Document Title

Summary of the toxicological and metabolism studies for fosetyl

Data Requirements

EU Regulation 1107/2009 & EU Regulation 283/2013
Document MCA
Section 5: Toxicological and metabolism studies

According to the guidance document SANCO/10181/2013 for preparing dossiers for the approval of a chemical active substance

Date

2016-12-05

Author(s)

[redacted]

Bayer CropScience

OWNERSHIP STATEMENT

This document, the data contained in it and copyright therein are owned by Bayer CropScience. No part of the document or any information contained therein may be disclosed to any third party without the prior written authorisation of Bayer CropScience.

The summaries and evaluations contained in this document are based on unpublished proprietary data submitted for the purpose of the assessment undertaken by the regulatory authority. Other registration authorities should not grant, amend, or renew a registration on the basis of the summaries and evaluation of unpublished proprietary data contained in this document unless they have received the data on which the summaries and evaluation are based, either:

- **from BayerCropScience; or**
- **from other applicants once the period of data protection has expired.**

Version history

Date (yyyy-mm-dd)	Data points containing amendments or additions[1] and brief description	Document identifier and version number
2015-09-04	Original Document MCA – Section 5 of Supplementary Dossier	M-531877-02-1
2016-05-02	Dossier update according to "Request for additional information on the supplementary dossier submitted by Bayer CropScience for the approval renewal of the active substance Fosetyl (2015-5865)" by RMS France on 2016-04-04: - Summaries of the studies used for the first approval of fosetyl and presented in the DAR and addenda to the DAR including detailed result tables have been added throughout Section 5. - Summary tables with additional information have been added to chapters CA 5.3.2, CA 5.5, CA 5.6.1, CA 5.6.2 and CA 5.8.2. Some of these additional information have been taken from two addenda to study report [redacted]; 1981; M-249664-02-1 therefore both addenda ([redacted]; 1983; M-234109-01-1 and [redacted]; 1983; M-159736-01-1) have been added to chapter CA 5.5. - Study KCA 5.3.2/02, [redacted]; 2013; M-459669-02-1, has been amended, new study report: [redacted]; 2016; M-459669-03-1. - ADME information on aluminium from EFSA's 2008 evaluation of aluminium in food has been paraphrased in chapter CA 5.1.1. - An analysis of the available MoA information regarding urinary bladder neoplastics lesions following the IPCS scheme has been added to chapter CA 5.8.2. - Study results were assessed according to the criteria of Regulation 1272/2008 throughout Section 5.	M-531877-03-1
2016-09-01	Dossier update according to "Request for additional information on the supplementary dossier submitted by Bayer CropScience for the approval renewal of the active substance Fosetyl (2015-5865)" by RMS France on 2016-04-04 and its follow up on 2016-06-02: - Historical control data for study [redacted]; 1981; M-249664-02-1 for neoplastics lesions, particularly for phaeochromocytomas, have been added to chapter CA 5.5 as KCA 5.5/10. - Statements regarding bone marrow exposure have been added to chapter CA 5.4.2.	M-531877-04-1
2016-12-05	Dossier update according to "Request for additional information on the supplementary dossier submitted by Bayer CropScience for the approval renewal of the active substance Fosetyl (2015-5865)" by RMS France on 2016-11-22: - Correction of dose groups in headline of Table 5.6.2- 7, it is 0, 50, 100 and 300 mg/kg bw/day instead of 0, 100, 315 and 1000 mg/kg bw/day - Historical control data provided in study [redacted]; 2000; M-205472-01-1 (KCA 5.6.2/04) have been added to the summary of this study in chapter CA 5.6.2.	M-531877-05-1

[1] It is suggested that applicants adopt a similar approach to showing revisions and version history as outlined in SANCO/10180/2013 Chapter 4 "How to revise an Assessment Report"

Table of Contents

CA 5 TOXICOLOGICAL AND METABOLISM STUDIES ON THE ACTIVE SUBSTANCE

Fosetyl was included in Annex I to Directive 91/414/EEC in 2006 (Directive 2006/64/CE of 18 July 2006, Entry into Force on 1 May 2007). This Supplementary Dossier contains only data which were not submitted at the time of the Annex I inclusion of fosetyl under Directive 91/414/EEC and which were therefore not evaluated during the first EU review. All data which were already submitted by Bayer CropScience (BCS) for the Annex I inclusion under Directive 91/414/EEC are contained in the DAR, its Addenda and are included in the Baseline Dossier provided by BCS. These data are only mentioned in the Supplementary Dossier for the sake of completeness and only general information (e.g. author, reference etc.) is available for these data. In order to facilitate discrimination between new data and data submitted during the Annex I inclusion process under Directive 91/414/EEC, the old data are written in grey typeface. For all new studies, detailed summaries are provided within this Supplementary Dossier. However, for a better understanding of the toxicological and metabolic behaviour of fosetyl, an overall summary is given below and additional short summaries including the results of all studies are given at the beginning of the relevant sections. Additional information requested by the RMS France on 2016-04-04 during the evaluation of the Supplementary Dossier is highlighted in yellow. Additional information requested by the RMS France on 2016-11-22 during the evaluation of the Supplementary Dossier is highlighted in grey.

Fosetyl is the ISO common name for ethyl hydrogen phosphonate (IUPAC). Due to the fact that the aluminium salt, a variant of fosetyl, is used in the formulated product, it should be noted that the presented data in this section belong to the variant fosetyl-aluminium (fosetyl-Al), unless otherwise specified.

