6/5/92

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

MEMORANDUM

SUBJECT: Second Carcinogenicity Peer Review of Aliette

FROM: Gary J. Burin, Ph.D., D.A.B.T.
Science Analysis and Coordination Branch
Health Effects Division (H7509C)

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Health Effects Division (H7509C)

TO: Susan Lewis
Registration Division (H7505C)

The Health Effects Division Peer Review Committee (PRC) for Carcinogenicity met on November 6, 1991 to discuss and evaluate the weight-of-the-evidence on aliette with particular reference to its classification for carcinogenicity. This was the second evaluation of aliette by the PRC. Although the Committee decided that aliette best fits the description of a Group C carcinogen according to the Agency Carcinogen Risk Assessment Guidelines, it also concluded that results observed in male rats at high levels of compound intake are expected to have little relevance to human exposure. The use of an RfD approach to risk assessment was recommended.

A. Individuals in Attendance:

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

Penelope Fenner-Crisp

William Burnam

Reto Engler

Karl Baetcke

Hugh Pettigrew

Gary J. Burin[1]

Julie Du

Jean Parker

Kerry Dearfield

George Ghali

Marion Copley

Yin-Tak Woo

Lucas Brennecke

Esther Rinde

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

Whang Phang[1] 6/24/92

Jim Rowe 6/24/92

3. Peer Review Members in Absentia: (Committee members who were unable to attend the discussion; signatures indicate concurrence with the overall conclusions of the Committee.)

Marcia Van Gemert

William Sette

Richard Hill

Jack Quest

Robert Beliles

4. Other Attendees: Jim Stone, Registration Division.

B. Material Reviewed:

The material available for review consisted of DER's,

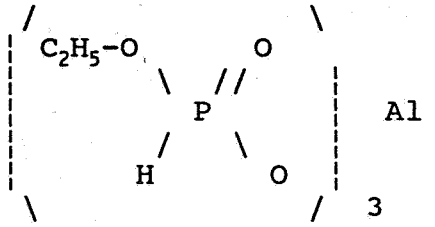
[1] Member of the Peer Review Committee for this meeting.

one-liners, and other data summaries prepared by Dr. Whang Phang and others, and the previous peer report. The material reviewed is attached to the file copy of this report.

C. Background Information:

Fosetyl-Al (aliette) is a fungicide used on vines, vegetable crops, ornamentals, hops, pineapple, avocado, rubber, cacao, and citrus. It has not been reviewed by the Joint FAO/WHO Meeting on Pesticide Residues or the International Agency for Research on Cancer.

The structure of aliette is:



Aluminum tris (O-ethyl phosphonate (aliette)

The Peer Review Committee first met on March 6, 1986 to evaluate the data base on aliette. At that time, the Committee concluded that aliette was associated with an increased incidence of urinary bladder tumors. The registrant had provided published articles to support the contention that urinary bladder tumors were caused by irritation and subsequent proliferation on the bladder epithelium due to the formation of urinary stones. However, the Committee noted that "neither mineralization nor urolithiasis were observed in the bladder of high dose male rats upon histopathological examination". The chemical was classified as a Group C carcinogen. The Committee 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."

The registrant has followed the Committee's recommendation and has specifically designed and conducted a 90-day feeding study in rats to investigate the hypothesis that bladder tumors seen in the chronic study were the result of a urinary tract irritant effect. The results of this 90-day study showed that large doses of aliette (30,000 and 50,000 ppm) produced urinary calcium imbalance, diuresis, a sharp drop in urine pH, formation of urinary calculi, and transitional cell hyperplasia in the renal pelvis, ureter, and urinary bladder. These changes occurred within two weeks of treatment; persisted to the end of treatment and were more predominant in males. In a letter to the Agency, the registrant

argued that, based upon all the available toxicology data on this chemical, aliette should not be classified as a group C carcinogen. The Committee evaluated the recently submitted study in conjunction with other relevant information to determine whether these data provide sufficient evidence for re-classifying the carcinogenic potential of aliette.

