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

JUN 29 1993

MEMORANDUM

OFFICE OF
PREVENTION, PESTICIDES AND
TOXIC SUBSTANCES

SUBJECT: Second and Third Carcinogenicity Peer Reviews of Fosetyl-Al (Aliette®)

FROM: Whang Phang, Ph.D. 5/26/93
Toxicology Branch II
Health Effects Division (H7509C)
and
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TO: Cynthia Parker
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Registration Division (H7505C)
and
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Special Review and Reregistration Division (H7508H)

The Health Effects Division Peer Review Committee (PRC) for Carcinogenicity met for the second and third times on November 6, 1991 and on July 29, 1992 to make a cancer weight-of-the-evidence determination on Fosetyl-Al (Aliette®). The PRC concluded that Fosetyl-Al was not amenable to classification using the current Agency cancer guidelines and chose to describe the weight-of-evidence using a narrative form. Based on a mechanistic evaluation of the only tumors seen, those that occurred at exceptionally high doses in the bladder of male rats (and possibly in the bladder and renal pelvis of female rats), it appears that humans are not likely to be exposed to doses of Fosetyl-Al that produce the urinary tract toxicity that precedes and seems to lead to the carcinogenic response in rats. In particular, anticipated human dietary and occupational exposures to Fosetyl-Al are far below the NOEL in rats for the apparent urinary tract tumor precursors (stone formation and attendant epithelial irritation). These effects are produced in rats at extremely high doses, under conditions not anticipated to occur outside of the experimental laboratory. The PRC concluded that pesticidal use of Fosetyl-Al is unlikely to pose a carcinogenic hazard to humans. Further information would be useful to strengthen this position.

A. Individuals in Attendance (at either meeting):

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

Penelope Fenner-Crisp	Penelope A. Fenner-Crisp
William Burnam	
Reto Engler	
Marcia Van Gemert	
Karl Baetcke	
Gary J. Burin[1]	—
Marion Copley	
Kerry Dearfield	
Julie Du	
George Ghali	
Richard Hill	
Jean Parker	
Hugh Pettigrew	
Esther Rinde	
Yin-Tak Woo	

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

Whang Phang[2]	5/26/93

[1]Gary Burin was a member of the Peer Review Committee in evaluating the carcinogenicity of this chemical. He wrote the original and a number of the subsequent drafts of this document. In February 1993, he left the Agency, and was unable to sign this document.

[2]Also a member of the PRC for this chemical; signature indicates concurrence with the peer review unless otherwise stated.

Jim Rowe

Lucas Brennecke[3]
(PAI/Clement)

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.)

William Sette

John Quest

4. Other Attendees:

Jim Stone, Registration Division.

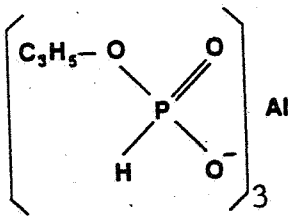
B. Material Reviewed:

The material available for review consisted of DERs, one-liners, additional data summaries prepared by Dr. Whang Phang and others, and the previous peer review document. 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 Fosetyl-Al [Aluminum tris (O-ethyl phosphonate)] is:



The PRC first met on March 6, 1986 to evaluate the data base on Fosetyl-Al. At that time, the PRC concluded that Fosetyl-Al

[3]Signature indicates concurrence with pathology report.

was associated with an increased incidence of urinary bladder tumors in male rats. The registrant provided published articles to support the contention that urinary bladder tumors were caused by irritation and subsequent proliferation of the bladder epithelium due to the formation of urinary stones. However, the PRC 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 PRC recommended that "the registrant pursue further studies to evaluate a possible urinary tract irritant effect of treatment resulting from either the urinary excretion of Fosetyl-Al per se, calcium, the aluminum portion of the Fosetyl-Al molecule, or the ethanol metabolite of Fosetyl-Al." Since that time, the Agency learned from further information submitted by the registrant that calculi had been found upon gross examination in the chronic rat study.

In addition, the registrant has followed the PRC'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 Fosetyl-Al in the diet 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; effects were reversible upon cessation of treatment. These changes occurred within two weeks of treatment, persisted to the end of treatment, and were much more predominant in males at levels of 30,000 and 50,000 ppm; effects in females were limited to the 50,000 ppm group. In a letter to the Agency, the registrant argued that, based upon all the available toxicology data on this chemical, Fosetyl-Al should not be classified as a Group C carcinogen. The PRC 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 Fosetyl-Al.

D. Evaluation of Carcinogenicity Evidence:

Some of the following summary is excerpted from the previous PRC report on Fosetyl-Al (Memorandum of J. Quest to H. Jacoby dated June 12, 1986).

1. Charles River CD Rat Feeding Carcinogenicity Study

Reference: Spicer, E.J.F. 1981. Fosetyl-Al: Chronic Toxicity (2-year) and Carcinogenicity Study in Rats. MRID No. 00098339. International Research and Development Corporation, Mattawan, MI. Study No. 147-016.

a. Experimental Design

Groups of Charles River CD rats (80/sex/dose) received Fosetyl-Al in Purina Lab Chow 5001 at dietary concentrations of 0, 2000, 8000, and 40,000/30,000 ppm (99.7% purity) for 2 years. Because red urine and staining of the abdominal fur were observed in males and females 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 Tumor Data

i. Adrenal pheochromocytomas

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 combined incidence of adenomas and carcinomas in mid (8000 ppm) and high-dose (40,000/30,000) male rats. The increased adrenal pheochromocytoma incidence was primarily due to an increase in adenomas. However, this original diagnosis was not confirmed by two other consulting pathologists who had evaluated the same slides. The PRC had evaluated this study in 1986 and considered the differences among the pathological diagnoses of pheochromocytomas to be due to the fact that there is a high degree of variability in the interpretation of adrenal medullary hyperplasia versus various adrenal medullary neoplasias. The PRC concluded that the available data did not provide sufficient evidence to indicate that Fosetyl-Al produced pheochromocytomas in male rats.

ii. Urinary bladder

The incidence of urinary bladder tumors (combined carcinomas and papillomas) 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 and hyperplasia in high-dose male rats. A second reading of the same slides by another pathologist found more tumors but confirmed the findings of the original pathologist. The tumor scoring system used in the initial reading of the slides is unknown; however, the second pathologist used the Hicks' criteria (S. Cohen, personal communication, 1993), which often result in more neoplastic diagnoses than do some of the other classification systems in current use. Specifically, this method results in increased diagnosis of neoplasia in preference to hyperplasia, and of carcinoma in preference to benign tumors. Transitional cell bladder tumors are usually rare in untreated animals. Some control and low-dose

animals showed urinary bladder tumors. Historical control information has not been received by the Agency.