In original reports study authors may have used different names or codes for metabolites of fosetyl-Al. In this summary, a single name or a single code is used for each metabolite. A full list containing structural formula, various names, short forms, codes and occurrences of metabolites is provided as Document N3.

As some pragmatic approach "phosphonic acid" formed as a major metabolite is reported in this Supplementary Dossier as the free acid for the sake of clarity and unequivocal handling. After application, aluminium tris-O-ethyl phosphonate (i.e. fosetyl-Al) dissociates into the O-ethyl phosphonate and aluminium ions. Any phosphonate formed from O-ethyl phosphonate in the following would never be present in the form of the free acid (i.e. phosphonic acid) under the conditions of the environment (pH 4 to 9). This conclusion is supported by the molecular structure and by the dissociation constant observed (dissociation constant for the first step of deprotonation: pKa = 2.0). Consequently, phosphonates in their fully protonated form are strong acids that spontaneously form salts in contact with soil or natural water with any suitable counter ion present (i.e. sodium, potassium, magnesium, calcium). With the ability to readily form salts in the environment phosphonates are, in terms of their acidic or alkaline character, similar to the salts of phosphoric acid (i.e. phosphates) in their environmental behaviour. Conclusively, the character of being a salt prevents effects that would be observed when the fully protonated form, i.e. the free acid, would be tested. Consequently, toxicological tests were performed with the sodium or potassium salts rather than with phosphonic acid.

OVERALL SUMMARY AND CONCLUSION

The following summary is based on the EFSA conclusion on the peer review of fosetyl (EFSA Scientific Report (2005) 54, 1-79, revised publication dated 12 June 2013). Some information on high dose and multiple dose tests supporting the overall picture is taken from the public DAR, Section B.6.1.4 (Summary of ADME) of the RMS France (February 2005).

Toxicokinetics and metabolism

Fosetyl-aluminium (fosetyl-Al) is rapidly and totally absorbed (by the rat after a single oral low dose of 100 mg/kg bw of ^{14}C-fosetyl-Al), based on expiration and urinary and faecal excretion of the ^{14}C-ethyl radiolabel within 48 hours after administration. The large amount of radioactivity expired as $^{14}CO_2$ shows the occurrence of an extensive metabolic transformation. The presence of ^{14}C-ethanol in urine suggested also the integration of the radiolabel in naturally occurring components from the ^{14}C-ethyl moiety of the parent (as confirmed by analysis of fractionated tissue extracts, e.g. lipids fatty acids, amino acids preparations). After repeated doses, absorption is also rapid and practically complete (> 90% 24 hours post administration, and excretion is rapid, > 70% in air and urine). The major component in urine is ^{14}C-fosetyl-Al, accounting for 22.6 to 26.3% of the dose, associated by other minor components, accounting for 0.07 to 1.11% of the administered dose.

After single oral high dose of 3000 mg/kg bw of ^{14}C-fosetyl-Al, elimination was shown to be very rapid, mainly occurring within 24 hours after administration, through exhaled air (ca. 50%) and urinary excretion (32 to 33% of the dose). Overall, mean values for faecal excretion were 1.85% in males and 3.30% in females within 168 hours from administration.

After repeated oral exposures, large amounts of $^{14}CO_2$ were expired indicating the removal of the radiolabelled ethyl group and the subsequent metabolism via acetaldehyde and acetate giving rise to already mentioned integration into naturally occurring molecules. The remaining moiety of fosetyl, the phosphonate anion is renally excreted as a major component in the urine (together with unchanged fosetyl).

Fosetyl-Al widely distributes to all tissues, with the highest amounts in kidneys, liver, lungs, spleen, fat, adrenal glands, gonads, tissues with a high metabolic activity. Tissue levels were between 1.2 and 6% of the dose 168 hours after administration and between 6.9 and 9.5% of the dose 72 hours after administration of a single oral dose.

Acute toxicity

The acute toxicity of fosetyl-Al was evaluated following oral, dermal, subcutaneous and inhalation routes of exposure (see Table 5.2- 1, page 29).

Fosetyl-Al was of low acute toxicity following oral, dermal and inhalation administration and is therefore **not classified for acute lethal effects according to the criteria of Regulation 1272/2008.** Fosetyl-Al was previously classified as a severe eye irritating agent (Eye Dam. 1, H318; [redacted]; 1997; M-179082-01-1), but a newer study ([redacted]; 2012; M-446501-01-1) demonstrates the reversibility of ocular effects and justify a **new classification as Eye Irrit. 2, H319**. Fosetyl-Al is not irritating to skin and not a skin sensitizer and is **not classified for these endpoints** in accordance with the respective CLP criteria.

Short-term toxicity

Short term toxicity of fosetyl-Al (see Table 5.3- 1, page 60) has been assessed in rodents (6-week oral study in mice; 90-day oral studies in rats and 28-day dermal studies in rats) and in dogs (90 days, oral).

Fosetyl-Al did not induce adverse effects after oral administration, except for a marginal increase in the incidence and severity of extramedullary haematopoiesis in the spleen at the top dose (1922 and 2499 mg/kg bw/day in males and females, respectively) in an old study ([redacted]; 1977; M-158836-01-1) whose results were considered doubtful.

Dermal application of fosetyl-Al (1050 mg/kg bw/day) for 28 days in rats caused dermal irritation; no signs of systemic toxicity were recorded ([redacted]; 1999; M-178986-01-1).