D. Evaluation of Carcinogenicity Evidence:

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

1. Two-Year Feeding Carcinogenicity Study in Rats (MRID No. 00098339)

a. Experimental Design

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[2]. Because red urine and staining of the abdominal fur were observed in animals dosed at 40,000 ppm, this level was reduced to 30,000 ppm after 2 weeks. Subsequent to reducing the dosage, the clinical signs were not observed.

b. Discussion of Nonneoplastic toxicity

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.

c. Discussion of Tumor Data

The original histopathologic examination showed an increase in the incidence of urinary bladder tumors and pheochromocytomas. The slides of this study were read by several pathologists. For 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 primarily due to an increase in the adenomas. However, this

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[2]Additional information concerning dietary composition has been requested. This information may assist in the interpretation of the increased urinary excretion of calcium in treated animals.

original diagnosis was not confirmed by two other consulting pathologists which had evaluated the same slides. The Peer Review Committee had evaluated this study in 1986 and considered the differences in pathological diagnosis of pheochromocytomas among the three pathologists to be 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 concluded that the available data did not provide sufficient evidence to indicate that Aliette produced pheochromocytomas in male rats.

The incidence of urinary bladder tumors was evaluated by two pathologists as indicated in Table 1. The evaluation of the original pathologist indicated a statistically significant increase in the incidence of transitional cell tumors in high dose male rats which were sacrificed at the termination of the study. A second reading of the same slides by another pathologist confirmed the findings of the diagnosis of the original pathologist. Transitional cell bladder tumors are usually very rare in untreated animals, and additional information concerning the historical control incidence of this tumor type has been requested] The second reading also showed a high ratio of carcinomas to papillomas and the presence of urinary bladder hyperplasia in high dose male rats (Table 1). No bladder stones were reported among animals in the study. This is consistent with the observation that bladder stones associated with this chemical decrease in size and number with time. The Peer Review Committee concluded that the increase in the urinary bladder tumor incidence in male rats was produced by aliette.

Table 1. Urinary Bladder Histology in Chronic Feeding Study of Aliette in Male Charles River CD Rats

Classification	Reviewing Pathologist	Dose (ppm) 0	2000	8000	30000
transitional	1	0/80	1/78	1/79	8/80*
cell papilloma	2	1/80	1/78	1/79	5/80
Carcinoma	1	2/80	0/78	0/79	7/80*
	2	2/80	2/78	1/79	16/80*
Papilloma+	1	2/80	1/78	1/79	15/80*
Carcinoma	2	3/80	3/78	2/79	21/80*
Hyperplasia	1	NA	NA	NA	NA
	2	5/78	7/78	5/80	29/79*

*$p<0.05$

d. Adequacy of Dosing for Assessment of Carcinogenic Potential

The Committee had previously concluded that the dose levels utilized in this study were high enough to assess carcinogenicity. The present Committee noted that the high dose was, in fact, above the limit dose used as guidance by HED (20,000 ppm for rats).

2. Rat Chronic Feeding/Carcinogenicity Study on Mono-Sodium Phosphite (a metabolite of aliette) (MRID 00098352)

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. Urinary volume and pH were not altered by treatment. No compound-related increase in the incidence of either neoplastic or non-neoplastic changes was found in the urinary bladder, the adrenal medulla, or at any other site at a dose in excess of the HED limit dose.

3. 90-Day Feeding Study in Rats (Special Study)(MRID 413152-01)

Groups of rats (70/sex/dose) received aliette 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 (ranging from 2 weeks to 13 weeks). Other subgroups were placed on a normal (compound-free) diet for different recovery periods (ranging from 8 weeks to 21 weeks). Dose levels of 30,000 and 50,000 ppm produced increased mortality. 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 body weight and food consumption were comparable to those of the controls. An increase in water consumption and diuresis also was seen in males at 30,000 and 50,000 ppm and in females at 50,000 ppm during dosing. Hematological changes related to diuresis were found in 50,000 ppm males and females.

Clinical chemistry data indicated increases in BUN, 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 persisted. A significant decrease in the urinary pH values in all treated animals after 2 weeks of treatment and an increase in urinary Ca^{++} at 30,000 and 50,000 ppm was noted. The source of the excess urinary calcium is unknown. The urinary phosphorous levels were decreased in 30,000 and 50,000 ppm males and females; this decrease was attributed to diuresis. Although the urinary aluminum level was found to be increased, this may have been due to contamination of the urine samples with feces. 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 suggested that aluminum was not absorbed via the intestinal tract.