Table 1. Urinary Bladder Histology in the Chronic Feeding Study of Fosetyl-Al in Male Rats

Classification	Reviewing Pathologist	Dose (ppm) 0	2000	8000	40,000/30,000
Transitional Cell Papilloma	1	0/78*	1/80	1/81	9/79*
	2	1/73*	1/75	1/78	5/78
Carcinoma	1	2/78*	0/80	0/81	7/79*
	2	2/73*	2/75	1/78	16/78*
Combined Papilloma/ Carcinoma	1	2/78*	1/80	1/81	16/79*
	2	3/73*	3/75	2/78	21/78*
Hyperplasia	1	1/78*	2/75	4/81	13/79*
	2	5/73*	7/75	5/78	29/78*

* $p < 0.05$ (Fisher's Exact Test or Cochran-Armitage trend test)

Significance of trend denoted at control.
Significance of pair-wise comparison denoted at dose level.

Pathologist 1: Original pathologist from IRDC
2: Second pathologist, who applied Hicks' criteria in classifying tumors.

Using the histological diagnoses of the second but not the first pathologist, female rats in the 2-year study showed a possible increase in the frequency of epithelial tumors of the bladder as a function of dose (Table 2). Tumor responses are much less significant than in dosed males. Hyperplasia showed dose-related increases in females for both pathologists.

Table 2. Urinary Bladder Histology in the Chronic Feeding Study of Fosetyl-Al in Female Rats.

Classification	Reviewing Pathologist	Dose (ppm) 0	2000	8000	40,000/30,000
Transitional cell Papilloma	1	0/80	1/78	1/76	1/81
	2	0/78	2/79	0/75	1/78
Carcinoma	1	0/80	0/78	0/76	1/81
	2	0/78*	1/79	0/75	5/78+*
Combined Papilloma/ Carcinoma	1	0/80	1/78	1/76	2/81
	2	0/78*	3/79	0/75	6/78*
Hyperplasia	1	0/80*	4/78	5/76*	6/81*
	2	0/78*	5/79*	3/75	9/78*

+ 1 female rat with adenosquamous carcinoma.
* $p < 0.05$ (Fisher's Exact Test or Cochran-Armitage trend test)

Significance of trend denoted at control.
Significance of pair-wise comparison denoted at dose level.

Pathologist 1: Original pathologist from IRDC
2: Second pathologist, who applied Hicks' criteria in classifying tumors.

iii. Renal pelvis

Given that the whole of the urinary tract is lined with transitional cell epithelium, animals in the chronic study were investigated for the presence of stones in the renal pelvis on gross examination and histological evidence of neoplasms at that site. Using the diagnoses of the initial pathologist, no increase in neoplasms was noted among male and female dose groups. The diagnoses of the second pathologist did not indicate a compound-related increase in the incidence of epithelial neoplasms of the renal pelvis in males (Table 3); however, they did provide some indication of an increase in epithelial neoplasms of the renal pelvis among female rats in the 40,000/30,000 ppm group (Table 3). Of 81 females, 7 animals had neoplasms in 9 renal pelves (1 had bilateral malignancies; another had a benign and a malignant neoplasm on opposite sides). At least 5/9 pelves with a tumor had stones in the same pelvis. One female had stones and tumors in both pelves and the bladder.

Table 3. Renal Pelvis Pathology in Male and Female Rats in the Fosetyl-Al Chronic Feeding Study

Classification	Pathologist	Dose (ppm) 0	2000	8000	40,000/30,000
		Males			
Uroliths[a]		1/80	0/80	1/81	0/81
Hyperplasia	1	1/80*	9/77*	9/80*	7/81*
	2	11/80	7/80	12/81	13/81
Neoplasm					
Benign (B)	1	0/80	0/80	0/81	1/81
	2	1/78	3/79	0/79	4/79
Malignant (M)	1	1/80	0/80	0/81	0/81
	2	1/78	1/79	1/79	0/79
Combined (B/M)	1	1/80	0/80	0/81	1/81
	2	1/78	4/79	1/79	4/79
		Females			
Uroliths[a]		0/80*	3/80	0/82	11/81[b]*
Hyperplasia	1	17/80	26/80	9/81	8/79
	2	6/79*	10/79	1/80	3/81
Neoplasm					
Benign (B)	1	0/80	0/80	0/81	0/79
	2	1/79	1/79	1/80	2/79
Malignant (M)	1	0/80	0/80	0/81	0/79
	2	0/79*	0/79	0/80	6/79*
Combined (B/M)	1	0/80	0/80	0/81	0/79
	2	1/79*	1/79	1/80	7/79[c]*

[a] Kidney pelvis with stone or gritty material on gross examination.

[b] Includes 1 animal with a cystic medulla containing gritty material and another animal with hydroureters and bilateral calculi.

[c] 1 animal had a benign and malignant neoplasm in opposite pelves; another animal had bilateral malignancies.

*$p < 0.05$ (Fisher's Exact Test or Cochran-Armitage trend test)

Significance of trend denoted at control.
Significance of pair-wise comparison denoted at dose level.

Pathologist 1: Original pathologist from IRDC
2: Second pathologist, who applied Hicks' criteria in classifying tumors.

iv. Association of neoplasms with bladder stones

Rats in the 2-year study were also investigated for the presence of bladder stones upon gross examination; few were noted. The formation of stones in the 2-year study correlated with the possible neoplasms for females; for males, although there were some stones associated with neoplasia, no real correlation was noted. Males receiving 40,000/30,000 ppm showed stones in 5 animals; only 2/21 animals with neoplasms had stones, while 19/21 animals had neoplasms without stones. Two stone-bearing animals had no histopathological lesion of the bladder, and one was autolyzed. Among females receiving 40,000/30,000 ppm of Fosetyl-Al, a nearly perfect association between stones and tumors was noted. All six of the high-dose females with epithelial neoplasms had stones; one other female had a bladder neck stone with no accompanying lesion of the bladder or bladder neck. None of the high-dose animals of either sex with bladder hyperplasia had bladder stones. No males or females had bladder stones in the control, 2000 or 8000 ppm groups.