The oral 90-day rat study by [redacted]; 1999; M-184588-01-1 was acceptable and showed no effects up to 1270 mg/kg bw/day and this would be the relevant short-term NOAEL. A recent 90-day feeding study in rats has been conducted with almost identical results ([redacted]; 2016; M-459669-03-1).
The EFSA experts, however, considered that, for the overall short-term NOAEL, also the mechanistic studies should have been taken into consideration. In these studies, increased calcium levels in urine were observed at 20 000 ppm and above and treatment-related changes in the urinary bladder, kidney and ureters were seen at 30 000 ppm and above. The NOAEL of 500 mg/kg bw/day from the 13-week rat study by [redacted]; 1989; M-160331-01-1 was considered to be an overall NOAEL for short-term toxicity, with histopathological changes in the kidney, impairment of calcium/phosphorus metabolism, calculi and hyperplasia in the urinary tract at higher doses.
No specific target organs were identified in any of the repeated-dose toxicity studies. Therefore, a **classification in the STOT RE hazard category is not warranted,** according to the criteria of Regulation 1272/2008.

Genotoxicity
Genotoxicity of fosetyl-Al was assessed in a battery of *in vivo* and *in vitro* tests (see Table 5.4- 1, page 88). All the studies gave negative results, clearly indicating the absence of any genotoxic potential for fosetyl-Al. Therefore, a **classification for germ cell mutagenicity is not warranted**, according to the criteria of Regulation 1272/2008.

Long-term toxicity and carcinogenicity
Long term toxicity of fosetyl-Al was tested in a 2-year oral study in dogs, in a 2-year oral rat study and in a 2-year oral study in mice (see Table 5.5- 1, page 119).
Mice did not show any treatment related effect, even at high doses (around 4000 mg/kg bw/day).
Testes were the main target organ in dogs. Males treated at high doses displayed testicular degeneration. The NOAEL of the study was 288 mg/kg bw/day.
In rats, effects in the urinary tract were recorded, such as calculi and hyperplasia of the urinary bladder epithelium in females at doses of 30 000 ppm; at the same dose, males showed a higher incidence of transitional cell papilloma and carcinoma in the urinary bladder. The NOAEL from this study was 348 and 450 mg/kg bw/day for males and females, respectively.
Two mechanistic studies were conducted to assess the mode of action of fosetyl-Al during long term exposures. It was hypothesised that the renal tumours could result from a chronic irritation rather than a true carcinogenic effect of fosetyl-Al. The ingestion of high doses of fosetyl-Al is a possible cause of functional alterations of renal excretion, while the formation of calculi may induce a chronic irritation of the urinary bladder epithelium, leading therefore to the development of transitional cell papilloma and carcinoma of the urinary bladder. With all long-term NOAELs and LOAELs being ≥300 mg/kg bw/day a **classification in the STOT RE hazard category is not warranted,** according to the criteria of Regulation 1272/2008.
The relevant NOAEL for long-term effects was discussed by the EFSA experts and it was agreed to be about 300 mg/kg bw/day, based on the 2-year studies in rats ([redacted]; 1981; M-249664-02-1; addenda [redacted]; 1983; M-234109-01-1 and [redacted]; 1983; M-159736-01-1) and dogs ([redacted]; 1981; M-159302-01-1).
In conclusion, no carcinogenic potential for fosetyl-Al is evident.

Reproductive and developmental toxicity
The reproductive toxicity of fosetyl-Al was evaluated in rats and rabbits (see Table 5.6- 1, page 145).
In a multi-generation rat study, fosetyl-Al did not induce any sign of reproductive toxicity. The NOAELs for maternal and reproductive toxicity are 1782 and 1997 mg/kg bw/day in males and females respectively (highest doses tested).
The overall results indicated that fosetyl-Al dose not induce teratogenic effects in rats and rabbits, with an overall NOAEL of 300 mg/kg bw/day from the rabbit study ([redacted]; 2000; M-205472-01-1).
According to the criteria of Regulation 1272/2008, **no classification as reproductive toxicant is required.**

Neurotoxicity
Fosetyl-Al did not show any evidence of neurotoxic potential (see Table 5.7- 1, page 162).

Studies on metabolites
Phosphonic acid is the major metabolite of fosetyl-Al in plants and in *in vivo* studies. Phosphonic acid and its potassium and sodium salts are of low acute oral, dermal and inhalation toxicity. It is not a skin irritant while it is a slight eye irritant, albeit with no resulting classification (see Table 5.8.1- 1 page 164). Phosphonates were also tested in some *in vitro* and *in vivo* genotoxicity assays, without showing any genotoxic potential (see Table 5.8.1- 3, page 165).
The NOAEL from a 90-day oral study with phosphonic acid in rats was 400 mg/kg bw/day, based on soft faeces, increased water intake and urinary sodium excretion at 2000 mg/kg bw/day. In a 117-week oral study in rats ([redacted]; 1981; M-159229-01-1), phosphonic acid did not induce any major effect at very high doses, and it is unlikely to pose a carcinogenic hazard to humans (see Table 5.8.1- 2, page 165). Further revision of this last result for phosphonic acid has been undertaken by EFSA after the peer-review[1]. **Considering that this 117-week rat study had been performed with the hydrated monosodium phosphonate, a correction has been made by EFSA experts for the content of water (25.9% of the tested material) and for the molecular weight of monosodium phosphonate (104 g/mol) versus phosphonic acid (82 g/mol). However, the correction for water content is inappropriate, since the study NOAEL had already reported as a dose of anhydrous monosodium phosphonate. Therefore the corrected NOAEL expressed as phosphonic acid is:**

$NOAEL(H_3PO_3)_{corrected}$ = 348 mg/kg bw/day * (82/104) = 274 mg/kg bw/day,

resulting in an ADI of 2.74 mg/kg bw/day by applying an uncertainty factor of 100.