Both gross and histopathology examinations found calculi in kidneys, ureters, and urinary bladder of 30,000 and 50,000 ppm males and in a few 50,000 ppm females. Calculi were found more commonly in the kidney and ureter than in bladder. In the kidneys, an increased incidence of 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 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 submucosal 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. Calculi found at the 2 week sacrifice were in greater number and size than those found at later times. More numerous and larger calculi were found in the urinary bladder than in the kidneys or ureters and males were affected to a much greater extent than females. The chemical composition of the urinary bladder calculi was approximately 23% phosphorous, 33% calcium, 0.2% magnesium, and less than 0.1% aluminum with the remainder not determined. This ratio of calcium and phosphorous is similar to that of monohydrogen phosphite ($Ca(HPO_3)$). However, the reported major urinary metabolite is monosodium (dihydrogen)phosphide. Additional information concerning the chemical composition of the stones has been requested.

An evaluation of the individual animal histopathology and the urinalysis data revealed that the presence of calculi was almost always (>90%) associated with urinary hypercalciuria, acidic urine, transitional cell hyperplasia and/or papillary hyperplasia of the urinary bladder, ureter, and kidney tubules. The incidences of urinary uroliths and simple transitional cell hyperplasia and papillary hyperplasia of the urinary bladder are summarized in the in Table 2, and Table 3 shows the correlation of the findings of uroliths and hyperplasia found in the urinary bladder.

Over the 13 week treatment phase there was a significant correspondence between stones and bladder hyperplasia. In the control and 8000 ppm male groups, no animals had stones, and no animal had bladder hyperplasia. In contrast, males in the 30,000 and 50,000 ppm groups had a high incidence of stones and hyperplasia that extended over the dosing period. Of a total of 76 males in the two high dose groups over the 13 week period, 8 lacked both stones and hyperplasia, while 50 had both bladder stones and hyperplasia. Only 5 males had bladder hyperplasia without stones; 7 had bladder stones without hyperplasia; and another 6 had stones but not in the bladder and hyperplasia. The association between

stones and hyperplasia was significant (phi coefficient = 0.47, p <.001). All males treated for 2 weeks or longer with bladder lesions had advanced to papillary hyperplasia except two that had simple transitional cell hyperplasia.

There was also strong evidence of reversibility of stones and hyperplasia when treated animals (30,000 and 50,000 ppm) were returned to basal diet. Whereas bladder stones had occurred in a total of 66% (50/76) of males over the 13 week dosing period, only 19% (10/52) had bladder stones in one of the three recovery groups regardless of the length of treatment or time of recovery. Likewise, bladder hyperplasia showed comparable reductions: 72% (55/76) of treated males over the 13 week treatment period showed bladder hyperplasia whereas only 18% (19/104) males showed it in the recovery groups. In addition, the severity of hyperplasia diminished during recovery; 97% (58/60) of males showed papillary hyperplasia during treatment while only 37% (7/19) retained papillary hyperplasia during recovery; the remainder of animals with lesions had returned to simple transitional cell hyperplasia during recovery or the lesions had totally disappeared.

Table 2. Incidence of uroliths and hyperplasia in urinary bladder of aliette-treated male rats

Time	2 Weeks				4 Weeks				8 Weeks				13 Weeks			
Groups	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4
Uroli.	0/10	0/10	5/10	3/10	0/10	1/10	7/10	5/10	0/10	0/10	10/10	5/9	0/10	0/10	6/10	6/6
P. hy.	0/10	0/10	7/10	8/10	0/10	0/10	9/10	7/10	0/10	0/10	9/10	7/9	0/10	0/10	5/10	6/6
T. hy .													0/10	0/10	2/10	0/6

B. Recovery groups

Time	8 Weeks Treatment & 8 Weeks Recovery				8 Weeks Treatment & 16 Weeks Recovery				13 Weeks Treatment & 21 WeeKs Recovery			
Groups	1	2	3	4	1	2	3	4	1	2	3	4
Uroli.	0/10	0/10	1/9	1/8	0/10	0/10	2/10	1/8	0/10	0/10	2/8	3/9
P. hy.	0/10	0/10	1/9	2/8	0/10	0/10	2/10	1/8	0/10	0/10	0/8	1/9
T. hy.	0/10	0/10	4/9	1/8	0/10	0/10	1/10	1/8	0/10	0/10	3/8	2/9

