,000 ppm occurred at week 19 of the treatment period which had missed the early critical periods of the growth curve of the test animals. However, the Committee believed that additional carcinogenicity testing in mice would not yield an increased understanding of the toxicity of this chemical because the high dose (30,000 ppm) tested in this study was sufficiently high (Attachment 1, page 9).

5. Two-Year Dog Toxicology Study

Aliette was administered to groups of dogs (6/sex/dose) at

dietary levels of 0, 10,000, 20,000, and 40,000 ppm. The NOEL was 10,000 ppm, and LEL was 20,000 ppm based upon the finding of the presence of giant cells in the lumen of the seminiferous tubules in 2/6 male dogs. Other changes were mainly in high dose animals consisting of a reduction in total serum proteins in males throughout the study and a reduced BUN in females at several study intervals. Additional toxicological effects were not found (Attachment 1, pages 9 & 10).

6. Reproduction and Teratology

Three-generation reproduction study: For each generation, groups of 25 rats/sex/dose received Aliette at dietary concentrations of 0, 6,000, 12,000, and 24,000 ppm. For F0 animals, the treatment began 90 days prior to mating. The parental animals (approximately 25/sex/dose) of subsequent generations were selected at 21 days post partum and reared on the test diet to an age of at least 90 days. The animals were then mated for 20 days. The results showed no reproductive or developmental effect. At 24,000 ppm, Aliette reduced body weight gains in males of all generations and in females of F1B and F2B generations. Necropsy and histopathology examinations showed urinary bladder changes in F1, F2, and F3 generation males and females of the 24,000 ppm group. The changes were described as "hemorrhage of the bladder wall, increased pelvic dilation... and papillary necrosis". In F3B animals, the changes also included "minimal epithelial hyperplasia and/or hypertrophy of the transitional epithelium, sometime associated with small papillary projections and or desquamation cells in the lumen of the urinary tract". These changes were "associated with the presence of crystalline or calcareous deposits". No urinary bladder changes were found in F0 rats (Attachment 4).

7. Teratology

Two teratology studies on Aliette were available. A rat teratology study was conducted by Huntingdon Research Centre, England, while the rabbit teratology study was performed by Centre de Recherches Rhône-Poulenc.

In the rat teratology study, groups of 20 pregnant females received Aliette by gavage at doses of 0, 500, 1,000, and 4,000 mg/kg from gestation days 6 to 15. On gestation day 20, the fetuses were delivered. Increased death rate and decreased body weight gains were seen in 4,000 mg/kg females. Reduced mean fetal weights, delayed ossification, and increased total resorptions were seen in fetal data of 4,000 mg/kg group. No evidence of teratogenic effect was seen. Based upon the data presented in the report, the values for maternal and develop-developmental NOEL were 1,000 mg/kg; LEL, 4,000 mg/kg (Attachment 5).

In the rabbit teratology study, groups of mated females received Aliette by gavage at doses of 0, 125, 250, and 500 mg/kg from gestation days 6 to 16. On gestation day 28, the fetuses were delivered. Under the conditons of the study, Aliette did not induce any developmental effects, and the NOEL for developmental toxicity was > 500 mg/kg (HTD). A slight reduction in both food consumption and body weight gains was seen in 250 mg/kg does. Additional toxicity was not seen (Attachment 6).

8. Metabolism

Four metabolism studies in rats were available; two studies on ^{14}C-Aliette and two studies on ^{32}P-Aliette. The results indicated that after oral ingestion, Aliette was extensively absorbed and hydrolyzed to phosphite and ethanol which was then oxidized via acetaldehyde and acetate to CO_2 and eliminated in the expired air. The CO_2 accounted for 60% of the administered radioactivity. The phosphite and some unchanged parent compound are excreted via urine without further oxidation to phosphate and accounted for approximately 26% of the administered dose. Only a minor amount (2-3%) of the administered radioacitivity was found in the feces.

9. Mutagenicity

There were 8 mutagenicity studies which consisted of 2 Ames assays using S. typhimurium (strains TA 1535, TA 1537, TA 98, TA 100, and TA 1538), 2 phage induction tests using E. coli, 2 micronucleus tests in Swiss mice and CD-1 mice, 1 DNA repair study using E. coli, and 1 saccharomyces cereviscae yeast assay. The results of all these studies were negative (Attachment 1, page 10).

TABLE 1+

Pathology Diagnoses of Urinary Bladder and Adrenal Medullary Tumors in Oncogenicity Feeding Study of Aliette in Male Charles River CD Rats

Tumor Site and Type	Reviewing Pathologist[a]	Dose (ppm) 0	2,000	8,000	40,000/ 30,000
Urinary Bladder:					
Adenoma	1	0/80(0%)	1/78(1%)	1/79(1%)	8/80(10%)
	3	1/80(1%)	1/78(1%)	1/79(1%)	5/80(6%)
Carcinoma	1	2/80(2.5%)	0/78(0%)	0/79(0%)	7/80(9%)
	3	2/80(2.5%)	2/78(2.5%)	1/79(1%)	16/80(20%)
Adenoma + Carcinoma Combined	1	2/80(2.5%)	1/78(1%)	1/79(1%)	15/80(19%)*
	3	3/80(4%)	3/78(4%)	2/79(2.5%)	21/80(26%)
Hyperplasia	1	NA	NA	NA	NA
	3	5/78(6%)	7/78(9%)	5/80(6%)	29/79(37%)*
Adrenal Medulla:					
Adenoma	1	5/80(6%)	7/78(9%)	15/79(19%)	16/80(20%)
Carcinoma	1	1/80(1%)	0/78(0%)	1/79(1%)	2/80(2.5%)
Adenoma + Carcinoma Combined	1	6/80(7%)	7/78(9%)	16/79(20%)*	18/80(22%)*
	2	17/80(21%)	15/78(19%)	19/79(24%)	21/80(26%)
	3	6/80(7%)	5/78(6%)	10/79(13%)	6/80(7%)
Hyperplasia	1	16/80(20%)	11/78(14%)	10/79(13%)	9/80(11%)
	2	5/80(6%)	3/78(4%)	5/79(6%)	4/80(5%)
	3	15/80(19%)	14/78(18%)	13/79(13%)	16/80(20%)
Adenoma + Carcinoma + Hyperplasia (Combined)	1	22/80(27%)	18/78(23%)	26/79(33%)	27/80(34%)
	2	22/80(27%)	18/78(23%)	24/79(30%)	25/80(31%)
	3	21/80(26%)	19/78(24%)	23/79(29%)	22/80(27%)

a - 1 = Dr. R. M. Kovatch (Original Pathology Report)
2 = Dr. W. R. Richter (Consultant; Examined Limited Slides)
3 = Dr. S. W. Thompson (Consultant; Examined All Slides Blindly).

* - $p < 0.05$ compared to controls (Note: Statistical evaluation of data was presented only for the diagnosis provided by pathologist No. 1).

NA - Information Not Avaialble

+ - TABLE EXCERPTED FROM MEMORANDUM FROM J. Quest To H. Jacoby, June 12, 1986. (Tox. Peer Review Document on Aliette).

6/12/86 ATTACHMENT 1 -9-

FILE COPY

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

JUN 12 1986

MEMORANDUM

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCE

SUBJECT: Peer Review of Aliette (Fosetyl-AL)

FROM: John A. Quest, Ph.D.
Team Leader, Scientific Mission Support Staff
Toxicology Branch/HED (TS-769)

TO: Henry Jacoby, Product Manager #21
Fungicide-Herbicide Branch
Registration Division (TS-767)

The Toxicology Branch Peer Review Committee met on March 5, 1986, to discuss and evaluate the data base on Aliette (Fosetyl-AL). Particular attention was focused on the oncogenic potential of the chemical in Charles River (CR)-CD rats.

A. Indivduals in Attendance:

1. Peer Review Committee: (Signatures indicate concurrence with the peer review unless otherwise stated).

William Burnam
Reto Engler
Richard Hill
Stephen Johnson
Louis Kasza
Albin Kocialski
Richard Levy
Bertram Litt
John A. Quest

2. Scientific Reviewers: (Non-committee members responsible for presentation of data; signatures indicate technical accuracy of panel report).

*Carolyn Gregorio
*Clint S. Skinner

*Both have left the Agency

13

(NOTE: Neither Ms. Gregorio nor Dr. Skinner were employees of the Toxicology Branch at the time this report was circulated for review by the individuals in attendance at the Peer Review meeting. The accuracy of the data presented is verified by Wm. Burnam, Deputy Chief of the Toxicology Branch, on their behalf.)

3. Peer Review Committee Members in Absentia: (Committee members who were not able to attend the discussion; signatures indicate concurrence with the overall conclusions of the Committee.)

Anne Barton

Diane Beal

Robert Beliles

Theodore M. Farber Theodore M. Farber 5/29/86

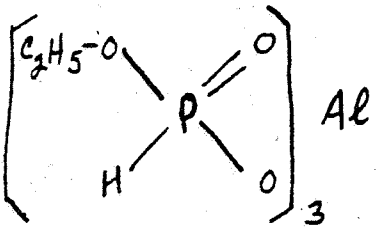
B. Material Reviewed:

The material available for review consisted of DER's of rat oncogenicity studies of Aliette and its metabolite, Monosodium phosphite; a mouse oncogenicity study of Aliette; metabolism studies of Aliette and its phosphite metabolite; and a memorandum of 11/5/85 by C. Gregorio evaluating the registrant's rebuttal to the oncogenic findings.

C. Overview of Toxicology Issues:

Aliette is a fungicide whose primary use is on pineapples. Toxicology Branch review of data on aliette resulted in a recomendation that the chemical be considered as a possible human oncogen based on findings of adrenal medullary pheochromocytomas and urinary bladder tumors in a study performed by IRDC in CR-CD rats. The registrant (Rhone-Poulenc Agrochimie, Lyon, France) provided a rebuttal to the oncogenic classification of Aliette indicating: 1) that the adrenal medullary tumors were unrelated to compound administration based on re-reviews of the pathology data by two other consulting pathologists; and 2) that the urinary bladder tumors were a reaction to the massive dose levels of the chemical that were administered, i.e., the result of a disturbance of the phosphorus/calcium balance due to an overloading of animals with phosphorus (Aliette is phosphorus-containing compound) which in turn led to an increased incidence of bladder stones and subsequent irritation and proliferation of the bladder epithelium.

STRUCTURE:



Aliette (Fostyl-AL)

(Aluminum Tris (-O-ethyl phosphonate))

D. Evaluation of the Evidence:

1. Rat Oncogenicity Study of Aliette:

Aliette was administered in the diet to 80 Charles River CD rats/sex/dose level at doses of 0, 2,000, 8,000 and 40,000/30,000 ppm for 2 years. The study was conducted by IRDC. (International Research and Development Corporation). The high dose level was reduced to 30,000 ppm after 2 weeks, following observations of staining of the abdominal fur and red coloration of the urine at 40,000 ppm. The incidence patterns of tumors that were described in the urinary bladder and in the adrenal medulla of male rats treated with Aliette are summarized in Table 1. The Table describes tumor diagnoses by several pathologists retained by the registrant. No tumors were observed in female rats.

TABLE 1

Pathology Diagnoses of Urinary Bladder and Adrenal Medullary Tumors in Oncogenicity Feeding Study of Aliette in Male Charles River CD Rats

Tumor Site and Type	Reviewing Pathologist [a]	Dose (ppm) 0	2,000	8,000	40,000/ 30,000
Urinary Bladder:					
Adenoma	1	0/80(0%)	1/78(1%)	1/79(1%)	8/80(10%)
	3	1/80(1%)	1/78(1%)	1/79(1%)	5/80(6%)
Carcinoma	1	2/80(2.5%)	0/78(0%)	0/79(0%)	7/80(9%)
	3	2/80(2.5%)	2/78(2.5%)	1/79(1%)	16/80(20%)
Adenoma + Carcinoma Combined	1	2/80(2.5%)	1/78(1%)	1/79(1%)	15/80(19%)*
	3	3/80(4%)	3/78(4%)	2/79(2.5%)	21/80(26%)
Hyperplasia	1	NA	NA	NA	NA
	3	5/78(6%)	7/78(9%)	5/80(6%)	29/79(37%)
Adrenal Medulla:					
Adenoma	1	5/80(6%)	7/78(9%)	15/79(19%)	16/80(20%)
Carcinoma	1	1/80(1%)	0/78(0%)	1/79(1%)	2/80(2.5%)
Adenoma + Carcinoma Combined	1	6/80(7%)	7/78(9%)	16/79(20%)*	18/80(22%)*
	2	17/80(21%)	15/78(19%)	19/79(24%)	21/80(26%)
	3	6/80(7%)	5/78(6%)	10/79(13%)	6/80(7%)
Hyperplasia	1	16/80(20%)	11/78(14%)	10/79(13%)	9/80(11%)
	2	5/80(6%)	3/78(4%)	5/79(6%)	4/80(5%)
	3	15/80(19%)	14/78(18%)	13/79(13%)	16/80(20%)
Adenoma + Carcinoma + Hyperplasia (Combined)	1	22/80(27%)	18/78(23%)	26/79(33%)	27/80(34%)
	2	22/80(27%)	18/78(23%)	24/79(30%)	25/80(31%)
	3	21/80(26%)	19/78(24%)	23/79(29%)	22/80(27%)

[a] - 1 = Dr. R. M. Kovatch (Original Pathology Report)
2 = Dr. W. R. Richter (Consultant; Examined Limited Slides)
3 = Dr. S. W.Thompson (Consultant; Examined All Slides Blindly)

* - $p < 0.05$ compared to controls (Note: Statistical evaluation of data was presented only for the diagnosis provided by pathologist No. 1).