Endocrine-disrupting properties
There are **currently no scientific criteria for classification as ED**. However, the **interim criteria for ED** classification laid down in Regulation 1107/2009 **are not fulfilled**, since fosetyl-Al is neither classified as Carc. 2 and/or Repr. 2 nor does it fulfil the criteria for such a classification. Designated studies on endocrine disrupting (ED) properties of fosetyl-Al have not been conducted. The existing body of data is sufficient to exclude relevant ED-like potential of fosetyl-Al.

Overall conclusion
Following oral administration, fosetyl-Al was rapidly and almost completely absorbed and metabolised into phosphonate and ethanol and rapidly eliminated within 24 hours.
Fosetyl-Al had low acute toxicity. The compound was classified as a severe eye irritating agent (Eye Dam. 1, H318; [redacted]; 1997; M-179082-01-1), but a newer study ([redacted]; 2012; M-446501-01-1) allows a **re-classification as Eye Irrit. 2, H319**. Fosetyl-Al was not irritating the skin and was not a skin sensitizer. It was not genotoxic both *in vitro* and *in vivo*. Fosetyl-Al is not expected to exhibit carcinogenic, reproductive and developmental hazards to humans. In addition, it was shown to be devoid of any neurotoxic potential.
The overall acceptable NOAEL for short-term toxicity is 500 mg/kg bw/day based on the results of a 90-day rat study ([redacted]; 1989; M-160331-01-1).
The overall acceptable NOAEL for long-term toxicity was 300 mg/kg bw/day (both sexes) based on the 2-year studies in rats ([redacted]; 1981; M-249664-02-1) and dogs ([redacted]; 1981; M-159302-01-1).

[1] EFSA (2013): Conclusion on the peer review of the pesticide risk assessment of the active substance fosetyl. EFSA Scientific Report (2005) 54, 1-79, revised version dated 12 June 2013.
Available at http://www.efsa.europa.eu/de/efsajournal/doc/54r.pdf

Acceptable Daily Intake (ADI)
Parent: Fosetyl-Al
The NOAEL of 300 mg/kg bw/day based on the 2-year studies in rats ([redacted]; 1981; M-249664-02-1) and dogs ([redacted]; 1981; M-159302-01-1) was selected as the relevant value to set the ADI. Applying a safety factor of 100, this leads to an **ADI for fosetyl-Al of 3 mg/kg bw/day**. Expressed as **fosetyl**, the ADI is **2.8 mg/kg bw/day.**

Metabolite: Phosphonic acid
The most relevant study to derive the ADI for phosphonic acid is the carcinogenicity rat study ([redacted]; 1981; M-159229-01-1) for which the NOAEL, expressed as phosphonic acid, is 274 mg/kg bw/day. Applying a safety factor of 100, this leads to an **ADI of 2.74 mg/kg bw/day**. Expressed as **fosetyl**, the ADI is **3.7 mg/kg bw/day.**

Acceptable Operator Exposure Level (AOEL)
The most relevant study for setting an AOEL for fosetyl-Al is the mechanistic rat study with an NOAEL of 500 mg/kg bw/day ([redacted]; 1989; M-160331-01-1), resulting in an **AOEL of 5 mg/kg bw/day** (SF 100). Fosetyl-Al was well absorbed (82-89%) when orally administered at dose levels of 100 and 1000 mg/kg bw. Accordingly, no adjustment for oral absorption was considered necessary when calculating the systemic AOEL. Expressed as **fosetyl**, the AOEL is **4.7 mg/kg bw/day.**

Acute Reference Dose (ARfD)
An ARfD for fosetyl-Al was not set because of the low acute toxicity and the absence of severe acute effects.

Drinking Water Limit (DWL)
The maximum admissible concentration of an active substance is 0.1 µg/L, as established by Council Directive 98/83/EC.
The DWL based on the ADI derived from dietary studies is several orders of magnitude higher. Taking into account that exposure through drinking water should not exceed 10% of the ADI and assuming that a 60 kg person consumes 2 L of water per day, the DWL values were calculated as follows:

$DWL_{fosetyl\text{-}Al}$ = [(3 mg/kg bw/day x 60 kg) / 2 L/day] x 0.1 = 9.0 mg/L

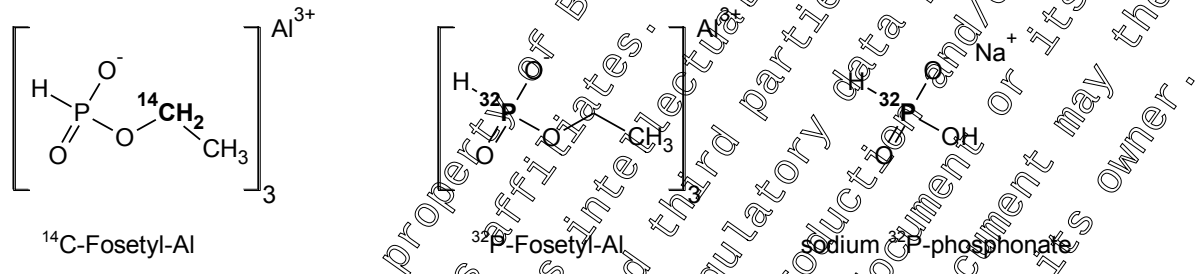
$DWL_{fosetyl}$ = [(2.8 mg/kg bw/day x 60 kg) / 2 L/day] x 0.1 = 8.4 mg/L