Uroli.: Uroliths in the urinary bladder
P. hy.: urinary bladder papillary hyperplasia
T. hy.: urinary bladder simple transitional cell hyperplasia
Groups: 1-control, 2-8000 ppm, 3-30,000 ppm, 4-50,000 ppm

Table 3. The Correlation of the Findings of Urolith and Hyperplasia in the Urinary Bladder of Aliette-Treated Male Rats

			Urolith: Control			Urolith: 8000 ppm			Urolith: 30,000 ppm			Urolith: 50,000 ppm		
			+	-	N	+	-	N	+	-	N	+	-	N
Hyperplasia	2 Wks T	+	0	0	10	0	0	10	5	2	10	5	3	10
		-	0	10		0	10		1	2		0	2	
	4 Wks T	+	0	0	10	0	0	10	8	1	10	5	2	10
		-	0	10		1	9		0	1		1	2	
	8 Wks T	+	0	0	10	0	0	10	9	0	10	6	1	9
		-	0	10		0	10		1	0		2	0	
	13 Wks T	+	0	0	10	0	0	10	5	2	10	6	0	6
		-	0	10		0	10		2	1		0	0	
	8 Wks T & 8 Wks R	+	0	0	10	0	0	10	3	2	9	1	2	8
		-	0	10		0	10		1	3		0	5	
	8 Wks T & 16 Wks R	+	0	0	10	0	0	10	2	1	10	1	1	8
		-	0	10		0	10		1	6		1	5	
	13 Wks T 21 wks R	+	0	0	10	0	0	10	3	0	8	2	1	9
		-	1	9		0	10		3	2		1	5	

Hyperplasia: Urinary bladder papillary and transitional cell hyperplasia
+: present T: treatment Wks: weeks
-: absent R: recover

4. Mouse Carcinogenicity Study (Accession No. 247168)

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, animals in the 20,000 ppm group were increased to 30,000 ppm due to the absence of any effect in the early part of the study. No evidence of a carcinogenic response or other forms of toxicity was found including any lesions of the urinary system. This study had been reviewed by the previous PRC meeting.

The Peer Review Committee concluded that this study could have been conducted at a higher dose level. Although the highest dose tested in this study was increased from 20,000 ppm to 30,000 ppm at week 19 of the treatment period, the higher level was not administered during the early critical periods of the growth curve of the test animals. However, the Committee was of the opinion that additional carcinogenicity testing in mice would not yield an increased understanding of the toxicity of this chemical. The limit dose for testing in mice is 12,000 ppm.

E. Additional Toxicity Data on Aliette

1. Two-Year Dog Study (MRID No. 00098340)

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 seminiferous tubules. 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. No effects were observed in the urinary tract.

2. Rat Reproduction Study (Accession No. 247174)

In a three-generation reproduction study, 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 postpartum and reared on the test diet to an age of at least 90 days. The animals were then mated. 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 (renal) papillary projections and or desquamation cells in the lumen of the urinary tract." These changes were "associated with the presence of (urinary) crystalline or calcareous deposits." No urinary bladder changes were found in F0 rats.

2. Developmental toxicity

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 the 4,000 mg/kg group. No alterations were seen in the urinary tracts of fetuses. The NOELs for maternal and developmental toxicity were 1,000 mg/kg.

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. 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 gain was seen at the 250 mg/kg dose.

3. Metabolism (Accession No. 247183)

Four metabolism studies in rats were available; two studies on ^{14}C-Aliette and two studies on ^{32}P-Aliette. 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 excreted via the urine without further oxidation to phosphate accounted for approximately 26% of the administered dose. Only a minor amount (2-3%) of the administered radioactivity was found in the feces.

4. Mutagenicity (Accession Nos. 247173 and 247186)

Two forms of aliette were tested for mutagenicity, the technical grade and a wettable powder containing 80% a.i.. Two acceptable tests for each form were submitted and found to be negative, a Salmonella assay and a mouse micronucleus test. Other tests for each form were submitted, but were found unacceptable. These tests, which were negative, included repair induction assays with E. coli and yeast assays with S. cerevisiae. Therefore, while the data do not suggest a mutagenicity concern, a data gap exists for the other genotoxic effects category.