The paucity of stones among the high-dose male animals with tumors in this 2-year study is noteworthy; however, a number of them showed hydronephrosis or dilatation of the ureter, presumptive indicators of past urinary tract obstruction. In addition, the special 90-day study in rats (section E.1 below) demonstrated that significant numbers of calcium and phosphorus-containing bladder calculi rapidly developed in males receiving ≥30,000 ppm Fosetyl-Al, then decreased in frequency and size; females developed some stones but only at the higher level, 50,000 ppm. The reason for the decrease in frequency of stones over the subchronic and chronic dosing periods is not known, but it is recognized that a constant ppm of an agent in the diet results in a reduction in dose on a mg/kg basis as the animal grows. In addition, the kidney may increase urine volume or decrease urinary pH over time as a means of dealing with the heavy load of Fosetyl-Al present in the diet, and calcium and phosphorus-containing stones are more soluble in a dilute urine and in an acid environment. The decrease in stones does not seem to be due to calcium being deposited in the urinary system, since the frequency of Alizarin red S staining of the kidneys and bladders of rats in the chronic study is not different across dose groups.

c. Non-neoplastic Lesions and Other Observations

Based upon the reported data, Fosetyl-Al 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 of study. The initial 1-2 weeks of administration of 40,000 ppm Fosetyl-Al

produced a decrease in body weights in males (-12%) and females (-9%) relative to the controls.

d. Adequacy of Dosing for Assessment of Carcinogenic Potential

The original PRC had previously concluded that the dose levels utilized in this study were high enough to assess carcinogenicity. The present PRC noted that the high dose was, in fact, above the limit dose used as guidance by HED (1 g/kg/d or 20,000 ppm for rats, EPA 1986).

2. Charles River CD Rat Chronic Feeding Carcinogenicity Study on Mono-Sodium Phosphite (a metabolite of Fosetyl-Al)

Reference: Spicer, E.J.F. 1981. Lifetime Chronic Toxicity and Carcinogenicity in Rats. MRID No. 00098352. International Research and Development Corporation, Mattawan, MI. Study No. 347-022.

Groups of Charles River CD rats (60/sex/dose) received mono-sodium phosphite (NaH_2PO_3) (98% purity), the major urinary metabolite of Fosetyl-Al in rats, at dietary concentrations of 0, 2000, 8000, or 32,000 ppm for 27 months (117 weeks). Results of this study 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. Charles River Mouse Feeding Carcinogenicity Study

Reference: Spicer, E.J.F. 1981. 24-Month Carcinogenicity Study in Mice. Accession No. 247168. International Research and Development Corporation, Mattawan, MI. Study No. 347-021.

a. Experimental Design

Charles River CD-1 mice (60/sex/dose) received Fosetyl-Al in Purina Lab Chow No. 5001 at concentrations of 0, 2500, 10,000, or 20,000/30,000 ppm (96.9% purity) 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.

b. Discussion of Tumor Data

No evidence of a carcinogenic response was found. This study was reviewed at the previous PRC meeting.

c. Non-neoplastic Lesions and Other Observations

No evidence of other forms of toxicity was found, including any lesions of the urinary system. This study was reviewed at the previous PRC meeting.

d. Adequacy of Dosing for Assessment of Carcinogenic Potential

The PRC 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 PRC was of the opinion that additional carcinogenicity testing in mice would not yield an increased understanding of the toxicity of this chemical. The HED limit dose for testing in mice is 1 g/kg/d or 7000 ppm (EPA 1986) and the doses administered to mice in this study were well in excess of this value.

E. Additional Toxicity Data on Fosetyl-Al

1. Sprague Dawley Rat Special Study

Reference: Osborne, B.E. 1989. A Maximum 13-Week Dietary Toxicity Study of Fosetyl-Al in the Albino Rat With a Maximum 21-Week Recovery Period. MRID No. 413152-01. Bio-Research, Ltd., Senneville, Quebec, Canada. Study No. 83040.

a. Experimental Design

Groups of Sprague Dawley (Crl:CD(SD)BR) rats (70/sex/dose) received Fosetyl-Al (97.3% purity) at dietary concentrations of 0, 8000, 30,000, or 50,000 ppm in Purina Chow. Each dose group was divided into 7 subgroups differing as to the length of chemical administration (2, 4, 8 or 13 weeks) and whether there was a recovery period when animals were placed on compound-free diet (0, 8, 16 or 21 weeks). Dose levels of 30,000 and 50,000 ppm produced increased mortality. A significant 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 was also seen in males at 30,000 and 50,000 ppm and in females at 50,000 ppm during dosing. Increases in RBC count, hemoglobin levels and platelet

counts, probably related to diuresis, were found in 50,000 ppm males and females.

b. Blood and Urinary Chemistry

Clinical chemistry data indicated increases in BUN, phosphorus, and CO_2 in 50,000 ppm males and to a lesser extent in females. During the recovery periods, CO_2 and phosphorus levels were comparable to those of the controls, but the increase in BUN persisted. A significant decrease in the urinary pH and phosphorus and an increase in urinary Ca^{++} at 30,000 and 50,000 ppm were noted during the dosing period. Urinary pH was also decreased at 8000-ppm. The methods used for the collection of urine and determination of urinary pH and other urinary parameters were not reported. The exact source of the excess urinary calcium (diet or bone) is unknown. 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. This suggests that more calcium is being absorbed from the gut in the higher dose groups; this source probably accounts for the elevated urinary calcium. More investigation could clarify this presumption. 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 essentially not absorbed via the intestinal tract. Although the urinary aluminum level was found to be increased, this may have been due to contamination of the urine samples with feces.

c. Pathological Effects

Both gross and histopathology examinations found calculi in kidneys, ureters, and urinary bladder of 30,000 and 50,000 ppm males and in a smaller number of 50,000 ppm females. Calculi were more commonly found in the kidney and ureter than in the bladder. In the kidneys, an increased incidence of hydronephrosis, pyelitis, pyelonephritis, papillary necrosis, dilatation of the collecting tubules, and simple transitional cell 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 all treatment durations, and some of these findings persisted into the recovery periods. Calculi found at the 2 week sacrifice were greater in 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. Females have a much shorter urethra than males, so that bladder stones are more easily passed in the urine.