$DWL_{phosphonic\ acid}$ = [(2.74 mg/kg bw/day x 60 kg) / 2 L/day] x 0.1 = 8.2 mg/L

CA 5.1 Studies on absorption, distribution, metabolism and excretion in mammals

The absorption, distribution, metabolism and excretion of fosetyl-aluminium (fosetyl-Al) by Sprague-Dawley rats have been investigated using both ^{14}C-ethyl radiolabel and ^{32}P-phosphonate radiolabel.
The ADME properties of its metabolite, phosphonic acid (H_3PO_3) which is formed in plants and the environment (water and soil) have also been investigated (see Figure 5.1- 1).
The available studies are listed in Table 5.1- 1. All ADME studies have been submitted and evaluated on the occasion of the Annex I inclusion of fosetyl under Directive 91/414. No new ADME studies have been performed.

Figure 5.1- 1: Radiolabelled substances used in the ADME studies



According to the data requirements published in the Commission Regulation (EU) No 283/2013 of 01 March 2013, a "comparative *in-vitro* metabolism study" should performed "on animal species to be used in pivotal studies and on human materials (microsomes or intact cell systems) in order to determine the relevance of the toxicological animal data".
However, no official test guideline or guidance exists at present. In such cases, waiving of this particular data requirement is considered acceptable according to the "Guidance document for applicants on preparing dossiers for the approval of a chemical new active substance and the renewal of approval of the chemical active substance according to regulation (EU) No 283/2013 and regulation (EU) No 284/2013" (SANCO/10181/2013-rev 2 of 2-May-2013).
In addition there is no scientific need for comparative study. Metabolism of fosetyl-Al across mammalian species is going to be very similar as it follows basic pathways (see Figure 5.1- 2).
There are also technical difficulties that would make an *in-vitro* study unfeasible, since expiration is the major route of excretion and this will be hard to follow in an *in-vitro* system.

Figure 5.1- 2: Metabolic pathway of fosetyl-Al

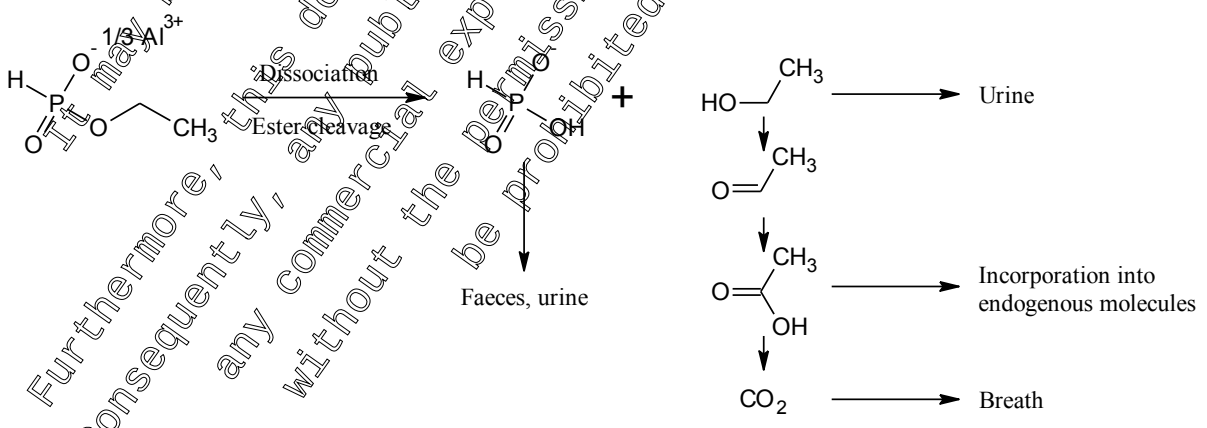


Table 5.1- 1: Overview of ADME studies (all studies via oral gavage, SD rats)

Annex point / reference number	Test substance Dosing regime	Scope of study	Reference
KCA 5.1.1/01	^{14}C-fosetyl-Al Single dose: 3000 mg/kg bw (♂+♀)	Distribution and excretion	[redacted]; 1982; M-161369-01-1
KCA 5.1.1/02	^{14}C-fosetyl-Al Single low dose: 100 mg/kg bw Single high dose: 1000 mg/kg bw (♂+♀)	Absorption, distribution, metabolism, and excretion	[redacted]; 2001; M-205381-01-1
KCA 5.1.1/03	^{14}C-fosetyl-Al Repeated dose: 250 mg/kg bw/day, 7 days (♂+♀)	Distribution and excretion	[redacted]; 1976; M-159160-01-1
KCA 5.1.1/04	^{14}C-fosetyl-Al Repeated dose: 250 mg/kg bw/day, 7 days (♂+♀)	Metabolism	[redacted]; 1976; M-161367-01-1
KCA 5.1.1/05	^{32}P-fosetyl-Al Repeated dose: 250 mg/kg bw/day, 7 days (♂+♀)	Distribution and excretion	[redacted]; 1977; M-158800-01-1
KCA 5.1.1/06	Sodium ^{32}P-phosphonate Repeated dose: 111 mg/kg bw/day, 7 days (♂+♀)	Distribution and excretion	[redacted]; 1977; M-158817-01-1
KCA 5.1.1/07	Sodium ^{32}P-phosphonate Repeated dose: 111 mg/kg bw/day, 7 days (♂+♀)	Metabolism	[redacted]; 1978; M-158849-01-1