NA - Information Not Avaialble

(a) Discussion of Urinary Bladder Tumors: The original diagnosis of urinary bladder tumors provided by the registrant indicated that there was a statistically significant increase in adenomas plus carcinomas combined in male rats at the highest (40,000/30,000) dose level that was tested (Table 1; pathologist No. 1). The elevated bladder tumor incidence was due to an increase in both adenomas and carcinomas and the tumors were mainly seen in surviving animals at terminal sacrifice. The registrant also submitted the results of a re-reading of the urinary bladder slides by a consulting pathologist (pathologist No. 3) who confirmed the findings of the original diagnosis of pathologist No. 1; i.e. urinary bladder adenomas plus carcinomas combined were increased at the highest dose level tested. Pathologist No. 3, however, found a higher ratio of carcinomas to adenomas than did pathologist No. 1, and also reported the presence of urinary bladder hyperplasia (Table 1) in high dose male rats. Based on the generally similar information provided by two independent pathologists, the Peer Review Committee concluded that Aliette produced an elevated incidence of urinary bladder tumors in male rats.

As indicated above (section C), the registrant interpreted the bladder tumors as being due to irritation and subsequent proliferation of the bladder epithelium due to the formation of kidney stones. The basic argument made by the registrant was that ingestion of the high dose level of Aliette (a phosphorus-containing compound) altered the normal calcium/phosphorus balance and thereby led to unbalanced calcium excretion in the urine. (This was supported by data from a one-month rat study (10,000, 20,000 and 40,000) in which male rats displayed increased urinary calcium excretion and reduced urinary phosphorus levels). The increased urinary calcium, in turn, was said to have led to mineralization and calculi formation and subsequent bladder tumors. The Peer Review Committee considered this hypothesis, but rejected it based on additional data provided by consultant pathologist No. 3 who did not observe the presence of either mineralization (controls, 1/78; low dose 1/78; mid dose 0/80; high dose 0/79) or stones (controls, 1/78; low dose, 1/78; mid dose, 0/80; high dose, 0/79) in the bladders of male rats. The Committee discussed at length the possible mechanism for the

formation of the bladder tumors and reached the following conclusions: (a) there was insufficient information to identify the cause of the tumors other than to note that they were not due to the urinary metabolite of Aliette, namely mono-sodium phosphite (see section D.2); (b) because of the presence of hyperplasia in the bladders of high dose males as described by pathologist No. 3 (see Table 1) the potential for an irritant effect existed; and (c) the registrant should attempt to further define the mechanism for this effect (this might include a further followup of a possible Ca^{++} irritant mechanism).

(b) Discussion of Adrenal Gland Tumors: The original diagnosis of adrenal tumors provided by the registrant indicated there was a statistically significant increase in pheochromocytomas (adenomas plus carcinomas combined) in male rats at the mid (8,000 ppm) and high (40,000/30,000) dose levels that were tested (Table 1; pathologist No. 1). The elevated pheochromocytoma incidence was primarily due to an increase in the adenomas; no elevated incidence of adrenal medullary hyperplasia was observed. Furthermore, when all 3 adrenal medullary lesions were combined (i.e., adenomas, carcinomas and hyperplasia), no significant dose-related effects were reported by pathologist No. 1. Based on this data, the Toxicology Branch reviewer initially concluded that Aliette produced an increased incidence of adrenal gland pheochromocytomas at 8,000 and 40,000/30,000 ppm in male rats.

The registrant rebutted the Toxicology Branch's initial conclusion regarding adrenal pheochromocytomas by providing information from two additional consulting pathologists who re-read the adrenal gland slides in male rats (Table 1; pathologists No. 2 and No. 3). In contrast to the findings originally reported by pathologist No. 1, neither of the consulting pathologists found significant dose-related increases in the incidence of pheochromocytomas (adenomas plus carcinomas combined) in male rats treated with Aliette. In addition, neither consulting pathologist reported increased incidences of adrenal gland hyperplasia, nor increased incidences of all 3 types of adrenal medullary lesions (i.e., adenomas, carcinomas, and hyperplasia) when they were combined (Table 1).

On the basis of the above data and other available information, the Committee concluded that Aliette did not produce an elevated incidence of adrenal gland pheochromocytomas in male rats for the following reasons:

1) An independent re-reading of the adrenal gland slides for male rats by two consulting pathologists (Nos. 2 and 3) did not confirm the initial diagnosis of an elevated incidence of pheochromocytomas (adenomas plus carcinomas combined) as reported by original pathologist No. 1 (see Table 1).

2) The Peer Review Committee regarded the differing opinions of original pathologist No. 1 vs. those of consulting pathologists No. 2 and 3 to be due to the fact that there is a high degree of variability in the interpretation of adrenal medulla hyperplasia and adrenal medulla neoplasia. That is, adenomas and hyperplasia are hard to differentiate histologically; both are proliferative lesions and there are no obvious changes in cellular morphology as cellular events progress from hyperplasia to adenomas. This variability in diagnosing the adrenal lesions is illustrated in Table 1 from the readings provided by pathologists No. 2 and 3; pathologist No. 2 concluded there was a relatively low incidence of hyperplasia and a relatively high incidence of adenomas plus carcinomas combined, whereas pathologist No. 3 reached the opposite conclusion. The Committee considered this difficulty in diagnosing hyperplasia vs. adenomas to be a primary reason to suspect that there was not an apparent treatment related effect of Aliette on the adrenal medulla as originally reported by pathologist No. 1. For this same reason it was concluded that a review of the data by an additional pathologist would not necessarily provide additional information over and above that already available. Similarly, it was also noted that no historical data on the incidence of adrenal gland tumors was provided by the test laboratory (IRDC), but that such data would be only of limited value due to the inherent difficulties in diagnosing adrenal hyperplasia vs. adenomas.

3) Another factor that led the Committee to suspect that the adrenal tumors might not be compound-related was the observation that none of the 3 pathologists involved in reading the adrenal gland slides reported an increased incidence of all 3 types of adrenal gland proliferative lesions (i.e. adenomas, carcinomas and hyperplasia) when they were combined (see Table 1).

4) Finally, the Committee noted that no adrenal gland tumors or adrenal gland hyperplasia were observed in an oncogenicity study of Mono Sodium Phosphite (urinary metabolite of Aliette) in Charles River CD rats at dose levels (i.e., 0, 2,000; 8,000, and 32,000 ppm) that were comparable to those tested in the Aliette Charles River CD rat oncogenicity study.

(c) Maximum Tolerated Dose (MTD) Considerations: The initial high dose level of Aliette tested on weeks 1-2 in rats (i.e., 40,000 ppm) produced red colored urine and staining of the abdominal fur, and decreased body weight gain (-9 to -12%) in males and females. This dose level most probably would have exceeded a MTD level had its use been continued for a longer period of time. The reduction of this dose level to 30,000 ppm after 2 weeks eliminated the above described toxic effects. However, the 30,000 ppm dose level was associated later on in the study with a dose and time related increase in albumin in the urine of male rats and with urinary bladder hyperplasia in male rats. Both these changes (especially the hyperplasia) appear to correlate with the bladder tumors seen in male rats and suggest that an MTD level was reached (but not exceeded) in male rats at the 30,000 ppm dose level. In addition, it is probable that the 30,000 ppm dose level was also close to the MTD level in female rats (where no tumorigenic responses were observed), because of the weight loss and urine discoloration seen in females at 40,000 ppm during the first 2 weeks of the study.

2. Rat Oncogenicity Study of Mono-Sodium Phosphite (Metabolite of Aliette):

Monosodium phosphite, the urinary metabolite of Aliette in the rat (see section E.2.); was administered in the diet to 60 Charles River-CD rats/sex/dose level at doses of 0, 2,000, 8,000 and 32,000 ppm for 27 months (117 weeks). The dose levels tested in this study were equivalent to those employed in the chronic oncogenicity study of Aliette described above. The study was conducted by IRDC. No evidence of an oncogenic response in the urinary bladder, the adrenal medulla, or at any other site was observed with monosodium phosphite.

The highest dose of monosodium phosphite tested in the study was associated with the following signs of toxicity: (a) a significant ($p < 0.05$) reduction in mean body weight gain in male rats (-13.8%) and female rats (-9.4%) throughout the study (this effect appeared to be compound-related for the the males since weight gain was

also reduced in low dose males (- 9.5%) and in mid dose males (- 15.4%) at the end of the study); (b) a reduction in the efficiency of food utilization for male rats only (this effect, which also occurred in the mid dose males, may have been related to the reduced rate of weight gain seen in male rats); (c) soft stools in male rats; (d) a slight reduction in urine pH (acidification) in male rats, and (e) significant ($p < 0.05$) increases in relative weights of the liver (male rats only), kidneys (male and female rats), and the heart (male and female rats). The Peer Review Committee considered these findings in assessing whether an MTD level had been reached in this study. It was concluded that although most of these changes would not satify the ususal criteria for meeting a MTD level, that this was not a major issue in the present study due to the fact that an unusual high dose of 32,000 ppm was tested.

3. Mouse Oncogenicity Study of Aliette:

Aliette was administered in the diet to 60 Charles River CD-1 mice/sex/dose level at doses of 0, 2,500, 10,000 and 20,000/30,000 ppm for 2 years. This study was conducted by IRDC. The high dose level was increased from 20,000 ppm to 30,000 ppm at study week 19 because of the absence of any effect in the early part of the study. No evidence of an oncogenic response was observed with Aliette, and no other toxicological changes were seen.

The highest dose of Aliette tested in this study (i.e., 20,000/30,000 ppm, or 3,000/4,500 mg/kg/day) did not approximate a MTD level. The registrant apparently set the dose levels in this study on the basis of those used in the chronic Aliette rat oncogenicity study (section D. 1.), since no subchronic toxicity tests were performed in mice for use in estimating the MTD level. The Committee also believed that this study was inadequate because of the fact that the high dose level was increased at week 19, following the early critical part of the animal's growth curve. Despite these shortcomings, the Committee did not believe that additional oncogenicity testing in mice would increase an understanding of the chemical's toxicity due to the magnitude of the high dose level that was tested.

E. Additional Toxicity Data:

1. Two-Year Dog Toxicity Study:

The Committee briefly reviewed the results of a 2-year study of Aliette in pure bred beagles that was conducted by IRDC. The chemical was administered in the diet to 6

dogs/sex/dose level at doses of 0, 10,000, 20,000 and 40,000 ppm. The NOEL was 10,000 ppm. The LEL was 20,000 ppm based on testicular changes (i.e., presence of spermatocytic and/or spermatidic giant cells in the lumen of the seminiferous tubules) in 2/6 males; this effect was also observed for 6/6 high dose male dogs. Other changes that were seen only at the high dose level consisted of a reduction in total serum proteins in male dogs throughout the study and a reduced BUN in female dogs at several study intervals. No other toxicological or histopathological effects were observed.

2. Metabolism:

Two studies were conducted in Sprague-Dawley (SD) rats with orally administered ^{14}C-Aliette (250 mg/kg/day X 7 days). The compound was rapidly metabolized to give mainly CO_2 (60%) which was recovered from exhaled air. The second major route of excretion was via the urine (approximately 26%) which contained some unchanged parent compound plus a larger amount of phosphite (phosphorus acid) as a metabolite, but no phosphate. Only minor amounts (3-4%) of administered radioactivity were found in feces and this consisted mainly of the phosphite metabolite. Two additional studies were conducted in SD rats with the ^{32}P labelled phosphite metabolite (111 mg/kg /day x 7 days). The phosphite was excreted unchanged in both the urine (59-65%) and the feces (30-32%). No unusual localization of either Aliette or the metabolite in tissues was observed. From the above results it appears that Aliette is essentially completely absorbed after oral ingestion and extensively hydrolyzed to phosphite and ethanol. The ethanol is oxidized via acetaldehyde and acetate to CO_2 and then excreted in expired air. The phosphite is excreted (along with some unchanged parent compound) directly into the urine without further oxidation to phosphate.

3. Mutagenicity:

Eight mutagenicity tests were performed with Aliette. All were acceptable to the Agency and all were negative. These included 2 Ames tests using S. typhimurium (strains TA 1535, TA 1537, TA 98, TA 100 and TA 1538), 2 phage induction tests using E. coli, 2 micronucleus tests in Swiss mice and CD-1 mice (no increase in the percentage of polychromatic erythrocytes with micronuclei was observed), 1 DNA repair test using E. coli, and 1 Saccharomyces cereviscae yeast assay.

4. Miscellaneous Information:

No reproduction/teratology data were available for review by the Committee. No SAR data on Aliette were available, but the individual chemical components and/or metabolites of Aliette (e.g., ethanol, phosphate, aluminum) are present in the human diet.

F. Weight of Evidence Consideration:

The Committee considered the following facts regarding toxicology data on Aliette to be important in a weight of the evidence determination of oncogenic potential.

1. Aliette was associated with a significantly elevated incidence of urinary bladder tumors (adenomas and carcinomas combined) at the highest dose level tested (40,000/30,000 ppm) in male Charles River CD rats. The tumors were mainly seen in surviving males at the time of terminal sacrifice. The original pathological diagnosis of these tumors was independently confirmed by another consulting pathologist, who also reported an elevated incidence of urinary bladder hyperplasia in high dose male rats. No urinary bladder tumors were produced in female rats.

2. There were insufficient data available to determine the mechanism for the production of the urinary bladder tumors in the high dose male rats in the Aliette oncogenicity study. The registrant claimed that the bladder tumors resulted from irritation and subsequent proliferation of the bladder epithelium due to the formation of urinary stones. However, neither mineralization nor urolithiasis were observed in the bladder of high dose male rats upon histopathological examination. In view of the fact that bladder hyperplasia was observed in high dose male rats, it was recommended that the registrant pursue further studies to evaluate a possible urinary tract irritant effect of treatment resulting from either the urinary excretion of Aliette *per se*, calcium, the aluminum portion of the Aliette molecule, or the ethanol metabolite of Aliette.