The chemical composition of the urinary bladder calculi was approximately 23% phosphorus, 33% calcium, 0.2% magnesium, and less than 0.1% aluminum; additional information concerning the chemical composition of the stones is lacking. This ratio of calcium and phosphorus is similar to that of monohydrogen phosphite ($Ca(HPO_3)$). However, the reported major urinary metabolite is monosodium (dihydrogen) phosphite. The mono and dihydrogen phosphite forms are interconvertible, but in an acidic urine one would expect more of the dihydrogen form.

i. Bladder: Stones/hyperplasia

An evaluation of the individual animal histopathology and the urinalysis data revealed that the presence of bladder calculi was almost always (>90%) associated with urinary hypercalciuria, acidic urine, or simple transitional cell or papillary hyperplasia of the urinary bladder, ureter, and kidney pelvis. The physiological basis for the hypercalciuria and concomitant stone formation is unknown, i.e., increased calcium mobilization from bone or increased calcium absorption from the gastrointestinal tract. The incidences of uroliths and simple transitional cell hyperplasia and papillary hyperplasia of the urinary bladder are summarized in Table 4.

Over the 13 week treatment phase, there was a significant correspondence between stones and bladder hyperplasia. Table 5 shows the correlation between the findings of uroliths and hyperplasia in the urinary bladder. In the control and 8000 ppm male groups, no animals had stones, and no animals had bladder hyperplasia. In contrast, males in the 30,000 and 50,000 ppm groups had a high incidence of stones and of hyperplasia that extended over the dosing period. Of a total of 75 males in the two high dose groups treated for up to 13 weeks without a recovery period, only 8 did not have either stones upon gross examination or hyperplasia, while 44 had both bladder stones and hyperplasia. Bladder hyperplasia without stones was seen in 16 males; 7 had bladder stones without hyperplasia. The association between bladder stones and hyperplasia was significant (phi coefficient = 0.23, $p < .05$). All males treated for two weeks or longer with bladder lesions had advanced to papillary hyperplasia except two that had simple transitional cell hyperplasia.

Table 4. Incidence of Stones and Hyperplasia in Urinary Bladder of Fosetyl-Al Treated Male Rats

A. Dosed groups

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
N	10	10	10	10	10	10	10	10	10	10	10	9	10	10	10	6
Uroli.	0	0	3	4	0	1	8	6	0	0	9	8	0	0	7	6
P. hy.	0	0	7	8	0	0	9	7	0	0	9	7	0	0	5	6
T. hy .													0	0	2	0

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
N	10	10	9	8	10	10	10	8	10	10	8	9
Uroli.	0	0	7	1	0	0	3	2	0	0	6	2
P. hy.	0	0	1	2	0	0	2*	1	0	0	0	1
T. hy.	0	0	4	1	0	0	1	1	0	0	3	2

Groups: 1=control, 2=8000 ppm, 3=30,000 ppm, 4=50,000 ppm

Uroli.: uroliths in the urinary bladder upon gross examination
P. hy.: urinary bladder papillary transitional cell hyperplasia
T. hy.: urinary bladder simple transitional cell hyperplasia
*: one of these also had a bladder papilloma
+: 3 of 4 animals which died prior to the scheduled sacrifice had stones and/or papillary hyperplasia.

Table 5. Correlation Between the Findings of Uroliths on Gross Examination and Hyperplasia in the Urinary Bladder of Fosetyl-Al Treated Male Rats

		Uroliths											
		Control			8000 ppm			30,000 ppm			50,000 ppm		
		+	-	N	+	-	N	+	-	N	+	-	N
Hyperplasia													
2 Wk T	+	0	0	10	0	0	10	2	5	10	4	4	10
	-	0	10		0	10		1	2		0	2	
4 Wk T	+	0	0	10	0	0	10	8	1	10	5	2	10
	-	0	10		0	10		0	1		1	2	
8 Wk T	+	0	0	10	0	0	10	9	0	10	6	1	9
	-	0	10		0	10		0	1		2	0	
13 Wk T	+	0	0	10	0	0	10	4	3	10	6	0	6
	-	0	10		0	10		3	0		0	0	
8 Wk T	+	0	0	10	0	0	10	5	0	9	1	2	8
8 Wk R	-	0	10		0	10		2	2		0	5	
8 Wk T	+	0	0	10	0	0	10	1	2	10	0	2	8
16 Wk R	-	0	10		0	10		2	5		2	4	
13 Wk T	+	0	0	10	0	0	10	3	0	8	2	1	9
21 Wk R	-	0	10		0	10		3	2		0	6	

Hyperplasia: Bladder papillary or simple transitional cell hyperplasia

+: present
-: absent
T: treatment
R: recovery
N: number examined
Wk: weeks

Among females in the 90-day study, similar findings in the bladder were noted as in males, but to a lesser degree. Like males, no bladder stones and hyperplasia were found in control and 8000 ppm animals, but females in the 30,000 ppm dose group also failed to display stones or lesions over any dosing period. In the 10 animals/group receiving 50,000 ppm Fosetyl-Al, bladder hyperplasia was found in 4, 4, 1 and 1 animals after 2, 4, 8 and 13 weeks treatment, respectively. Although 1 of these females had simple transitional cell hyperplasia, the other 9 with bladder lesions had papillary hyperplasia. Only 1/10 females with hyperplasia had a bladder stone upon gross examination, but all of them had indications of stones in the renal pelvis (and occasionally in the ureter).

ii. Renal pelvis: Stones/hyperplasia

Upon gross examination, an increase in the incidence of stones in the renal pelvis was found in 50,000 ppm males over the treatment durations. Of the 40 rats that received 50,000 ppm

Fosetyl-Al for 2, 4, 8, or 13 weeks, 27 had stones in the renal pelvis. Four male rats of the 50,000 ppm group died prior to terminal sacrifice, and they all had stones in the renal pelvis. The incidence of the simple transitional cell hyperplasia was also increased in 50,000 ppm males, and this increase was particularly marked in 8 and 13 week treatment groups (controls, 0/10; 8 week, 7/9; 13 week, 6/6). No renal papillary hyperplasia finding was reported.