5. Structure-Activity Correlations

Except for the sodium phosphite metabolite (see D.2., above) no information was located concerning structurally related chemicals.

F. Weight of Evidence Considerations

The Committee considered the following facts regarding the toxicology data on aliette to be of importance in a weight-of-the-evidence determination of carcinogenic potential:

1. Aliette was found to induce urinary bladder tumors (transitional cell papillomas and carcinomas) in male but not female Charles River CD rats after dietary exposure at a level of 30,000/ 40,000 ppm.

2. Aliette did not produce tumors at any site in the mouse or at any site other than the urinary bladder in the rat. Although a sufficiently high dose level may not have been utilized in the mouse carcinogenicity study, further testing in this species was considered to be unlikely to better elucidate the carcinogenic potential of this chemical. It is also noted that the doses utilized in both the rat and mouse carcinogenicity studies were above the limit doses for these species.

3. The available evidence does not indicate a genotoxic potential for aliette. Studies considered acceptable include micronucleus tests in the mouse and a reverse mutation (Ames) assay in S. typhimurium. Phage induction assays using E. coli, DNA repair tests in E. coli and an assay in S. cerevisae, although negative, could have been conducted at higher concentrations.

4. A short-term (90 day) study in the rat found that a papillary transitional cell hyperplasia of the bladder was induced at dose levels of 30,000 and 50,000 ppm but not at 8000 ppm. This hyperplasia was considered to be secondary to mechanical irritation produced by calculi in the bladder based upon an animal-by animal correlation of the two findings. The calculi were decreased in incidence and size with continued exposure and were found predominantly in male animals. Upon cessation of high-dose exposure and return to basal diet there was a profound decrease in the incidence of bladder stones and hyperplasia. In addition, in animals where hyperplasia persisted it had largely transformed from papillary to simple hyperplasia. Control and 8,000 ppm males lacked both bladder stones and bladder hyperplasia.

5. Aliette is well absorbed after oral administration and is extensively metabolized to phosphite, ethanol and CO_2. Monosodium phosphite has been assayed for carcinogenic activity and has not been shown to induce neoplasia or

hyperplasia of the urinary bladder in the rat. It also does not result in diuresis or changes in urinary pH in treated animals. Aliette, on the other hand, produces a profound lowering of the urinary pH in the high dose in the 90 day study accompanied by diuresis, elevated urinary calcium levels and reduced urinary phosphorus concentrations. The stones are composed of 33% calcium and 23% phosphorus. The source of the increased urinary calcium and composition of the remainder of the stone is unknown.

6. The chronic study in the rat found that an increase in urinary bladder tumors occurred only at a dose level which was associated with an increased incidence of hyperplasia. A prolonged increase in the rate of proliferation of cells of urothelium has been proposed to be an important step in the induction of urinary bladder tumors (Cohen and Ellwein, 1989; 1990a).

7. The tumors associated with administration of aliette at dose levels of 30000/40000 ppm in the male rat were considered by the Committee to be likely be due to prolonged mechanical irritation by bladder calculi. These calculi appear to result from alterations in the homeostasis of calcium and phosphate. The more common observation of the calculi in male rats than in female rats is probably due to either the narrower urethral opening in male rats which limits the passage of calculi or the presence of certain proteins or other factors in the male urine which enhance or precipitate calculus formation.

8. Aliette is similar to other chemicals which induce carcinogenesis through irritation of the urothelium at high levels of exposure. Cyromazine and its melamine metabolite were reviewed by the PRC on November 27, 1991. The PRC concluded that "It is likely that the melamine-induced bladder tumors in rats result from mechanical irritation caused by stones via stimulation of cell division." Saccharin induces urinary bladder tumors via the production of an abrasive silicate crystal in male rats (Cohen and Ellwein, 1990b) and sodium ortho-phenyl phenol induces tumors through the production of calculi in the bladder and certain reactive metabolites at high doses (Fujii et al, 1987).