3. Aliette was initially reported to produce a significantly elevated incidence of pheochromocytomas (adenomas and carcinomas combined) at the mid (8,000 ppm) and highest (40,000/30,000 ppm) dose levels tested in male Charles River CD rats. The elevated pheochromocytoma incidence

was primarily due to an increase in the adenomas. This conclusion was based on the diagnosis of the original pathologist at the test laboratory (IRDC) where the study was performed. However, this original diagnosis was not confirmed by two other consulting pathologists who reevaluated the same data. The difference in the pathological diagnosis of pheochromocytomas by different groups of pathologists was attributed to the fact that a high degree of variability exists in the interpretation of adrenal medullary neoplasia vs. adrenal medullary hyperplasia (see section D.1.b. for details). Based on the information available, the Committee concluded that Aliette did not produce pheochromocytomas in high dose male rats. No adrenal gland tumors were produced in female rats.

4. The highest dose level of Aliette tested in male Charles River CD rats (40,000/30,000 ppm) appeared to approximate a MTD level based on the finding of urinary bladder hyperplasia at this dose. Similarly, a MTD level appeared to be satisfied in female Charles River CD rats at the high dose level of 40,000/30,000 ppm, because of the weight loss (about 10%) incurred at 40,000 ppm during the first two weeks of the oncogenicity study before the dose level was reduced to 30,000 ppm.

5. Aliette was not oncogenic when administered in the diet to Charles River CD mice at dose levels ranging from 2,500 to 30,000 ppm. Similarly, the urinary metabolite of Aliette, namely monosodium Phosphite, was not oncogenic when administered in the diet to Charles River CD rats at dose levels ranging from 2,000 to 32,000 ppm. These dose levels were similar in magnitude to those employed in the chronic oncogenicity study of Aliette in Charles River CD rats where urinary bladder tumors were observed.

6. No adverse effects on the urinary bladder or the adrenal gland were produced by Aliette in a 2-year chronic toxicity study performed in Beagle dogs at dose levels ranging from 10,000 to 40,000 ppm.

7. Aliette was not found to be mutagenic in 8 genotoxicity assays considered to be acceptable to the Agency and which were determined to be generally adequate for detecting an oncogenic potential of a chemical (Ames Mutagenicity Assays, E. coli phage induction tests, micronucleus tests in mice, DNA repair tests using E. coli, and the Saccharomyces cerevisiae yeast assay).

8. Metabolism data available for Aliette in Sprague-Dawley rats indicated that the compound is almost completely absorbed following oral administration, and then hydrolized to ethanol which is excreted in expired air as CO_2 (the primary route of excretion) and to phosphite which is excreted in the urine (the secondary route of excretion). In addition, some of the orally administered Aliette is also excreted unchanged in the urine.

9. No reproduction/teratology data or structure-activity data related to Aliette were available for evaluation.

G. Classification of Oncogenic Potential:

The Committee concluded that the data available for Aliette provided limited evidence of oncogenicity for the chemical in male rats. According to EPA proposed guidelines (CFR, November 23, 1984), the Committee classified Aliette as a Category C oncogen (possible human carcinogen with limited evidence of carcinogenicity in animals in the absence of human data). That is, Aliette produced urinary bladder tumors (adenomas and carcinomas combined) at the HDT in only one sex and species of experimental animal (i.e. male Charles River CD rats) and in only one experiment. The urinary bladder tumors were evaluated by 2 different pathologists and both pathologists confirmed an increase in adenomas plus carcinomas combined at the HDT. However, one of the pathologists indicated that both adenomas and carcinomas were elevated to a similar extent whereas the other pathologist found a higher ratio of carcinomas to adenomas. In addition, Aliette did not show any positive response in a variety of short-term tests for mutagenicity. Finally, the information that was provided by the registrant regarding possible mechanisms for the induction of the urinary bladder tumors in Charles River CD rats did not adequately assist the Committee in classifying the oncogenic potential of Aliette (e.g., the registrant claimed that bladder stones were responsible for the observed tumors but neither bladder stones nor mineralization changes were observed in male rats upon histological examination). None of the criteria specified in the EPA proposed guidelines for classifying Aliette as a Category B_2 carcinogen were met based on the data available to the Toxicology Branch Peer Review Committee.

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December 1, 1989

Ms. Susan Lewis
Product Manager (21)
Office of Pesticide Programs
Environmental Protection Agency
Crystal Mall, Building 2
Arlington, Virginia 22202

Dear Ms. Lewis:

SUBJECT: Fosetyl-Al EPA Reg. No. 264- 466
Rebuttal to Oncogenicity Classification

Based upon the finding of bladder tumors in male rats, fosetyl-al is currently classified as a Category C oncogen. At the time fosetyl-al was classified as an oncogen, the Environmental Protection Agency (EPA) encouraged Rhône-Poulenc to investigate possible mechanisms for the production of the bladder tumors. We have recently completed a study which supports that bladder tumors in the chronic study were the result of acute renal injury induced by massive doses of fosetyl-al and subsequent chronic irritation of the urinary tract. The purpose of this letter is to present all of the evidence which documents that fosetyl-al should not be classified as an oncogen and to submit the following data:

> A Maximum 13-Week Dietary Toxicity Study of Fosetyl-Al in the Albino Rat With a Maximum 21 Week Recovery Period. B.E. Osborne. Bio-Research Ltd. August 9, 1989. Project No. 83040.

In a 1981 chronic toxicity/oncogenicity study in the rat (MRID No. 00098339) conducted by International Research and Development Corporation (IRDC), increased incidences of tumors (adenomas and carcinomas combined) and epithelial hyperplasia in urinary bladders were reported for male Charles River CD rats fed fosetyl-al in the diet at 40,000/30,000 ppm (the high dose was lowered to 30,000 ppm after two weeks due to clinical signs of toxicity). Epithelial hyperplasia but not urinary bladder tumors were observed in females at 40,000/30,000 ppm. No treatment-related tumors were found at the lower dietary levels of 2000 and 8000 ppm. A re-examination of the bladder slides by a consulting pathologist confirmed these findings (MRID No. 00153174).

In another chronic study conducted by IRDC, monosodium phosphite, the primary urinary metabolite of fosetyl-al in rats, was fed to Charles River CD rats at dietary levels of 0, 2000, 8000, and 32,000 ppm for 27 months (MRID No. 00098352). The highest dietary level did not exceed a Maximum Tolerated Dose (MTD). No evidence of a carcinogenic response was observed in the bladder at any dose level. Further, no treatment-related histopathological changes were observed at any other tissue site.

After reviewing the results of the chronic rat studies, Dr. Conrad D. King, a pathologist consulting for Rhône-Poulenc, hypothesized that massive doses of fosetyl-al altered the

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normal calcium/phosphorous balance leading to changes in renal excretion and the physical/chemical composition of the urine. (Fosetyl-al readily decomposes into phosphorous acid and ethanol.) According to Dr. King, such changes resulted in chronic irritation of the urinary tract and subsequent bladder tumors. Thus, he proposed that the bladder tumors were the result of a chronic toxic reaction rather than a true carcinogenic effect. This argument was submitted to EPA in 1985 as part of Rhône-Poulenc rebuttal of the oncogenic classification of fosetyl-al (MRID No. 00153174).

In 1986, the Toxicology Branch Peer Review Committee of EPA rejected the above hypothesis citing the lack of microscopic observations of mineralization or stones in the urinary bladders of rats in the IRDC fosetyl-al study which would support the presence of chronic irritation. (However at 40,000/30,000 ppm, an increased number of calculi in the bladder was noted at gross necropsy of males and females.) The Peer Review Committee did indicate that the epithelial hyperplasia in the bladder suggested a possible irritant effect. At that time, EPA encouraged Rhône-Poulenc to further investigate an altered calcium/phosphorous balance as a possible mechanism for the bladder tumors. Therefore, in 1987 Rhône-Poulenc initiated a study at Bio-Research Ltd. to closely monitor urine biochemistry, development of calculi, and subsequent gross and histopathological changes in the kidneys and urinary tract. The details and results of the Bio-Research study are summarized in the following paragraphs.

Male and female rats received fosetyl-al admixed in the diet at 0, 8000, 30,000, or 50,000 ppm (70 rats/sex/group) for a maximum of 13 weeks. A high dose of 50,000 ppm was selected because dietary analyses for fosetyl-al in the IRDC study indicated that animals in the high dose group actually received dietary concentrations ranging from approximately 38,000 to 61,000 ppm during the first 32 weeks of the study. The mid-dose level of 30,000 ppm was selected to replicate the intended dietary concentration for the high dose group in the IRDC study. The low dose of 8000 ppm corresponds to the No Observed Effect Level for the development of bladder tumors in the IRDC chronic rat study. Ten animals/sex/ group were sacrificed after 2, 4, 8, and 13 weeks of test material administration. The remaining rats were allowed recovery periods of either 8 or 16 weeks following 8 weeks of exposure to the test material or a 21 week recovery after 13 weeks of exposure. All animals received basal diet for the recovery periods. During the study, animals were observed daily for clinical signs, and bodyweights and food consumption were measured weekly. Water consumption was measured at Weeks 3, 7, 12, 15 (8 week recovery group), and 33 (21 week recovery group). Hematology, clinical chemistry, urinalysis, and urinary and fecal calcium, phosphorous, and aluminum levels were determined for all animals surviving to their scheduled sacrifice. A gross necropsy and histopathological examination of the bladder, kidneys, ureters, and thyroid with parathyroids were performed on all animals.

Overt toxicity was observed at 50,000 ppm. Marked diuresis and red/brown urine and stained fur were noted at 50,000 ppm and to a lesser extent at 30,000 ppm. Diuresis subsided after approximately 4 weeks of dosing but the stained fur continued to be observed at 50,000 ppm even during the recovery periods. At 30,000 and 50,000 ppm, respectively, 3 and 10 males either died or were sacrificed moribund. At postmortem examination, urinary calculi and dilatation of renal pelves, ureters, and urinary bladder

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were observed in these animals. Histopathological evaluation showed hydronephrosis and transitional cell hyperplasia in the kidneys and papillary hyperplasia in the bladder.

Bodyweights for males and females at 50,000 ppm were approximately 20 to 40% below controls for most of the treatment period. Compared to controls, food consumption was lower in the high dose group during the treatment period only. At 30,000 ppm, bodyweights for males were slightly depressed during treatment, but returned to control levels during recovery. Although animals in the high dose group generally exhibited the greatest weight gains in the recovery periods, bodyweights for these animals remained below control levels throughout recovery.

Water consumption was significantly increased for mid and high dose males and for high dose females at Weeks 3, 7, and 12. During the recovery period, water consumption was statistically increased for the 50,000 ppm group at Week 15 only. Slight increases in red blood cells and hemoglobin were recorded for 50,000 ppm males at Weeks 2 and 16 (i.e. 8 weeks of treatment followed by 8 weeks of recovery). A slight increase in platelets was noted for high dose animals at Weeks 2 and 4. In general, increased blood urea nitrogen (BUN) and phosphorous levels and decreased albumin and total protein levels were noted at 50,000 ppm throughout the treatment period. For high dose animals, calcium levels were slightly lower at Weeks 4 and 8 and carbonate levels were higher than control at Weeks 8 and 13. At 30,000 ppm, phosphorous levels were increased at Weeks 2 and 4 and albumin, total protein, and calcium (males only) were decreased at Week 4. Plasma aluminum was generally below the limit of detection. BUN levels for high dose males remained elevated throughout the recovery periods. All other parameters were comparable to controls during recovery.

Urinalysis revealed decreased sodium, potassium, phosphorous, and pH and increased calcium and total volume at 50,000 ppm. In general, phosphorous was 50 to 100 times lower than control while calcium was 6 to 8 times higher than control. Similar but less marked effects were observed at the mid dose. Only urinary pH was decreased at the low dose. Specific gravity and oxalate were sporadically decreased for high dose males. Although urinary aluminum levels tended to be higher at 30,000 and 50,000 ppm, statistically significant differences were observed only at Weeks 2 and 8. All parameters were similar to control during recovery. With the exception of an initial increase at Week 2, fecal calcium levels were slightly decreased for high dose males during treatment only. For 50,000 ppm males and females, fecal phosphorous levels were slightly higher than control during treatment. Fecal aluminum levels tended to be increased at all fosetyl-al dose levels during the treatment period.

At necropsy, calculi in the bladder were found in high and mid dose males as early as Week 2 and persisted throughout the recovery period. The largest number and size of bladder calculi were observed at the Week 2 sacrifice. At each subsequent sacrifice interval, calculi decreased progressively in number and size. This decrease was most likely related to the decreased urinary pH which caused dissolution of the calculi. Relatively few bladder calculi were found for high dose females. A high incidence of calculi in the kidneys was reported for high dose males and females throughout the study. Calculi were also found in the ureters of high dose animals but only during the treatment period. Analysis of the calculi from high and mid dose animals revealed high calcium and phosphorous content and

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low aluminum and magnesium content, i.e. less than 0.05%. Dilatation of the kidneys and ureters was observed for 50,000 ppm animals and 30,000 ppm males throughout the study.

Histopathology revealed papillary hyperplasia and uroliths in the urinary bladders of high and mid dose males throughout the treatment period. These lesions were also found in recovery males from these groups but at a lower incidence than during the treatment period. Papillary hyperplasia was also observed at a relatively low incidence in high dose females during the treatment period. Dilatation of the ureters, chronic interstitial nephritis, hydronephrosis, and renal uroliths were observed at 30,000 (males only) and 50,000 (males and females) ppm throughout the study. Transitional cell hyperplasia was found in the kidneys of high dose animals throughout the study. During recovery, an increased incidence of dilatation of the collecting tubules was noted at 50,000 ppm.

The following results of the Bio-Research study support the arguments made by Rhône-Poulenc in 1985 that the bladder tumors found in male rats in the IRDC study were due to chronic irritation from calculi, and that these calculi were formed as a result of an imbalance in calcium/phosphorous homeostasis induced by massive doses of fosetyl-al.

