(5-30-86)

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

MEMORANDUM

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

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.

(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

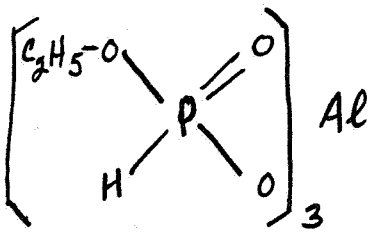
B. Material Reviewed:

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.

#10 3/31/86 sb
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