9. The disruption of calcium and phosphorus homeostasis in male rats at high levels of aliette exposure results in the formation of calculi, which induces hyperplasia of the urothelium and neoplasia after prolonged exposure. Levels which do not induce calculi are not expected to result in either hyperplasia or neoplasia. In the 90 day rat study stone and hyperplasia were noted at 30,000 ppm in the diet but not at 8,000 ppm. In the rat 2-generation reproduction study bladder lesions were found at 24,000 ppm but not at 12,000

ppm. Finally, in the 2 year rat study bladder hyperplasia and neoplasia occurred at 30,000 ppm but not at 8,000 ppm. It then seems that at doses of aliette of 8,000 to 12,000 ppm there are no stones, hyperplasia or neoplasia, but at doses of 24,000 to 30,000 ppm, treated males develop stones, bladder irritation, hyperplasia and neoplasia. The maximum dietary exposure to humans is estimated to be 1.2 ug/kg bw/day and this is approximately one million-fold less than the NOEL for stone formation in the rat.

G. Classification for Carcinogenic Potential

Criteria contained in the EPA Guidelines [51FR: 33992-34003, 1986] were considered. The majority of the PRC concluded that the available data for this compound best fit the 1986 Guidelines Classification of Group C (possible human carcinogen) based upon the finding of carcinogenicity only in male rats in a well conducted assay. Quantification by the RfD approach was recommended based upon the absence of genotoxicity potential and the limited weight assigned to the evidence of carcinogenicity. PRC members supporting the classification as Group C agreed that the response observed in the male rat urinary bladder at very high levels of exposure i.e 30000 ppm (equivalent to 1.5 g/kg) was expected to have little relevance for humans. For example, the estimated maximum exposure resulting from current tolerances of aliette is about one million fold less than lowest the dose levels which produced stone formation in male rats. The Committee members supporting a C designation felt somewhat constrained by the wording in the EPA guidelines and noted that the available data for this chemical would be classified as "Category 6: Carcinogenic Activity in Animals; Probably not a Human Cancer Hazard" using the system proposed by Ashby et al., 1990. This category was intended for chemicals with either sufficient or limited evidence for carcinogenicity in animal studies which are considered unlikely to be predictive of a human response due to quantitative or qualitative factors.

Several Committee members concluded that classifications as Group D (inadequate evidence of carcinogenicity) or Group E (no evidence of carcinogenicity) would have been appropriate. The classification of aliette as Group D is supported by a number of uncertainties in the human and animal evidence, including the following: rats and mice responded differently to the stone-induced irritation and the response of the human urothelium is not known with certainty; the level of intake which would be associated with stone formation in humans is not known; and it is possible that the presence of stones in animals with tumors is coincidental rather than causative. In addition, tumors were only observed at a dose level which exceeded the OPP guidance limit dose, which calls into question the study findings at that dose level. The

next lower dose level in the chronic rat bioassay, 8000 ppm, was clearly less than the MTD and limit dose. Because of the above uncertainties and problems in study design, a decision about potential human carcinogenicity can not be made.

A Group E classification would be supported by the conclusion that this chemical has been adequately tested and that the only tumor type which is associated with compound administration under the conditions of the rat bioassay, that of the urinary bladder, is not predictive of a carcinogenic potential for man. The tumors appear to be due to events stimulated by the presence of urinary stones (Appendix 1), and these stones are unlikely to be formed in humans from the predicted levels of exposure to aliette. Furthermore, bladder stones do not appear to be a significant risk factor in the induction of human bladder cancer (Appendix 2). A Group E classification would be derived from the conclusion that the extreme laboratory conditions needed to produce cancer in the male rat by aliette are not germane to human hazard and risk. This position is in keeping with the determination made by the Committee on cyromazine/melamine, another case where stones were associated with bladder tumors. The logic for a Group E classification differs from that of a Group C or Group D. Using the 3-step process in the EPA cancer guidelines the following determinations would be made in arriving at a Group E classification: 1) There is no evidence of carcinogenicity in human studies and limited evidence in animals (one sex of one species). 2) Considering that information, a tentative designation of Group C is made. 3) All the relevant supporting information on aliette is reviewed to arrive at a conclusion regarding the relevance of the carcinogenic response under the conditions of the study to human carcinogenic hazard. In the final step, the evaluation of the supporting evidence would lead to a change from the tentative designation to Group E.