1. The dietary level of 50,000 ppm clearly exceeded a Maximum Tolerated Dose. Ten males were found dead or sacrificed moribund in this group, and bodyweights for males and females were 20 to 40% below control during the treatment period. In the chronic rat study with fosetyl-al, IRDC believed that the high dose was lowered to 30,000 from 40,000 ppm after the first two weeks of the study. However for the first 32 weeks of the IRDC study, dietary levels at the high dose actually ranged from 38,000 to 61,000 ppm which exceeded the MTD. In a chronic rat study with monosodium phosphite, the high dose of 32,000 ppm did not exceed and MTD, and no treatment-related tumors were observed in this study

2. Although urinary aluminum levels appeared to have been increased, aluminum from fosetyl-al was excreted primarily in the feces. A lack of aluminum absorption in the gastrointestinal tract is supported by the extremely low levels of aluminum found in the plasma and calculi. The aluminum in the urine was most likely due to contamination from either food or feces.

3. Clearly, the calcium/phosphorous balance and composition of the urine were altered by fosetyl-al. Urinary calcium was 6 to 8 times higher than control while phosphorous was 50 to 100 times lower than control. Calculi analyzed from the 30,000 and 50,000 ppm groups contained high amounts of calcium and phosphorous indicating that the stones were formed as a result of the imbalance.

4. Fosetyl-al clearly induced acute renal injury at 30,000 and 50,000 ppm. Histopathological changes in the kidneys, ureters, and urinary bladder were related to this acute injury and the presence of calculi in these tissues. The urolithiasis induced by fosetyl-al caused sufficient irritation in the urinary tract to induce hyperplasia within a relatively short period of time. A low incidence of hyperplasia was observed in the urinary bladders of high dose females which correlates with their low incidence of bladder calculi. As the calculi in the urinary bladder of males tended to disappear during

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recovery, the incidence of papillary hyperplasia decreased as well suggesting partial reversibility of these effects. Irritation and hyperplasia are clearly related to the presence of calculi. Under conditions of chronic exposure, these effects would lead to the formation of bladder tumors as seen in the IRDC fosetyl-al study. Thus, the bladder tumors induced by fosetyl-al were the result of acute renal injury followed by a chronic toxic reaction rather than a true carcinogenic effect. Based upon the results of the Bio-Research study, fosetyl-al should not be classified as an oncogen.

If you have any questions or if any further information is needed, please let me know. My phone number is 919-549-2372.

Sincerely,

Nick Somma
Registration Manager

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PESTICIDES AND TOXIC
SUBSTANCES

MEMORANDUM

SUBJECT: Allette: Review of a non-guideline 90-day feeding study in rats

TO: S. Lewis / J. Stone, PM Team 21
Registration Division (H7505C)

FROM: Whang Phang, Ph.D. 12/13/90
Pharmacologist
Tox. Branch II / HED (H7509C)

THROUGH: James Rowe, Ph.D. 12/13/90
Section Head
and
Marcia van Gemert, Ph.D. 12/18/90
Branch Chief
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Introduction: In 1986, the Toxicology Branch Peer Review Committee evaluated the toxicology data on fosetyl-Al. Based upon the findings that fosetyl-Al induced an increase in the incidence of urinary bladder tumors (adenomas and carcinomas combined) in male Charles River CD rats which received the test article at dietary concentration of 30,000 ppm for 2 years, fosetyl-Al was classified as a Category C oncogen with qut risk assessment. This 90-day feeding study has been specially designed and conducted to provide evidence for the registrant's rebuttal that the urinary bladder tumors were "the result of a disturbance of the phosphorus/calcium imbalance due to an overloading of animals with phosphorus ... which led to an increased incidence of bladder stones and subsequent irritation and proliferation of the bladder epithelium" (Memorandum: J. Quest to H. Jacoby, June 12, 1986).

Discussion: The 90-day feeding study has been evaluated and the conclusion is as follows:

Groups of rats (70/sex/dose) received Fosetyl-Al at dietary concentrations of 0, 8,000, 30,000, and 50,000 ppm. Each dose group was further divided into 7 subgroups each of which received the test article for a different length of time (ranged from 2 weeks to 13 weeks), and some subgroups were then placed on the

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normal diet for different recovery periods (ranged from 8 weeks to 21 weeks). The results established that, in male rats, Fosetyl-Al at 30,000 ppm or more produced diuresis, hypercalciuria, acidic urine, uroliths in the kidneys, ureters, and bladder, and histopathological changes in the urinary system. The histopathological changes were characterized by the following:

kidneys: pyelitis, pyelonephritis, papillary necrosis, dilatation of collecting tubules, and transitional hyperplasia.

Ureters: dilatation, ureteritis, and transitional cell hyperplasia.

Urinary bladder: submucosal edema, papillary hyperplasia, cystitis, and transitional cell hyperplasia.

The results also indicated that the histopathological changes in the urinary system were related to the presence of uroliths.

This study is a non-guideline study which is specifically designed to show that the histopathological changes in the urinary bladder seen in an earlier 2-year chronic/oncogenicity study were related to the calculi formation in the bladder. Therefore, it will not be classified according to the Core Classification Guidelines for toxicology studies. However, it is scientifically acceptable.

The results of this study and all available toxicology data on Fosetyl will be presented to the Peer Review Committee on Carcinogenicity of HED which will re-consider the carcinogenic potential of this chemical.

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Reviewer: Whang Phang, Ph.D. Whang Phang 12/13/90
HFAS/Tox. Branch II/HED (H7509C)

Secondary Reviewer: James Rowe, Ph.D. James Rowe 12/13/90
Section Head
HFAS/Tox. Branch II/HED (H7509C)

DATA EVALUATION REPORT

Chemical: Fosetyl-Al; Aluminum tris(-O-ethyl phosphonate); Aliette[R]

Study Type: 90-Day feeding study (rats) (Non-guideline, special study)

Note: In 1986, the Toxicology Branch Peer Review Committee evaluated the toxicology data on fosetyl-Al. Based upon the findings that fosetyl-Al induced an increase in the incidence of urinary bladder tumors (adenomas and carcinomas combined) in male Charles River CD rats which received the test article at dietary concentration of 30,000 ppm for 2 years, fosetyl-Al was classified as a Category C oncogen with out risk assessment. This 90-day feeding study has been specially designed and conducted to provide evidence for the registrant's rebuttal that the urinary bladder tumors were "the result of a disturbance of the phosphorus/calcium imbalance due to an overloading of animals with phosphorus ... which led to an increased incidence of bladder stones and subsequent irritation and proliferation of the bladder epithelium" (Memorandum: J. Quest to H. Jacoby, June 12, 1986).

Caswell No.: 12B **HED Proj. No.:** 0-0469

MRID No.: 413152-01 **EPA ID No.:** 264-266

EPA Record No.: 257775

Sponsor: Rhône-Poulenc Ag Co.

Testing Laboratory: Bio-Research Ltd.
87 Senneville Rd
Senneville, Quebec H9X 3R3
Canada

Citation: Osborne, B.E. (1989), A maximum 13-week dietary toxicity study of Fosetyl-Al in the Albino rats with a maximum 21 week recovery period. Bio-Research Ltd.; Lab. Proj. No. 83040. Aug. 9, 1989.

Conclusion: Groups of rats (70/sex/dose) received Fosetyl-Al at dietary concentrations of 0, 8,000, 30,000, and 50,000 ppm. Each dose group was further divided into 7 subgroups each of which received the test article for a different length of time (ranged from 2 weeks to 13 weeks), and some subgroups were then placed on the normal diet for different recovery periods (ranged from 8 weeks to 21 weeks). The results established that, in male rats, Fosetyl-Al at 30,000 ppm or more produced diuresis, hypercalciuria, acidic urine, uroliths in the kidneys, ureters, and bladder, and histopathological changes in the urinary system. The histopathological changes were characterized by the following:

kidneys: pyelitis, pyelonephritis, papillary necrosis, dilatation of collecting tubules, and transitional hyperplasia.

Ureters: dilatation, ureteritis, and transitional cell hyperplasia.

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The results also indicated that the histopathological changes in the urinary system were related to the presence of uroliths.

This study is a non-guideline study which is specifically designed to show that the histopathological changes in the urinary bladder seen in an earlier 2-year chronic/oncogenicity study were related to the calculi formation in the bladder. Therefore, it will not be classified according to the Core Classification Guidelines for toxicology studies. However, it is scientifically acceptable.

Materials and Methods

Test article: Fosetyl-Al was a fine white powder with Lot No. DA 497, additional Code No. LS 74 783 Ca (39148-24-8); 97.3% purity.

Animals: Sprague-Dawley rats, Crl:CD^{R} (SD) BR strain (330 males and 331 females) were obtained from Charles River Canada Inc., St. Constant, Quebec, Canada. At the initiation of the study, the animals were approximately 6 weeks old, and body weights were 165 to 229 gm for males and 123 to 179 gm for females.

Study Design

It should be reiterated that this study was specially designed to investigate urinary bladder changes seen in rats of an earlier 2-year rat study. In this study, the rats were treated with Fosetyl-Al at dietary levels ranged from 8,000 to 50,000 ppm for up to 13 weeks followed by a recovery period of up to 21 weeks. The

urinary biochemical profile, the development of urinary calculi, and any gross and histopathological changes in the kidneys and urinary tract were examined to establish a possible cause-effect relationship for the increase in the incidence of urinary bladder tumors.

Prior to the initiation of the treatment, 5 rats/sex were randomly selected from the total number of animals for analyzing the baseline values of biochemical parameters of blood, feces, and urine. The animals were then randomly divided into 4 dose groups (70 rats/sex/dose). The dietary concentrations of Fosety-Al tested were 0, 8,000, 30,000, and 50,000 ppm. Each dose group was then further divided into 7 subgroups which were treated for various times and placed on the control diet for different recovery periods. The experimental scheme and the sacrifice schedules are presented below:

No. of animals per subgroup	Treatment Period Weeks	Recover Period Weeks	Sacrifice Week
10	2		2
10	4		4
10	8		8
10[b]	13		13
10[a]	8	8	16
10[a]	8	16	24
10[b]	13	21	34

a: In the report, these groups are referred to as Subpopulation 1.
b: These groups are referred to as Subpopulation 2, in the report.

Test diet preparation: The test diet was prepared weekly by mixing appropriate amounts of the test article with the control diet. The prepared test diet was stored at room temperature. Samples of the test diet were collected and analyzed for the stability and homogeneity.

Clinical Observations: All test animals were examined twice daily for signs of mortality and toxicity.

Body weights: All test animals were weighed weekly during the acclimation and treatment periods. At the scheduled sacrifice, the fasted body weights of the test animals were measured.

Food consumption: The food consumptions were reported to be determined weekly.

Compound intake: The chemical intake was calculated for each interval using the group mean values of the food consumption and the following formula:

$$\text{Average achieved intake (mg/kg/day)} = \frac{\text{dietary con. (mg/kg)}}{\text{mid-wk group mean body weight}} \times \frac{\text{group mean food intake (gm/rat/wk)}}{7}$$

Water consumption: Water consumption was measured on various groups of the test animals.

Hematology & Clinical chemistry: Prior to the initiation of the study, blood samples were collected from 5 rats/sex . During the study period, blood samples were collected from either 5 or 10 rats/sex/dose at various treatment periods.

a: Hematology: The following hematological parameters were examined:

- hematocrit
- hemoglobin
- red blood cell counts
- white blood cell counts (total & differential)
- platelet counts
- Wintrobes constants (MCV, MCHC, & MCH) (calculated)
- blood smear

b. Clinical chemistry: The following clinical chemistry parameters were measured:

- blood urea nitrogen (BUN)
- total protein
- creatinine
- glucose
- albumin
- sodium
- potassium
- calcium
- total CO_2
- inorganic phosphorous
- aluminum

Urinalysis: Urine samples were collected at various times from 5 rats/sex/dose for analyses. The following parameters were assayed:

- color and appearance
- pH
- glucose
- ketones
- blood
- volume
- specific gravity (SG)
- protein
- bilirubin
- urobilinogen
- sodium
- potassium
- calcium
- total phosphorous
- aluminum
- oxalate
- microscopy of centrifuge deposit
- nitrite

Fecal examinations: Fecal samples were collected and analyzed for the following parameters:

aluminum calcium phosphorous

Urinary calculi examination: The details of the experimental methods were not reported, but the following elements were analyzed:

aluminum calcium phosphorous magnesium

Gross pathology: Prior to the initiation of the study, 5 rats/sex were sacrificed, and gross pathological examinations were performed to obtain a baseline evaluation. During the study, gross pathological examinations were conducted in all test animals when possible. " For each animal, necropsy consisted of an external examination, including identification of all clinically recorded lesions, as well as a detailed internal examination. The abdominal cavity was opened and urinary bladder was inflated (but not distended) with Zenker's fluid".

Kidneys and thyroid with parathyroid were removed and weighed. In addition, kidneys, ureters, thyroids with parathyroids, and bones were removed and fixed in buffered 10% formalin. The urinary bladder was fixed in Zenkers's fluid.

Histopathology: Histopathological examinations were conducted on bladder, kidneys, ureters, and thyroids of all animals. In addition, the urinary bladder was examined for any crystalline formation. Representative calculi found in the urinary bladder were photographed. The urinary calculi from 10 rats were removed and assayed.

Statistical analyses: Certain data were analyzed for homogeneity of variance using Bartlett's test. Dunnett's "t" test was used to assess the significance of inter-group differences. Kruskal-Wallis test was used to analyze heterogeneous data.

Quality assurance: A quality assurance statement was signed and included in the report.