Four studies were conducted using the Sprague-Dawley (SD) rat and orally administered ^{14}C-fosetyl-Al. The animals received either one single dose ([redacted]; 1982; M-161369-01-1; [redacted]; 2001; M-205381-01-1) or repeated doses of 250 mg/kg bw/day for 7 days ([redacted]; 1976; M-159160-01-1; [redacted]; 1976; M-161367-01-1).
A repeated-dose ADME study (250 mg/kg bw/day for 7 days) was also performed using ^{32}P-fosetyl-Al using the SD rat ([redacted]; 1977; M-158800-01-1).
Another repeated-dose study (111 mg/kg bw/day for 7 days) study was performed using a salt of the phosphonic acid in the form of sodium ^{32}P-phosphonate ([redacted]; 1977; M-158817-01-1; [redacted]; 1978; M-158849-01-1).
The fate of ^{14}C-fosetyl-Al, ^{32}P-fosetyl-Al and sodium ^{32}P-phosphonate appeared to be similar in both sexes in all studies.

Absorption
The oral absorption of ^{14}C-fosetyl-Al, as calculated by the sum the radioactivity found in the urine, expired air and tissues, was almost complete, even at very high dose rates: 82 to 101% of dose over a range of dose rates covering 100 to 3000 mg/kg bw for single oral doses. Similar figures were obtained following seven repeat doses at 250 mg/kg bw/day: 94 to 96% of dose.
Due to this complete oral absorption intravenous administration or a bile cannulation study is not needed for determination of the bioavailability of fosetyl-Al.

Distribution
Following both single and repeated administration of ^{14}C-fosetyl-Al, the radioactivity was found to be widely distributed. In terms of % of administered radioactivity the levels found in the tissues were between 1.2 and 6% at 168 hours following a single oral administration (100 to 3000 mg/kg). Following seven repeat doses at 250 mg/kg bw/day, the tissues levels were 6.9 to 9.5% of dose at 72 hours after the last administration.

In terms of tissue concentrations, there appeared to be a dose proportionality between the 100 mg/kg and the 1000 mg/kg dose groups. This was not seen between the 100 mg/kg group and the 3000 mg/kg dose group which appears to be a reflection of the slightly higher elimination rates observed at 3000 mg/kg. In the most recent study ([redacted]; 2001; M-205381-01-1) the highest tissue concentrations were observed in the fat, adrenals and skin. In the earlier single oral dose study ([redacted]; 1982; M-161369-01-1) the higher levels (above 200 µg equiv./g) were observed in the skin, fur, fat, adrenals, testes and kidneys. In the repeated-dose study ([redacted]; 1976; M-161367-01-1) the highest concentrations were found in the fat and kidney.
A generalised distribution of radioactivity was also observed following seven 250 mg/kg/day administrations of ^{32}P-fosetyl-Al. In this study, the highest concentrations were observed in the spleen (ca 40 µg equiv./g) and the lowest levels in the fat (ca 6 µg equiv./g).
A generalised distribution of radioactivity was also observed following seven 111 mg/kg/day administrations of sodium ^{32}P-phosphonate. In this study the highest concentrations were, again, observed in the spleen (ca 11 to 12 µg equiv./g) and the lowest levels in the fat (ca 1 to 2 µg equiv./g).

Metabolism
^{14}C-fosetyl-Al was rapidly metabolised to give mainly $^{14}CO_2$ which was eliminated in the expired air. The urine was found to contain unchanged parent material as the major component following administration of ^{14}C-fosetyl-Al although ^{14}C-ethanol has also been detected at early time points. Determination of phosphonate levels by GC-FTD indicated that the phosphonate anion was also a major component being eliminated in the urine. Phosphonate levels in the tissues were found to be either below the detection limit or at trace levels. Analysis of fractionated tissue extracts demonstrated the association of radioactivity with fractions involving the neutral lipid, free fatty acid and amino acid preparations. In no instance the parent compound or possible metabolites were detected.
Following seven administrations of 111 mg sodium ^{32}P-phosphonate/kg bw/day the urine was found to contain one radioactive component which was identified as the ^{32}P-phosphonate anion. The aqueous extracts of the faeces also contained the ^{32}P-phosphonate anion as the major component and lower amounts (up to 35% of the extracted radioactivity) of the ^{32}P-phosphate anion. Analysis of the tissue extracts revealed the presence of trace levels of the phosphonate anion in the kidney and intestinal tract samples. Trace levels were also observed in the female muscle samples.

Elimination
The major route of elimination following oral administration of ^{14}C-fosetyl-Al was the expired air (ca 56% of dose was expired as $^{14}CO_2$) followed by the urine (ca 30% of dose). The faeces proved to be a relatively minor route of elimination and accounted for only ca 4% of dose. The rate of elimination was rapid with the majority being completed within 24 hours of dose administration.
Blood kinetics following repeated dosing suggested a biphasic elimination with an initial rapid phase (half-life of 1 to 2 hours) followed by a longer elimination phase for the remaining low levels of radioactivity.
The major route of elimination following repeated administration of ^{32}P-fosetyl-Al was with the faeces (ca 54% of dose) followed by the urine (ca 36 to 38% of dose).
The major route of elimination following repeated administration with sodium ^{32}P-phosphonate was with the urine (ca 65 to 69% of dose) followed by the faeces (ca 30 to 32% of dose).