In female rats, simple transitional cell hyperplasia was increased in the renal pelvis of 50,000 ppm groups treated for 2, 4, 8 and 13 wk; no animal showed papillary hyperplasia. Of 40 dosed females, 22 had hyperplasia, and 26 had stones upon gross examination. No increases in hyperplasia or stones were observed at doses of 30,000 ppm and below. The presence of stones and hyperplasia of the renal pelvis at 50,000 ppm in the 90-day study is consistent with a possible carcinogenic effect at 40,000/30,000 ppm among females in the chronic study. It should be noted, however, that males also demonstrated stones, irritation and hyperplasia of the renal pelvis in the 90-day study, but they failed to develop an increase in neoplasms of the renal pelvis following chronic administration.

iii. Reversibility of bladder effects

There was also strong evidence of reversibility of bladder stones and bladder hyperplasia when treated male animals (30,000 and 50,000 ppm) in the 90-day study were returned to basal diet. Whereas bladder stones on gross examination had occurred in a total of 59% (44/75) of males over the dosing periods, 40% (21/52) 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: 80% (60/75) of treated males over the treatment periods showed bladder hyperplasia whereas only 37% (19/52) 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. Interestingly, one 30,000 ppm male in the 8-week treatment/16-week recovery group had a urinary bladder papilloma.

2. Two-Year Dog Chronic Feeding Study

Reference: Spicer, E.J.F. 1981. Fosetyl-Al: Two Year Dietary Toxicity Study in Dogs. MRID No. 00098340. International Research and Development Corporation, Mattawan, MI. Study No. 347-023.

Fosetyl-Al was administered to groups of dogs (6/sex/dose) in Purina Canine Diet at concentrations of 0, 10,000, 20,000, or

40,000 ppm (99.6% purity). 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.

3. Rat Reproduction Study

Reference: (Accession No. 247174)

In a three-generation reproduction study, groups of 25 rats/sex/dose received Fosetyl-Al in Spratts Lab. Animal Diet No. 2 (powdered rodent diet) at concentrations of 0, 6000, 12,000, and 24,000 ppm (98% purity). 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, Fosetyl-Al 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 or in groups receiving 6000 or 12,000 ppm Fosetyl-Al.

4. Metabolism

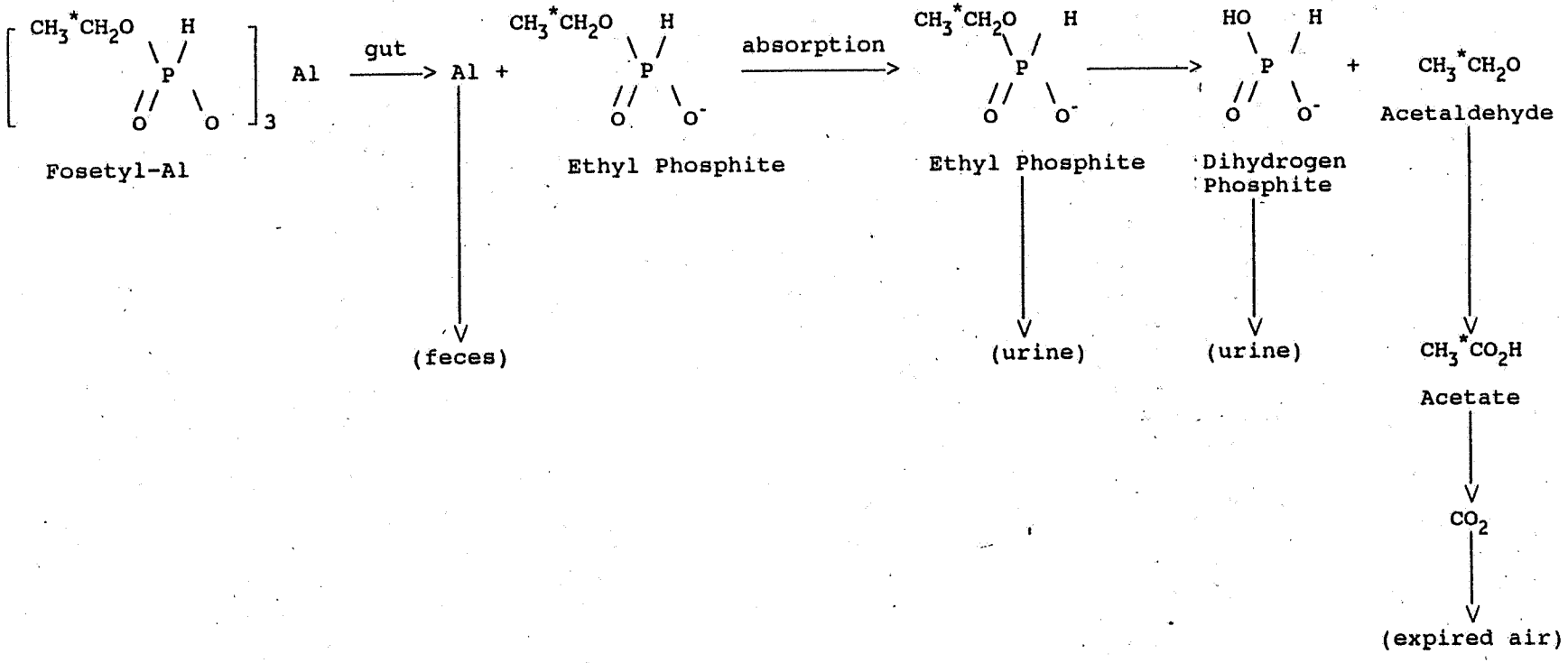
Reference: Unsworth, J.B. 1976. Aluminum Ethyl Phosphate (LS 74.783): Metabolism Study in Rats. Accession NO. 247183. May and Baker, Ltd., Research Laboratories, Essex, England. Study No. RES