Results

a). Mortality: During the study, 10 males of 50,000 ppm and 3 males of 30,000 ppm group died or were sacrificed *in extremis*. Before death, these animals showed signs of "weakness, skin and eye pallor, tremors, hypothermia, and abdominal distension". Wetness and red/brown staining of the abdominal fur were consistently seen, and these signs were related to diuresis which was also observed. These gross pathology examinations showed the presence of calculi in the urinary system (kidneys, ureters, and urinary bladder) and dilatation of the bladder, ureters and/or pelves of kidneys. The histopathology findings were urolithiasis, papillary hyperplasia in the bladder, hydronephrosis, nephritis,

papillary necrosis, and pelvic transitional cell hyperplasia in the kidneys and occasional transitional cell hyperplasia in the ureters. No death was reported in 8,000 ppm rats.

b). Clinical observation: Clinical signs consisted of abdominal wetness, red/brown fur staining and/or yellow fur staining, and diuresis were seen in 30,000 and 50,000 ppm males at various treatment periods. However, the above clinical signs were seen to a lesser extent in females of similar dose groups. The diuresis was reported to subside slightly after 4 weeks or 13 weeks, but the staining of the abdominal fur persisted into 8 or 13 weeks of the recovery periods. Rats which received 8,000 ppm test article did not exhibit these clinical signs.

c). Body weights: The relevant group mean body weight data were excerpted from the report and presented in Table 1. Both male and female rats of 50,000 ppm groups showed statistically decreases in body weights at various treatment periods, and the decreases persisted through the 21 week recovery period (Table 1). A less dramatic decrease in body weights was also seen in 30,000 ppm males, but during the recovery periods, the body weights of these animals were comparable to those of the controls.

The body weights of 30,000 ppm females and of 8,000 ppm males and females were comparable to those of the controls (Table 1).

c). Food consumption: Food consumptions in both 50,000 ppm males and females were significantly decreased at various treatment periods relative to those of the controls. However, after the treatment stopped, the food consumptions returned to the level of the controls (Figure 1). On some occasions of the first 6 weeks of the treatment period, a decrease in food consumptions was seen in 30,000 ppm males. A decrease in food intake in 8,000 ppm males was seen only on isolated occasions. A persistent decrease in food intake was not seen in females of 30,000 or 8,000 ppm groups.

d). Compound intake: The average compound intake during the treatment periods was calculated as follows:

Dietary Concentration of Fosetyl-AL ppm	Average Compound Intake gm/kg/day Male	Female
8,000	0.5	0.6
30,000	2.1	2.5
50,000	3.5	4.2

e). Water consumption: A significant increase in water consumption

was seen in 50,000 ppm males and females and in 30,000 ppm males. This effect persisted in 50,000 ppm males and females after the treatment was ceased. Notable changes were not seen in other dose levels.

f). Hematology: At 2 and 8 weeks of the treatment periods, an increase in the levels of erythrocyte counts, hemoglobin, and platelets was seen in 50,000 ppm males and females. These hematological changes were likely caused by diuresis.

g). Clinical chemistry: The clinical chemistry results at various analysis periods were excerpted from the report and presented in Tables 2A, 2B, 2C, 2D, and 2E. A statistically significant increase in the levels of BUN, phosphorous, and CO_2 was found in 50,000 ppm males and females at various periods of treatments (Tables 2A to 2E). The increase in the BUN level persisted during the treatment and the recovery periods although females showed a slight recovery. The levels of phosphorous and CO_2 returned to the level of the controls at the recovery periods.

An increase in the phosphorous, BUN, and CO_2 levels was also seen in 30,000 ppm animals during the initial periods of the study (Tables 2A and 2B). Significant changes were not seen in other dose levels.

h). Urinalysis: The relevant urinalysis data at various treatment and recovery periods were excerpted from the report and presented in Tables 3A through 3E. All treated males and females showed a decrease in urinary pH and an increase in the urinary Ca++ levels while receiving the test compound. The urinary phosphorous levels were decreased in the treated males and females relative to those of the controls, and this decrease might due to diuresis. Urinary aluminum levels were also elevated in all treated animals during the treatment periods. Urine volumes were consistently increased in 50,000 ppm males and females at different sacrifice periods. When the treatment ceased, some of the changes in the urinary electrolytes virtually returned to the control levels (Table 3E).

i). Fecal analysis: The relevant results of fecal analyses were excerpted from the report and presented in Tables 4A to 4E. There was an overall decrease in fecal Ca++ levels in 50,000 ppm males and females despite an initial increase (at 2 weeks of treatment period) in 50,000 ppm males. A consistent and dose-related increase in fecal aluminum level was seen in all treated males and females. The phosphorous levels were also increased in 50,000 ppm males and females at different treatment durations. However, all these fecal electrolyte changes returned to the control levels after the treatment was stopped (Tables 4A through 4E).

j). Gross pathology: The major gross pathology findings were associated with the presence of calculi in the urinary system. Calculi were found in kidneys, ureter, and urinary bladder of 30,000 and 50,000 ppm males. The kidney calculi were mainly found in the pelves. The ureter calculi often "seen to be causing a virtual obstruction of the duct"; the registrant presented photographic results to support this observation (pages 1387-1402 of the report). The urinary bladder calculi were reported to be in greater number and larger size than the calculi found in the kidneys and ureter. In addition, the number and size of the urinary bladder calculi were also greatest at the 2 week sacrifice; at each subsequent sacrifice the size and number of the urinary bladder calculi progressively decreased. Therefore, the selective gross pathology data of 2 and 4 weeks sacrifices were excerpted from the report and presented in Tables 5A and 5B.

The reduction in the size and number of urinary bladder calculi after 2 weeks of treatment might be associated with the decrease in the pH of the urine causing the dissolution of the urinary bladder calculi.

The recovery animals showed a decrease in the incidence of urinary bladder calculi. The calculi were not found in 8,000 ppm and the control animals. The incidence of urinary bladder calculi was very few in 50,000 ppm females and essentially none in lower dose group females despite the presence of calculi in the kidneys and the ureters (Tables 5A and 5B).

The kidneys of the affected animals often showed dilatation, irregular surface, enlargement, and discoloration. Dilatation of the ureter was frequently seen in the affected animals.

k). Organ weights: The absolute kidney and thyroid weights were not substantially different from the controls in animals treated for less than 13 weeks. The relative kidney weights (organ/body) were increased in 50,000 ppm males and females at various sacrifice periods prior to 13 weeks, but this increase was mainly due to a decrease in the body weights of these animals.

After 13 weeks of treatment, the absolute kidney weights were increased in 30,000 and 50,000 ppm males and 50,000 ppm females relative to those of the controls as indicated below:

	Absolute Kidney Weights (gm)		
	Control	30,000 ppm	50,000 ppm
Males:	2.90 ± 0.39	3.41 ± 0.39a	3.75 ± 0.32b
Females:	1.86 ± 0.14	1.90 ± 0.21	2.10 ± 0.24a

a: $p < 0.05$; b: $p < 0.01$

The kidney weights of recovery animals were essentially comparable to those of the controls.

l). Urinary calculi analysis: Calculi were collected from 30,000 and 50,000 ppm males and females at 2-week sacrifice and analyzed for phosphorous, magnesium, aluminum, and calcium contents. The results are excerpted from the report and presented in Table 6. The results indicated that these calculi consisted of approximately 23% phosphorous, 33% calcium, 0.2% magnesium, and less than 0.1% aluminum (Table 6). The remaining portion was thought to be oxalate which was not analyzed. In this reviewer's opinion, the calculus analysis was scientifically insufficient. The analysis on oxalate, uric acid, protein, and the entire unit of the test chemical should have been included to properly characterize the composition of a calculus.

m). Histopathology: The histopathology findings in the Fosetyl-Al treated animals were closely associated with the presence of the calculi or uroliths in the urinary system. The findings on kidneys, ureter, and urinary bladder at different sacrifice periods were excerpted from the report and presented in Tables 7A through 7E.

1). Kidneys: In the kidneys, an increase in the incidence of uroliths, chronic interstitial nephritis, hydronephrosis, pyelonephritis, transitional cell hyperplasia, and papillary necrosis was commonly seen in 30,000 and 50,000 ppm males and 50,000 ppm females at different sacrifice periods. Some of these incidences were also increased in the recovery males and females (Tables 7A to 7E). The author of the report believed that hydronephrosis and other findings in kidneys were likely to be induced by urine stasis resulting from the obstruction of the urine flow.

The report also noted that a benign transitional cell papilloma was found in a 50,000 ppm male which received Fosetyl-Al for 8 weeks followed by 16 weeks of recovery, but this rat died in week 22.

2). Ureters: An increase in the incidence of ureteritis, the presence of uroliths, dilatation, was seen in

30,000 and 50,000 ppm males and 50,000 ppm females whereas transitional cell hyperplasia was seen predominantly in 50,000 ppm males (Tables 7A through 7E).

3). Urinary bladder: In the urinary bladder, an increase in the incidence of submucosa edema, papillary hyperplasia, the presence of uroliths, and cystitis was seen in 30,000 and 50,000 ppm males at different treatment periods. Some of these findings were also seen during the recovery periods (Table 7E). In contrast, the above findings were rather low in females of similar dose groups; for example, the presence of uroliths was seen in 1/10 females verses 5/9 males which received 50,000 ppm test compound and sacrificed at 8 weeks of treatment (TAble 7C).

Papillary hyperplasia was seen mainly in 30,000 and 50,000 ppm males and 50,000 ppm females, and none was reported in 30,000 ppm females or 8,000 ppm males and females. Papillary hyperplasia was characterized by the "folded processes or expansion of the lamina propria covered by a hyperplastic urothelium of varying thickness and were generally accompanied by a minimal inflammatory infiltrate". When the treat-treatment stopped, a significant decrease in these proliferative changes was found.

Discussion and Conclusion

Groups of rats (70/sex/dose) received Fosetyl-Al at dietary concentrations of 0, 8,000, 30,000, and 50,000 ppm. Each dose group was further divided into 7 subgroups each of which received the test article for a different length of time (ranged from 2 weeks to 13 weeks), and some subgroups were then placed on the normal diet for different recovery periods (ranged from 8 weeks to 21 weeks). The results indicated that Fosetyl-Al at 30,000 and 50,000 ppm produced death and diuresis in males and some females. A decrease in body weight and food consumption was consistently seen in 50,000 ppm males and females and to a lesser extent in 30,000 ppm males. However, during the recovery period the body weights and food con-sumptions were comparable to those of the controls. An increase in water consumption was also seen in 30,000 and 50,000 ppm males and in 50,000 ppm females. Hematological changes were found in 50,000 ppm males and females, but the changes were related to diuresis.

Clinical chemistry data indicated increases in BUN nitrogen, phosphorous, and CO_2 in 50,000 ppm males and females. During the recovery periods, CO_2 and phosphorous levels were comparable to those of the controls, but the increase in BUN level persisted.

The urinalysis results indicated a decrease in the pH values in all treated animals after 2 weeks of treatment and an increase in urinary Ca++ levels in 30,000 and 50,000 males and females. The urinary phosphorous level was decreased in 30,000 and 50,000 ppm males and females; this decrease was related to diuresis. Urinary aluminum level was increased, and it was thought to be due to contamination of the urine samples since the blood level of aluminum was low.

Fecal analysis revealed that there was an overall decrease in the Ca++ level in 50,000 ppm males and females relative to that of the controls. The fecal aluminum level was consistently higher in the treated animals, and this increase was dose-related. The normal level of blood aluminum and the dose-related increase in the fecal aluminum level indicated that aluminum was not absorbed via the intestinal tracts.

Both gross pathology and histopathology data showed that calculi or uroliths were present in kidneys, ureters, and urinary bladder of 30,000 and 50,000 ppm males and of a few 50,000 ppm females. The chemical compositions of the urinary bladder calculi were approximately 23% phosphorous, 33% calcium, 0.2% magnesium, and less than 0.1% aluminum. In the kidneys, the increased incidence of urolithiasis, hydronephrosis, pyelitis, pyelonephritis, papillary necrosis, dilatation of the collecting tubules, and transitional hyperplasia of the pelvis was seen in 30,000 and 50,000 ppm males and 50,000 ppm females. In the ureters, an increase in the incidence of urolithiasis, ureteritis, and dilatation was found in 30,000 and 50,000 ppm males and 50,000 ppm females. In the urinary bladder, an increase in the incidence of urolithiasis, submucosa edema, papillary hyperplasia, and cystitis was seen in 30,000 and 50,000 ppm males at different treatment durations, and some of these finding persisted to the recovery periods. After 13 weeks of treatment, the absolute kidney weights of 30,000 and 50,000 ppm males and 50,000 ppm females were significantly increased ($p < 0.05$).

Based upon the data presented in this report, the histopathological findings in the kidneys, ureters, and urinary bladder were related to the presence of uroliths in these organs because, in 8,000 ppm males and females and 30,000 ppm females, uroliths were virtually absent in their urinary systems, and accordingly the histopathological changes were not seen. Most importantly, an evaluation of the individual animal histopathology and the urinalysis data revealed that the presence of calculus was almost always associated with urinary hypercalciuria, acidic urine, transitional cell hyperplasia and/or papillary hyperplasia of the urinary bladder, ureter, and kidney tubules.

The report did not attempt to offered any explanation for the substantially lower incidence of calculi formation in 50,000 ppm females relative to that of the males.

This study is a non-guideline study which is specifically designed to show that the histopathological changes in the urinary bladder seen in an earlier 2-year chronic/oncogenicity study were related to the calculi formation in the bladder. This study, however, will not be classified according to the Core Classification Guidelines for toxicology studies, but the results of this study and all available toxicology data on Fosetyl will be presented to the Peer Review Committee on Carcinogenicity of HED which will re-consider the carcinogenic potential of this chemical.

Aliette toxicology review

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Pages 15 through 39 are not included in this copy.

The material not included contains the following type of information:

___ Identity of product inert ingredients

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___ A draft product label

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X FIFRA registration data

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The information not included is generally considered confidential by product registrants. If you have any questions, please contact the individual who prepared the response to your request.