The Committee members recommending a Group C classification noted that Group D (inadequate evidence) would not be appropriate in this case because further testing is unlikely to better elucidate the carcinogenic potential of this chemical and the classification as Group E (evidence of noncarcinogenicity for humans) is used when there are negative studies in two animals species. This is not the case for aliette where tumors were found in treated male rats. After extensive discussion the consensus of the PRC was to classify aliette as Group C (a possible human carcinogen) and to use the RfD approach for risk asssessment.

H. Recommendations:

An in vivo/in vitro unscheduled DNA synthesis assay in rats is recommended to fulfill the data gap for genotoxicity testing.

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Appendix 1- Bladder stones in rodents

The etiology of transitional cell tumors of the urinary bladder has been subject to extensive investigation in laboratory animals. Studies over the last quarter of a century have led to the knowledge that urinary stones commence steps which can include increases in urinary epithelial cellular proliferation, cellular hyperplasia, benign and malignant neoplasms. It appears that it is the presence of a physical body (and urine) in the bladder, rather than the chemical composition of the physical body which is essential for tumor induction in these cases i.e. the tumors are secondary to the presence of stones. A series of examples are available which typify this type of carcinogenic process in the bladder and which serve to elucidate the various mechanisms by which tumors are formed in treated animals.

It has long been known that the implantation of inert material such as glass beads or wax pellets into the urinary bladder of rodents lead to events terminating in bladder cancer development (Chapman et al., 1973). The presence of mechanical irritation for at least 6 months appears to be necessary for the induction of neoplasia (Roe, 1964). The surface characteristics of the foreign body also influence the likelihood of tumor development; rough glass beads resulted in a higher tumor incidence than did smooth glass beads (DeSesso, 1989).

A number of other factors have been identified as influencing the induction of bladder tumors. Increased incidences of urinary bladder tumors are observed with some chemicals at a high pH and lowering of urinary pH decreases or eliminates carcinogenic activity. Saccharin appears to induce urinary bladder tumors via the production of an abrasive silicate crystal in male rats (Cohen and Ellwein, 1990) and sodium ortho-phenyl phenol induces tumors through the production of calculi in the bladder (Fujii et al ., 1987). The induction of tumors by each of these chemicals is pH-dependent. However, the induction of bladder tumors by other chemicals is not enhanced by decreased pH and this is most likely due to the nature of the urinary precipitate e.g. precipitation of phosphate salts or silicate salts may occur at a low pH whereas other chemicals such as uric acid or cysteine may precipitate at a higher pH. Elevated sodium ion concentration has also been shown to increase the rate of cellular proliferation and resultant tumor formation following the administration of ortho-phenyl phenol to rats (Shibata et al., 1989). Decreased osmolality and increased urine volume have also been associated with increased bladder tumor formation (Munro et al., 1975).

Male rodents appear to be more susceptible to stone formation than do female rodents (Teelman and Nieman, 1979). This may be due to the longer and narrower urethra of the male rodent which results in retention of calculi and stasis of the urine. The chemical constitution of urine also differs between the sexes and the presence of higher protein levels in male urine may also serve to catalyze the formation of calculi. The rat

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appears to be more sensitive to the induction of bladder tumors by mechanical irritation than do other species such as the mouse and guinea pig. The basis for this difference in species sensitivity is as yet unexplained. Humans appear to be relatively insensitive to tumor induction by urinary stones (see Appendix 2).

Studies of experimental bladder carcinogenesis have suggested an interaction of genotoxic and nongenotoxic events as critical components (Cohen & Ellwein 1989, 1990). Stimulation of cell division is one of the elements. While genotoxic events represent the second. For a review of some of the genetic events associated with human bladder cancer, see Raghavan et al. (1990). Some chemical substances that have produced bladder tumors in rodents appear both to produce mutations and stimulate cell division (e.g., N-[4-(5-nitro-2-furyl)2-thiazolyl]-formamide), whereas others, like saccharin, seem to influence only cell division. In the latter case, genetic events might be considered to be "spontaneous" and not associated with saccharin per se, but possibly to other factors or exposures to the chemical which has induced cell division, a metabolite of that chemical or a natural constituent of the diet or its metabolite. Certainly the finding of bladder tumors following the implantation of glass or wooden beads or wax pellets would also fall into this category, since they seem to irritate the bladder wall and stimulate cell turnover without having genotoxic activity.