Four metabolism studies in rats were available: two studies on ^{14}C-Fosetyl-Al and two studies on ^{32}P-Fosetyl-Al. While there are no direct measurements of the gastrointestinal absorption of Fosetyl-Al, based upon reported recovery data of orally-administered labeled compound (250 mg/kg/d for 7 d), it appears that the ethyl phospite moiety is well absorbed and that aluminum is not significantly absorbed. After oral ingestion, a majority

of the radiolabeled Fosetyl-Al was hydrolyzed to dihydrogen phosphite and ethanol which was then oxidized via acetaldehyde and acetate to CO_2 and eliminated in the expired air and approximately 26% of the orally-administered Fosetyl-Al was eliminated in the urine. The CO_2 accounted for 60% of the administered ^{14}C radioactivity. From a diet containing 30,000 ppm of Fosetyl-Al, the amount of ethanol consumed by rats on a daily basis would be equivalent to a human, on a mg/kg basis, consuming 1.75 ounces of ethanol.

Phosphite and ethyl phosphite moieties were eliminated via the urine without further oxidation to phosphate; they accounted for approximately 73% and 26%, respectively, of the radioactivity in the urine. Only a minor amount (2-3%) of the administered radioactivity was found in the feces. Aluminum does not appear to be absorbed to a significant extent from the gastrointestinal tract. The proposed metabolic pathway for Fosetyl-Al is shown in Figure 1.

Figure 1. Metabolism of Fosetyl-Al.



* ^{14}C-label

Note: Aluminum atoms were included for completeness. Most aluminum is eliminated in the feces; a small component may be absorbed and eliminated as counter ions with phosphite or ethyl phosphite; others may follow different elimination paths.

5. Genotoxicity

Reference: (Accession Nos. 247173 and 247186)

There is nothing in the structure of Fosetyl-Al that would alert one to potential genotoxic effects relevant to carcinogenicity. Two forms of Fosetyl-Al were tested for genotoxicity, the technical grade and a wettable powder containing 80% active ingredient (a.i.). Two acceptable tests for each form were submitted and found to be negative, a Salmonella assay and a mouse bone marrow micronucleus test. Other tests for each form were submitted, but were found unacceptable because of inadequate dosing; these tests, which were negative, included phage induction assays using E. coli, DNA repair tests in E. coli and in yeast assays with S. cerevisiae measuring convertants, revertants, or recombinants. Therefore, while the data do not suggest a genotoxicity concern, a data gap exists for the other genotoxic effects category.

6. Structure-Activity Correlations

Except for the sodium dihydrogen phosphite metabolite (see D.2., above), no information was located concerning structurally related chemicals. The composition of Fosetyl-Al can be considered to be aluminum, phosphite and ethanol. There is no evidence for the carcinogenicity of these components in the bladder although consumption of ethanol-containing beverages has been associated with gastrointestinal and liver cancer in humans (IARC 1988, Rothman 1975).

F. Weight of Evidence Considerations

The PRC considered the following findings regarding the toxicology data on Fosetyl-Al to be of importance in a weight-of-the-evidence determination of carcinogenic potential:

1. In the 2-year feeding carcinogenicity study in Charles River CD rats, Fosetyl-Al induced urinary bladder tumors in males (transitional cell papillomas and carcinomas) at a level of 40,000/30,000 ppm, a dose which led to bladder stone formation in males in a subchronic study. In the 2-year study in females at 40,000/30,000 ppm, there was a possible increase in transitional cell neoplasms of the bladder and renal pelvis, accompanied by bladder stones. However, the tumor increases in the female bladder and renal pelvis were noted by one pathologist but not by another. The formation of stones in the 2-year study correlated with the possible neoplasms for females; for males, although there was some stone formation associated with neoplasia in 2 out of 21 tumor-bearing animals, significant association was not found.

Fosetyl-Al did not produce tumors at any site other than the urinary tract in the rat. It is noted that the high dose used in this study was above the OPP limit dose for a carcinogenicity study in rats.

2. In a chronic feeding/carcinogenicity study with mono-sodium dihydrogen phosphite, a metabolite of Fosetyl-Al, no evidence of neoplastic or non-neoplastic changes in Charles River CD rats at doses up to 32,000 ppm was noted; there was no indication of diuresis, changes in urinary pH, or of neoplasia or hyperplasia in the urinary bladder.

3. Fosetyl-Al at doses up to 20,000/30,000 ppm did not produce tumors at any site in mice in a 2-year feeding/carcinogenicity study, and the mice failed to demonstrate any target organ toxicity in the urinary system. 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 because the administered doses were well in excess of the HED limit dose.

Similarly, no urinary tract effects were observed in the 2-year dog study at dose levels of Fosetyl-Al up to 40,000 ppm. Male rats, therefore, appear to be more sensitive to stone and tumor formation than female rats, and rats are much more sensitive than mice or dogs of either sex.

4. In a short-term (90-day) study in the rat, papillary transitional cell hyperplasia of the bladder was induced at dose levels of 30,000 and 50,000 ppm in males, and 50,000 ppm in females. Hyperplasia developed within a 2-week dosing period and was considered to be secondary to mechanical irritation produced by calculi based upon an animal-by-animal correlation of bladder calculi and bladder hyperplasia. The calculi were decreased in incidence and size with continued exposure. There was also strong evidence of reversibility; upon cessation of high-dose exposure and return to control 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 8000 ppm males failed to show either bladder stones or any indication of bladder hyperplasia. Calculi and hyperplastic effects in females were less pronounced than in males and occurred only at the 50,000 ppm level.

The more common observation of the urinary bladder calculi in male rats than in female rats may be due in part to either the longer narrower urethral opening in male rats which limits the passage of calculi, and/or the presence of certain proteins or other factors which enhance precipitate or calculus formation in the urine of males.

Fosetyl-Al induced increases in blood phosphorus and CO_2 and produced a profound lowering of the urinary pH in the high-dose males in the 90-day study accompanied by diuresis, elevated urinary calcium levels and reduced urinary phosphorus concentrations. The bladder stones are composed of 33% calcium and 23% phosphorus. The precise mechanism and source of increased urinary calcium and the complete composition of the stones are unknown.