ATTACHMENT 4 — 64 — REC 9-7-82 (2) ✓

DATA EVALUATION RECORD

CHEMICAL:	Aluminum tris (O.ethyl phosphonate) Trade name: Foseytl-Al
FORMULATION:	Technical
CITATION:	Palmer, A.K., Bottomley, A.M., Barton, S.J., Clark, R., Offer, J.M., 1981 Effect of LS 74-783 on reproductive function of multiple generations in the rat
CONTRACTING LAB.:	HUNTINGDON RESEARCH CENTRE
SPONSOR:	RHONE-POULENC AGROCHIMIE, LYON, FRANCE
REPORT NO.:	HUNTINGDON RESEARCH RNP/89/80745 of January 22, 1981
	EPA Reg. No. Acc. No. 247174
REVIEWED BY:	A. F. PELFRENE 09/13/1982 C. Gregorio 9-13-82
REVIEWED ON:	August 2, 1982
TEST TYPE:	MULTIGENERATION FEEDING STUDY
TEST MATERIAL:	FOSETYL-AL technical Purity 97.3% Batch No. DA 73

MATERIALS AND METHODS

ANIMALS AND MAINTENANCE

Specific pathogen free rats of the CFY strain, supplied by Anglia Laboratories, Alconbury Huntingdon, England, with body weight range of 60 to 70 g for both sexes were used to start in the first generation Fo. After an acclimatization period of 7 days, they were weighed and allocated to 4 groups. The animals were maintained in a temperature and humidity controlled room (21±4°C and 50±5% relative humidity) with 12 to 14 air changes per hour. Natural light in the room was supplemented by artificial light between 8 a.m. and 8 p.m.

During the premating periods the rats were housed 5 to a cage in suspended galvanized metal cages. Cages of males were interspersed among those holding females to promote the development of oestrous cycles.

During the mating periods the rats were housed in plastic breeding cages. At the end of the mating periods the males were re-housed to their original cages. Females were retained in the plastic breeding cages until sacrifice of the litter (first mating) or termination (second mating).

TEST MATERIAL ADMINISTRATION

Treatment groups (FO generation) were as follows:

Treatment (dietary concentration)	No. of Rats (FO gen.) Male	Female
Control	25	25
6000 ppm	25	25
12000 ppm	25	25
24000 ppm	25	25

Test diets were prepared freshly each week. Homogeneity and concentrations of test material in feed verified in the 6 samples taken from the 6,000 ppm batch were found to be in the range of 4,170 - 5,870 with a mean of 5120 ppm and a standard deviation of 559 ppm. The 6 samples taken from the 24, 000 ppm batch were in the range of 20,6000 - 23,000 ppm with a mean of 22,300 ppm and a standard deviation of 875.

Stability of Foseytl-Al in the feed was verified from day 1 through day 7 on samples taken from 6,000 ppm batch. The concentrations found were in the range of 4,950 - 5,640 ppm with a mean of 5,180. Concentrations of Fosetyl-Al in day 1 through day 7 samples taken from the 24,000 ppm batch

were found to be in the range of 21,000 - 25,800 ppm with a mean of 23,200 ppm. It is concluded that Fosetyl-Al is stable in the feed for one-week periods when stored at room temperature.

Concentration analysis in feed: The Fosetyl-Al concentrations in the feed averaged 5,481 - 11,140 and 23,942 ppm and were in the ranges of 4,690 - 6,300, 9,680 - 11,900 and 21,900 - 26,000 ppm corresponding to respective nominal concentrations of 6,000 ppm - 12,000 ppm and 24,000 ppm.

Animals of the F0 generation were maintained on their respective diet for 90 days prior to mating. The animals were then mated on a one male to one female basis for a period of 20 days. Resulting litter (F1A) were reared to 21 days post partum. The F1A pups were then sacrificed and subjected to post mortem examination for detection of macroscopic changes.

Approximately 10 days after the weaning of the F1A litters, the F0 generation was remated for a period of 20 days employing different male and female parings. Five pregnant females in each group were sacrificed on day 20 of gestation for teratological examination, (day 0 being the day of appearance of sperm in the vaginal smear).

The remaining dames were allowed to rear their young to 21 days post partum when 25 males and 25 females were selected from each group to form the basis of the F1B generation. Parent (F0) animals and surplus of F1B pups were killed and examined macroscopically.

The F1B generation were reared on their respective diets to an age of at least 90 days and then mated for a period of 20 days. The resulting F2A generation was reared 21 days post partum, sacrificed and examined macroscopically.

Approximately 10 days after weaning of the F2A litters, the F1B generation were remated for 20 days. Ten pregnant females in each group were sacrificed for teratological examination. The remaining F1B dames were allowed to rear their litters to day 21 post partum when 27 males and approximately 39 females from each group were selected, of these 15 males and 15 females were reared on their respective diets for at least 91 days after which time they were subjected to detailed macroscopic examination and representative tissues were retained for microscopic examination.

The remaining 12 males and 24 females were reared on their respective diets for 91 days (housed 6 to a cage) after which two matings with rearing to day 21 post partum were permitted. Mating was on the basis of 1 male to 2 females, employing different partners at the second pairing. All F3A pups were sacrificed and discarded following macroscopic examinations. Ten male and ten female F3B pups from all groups were subjected to organ weight measurements with histopathological examination initially restricted as per protocol to control and high dosage groups.

INTERIM SACRIFICE (for teratology purposes)

On day 20 of pregnancy selected F0 and F1B generation dames were killed by CO_2 asphyxiation, dissected, and examined for abnormalities, and macroscopical changes in internal organs. The overies and uteri were immediately examined for:

Number of corpora lutea
Number and distribution of live young
Number and distribution of embryonic/fetal deaths
Litter weight from which the mean pup weight was calculated
Fetal abnormalities

Embryonic and fetal deaths were classified as: Early (only placenta visible) and late (both placenta and embryonic remnants visible).

Live young were examined externally and weighed. Half the pups in each litter were preserved in Bouin's solution for subsequent researching for visceral abnormalities (Wilson's technique). The remainder were fixed for skeletal examination. Structural deviations were classified as major malformations, minor malformations, and variants.

GROSS AND HISTOPATHOLOGY OF F3B AND NON-MATED F2B GENERATIONS

Animals of the non-mated F2B generation and selected pups of the F3B generation were killed by CO_2 asphyxiation and subjected to detailed post mortem examination for macroscopic pathological changes.

For selected pups of the F3B generation only, the following organs were weighed: BRAIN, HEART, LIVER, KIDNEY, LUNGS, SPLEEN, THYMUS.

The following tissues were preserved and subsequently examined:

Routinely examined microscopically		Preserved but not examined in the first instance
brain	salivary gland	aorta
eye	pancreas	trachea
heart	lymph nodes	oesophagus
lung	(cervical & Mesenteric)	jejunum
liver	thymus	mid-colon
spleen	thyroid	mammary gland
kidney	pituitary	skin
urinary bladder	adrenals	prostate
stomach (gladular & non-glandular)	testes	tongue
duodenum	seminal vesicle	second eye
ileum	ovaries	sciatic nerve
caecum	uterus	
	any other tissue macroscopically abnormal	

The following observations and tests were performed throughout the study:

PARENT ANIMALS

General observation for signs of overt toxicity, mortality was performed daily.

URINALYSIS:

Urinalysis was undertaken following the death of 5 animals from group 4 (high dose level, 24,000 ppm) when autopsy revealed kidney damage. Urinalysis was performed during week 7 of the F1B generations, on individual urine samples collected overnight from 10 males each from group 1 (control) and 4 (high dose - 24,000 ppm).

FOOD AND WATER CONSUMPTION:

Food intake was recorded weekly during the first pre-mating phase of each generation. Water intake was measured during the first and penultimate weeks of the same premating phase.

BODY WEIGHTS:

Body weight changes of each rat of the F0 generation were taken initially and at intervals of 1 week thereafter Animals of all subsequent generations were weighed at birth, 4, 8, 12 and 21 days and at weekly intervals thereafter. Mating performance, pregnancy rate, gestation period (time between the day of successful mating as indicated by presence of spermatoza in vaginal smear and parturition) were recorded.

TERMINAL AUTOPSY:

After the second litters had been weaned, parent animals were sacrificed and macroscopically examined.

LITTER DATA

As soon as possible after birth, all young were counted, identified by toe amputation and examined for external abnormality. All litters were examined daily up to day 21 post partum for dead and malformed young. Pups were weighed individually at 1, 4, 8, 12 and 21 days post partum. Surplus pups were sacrificed at day 21 and examined externally and internally for abnormalities. Sex was determined by gonadal inspection.

Other litter data were: Litter size, litter and mean pup weight, pup mortality, abnormalities.

Tissues were generally preserved in 10% buffered formalin, except eyes which were preserved in Davidson's fluid. For microscopic examination tissues were processed, embedded in paraffin wax, sections cut a 5 µ and stained with haematoxylin and eosin. Frozen cryostat sections of liver and kidney, previously fixed in 10% buffered formalin, were sectioned at 12 µ and stained for fat with Oil Red O.

Microscopic examination was restricted to Groups 1 and 4 except for urinary bladder for which all groups were examined.

The tissues listed above were also preserved for the non-mated F2B animals but were not processed.

ASSESSMENT AND ANALYSIS OF RESULTS

In respect of litter data, group mean values generally were calculated in two ways viz:

Mean A: Generally includes all animals showing evidence of pregnancy either at day 20 of pregnancy (sacrificed dams) or at birth (dams rearing young).

Mean B: Generally only includes dams with viable young either at day of pregnancy (sacrificed dams) or at day 21 post partum (dams rearing young).

Mean B has more meaning when group size is low, in which case mean values would be unduly influenced by the presence of a single animal with total litter loss. Mean A is a more accurate index with large group sizes or when several litter are totally lost.

Litter weights, mean pup weights and incidences of abnormality are only calculated as Mean B values. For all values expressed as a ratio e.g. pre- and post-implantation losses, group mean values are derived as the mean of percentages within individual litters.

As litter values do not follow a "normal" distribution, intergroup differences are analysed by non-parametric statistical methods using the litter as the basic sample unit.

Organ weights of the F3B generation were analysed by analysis of variance adjusting for bodyweight at sacrifice as covarite, provided there was found to be a significant relationship (F-test; P 0.1). Treatment means were compared with control values by the method of L.S.D.'s in conjunction with Williams' test.

RESULTS

INTAKE OF TEST COMPOUND (Remark)

Estimation of dosage in terms of mg/kg bodyweight/day in studies employing dietary incorporation as a means of administration to individual animals, particularly in multigeneration studies where animals progress through a marked range of physical and physiological development. Comparisons, therefore, may only be made when appreciable inter-group or in this case intergeneration differences occur.

Allowing for inaccuracies it was evident, nevertheless, that for each dietary concentration employed, the dosage of LS 74-783 in terms of mg/kg bodyweight/day was considerably greater for the F1B and F2B generations than for the F0 generation. It was considered that these differences were largely responsible for the greater responses, particularly in respect of urinary tract changes and bodyweight gains seen in the second and third generations and described in the following sections.

GENERAL OBSERVATIONS:

The general appearance and condition of the animals were unaffected by the test compound.

The following deaths were recorded:

GROUP	MORTALITIES BY SEX AND GENERATION					
	MALES			FEMALES		
	F0	F1B	F2B	F0	F1B	F2B
Control	1	-	-	-	-	-
6000 ppm	-	-	-	2	1	-
12,000 ppm	1	1	1	-	-	2
24,000 ppm	-	7	3	2	1	-

URINALYSIS:

A significant ($p<0.01$) lower specific gravity in the 24,000 ppm male group was observed. In addition, an increase in epithelial or polymorphonuclear cells was observed in the urine of 4/10 males at the 24,000 ppm dose level as compared to 0/10 in the control males.

No other treatment related urinary effect was reported.

WATER CONSUMPTION:

For all generations, water consumption for the 24,000 ppm males and females was greater than that of the controls as seen in the table below:

GROUP ppm	GROUP MEAN WATER CONSUMPTION (G/RAT/DAY)					
	F0	F1B	F2B	F0	F1B	F2B
	Week 1	Week 1	Week 1	Week 12	Week 9	Week 12
Males						
Control	24.3	20.3	19.4	38.9	36.5	43.2
6000	26.8	23.1	17.3	42.5	42.7	46.8
12000	24.3	23.0	16.5	42.7	39.6	39.6
24000	27.5	25.0	19.6	40.0	40.5	48.5
Females						
Control	23.5	20.8	16.8	31.2	32.3	31.0
6000	24.2	22.1	16.5	31.7	35.2	35.9
12000	23.2	20.5	14.8	29.9	31.1	30.5

FOOD CONSUMPTION:

No changes in food consumption were evident when treated groups were compared to the control groups of males and females.

PARENTAL BODY WEIGHTS:

Mean body weights (in grams) was similar in the 6,000 and 12,000 ppm treatment groups when compared to the control group. The 24,000 ppm group (males and females) also gained weight. The F_o generation 24,000 ppm dose animals showed that female weight gain to be slightly superior to that of the respective control dams and male weight gains slightly lower than respective controls animals as shown in the following table:

F_o GENERATIONS (25 ANIMALS/SEX/GROUP)
MEAN BODY WEIGHT (GRAMS)

	CONTROL		24,000 ppm	
Study week	Males	Females	Males	Females
0	111	103	110	102
13	552	307	537	339
22	663	355	653	368
30	724	384	715	390

The F1b generation, 24,000 ppm dosed females and males weight gains were approximately 9% lower than that of their respective controls as seen in the following table:

F1b GENERATION (25 males/sex/group)
MEAN BODY WEIGHT (GRAMS)

	CONTROL		24,000 ppm	
Study week	Males	Females	Males	Females
0	79	77	47	48
11	499	307	427	280
20	609	365	539	334
26	644	415	578	355

The F2b generation 24,000 ppm dosed females and males weight gains were approximately 8.5% lower than that of their respective controls as seen in the following table:

F_2b GENERATION (25 ANIMALS/SEX/GROUP)
MEAN BODY WEIGHT (GRAMS)

	CONTROL		24,000 ppm	
Study week	Males	Females	Males	Females
0	68	64	51	48
14	542	308	498	313
22	686	360	601	333
30	746	445	653	371

MATING PERFORMANCES AND PREGNANCY RATES:

As can be seen in the following tables, from the most part mating performance assessed by the number of females becoming pregnant, was comparable for all groups at both matings of each generation. One exception was the lower pregnancy rate recorded at 6000 ppm for both matings of the F_2b generation. This is considered as coincidental in the absence of a similar reduction at higher concentrations.