The chemicals which induce carcinogenesis through irritation of the urothelium at high levels of exposure present little or no increase in tumor incidence until a fixed level of exposure is exceeded (Cohen and Ellwein, 1989). The disruption of homeostasis in the male rat at high levels of exposure to such compounds results in the presence of calculi, which induce hyperplasia of the urothelium and neoplasia after prolonged exposure. Exposure to levels of these chemicals which do not induce calculi are not expected to result in either hyperplasia or neoplasia.

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Appendix 2- Human bladder cancer

The urinary bladder is lined with transitional epithelium as are the renal calyces and pelvis and the ureters. Under certain stresses the epithelium undergoes metaplasia to stratified squamous epithelium. Cancer statistics are not collected in a manner that would allow for an inclusive evaluation of all these structures; therefore, urinary bladder is usually investigated separately from the upper part of the urinary system.
Urinary stones (calculi) can form anywhere along the urinary collection system from the calyces and pelvis to the ureters and bladder. Stones are quite common and are seen in about 1% of autopsies (Smith, 1982). Unless they produce stasis by occluding urinary flow or cause infection, they are usually clinically silent. About 1 in 1000 adults is hospitalized annually with stones. The incidence of stones varies significantly in different geographic regions of the world, probably due to dietary and other factors (Smith, 1982). Males are much more frequently affected than females. Over 90% of stones are composed of calcium or in some cases magnesium along with oxalate and phosphate. Most of the remainder are organic in composition and contain uric acid or cystine (Smith, 1963; Cheng, 1980).

Most bladder cancers in humans are transitional cell carcinomas (over 90%) while the remainder are squamous cell carcinomas and other types (Silverman et al., 1992). Bladder cancer constitutes 7% of all cancer cases in men and 4% in women, while bladder cancer deaths as a part of the total are 2% and 1%, respectively (Boring et al., 1991). It is largely a disease of the elderly, with about 2/3 of the cases occurring at 65 years or older. Among race-sex groups in the U.S., the lifetime risk of bladder cancer is highest among white males (nearly 3%), and the male to female ratio is about 3 to 4 (Silverman et al., 1992).

Many epidemiologic studies have identified risk factors in the development of bladder cancer. Many of the associations involve exposure to genotoxic chemicals, notably the aromatic amines. These associations include such things as smoking and certain occupations like dye, leather, and rubber workers, painters and truck drivers. Certain other associations have not been established, like those for coffee drinking and artificial sweeteners (Silverman et al., 1992).

Intercurrent urologic conditions also have been investigated as potential risk factors for bladder cancer without developing a clear cut position (Matanoski and Elliott, 1981; Silverman et al., 1992). There is some evidence suggesting that urinary stasis from various causes, stones and infection (bacteria and Schistosoma haematobium) may be related to cancer, but more work is needed in these areas. For instance, as to the role of stones per se in cancer, several studies fail to show any link (Waller & Hamer, 1950; Thompson, 1959; Morris & Hemminger, 1962). In a hospital bladder cancer case control study with 350 cases and an equal number of controls, bladder stones were noted in 4.6% of

the cancer patients and 2.3% of controls, a suggestive but non-significant difference (Wynder et al., 1963). The average time between observation of the stones and the cancer was 15 years. Supposedly these patients had not had bladder infections. In a follow-up study, these authors questioned the influence of stones on cancer development (Wynder and Goldsmith, 1977).

The other indication of a potential role of stones as a risk factor for bladder cancer in humans comes from an analysis of data on nearly 3000 new bladder cancer cases and double that number of controls who were administered a questionnaire that requested information on urinary stones and infections that had occurred more than 1 year before the interview. Relative risks were significantly increased for bladder stones, with or without infection (RR=2.0 and RR= 1.8, respectively). The time between the finding of stones and cancer was not given. Kidney stones showed no increased risk for bladder cancer (Kantor et al., 1984).

The accumulated evidence for urinary stones being a significant factor in bladder cancer risk is marginal. Given the high frequency of stones in the population and the limited evidence of an association, it would seem that bladder stones play only a minor role, if any, in conditioning human bladder cancer. Certainly humans appear to be much less sensitive to the impact of stones on bladder carcinogenesis than are laboratory rodents.

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