5. Four metabolism studies in rats were available: two studies on ^{14}C-Fosetyl-Al and two studies on ^{32}P-Fosetyl-Al. While there are no direct measurements of the gastrointestinal absorption of Fosetyl-Al, based upon reported recovery data of orally administered labeled compound, the ethyl phosphite moiety appears to be well-absorbed. After oral ingestion, radio-labeled Fosetyl-Al was hydrolyzed to dihydrogen phosphite and ethanol which was then oxidized via acetaldehyde and acetate to CO_2 and eliminated in the expired air. The phosphite and some of the ethyl phosphite moiety were eliminated via the urine without further oxidation to phosphate. Aluminum does not appear to be absorbed to a significant extent from the gastrointestinal tract.

6. The available evidence does not indicate a genotoxic potential for Fosetyl-Al. There is nothing about the structure of the compound that would alert one to potential genotoxic effects relative to carcinogenicity. Negative genotoxicity studies considered acceptable include a Salmonella assay and a mouse bone marrow micronucleus test. Phage induction assays using E. coli, DNA repair tests in E. coli and in yeast assays with S. cerevisiae measuring convertants, revertants, or recombinants, although negative, were found unacceptable because of inadequate dosing. Additional genotoxicity information, including studies of DNA damage and repair in the rat bladder, is desirable.

7. At doses of 8000 to 12,000 ppm of Fosetyl-Al, there are no stones or hyperplasia, but at doses of 24,000 ppm and above, treated male rats develop stones, bladder irritation, and hyperplasia after short-term exposure. After chronic exposure, doses of 40,000/30,000 ppm are associated with bladder tumor development in male and possibly female rats and possibly in the renal pelvis of female rats. However, the doses leading to stone formation are in excess of the OPP limit dose for rat cancer studies (1 g/kg/d or 20,000 ppm, EPA 1986).

The PRC considered the bladder tumors in male rats and possibly the bladder and renal pelvis tumors in females at dose levels of 40,000/30,000 ppm associated with chronic administration of Fosetyl-Al to be likely due to prolonged mechanical irritation by bladder calculi and attendant hyperplasia (see Appendix 1). These calculi appear to result from alterations in the homeostasis of calcium and phosphorus.

An increase in urinary bladder tumors in the 2-year rat study occurred in females only at a dose level which was associated with an increased incidence of stones and 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). The paucity of stones among the high-dose male animals with tumors in the chronic study is noteworthy; however, a number of them showed hydronephrosis or dilatation of the ureter, presumptive indicators of past urinary tract obstruction. In addition, the special 90-day study in rats demonstrated that significant numbers of calcium and phosphorus-containing bladder calculi rapidly developed in males receiving ≥30,000 ppm Fosetyl-Al, then decreased in frequency and size; females developed some stones but only at the higher dose, 50,000 ppm.

Levels of Fosetyl-Al which do not induce calculi are not expected to result in either hyperplasia or neoplasia. For instance, in the 90-day rat study, stones and hyperplasia were noted at 30,000 ppm but not at 8000 ppm. Also, in the 2-generation rat reproductive study calculi/hyperplasia were found at 24,000 ppm but not at 12,000 ppm. Finally, in the 2 year rat study, bladder stones, or hyperplasia and/or neoplasia occurred at 40,000/30,000 ppm but not at 8000 ppm.

In summary, the carcinogenic effects in the male rat bladder (and possibly the female rat bladder and renal pelvis), appear to be contingent upon the formation of urinary stones and the development of epithelial irritation. These effects are produced in rats at extremely high doses under conditions not anticipated to occur outside of the experimental laboratory. However, before making a judgment on the overall carcinogenic potential of Fosetyl-Al to humans, it is important to evaluate potential exposure due to pesticidal use of the chemical.

G. Human Exposure Considerations

A formal exposure assessment of Fosetyl-Al has not been performed, but a preliminary evaluation indicates that human exposures may be quite low. There was no evidence of stones in rats except at the 24,000 ppm dose level or above. The highest NOEL is 12,000 ppm, which is equivalent to about 600 mg/kg/d [assuming a reference food factor of 0.05 for rats (EPA 1986)]. It would seem that human dietary exposure is only about 1.2 μg/kg/d, a daily dose about 500,000-fold lower than the NOEL for stone formation in rats. Likewise, for applicators who receive most of their exposure dermally, it is highly unlikely that there would be enough absorption from dermal exposure to result in stone formation, especially considering that Fosetyl-Al is an aluminum phosphonate compound which is known to readily dissociate, at least in the gut.

H. Characterization of Human Carcinogenic Risk Potential

Criteria contained in the current EPA Guidelines [51FR: 33992-34003, 1986] were considered. The PRC recognized that on the one hand, the guidelines emphasize the use of a weight-of-the-evidence evaluation in determining the carcinogenic hazard of an agent; but on the other hand, they also lay out rather rigid criteria for the evidence associated with each of the categories in the classification system. Due to these different emphases, the PRC presents a description of the hazard potential of Fosetyl-Al in a narrative form that is not associated with the categories per se in the EPA guidelines.

The PRC concluded that this chemical has been adequately tested for carcinogenicity in rats and mice and for chronic effects in dogs. The only tumor type which is associated with compound administration under the conditions of the bioassays, that of the urinary bladder in the male and possibly female rats, appears to be due to bladder stone formation, bladder wall irritation, profound proliferation of bladder epithelium (see Appendix 1) and finally, neoplastic conversion. Similarly, female rats may also show an increase in epithelial tumors of the renal pelvis that are associated with stone formation and epithelial irritation. Male rats are more sensitive than female rats to stone formation in the urinary bladder, while mice and dogs show absolutely no stone formation or any other urinary tract effects in chronic studies.

Besides stone formation, there is no other apparent cause for the induction of urinary tract tumors in rats. The aluminum in Fosetyl-Al is not significantly absorbed from the gut, while the organic moiety is easily absorbed and rapidly broken down into simple molecules, none of which has historically been associated with bladder carcinogenesis. Fosetyl-Al was also negative for gene and chromosomal mutations in the short-term tests performed to date and judged adequate, and its structure contains no entities that would alert one to a possible genotoxic effect.