MATING PERFORMANCE AND PREGNANCY RATE

F_2 GENERATION
FIRST AND SECOND MATINGS

Group (ppm)	Paired	Mated	Pregnant	Mating index	Pregnancy index
Control	25	21	21	0.84	1.00
6000	25	24	24	0.96	1.00
12000	25	24	24	0.96	1.00
24000	25	22	22	0.88	1.00
Control	25	20	15	0.80	0.75
6000	25	22	16	0.88	0.72
12000	25	25	20	1.00	0.80
24000	23	18	13	0.78	0.72

F_1b GENERATION
FIRST AND SECOND MATING

Group	Paired	Mated	Pregnant	Mating Index	Pregnancy Index
Control	25	19	19	0.76	1.00
6000	25	18	18	0.72	1.00
12000	25	21	21	0.84	1.00
24000	25	20	20	0.80	1.00
Control	25	21	11	0.84	0.52
6000	24	16	6	0.66	0.37
12000	25	20	11	0.80	0.55
24000	25	22	12	0.88	0.54

F_2b GENERATION
FIRST AND SECOND MATING

Group	Paired	Mated	Pregnant	Mating Index	Pregnancy Index
Control	24	18	18	0.75	1.00
6000	24	13	13	0.54	1.00
12000	24	20	20	0.83	1.00
24000	22	19	19	0.86	1.00
Control	24	18	18	0.75	1.00
6000	24	14	14	0.58	1.00
12000	22	16	16	0.72	1.00
24000	24	20	20	0.83	1.00

GESTATION PERIOD:

The gestation time was comparable for all groups.

LITTER LOSS:

The incidence of litter loss was scattered and limited and therefore not treatment related.

LITTER SIZE AND PUP MORTALITY:

Mean litter size and viability of the pups were comparable for all groups.

LITTER AND MEAN PUP WEIGHTS:

For both matings of each generation, values for litter and mean pup weights were essentially comparable for all groups at birth and day 4 post partum. Subsequently however, pup weight gain at 24,000 ppm was consistently retarded, leading to lower values for litter and mean pup weights during the latter part of lactation. Divergence from control values was most marked at 21 days but was also frequently discernible at 12 days and sometimes as early as 8 days post-partum. Differences frequently attained statistical significance.

A similar but less marked and consistent effect on litter and mean pup weights was also seen at 12,000 ppm.

There was no conclusive evidence of a similar effect at 6000 ppm.

The effects on litter and mean pup weights were considered to be related to lower maternal weights gain during lactation.

PATHOLOGY

F0 GENERATION:

Gross necropsy and histopathology of the F0 males and females showed no treatment related effects.

F1 GENERATION:

Gross necropsy and histopathology of the F1 animals demonstarated urinary bladder changes described as "hemorrage of the bladder wall, increased pelvic dilation, interstitial nephritis and papillary necrosis" in males at the following incidences: 0/25, 2/25, 0/25 and 8/25 in males and in females: 0/25, 3/25, 3/25 and 10/25 for the controls - 5,000, 12,000 and 24,000 ppm dose levels respectively. No other pathological changes were seen.

F_2 GENERATION:

Gross necropsy and histopathology of the F_2 generation animals demonstrated similar urinary bladder and kidney changes (described above) in males 1/25, 0/25, 1/25 and 5/25 and in females 0/25, 0/25, 1/25 and 5/25 for the controls, 6,000-12,000 and 24,000 ppm dose levels respectively. No other pathological treatment related effects were seen.

F_3 GENERATION:

Minimal epithelial hyperplasia and/or hypertrophy of the transitional epithelium, sometimes associated with small papillary projections and/or desquamation cells in the lumen of the urinary tract were observed. These were associated with the presence of crytalline or calcareous deposits usually in the lumen but also in individual cases, in the serosa the mucoid epthelium or tubules of the seminal vesicles. These changes were observed in 8/10 males and 8/10 females at 24,000 ppm and 1/10 males at the 12,000 ppm level. However, young rats are known to be prone to have spontaneous inflammatory lesions of the urinary tract and to easily develop such lesions in presence of crystalline deposits (1-2-3). In the present case, the very high phosphorus intake has been shown to induce increase calciuria (4) in the treated animals and therefore the changes observed in the urinary tract are most likely related to the presence of crystalline calcareous deposits.

Site reference

PATHOLOGY OF FETUSES

No treatment related effects were observed in fetuses from the F_0 (2nd generation) and F_1b dams sacrificed at day 20 of gestation.

ORGAN WEIGHTS

All organs of both sexes showed a marked correlation with bodyweight.

In case of males significant deviation from control values was recorded with respect to lower liver, spleen and thymus weights of all test groups and higher lung weight of males at 24,000 ppm. For females a similar pattern was evident for thymus, spleen and lung weight but differences from controls attained statistical significance only with respect to thymus weight. The corresponding values are presented in the following table.

The biological reference of the differences was considered uncertain in view of the lack of marked dosage-related trends within test groups, the marked effect at 24,000 ppm and 12,000 ppm on maternal body weight gain and pup weight gain during lactation and most important the absence of significant dosage-related histopathological findings in these organs.

1) CHENG, L.. J. ENVIRON. PATHOL. TOXICOL. 1980; 4:317-349.
2) ROBERTSON, J.L.. TOXICOL. PATHOL. 1980; 8:9-13.
3) CLAYSON, D.B.. J. NAT. CANCER INST. 1974; 52:1685-1689.
4) KALIFAT, R. et al: unpublished report. RHONE-POULENC. C.R. Vitry/C.N.G. No. 20765-E of 1.29.1981.

MEAN GROUP ORGAN WEIGHTS OF F_3B ANIMALS
(grams)

GROUP (ppm)	LIVER		LUNG		SPLEEN		THYMUS	
	MALES	FEMALES	MALES	FEMALES	MALES	FEMALES	MALES	FEMALES
CONTROL	3.870	3.303	0.648	0.644	0.338	0.277	0.308	0.322
6000	3.513	3.293	0.725	0.681	0.277	0.255	0.250	0.226
12000	2.963	2.842	0.600	0.576	0.229	0.220	0.199	0.210
24000	2.884	2.460	0.653	0.537	0.184	0.200	0.191	0.210

CONCLUSION

Considering the three generations there was no evidence of an adverse effect on Fertility or Reproduction at any dosage. Similarly there was no indication of an adverse effect on in utero or development of young. Parental animals were adversely affected at the highest concentration (24,000 ppm) and to a lesser extent at 12,000 ppm but not at 6,000 ppm.

At 24,000 ppm the most remarkable effects were: 1) lower body weight gain for males of all generations, and females of the F1B and F2B gerations; the more marked deviations of the F1B and F2B generations being associated with both higher ingestions of material and lower weight at weaning. 2) for all generations a specific effect on the pattern of maternal weight changes during lactation leading to retarded mean pup weight gain and lower litter and mean pup weights in mid- and late lactation. 3) A high incidence of animals showing pathological changes in the urinary tract, particularly for the F1B and F2B generations. This was associated with an increased incidence of male but not female deaths for the F1B and F2B generations. 4) Correlating with the urinary tract changes observed in adults, the more detailed microscopic examination at weaning of 10 male and 10 female per group (F3B generation) revealed crystalline or calcareous deposits in the lumen of the urinary bladder of most animals. The presence of these deposits was frequentl

associated wiht minimal hyperplasia/hypertrophy of transitional epithelium and sometimes also with papillary projections and/or desquamation of epithelial cells. These epithelial abnormalities are most likely reactive to the presence of crystalline, calcareous deposits in the bladder.

At 12,000 ppm similar but much less marked effects were evident principally in respect of lower overall weight gains of the F2B generation, lower litter and mean pup weight in late lactation, and the recording of urinary tract changes in occasional adults andl/10 weanling males of the F3B generation.

The No Observable Effect Level (NOEL) in this study is considered to be 6,000 ppm in adult and young rats. (300 mg/kg and 600 mg/kg respectively).

Classification: Minimum

ATTACHMENT 5

9-1-82
(12)

DATA EVALUATION RECORD

CHEMICAL: Aluminum tris (O-ethyl phosphonate)
Trade name: Fosetyl-Al

FORMULATION: Technical

CITATION: PALMER, A.K. and JAMES, R.W., 1977
Effect of LS74 783 on pregnancy of the rat

CONTRACTING LAB.: HUNTINGDON RESEARCH CENTRE
HUNTINGDON - ENGLAND

SPONSOR: RHONE-POULENC AGROCHIMIE, LYON, FRANCE

REPORT NO.: RNP/33/76939 of 06/23/1977

EPA Reg. No Acc. No. 247174

REVIEWED BY: A.F. PELFRENE, MD, PhD, ATS,
Director of Toxicology
RHONE-POULENC INC.
09/13/1982

C. Caracoix
9-13-82

REVIEWED ON: JUNE 9, 1982

TEST TYPE: TERATOGENICITY

TEST MATERIAL: FOSETYL-AL technical
Purity 99.8%
Batch No. FR 794/795

MATERIAL AND METHODS

ANIMALS AND MAINTENANCE

Eight sexually mature CFY strain (Anglia laboratory animals, Huntingdon, England) SPF female rats weighing between 180 and 250 g were used in this study. They were supplied timed mated by the breeding laboratory.

The day of mating as judged by the appearance of sperm in the vaginal smear or by the presence of a vaginal plug was considered day 0 of pregnancy.

On arrival, the animals were arbitrarly assigned to 4 groups, 5 per cage and ear marked.

They were given free access to Spratt's laboratory diet n° 1 and to tap water.

TEST PROCEDURE

The following treatment groups were set up:

Group	Treatment Fosetyl-Al	Concentration of solution % w/v	Dosage volume (ml/100g)	Number of Females
Gr. 1	Control	-	2.0	20
Gr. 2	500 mg/kg	2.5	2.0	20
Gr. 3	1000 mg/kg	5.0	2.0	20
Gr. 4	4000 mg/kg	20.0	2.0	20

The pregnant females were treated according to the above regimen from day 6 to day 15 of pregnancy inclusively by oral gavage of 2.0 ml/100g of the appropriate concentration.

The test solution was prepared fresh on a daily basis.

OBSERVATION

The animals were observed daily for signs of overt systemic toxicity or reaction to treatment and weighed on days 1-3-6-10-14-17 and 20 of the study.

On day 20 of pregnancy, all animals were sacrificed by CO_2 asphyxiation, dissected and observed for macroscopic pathological changes in maternal organs.

The ovaries and uteri were examined immediately to determine:

- number of corpora lutea
- number and distribution of live youngs
- number and distribution of embryo/fetal deaths
- litter wieght from which the mean pup weight was calculated
- fetal abnormalities.

Uteri or individual uterine horns without visible implatations were stained with a 10% solution of ammonium sulphide to reveal evidence of embryo resorption at very early stages of implantation.

Live young were examined externally and weighed.
Half the pups in each litter were preserved in Bovin's solution for subsequent free-hand sectioning (Wilson's technique) in order to evaluate any eventual visceral malformations. The remaining half of the litter was fixed in methylated spirit for subsequent macroscopic examination, evisceration and determination of sex prior to clearing and alizarin red S staining (modified Dawson's technique) for skeletal examination.

Structural deviation were classified as: major malformations, minor anomalies and variants.

STATISTICS

All numerical data were evaluated on a litter basis using non-parametric methods (Kruskal and Wallis (1) - Jonckheere (2))

RESULTS

PARENT ANIMALS

*General Behavior and Mortality:

Deaths were recorded in the 500 and 1,000 mg/kg/day dose groups (1 and 2 respectively). These deaths were not related ~~with an effect of the~~ caused compound since they resulted from "dosing errors" (i.e. mis administration). At the high dose level (4,000 mg/kg/day), 5/20 mothers died or had to be sacrificed at days 9, 10 and 11. Post mortem examination of each animal showed marked gastric dilation and fluid retention. Prior to death, animals lost weight and 3 animals showed chromodacryorhea.

No deaths were reported in the control group.

(1) KRUSKAL, W.H. and WALLIS, W.A., 1952 - J.AMER. STAT. ASSOC. 47: 583-621

(2) JONCKHEERE, A.R., 1954 - BIOMETRIKA 41: 133-145

*Body Weight of Dams

A dose related retardation of weight gain was observed during the first four days of dosing in all the treatment groups when compared to the controls. The weight gain retardation was only significant at the high dose level (4,000 mg/kg/day) as seen in the following table:

Group mean body weight of dams

Dose (mg/kg/day	Body Weight (gram) at day 1	6	10[a]	17	20
0 Control	212	246	269	322	371
500	219	257	278	331	375
1,000	220	259	277	333	384
4,000	218	257	260	310	365

(a) 4 days of dosing.

As also observed in this table, the high dose dams never regain their body weight momentum which was seen prior to dosing.

*Pregnancy rate

In the controls, 500 and 1,000 mg/kg/day groups, 1/20 mated animals did not become pregnant. All females from the high dose level group were pregnant.

REPORTED LITTER DATA

*Litter size and post implantation sites

All treatment groups were comparable to controls with respect to litter siz

There was a statistically significant ($p<0.05$) increase of total resorptions when the 4,000 mg/kg/day (1.3 total resorptions) and the 500 mg/kg/day (0.9 total resorptions) were compared to controls (0.5 total resorptions).

*Litter and mean fetal weights

Values for litter and mean fetal weights at 500 and 1000 mg/kg/day were essentially comparable with those of controls. At 4,000 mg/kg/day, a combination of slightly lower size and slightly lower fetal weight led to a low litter weight which was significantly ($p<0.05$) different from the control values as seen in the following table:

Group mg/kg/day	Litter weight (grams)	Mean fetal weight (grams)
Control	48.05	3.79
500	42.07	3.74
1,000	48.50	3.72
4,000	39.71(a)	3.46(a)

(a) significant at p 0.05 (KRUSKAL-WALLIS)

*Fetal malformations

Examination for fetal malformations and developmental variations was performed on 19,18,17 and 14 litters for the control,500,1,000, and 4,000 mg/kg/day groups respectively

A total of 4 kinds of malformations, and 11 kinds of varitions were observed. The numbers of exposed fetuses with soft tissues and skeletal variations were comparable to controls in the 500 and 1,000 mg/kg/day dose groups.