Conclusion
Orally administered ^{14}C-fosetyl-Al to rats is essentially completely absorbed, widely distributed, extensively metabolised and then rapidly excreted. The metabolic steps involve dissociation and hydrolysis of fosetyl to phosphonate and ethanol. The ethanol is oxidised via acetaldehyde and acetate to CO_2 and then excreted with the expired air. The phosphonate is partly excreted (along with some unchanged fosetyl) with the urine in unchanged form. The major portion of phosphonate is excreted with the faeces, partly oxidized microbially to phosphate. A small proportion of the administered radioactivity associated with the two-carbon metabolites (ethanol, acetate) would have been available for introduction into normal anabolic processes and thus account for the appearance of radioactivity in endogenous molecules.

CA 5.1.1 Absorption, distribution, metabolism and excretion by oral route

All studies on ADME following oral administration have been previously submitted and evaluated for the Annex I inclusion of fosetyl under Directive 91/414/EEC.

Report:	KCA 5.1.1/01 [redacted] ; 1982; M-161369-01-1
Title:	Fosetyl-Aluminium: Rat metabolism study - Single oral dose
Report No.:	R001824
Document No.:	M-161369-01-1
Guideline(s):	not specified
Guideline deviation(s):	not applicable
GLP/GEP:	**no**

I. MATERIALS AND METHODS

One group of 10 (5/sex) young adults CORS (Sprague-Dawley derived) rats (bw at dosing ranging from 163 to 190 g; average bw for 2 sexes = 181 g) were given a single oral administration of 1 mL of an aqueous suspension[2] of ^{14}C-fosetyl-aluminium at the rate of 3000 mg/kg bw.
Test material: Batch no. KWC 1053; specific activity: 1.91 mCi/mmol; radiochemical purity > 98%, free of significant radiochemical impurities (TLC).
13 mg ^{14}C-fosetyl-aluminium dissolved in water to which non-radioactive fosetyl-aluminium (fosetyl-Al) was added: the concentration of the resulting suspension was approximately 5.2 mg/L with a specific activity of 39.4 and 37.6 mCi/mol for the male and females, respectively.
The rats were housed singly for the duration of the experiment (168 hours) in metabolic cages which allowed for the total collection of urine, faeces, exhaled CO_2 and exhaled ethanol.
Exhaled ethanol and CO_2 were trapped in either ethanol or 4N aqueous potassium hydroxide, respectively (ethanol traps were renewed at 24 hour intervals up to and including 72 hours post-dose for the males and 48 hours for the females and then left until 144 hours before being disconnected; similarly, CO_2 traps were renewed at 24 hour intervals up to and including 72 hours and the left until 144 hours before being renewed for the 2 sexes).
Urine and faeces were collected at 0 to 24; 24 to 48; 48 to 72; 72 to 96; 96 to 120; 120 to 144 and 144 to 168 hours after dosing.
Blood samples were taken from each rat at approximately 0.25, 0.5, 1, 2, 4, 6, 24 hours post-dosing and at 24 hour intervals thereafter (it was primarily intended to collect sufficient blood for separate analysis of erythrocytes and plasma but this was disregarded because rats exhibited peripheral ischemia immediately after dosing).
Cage washings were done at 168 hours after administration.
All rats were sacrificed end-of-test (168 hours) post-dosed and tissues were taken or sampled for either immediate assay for radioactivity or stored (residual carcass was also retained for analysis). Sampled tissues included liver, kidneys, heart, lungs, brain, fat, skeletal muscle, adrenals, spleen, small intestinal + contents, large intestine + contents, caecum + contents ovaries and uterus, testes, eyes, Harderian glands, thyroid, skin and fur).
Radioactivity in urine, cage washes and trap exhaled ethanol and CO_2 solutions was measured directly using LSC; samples of faeces and tissues, homogenized in water, were combusted in an oxidizer and the evolved CO_2 was absorbed and total radioactivity was measured using LSC. Blood samples and residual carcass were solubilised prior to assay by LSC. Skin and fur were combusted prior to assay by LSC.

[2] Storage stability, stability and homogeneity in vehicle were not considered as applicable