Absorption following high doses of Fosetyl-Al produces an increase in plasma phosphorus and an acidosis probably due to metabolism of the ethanol moiety to carbon dioxide; this leads to an acidic urine. Calcium enters the circulation in response to the hyperphosphatemia and is lost in the urine in increased amounts. Calcium and phosphorus-containing stones form which irritate the bladder epithelium and result in simple and papillary hyperplasia; these processes are reversible upon cessation of chemical treatment. However, should they continue, transitional cell tumors arise. Doses not sufficient to produce stones do not produce irritation and hyperplasia and, most likely, neoplasia. Thus, in the absence of stone formation there

should be no potential to produce the bladder irritation, hyperplasia and neoplasia.

Existing information from human case studies and epidemiologic investigations indicate that humans may be significantly less susceptible to the influence of stones on bladder carcinogenicity than rodents (Appendix 2). Given that (a) tumors develop in rats under extreme conditions that are unlikely to be achieved other than under laboratory conditions (at a dose in excess of the OPP dose limit for carcinogenicity studies); (b) tumors in rats are believed to develop only at doses that produce stones; (c) human dietary exposure to Fosetyl-Al is only about one-500,000th of the NOEL for stone formation in the rat (the most sensitive experimental model); and (d) the dose of Fosetyl-Al which can be absorbed dermally by applicators is also probably too low to result in stone formation, it would seem that the pesticidal use of Fosetyl-Al is unlikely to pose a carcinogenic hazard for humans. Further information to clarify the uncertainties listed below would be useful to strengthen this position.

The uncertainties which preclude a more definitive conclusion concerning the carcinogenic hazard to humans are as follows. An important uncertainty is the extent to which direct genetic damage may influence the development of tumors. Although Fosetyl-Al is not positive in short-term tests for point and chromosomal mutations, no information is available on the target cells in the rat urinary bladder. In addition, questions remain concerning the mechanism of stone formation in rats. The molecular composition of the stones has not been fully characterized and the source of the increase in urinary calcium (e.g., absorption from the gut or resorption of bone) and the mechanism which causes the increase (e.g., effects on the parathyroid glands or parafollicular cells of the thyroid) have not been determined. Finally, the incidence of transitional cell tumors in the control animals in the rat chronic/carcinogenicity study was higher than is normally seen in this strain of rat.

I. Recommendations

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

The registrant should develop a more complete analysis of the composition of the bladder stones and further information concerning the mechanism leading to calculus formation.

Historical control data for rat transitional cell bladder tumors are needed from the test laboratory.

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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 can lead to irritation of urothelium, increases in urinary epithelial cellular proliferation, cellular hyperplasia, and benign and malignant neoplasms. It appears that it is the presence of a physical body (and urine) in the bladder, rather than its chemical composition which is essential for tumor induction; that is, the tumors are secondary to the presence of stones. Examples are available which typify this type of carcinogenic process in the bladder and 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 leads 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 result in a higher tumor incidence than do smooth glass beads (DeSesso 1989).

A number of other factors have been identified as influencing the induction of bladder tumors. Increased incidences are observed with some chemicals at a high urinary pH and lowering of urinary pH decreases or eliminates carcinogenic activity e.g. saccharin (Cohen and Ellwein 1990) and sodium ortho-phenyl phenol (Fujii et al. 1987). Uric acid and cysteine may precipitate and form stones at low pHs, while calcium and phosphorus-containing stones form in an alkaline medium. 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, in part, 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 and possibly other constituents in male urine may also serve to catalyze the formation of calculi. The rat 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, including cell division, as critical components (Cohen and Ellwein 1989 1990). 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. Certainly the finding of bladder tumors following the implantation of inert materials like glass or wooden beads or wax pellets would also fall into this category, since they 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 are expected to 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 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 would not be 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 pelves and the ureters. Under certain stresses like inflammation, 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 urinary structures. Consequently, the urinary bladder is usually investigated separately from the upper part of the urinary system. However, urinary stones (calculi) can form anywhere along the urinary collection system from the calyces and pelvis to the ureter and bladder.

Stones of the urinary tract are quite common and are seen in about 1% of autopsies (Smith 1982). Unless they produce stasis by occluding urinary flow or causing 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). In the U.S. most stones are found in the upper urinary tract, with fewer in the urinary bladder. 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 cysteine (Cheng 1980, Smith 1963).

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 total cancer deaths are 2% and 1%, respectively (Boring et al. 1991).

It is largely a disease of the elderly: about 2/3 of the cases occur 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).

A number of 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 and compounds in cigarette smoke. Occupational associations include workers exposed to dyes, working in the leather and rubber industries, being a professional painter and being a professional truck driver. Certain other associations have not yet been conclusively 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. There is some evidence suggesting that urinary stasis from various causes, such as stones and infection (bacteria and Schistosoma haematobium) may be related to cancer, but more work is needed in these areas (Matanoski and Elliott 1981, Silverman et al. 1992). For instance, as to the role of stones in the induction of all types of bladder cancer, several studies fail to show any link (Morin and Hemminger 1962, Thompson 1959, Waller and Hamer 1950). However, based on a review of the clinical literature, there may be some associations between urinary tract stones and cancer. In these studies, stones appear to be associated with the development of squamous metaplasia and squamous cell carcinoma, an uncommon form of urinary tract cancer (Peterson 1992).

The existing epidemiologic literature fails to establish stones as a significant risk factor. 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). Three other epidemiologic studies with about 300 bladder cancer cases each also fail to show a significant relationship between bladder stones and bladder cancer: Dunham et al. (1968) had relative risks of 2.4 and 2.1 for males and females, Kjaer et al. (1989) showed a relative risk of 1.5, and La Vecchia et al. (1991) showed a relative risk of 1.0.

The only positive epidemiologic indicator of a potential role for 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. Subjects 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).

In summary, the accumulated evidence for urinary bladder stones as a significant factor in bladder cancer risk is marginal. In the U.S. most urinary tract stones are not found in the bladder. Epidemiologic studies provide little evidence for a major contribution by stones; from the clinical literature, it appears that stones may be associated with squamous cell cancer, an uncommon form of bladder cancer. Thus, the overall effect of

stones on bladder cancer seems minimal. Certainly humans appear to be much less sensitive to the impact of stones on bladder carcinogenesis than are laboratory rodents.

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