At the 4,000 mg/kg/day there was slight increase in malformations when compared to controls (5/161-3.1%-fetuses examined as compared with 2/242-0.8% - in the controls). These malformations at the 4-000 mg/kg/day level were described as "thoracic asymmetry-caudal displacement of the right kidney, moderate internal hydrocaphaly and transposition of the azygos vein, aortic arch and ductus arteriosus". A non significant number of minor visceral anomalies at the 4,000 mg/kg/day dose level were reported in 8/78-(10.2%) fetuses examined when compared to 8/119 (6.7%) fetuses examined in the control group. These were described as "mild to moderate subcutaneous edema and medial displacement of the left testis". Skeletal variations at the 4,000 mg/kg/day dose level were statistically different ($p<0.05$) from controls (7/78 fetuses examined as compared to 9/121 respectively). The skeletal variations were described as retarded ossification which correspounds to the lower mean fetal weight described in the litter and mean fetal weight section).

CONCLUSION:

Treatment with Fosetyl-Al in rats, caused maternal toxicity (5/20 pregnant dams died, retarded body weight gain) at the highest dose tested (4,000 mg/kg/day), ~~where~~ when administered orally by gavage from day 6 to day 15 of pregnancy, inclusively. As a result of the maternally toxic effect at this dose level, litter and mean fetal weights were reduced, total resorptions were increased and delayed ossification of fetuses were observed.

The NOEL is 1,000 mg/kg/day
The LEL is 4,000 mg/kg/day

Classification: Minimum

From the study

Skeletal Variations

Control

9/121 (7%)

4,000 mg/kg

7/78 (9%)

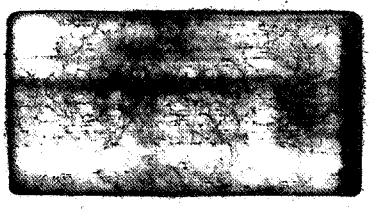
Minor visceral anomalies

Control

8/119 (7%)

4,000 mg/kg

8/78 (10.2%)



DATA EVALUATION REPORT

CHEMICAL : Aluminum tris (O-ethyl phosphonate)
Trade name Fosetyl-Al

FORMULATION : Technical

CITATION : PASQUET,J. and LEBAIL,R. 1976 -
Compound LS 74 783 -
Oral teratogenicity in the rabbit.

CONTRACTING LAB : CENTRE DE RECHERCHES RHONE-POULENC
VITRY sur SEINE - FRANCE

SPONSOR : RHONE-POULENC AGROCHIMIE - FRANCE

REPORT N° : RP/PD/CNG N° 18.917-E of 12/06/1976

EPA Reg. N° Acc. N° 247174

REVIEWED BY : A.F. PELFRENE, MD, PhD, ATS,
Director of Toxicology
RHONE-POULENC INC
10/20/82

C. Grebovic
10-20-82

REVIEWED ON : MAY 27, 1982

TEST TYPE : Teratogenicity

TEST MATERIAL : FOSETYL-Al technical
Purity 98 %
Batch : N° FT 795

MATERIAL AND METHODS

ANIMALS

Eighty-eight NZW derived Ju/RP strain virgin female rabbits, supplied by Elevage Junod, 10160 AIX-en-OTHE - France, and 11 males from the same strain and origine were used in this study. The body weight range at start of the study was from 2.6 to 3.4 kg.

Upon arrival to the testing facilities, the animals were identified with a numbered metal ring tag fitted around one of the hind legs.

MAINTENANCE

The does were individually caged in an environmentally controlled room (temperature : 22 ± 2°C - relative humidity : 30-50 % - artificial lighting from 7:00 am to 7:00 pm - air changes 14-15 times/hour). Those environmental parameters were not permanently recorded.

The animals were fed on UAR N°111 rabbit diet (200 g/day) and had free access to tap water.

All animals were acclimated to the laboratory condition for at least two weeks prior to mating.

TEST PROCEDURE

- Insemination : the mating was made under direct visual supervision of a trained technician on the basis of on male to one female being kept together for approximately 15 minutes. The breeding period took place between July 9 and September 6, 1976. Altogether 11 males were used to breed 88 females, thus each male was used several times during this period.

The day of successful mating was considered as Day 0 of pregnancy.

- Test groups : the mated females were randomly allocated to the treatment or control groups as the mating proceeded, their body weights on day 0 being taken into consideration for group homogeneity.

From day 6 to day 16 inclusively, the pregnant females were orally (via stomach tube) 5 ml/kg b.w. of Fosetyl-Al as a suspension in a 10% aqueous solution of gum arabic at the following daily dose level : 500 - 250 - 125 mg/kg b.w.

The highest dose level was selected from the results of a preliminary 11 day subacute study in non-pregnant female rabbits from the same origine.

One control group received only 5 ml/kg b.w. of the vehicle only under the same regime as the treated ones.

The number of pregnant females per dose level was as follows :

. Control		: 20
. Low dose	(125 mg/kg b.w. day)	: 23
. Intermediate dose	(250 mg/kg b.w. day)	: 23
. High dose	(500 mg/kg b.w. day)	: 22

TEST ARTICLE PREPARATION

Suspensions of test article in the vehicle at any given concentration were freshly prepared daily and administered to the rabbits within on hour of preparation. Therefore no stability analysis was performed on one hand ; on the other hand it was known from data obtained from other studies that Fosetyl-Al technical is quite stable for long periods of time under normal storage conditions at room temperature. Therefore it is highly unlikely that under the conditions of this test the suspension used were not satisfactory.

OBSERVATIONS

The does were weighed on days 0-6-16 and 28 of pregnancy and their daily food intake was recorded daily from day 0 to day 27.

The pregnant females were observed daily for signs of overt toxicity, mortality, premature abortion - general appearance and behavior.

On day 28 of pregnancy, the does were sacrified by cervical dislocation and immediately autopsied in order to examine the genital tract and to count the number of implantation sites, resorptions, and live and dead foetuses.

Live foetuses were weighed and given a careful external examination ; they were then sacrified and autopsied. Autopsy consisted of a detailed examination of the thoracic organs (after sectionning the left ribs and lifting the sternum and ribs sideways) and of the abdominal organs (including sex determination), examination of the eye (eyeball and lens enucleated) and of the brain (after cutting round and lifting backwards the skull cap). Lastly, all skeletons were stained with alizarin red S for examination of the bone segments.

STATISTICS

The results from each treated group were compared statistically with those from the control group, using the Chi-2-test in the case of percentages. In the case of means, either Student's t test or the analysis of covariance was used for roughly normal distributions and the Mann, Whitney and Wilcoxon test was used for asymmetrical distributions.

RESULTS

MATERNAL DATA

- Mortality :

From the 88 does which were mated, 15 were excluded from the study for incidental reasons (accidental death during compound administration or intercurrent infection). See annex

In the remaining females, pregnancy occurred in 12 control rabbits, 14 high dose (500 mg/kg b.w.), 15 intermediate dose level (250 mg/kg b.w.) and in 15 low dose (125 mg/kg b.w.) rabbits.

- Mean body weights of pregnant dams :

A significantly ($p = 0.05$) lower weight gain was observed in the high and intermediate dose levels when compared to the controls. During the treatment period (day 6 to day 16 of pregnancy), the weight gains being + 0.28 kg / + 0.21 / + 0.12 / + 0.15 for the control, low, intermediate and high dose groups respectively.

- Mean daily food consumption :

was comparable to that of the controls for the low and intermediate groups and slightly lower for the high dose group. This decrease was statistically significant ($p = 0.05$) from day 9 to day 16 of the treatment period.

- Pregnancy rate :

was comparable between all three treated groups as well as with that of the control.

Pregnancy occured in 12 out of 18 females (66 %), in the control group, in 14/18 (77 %) in the low dose group, in 15/18 (83 %) in the intermediate dose group and in 15/19 (79 %) in the high dose group.

No case of total loss of litter or total resorption were observed.

LITTER DATA

- Mean number of implantation sites :

was comparable in all groups : 8.3 in control / 8.6 in high dose / 9.3 in intermediate and 7.4 in low dose.

- The mean number of live foetuses :

was comparable in all treated and control group (7.7 / 7.6 / 8 / 6.9 from control to high dose groups respectively).

There was no evidence of an adverse effect on fetal growth in utero as shown by the mean weight of live foetuses (33.8 ± 0.9 g in control, 34.1 ± 2.5 g in the high dose group, 33.2 ± 1.2 g in the intermediate dose group and 36.6 ± 1.3 g in the low dose group).

- Embryotoxicity :

there was no evidence of an embryotoxic effect in any treatment group. The mean number of fetal losses (resorptions & dead foetuses) was not significantly different from one treatment group to the other and when treatment groups were compared to the control.

Also skeletal ossification was comparable.

- Teratogenicity :

no evidence of a teratogenic effect was observed. No malformed pup was found in any of the high or low dose groups and there were 3 malformed young in the control group and 2 in the intermediate dose group. These major malformations in the latter group (caridovascular, vertebral and brain malformation) are known to occur in untreated NZW rabbits. See annex.

All pregnant females carried to term and delivered normally ; therefore the above observations are drawn from respectively 12-14-15 and 15 litters for the control, low, intermediate and high dose groups.

All live foetuses were weighed and autopsied and a detailed gross examination of the thoracic and abdominal cavities, as well as of the brain and eyes was performed. All skeletons were stained and examined. The total numbers examined of foetuses per group were 92 in the control - 107 in the high dose group - 120 in the intermediate group and 103 in the low dose group.

Description of the few major abnormalities seen (3 in the control and 2 in the intermediate group) is given in the annex, along with the incidences reported in the literature for similar malformations in the same strain of rabbits.

CONCLUSION

Fosetyl-Al did not induce any embryotoxic, fetotoxic or teratogenic effects when orally administered to pregnant NZW rabbits at dose levels of 500 - 250 and 125 mg/kg b.w. daily from day 6 to day 16 of pregnancy.

NOEL : $\geq$ 500 mg/kg/day.

CLASSIFICATION : minimum

ANNEXES

INCIDENTAL DEATHS

Daily dose level (mg/kg po)	Doe N°	Time of death or sacrifice	Observations
Controls	164	Day 28	Bilateral pyometra
	173		Unilateral pyometra
500	265-a	Day 16	Accidental death (maladministration of the compound)
	267	Day 28	Unilateral uterine torsion (of the upper 1/3 of the horn)(a)
	279		Bronchial pneumonia
250	361-a	Day 13	Accidental death (maladministration of the compund)
	365	Day 11	
	365-a	Day 13	
	366-a	Day 11	
	378	Day 13	Found death with advanced autolysis on day 14; had presented the previous evening with almost total anorexia, dyspnoea and a frothy discharge with traces of blood.
125	465	Day 11	Accidental death (maladministration of the compound)
	465-&	Day 12	
	466-a	Day 7	
	467-a	Day 8	
	471-a	Day 21	Bronchial pneumonia

(a) From day 26, this doe showed a reduced food intake, then anorexia and, on day 28, a vaginal discharge with traces of blood. The horn showing the torsion was severly congested and contained 3 dead and 2 live foetuses, the other horn contained 2 dead and 2 live foetuses, all foetuses apparently being normal.

SUMMARY OF STUDY

Determinations		Control	Daily dose level (mg/kg p.o.) 500	250	125
Mated rabbits kept throughout the study (a)		18	18	18	19
Pregnant does (b)		12 (67)	14 (78)	15 (83)	15 (80)
Number of implantation sites	Total number	99	120	139	111
	Mean number (c)	8,3 ± 0,4	8,6 ± 0,5	9,3 ± 0,3	7,4 ± 0,4
Foetal losses (resorptions + dead foetuses)	Total number	7 (7 + 0)	13 (12 + 1)	19 (18 + 1)	8 (8 + 0)
	Mean number	0,6	0,9	1,3	0,5
Number of does which carried to term		12	14	15	15
Live foetuses	Total number	92	107	120	103
	Mean number (a)	7,7 ± 0,4	7,6 ± 0,6	8,0 ± 0,5	6,9 ± 0,5
	Male (b)	42 (46)	61 (57)	61 (51)	42 (41)
	Female (b)	50 (54)	46 (43)	59 (49)	61 (59)
	Mean weight (g) (c)	33,8 ± 0,9	34,1 ± 2,5	33,2 ± 1,2	36,6 ± 1,3
Foetuses showing no malformation	Hypotrophic (b)	0	0	2 (1,7)	0
	with one minor abnormality (b)	0	1 (0,9)	0	0
	ossification stage	Normal	Normal	Normal	Normal
Malformed foetuses (b)		3 (3,3)	0	2 (1,7)	0

(a) Not including rabbits excluded from the study
(b) Number (and %).
(c) ± standard error.

MALFORMED FETUSES

Daily dose level (mg/kg p.o.)	Litter No.	Number per litter	Findings
Controls	162	2	Foetuses with cardiovascular malformation (dilatation of the aortic arch and pulmonary artery).
	168	1	Talipes, front left paw
500	No malformed foetuses.		
250	364	1	Cardiovascular malformation (dilatation of the aortic arch) and vertebral malformation (dorsal spondylolisthesis + 2 fused ribs).
	372	1.	1 foetus with malformed face (cyclocephalus).
125	No malformed foetuses.		

Note: The frequencies of spontaneous cardiovascular and vertebral malformations and cyclocephalus in the New Zealand rabbit are 0.29%, 0.16%, and 0%, respectively for our strain, and 0.06%, 0.10%, and 0.02% according to A.K. Palmer [Lab. Animal, 2, 195-206, 1968: "Spontaneous malformations of the New Zealand white rabbit: the background to safety evaluation tests."]