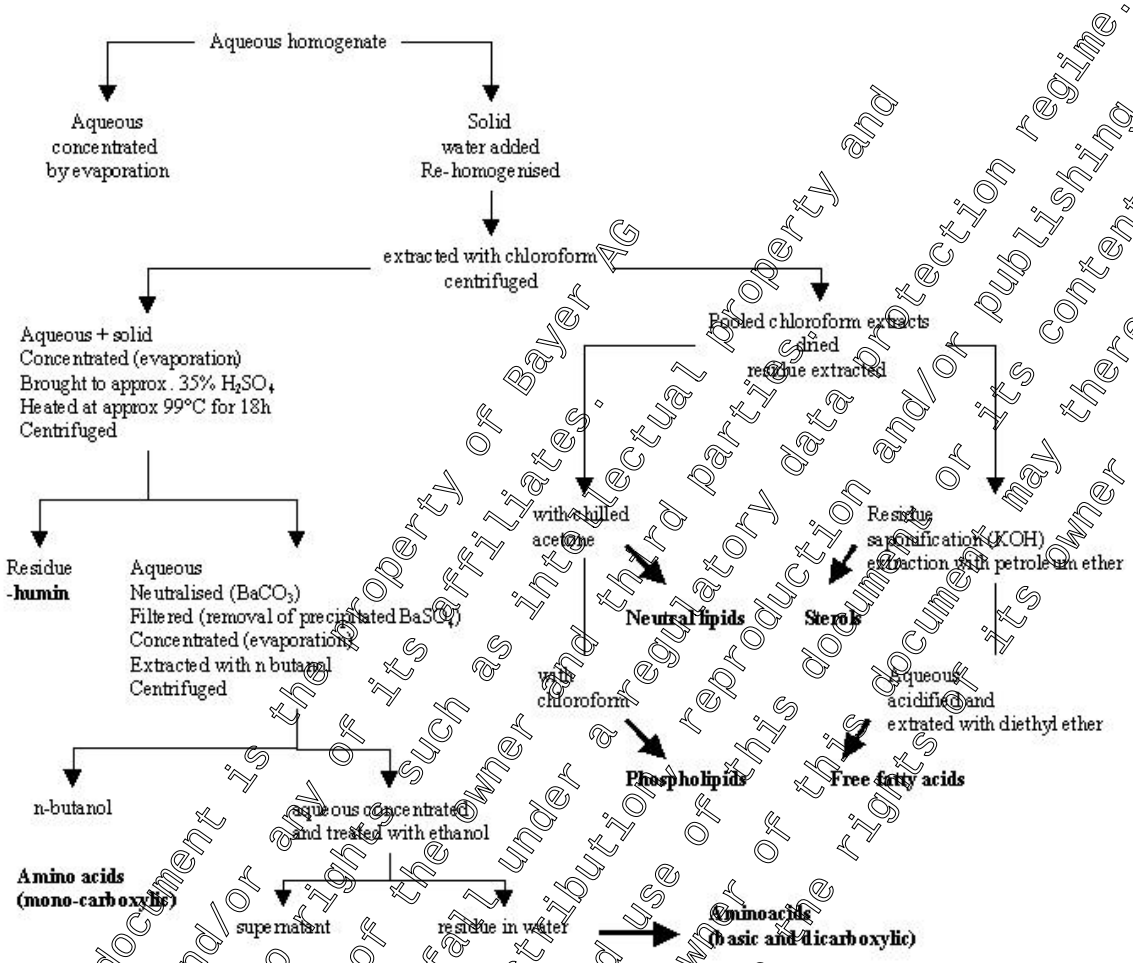
Fractionation, extraction and chromatographic procedures included:
For urine:
Chromatography on silica plates using 2 system solvents (in methanol/water/ammonia and acetonitrile/water mobile phase) and radio-gas chromatography.
Extraction of pooled urine samples from each time interval for both sexes with ethyl acetate and hexane for separate analysis of the organic and aqueous phase.
Extraction and isolation of the radiolabeled components from the 24-, 48- and 72 hours pooled urine samples for both sexes, using a solvent partition/salting out procedure (addition of ammonium sulphate and extraction with diethylether/ethanol). Following TLC analysis of extracts (in methanol/water/ammonia mobile phase), radiolabeled components were located by scanning and extracted with ethanol and further eluted on an ion exchange resin with hydrochloric acid; resultant residues were examined by nuclear magnetic resonance spectrometry and radio-gas chromatography.

For faeces:
Extraction of pooled faeces samples from each time interval for both sexes with ethyl acetate and hexane for separate analysis of the organic and aqueous phase.
Analysis of organic extracts and aqueous supernatants by TLC (in methanol/water/ammonia mobile phase).

For tissues:

- Fractionation using a multi-step procedure of the major part of the aqueous homogenate of pooled "suitable" tissues from each sex (see Figure 5.1.1-1), as indicated by radio-labeled content (treated tissues: liver, kidney, muscle, fat, heart, lung, brain, ovary (+ uterus) and testes).
- Water soluble fraction was concentrated and analysed by TLC (as for urine)
- Solid phase was extracted twice with chloroform. Chloroform extracts were used as a source of tissue lipids using acetone extraction (neutral lipids), chloroform extraction (phospholipids), saponification with KOH followed by extraction with petroleum ether (sterols) and acidification of the caustic solution followed by extraction with diethylether (free fatty acids); TLC analysis of lipidic fractions using various solvent systems known for their separating properties with respect to the different classes of lipids. Aqueous and solid phases serve as source of tissue proteins: after concentration and acidification by addition of sulfuric acid, samples were heated at 99 °C for 1h hour and centrifuged and then processed for separation humins and amino-acids. Amino-acids fractions were assayed by TLC (in n-butanol/glacial acetic acid/water mobile phase) as well as aqueous phases for which a second solvent system was used (chloroform/methanol/ammonia).

Figure 5.1.1- 1: Tissue fractionation scheme



II. RESULTS AND DISCUSSION

The total administered radioactivity was excreted in exhaled air, urine and to a much lesser extent in faeces within 168 hours after dosing; the mean recovery rates were 103.18% (range 94.65 to 111.56%) and 100.12% (range 91.36 to 108.07%) of the dose in males and females, respectively.
Excretion was rapid and occurred predominantly within the first 24 hours after dosing. The radioactivity was predominantly eliminated via the exhaled air as CO_2 with no significant difference between males and females. 24 hours after treatment, the mean $^{14}CO_2$ excretion accounted for 49.50% (males) and 49.70% (females), whereas insignificant amounts were found in ethanol traps (0.10 and 0.04% of dose in males and females, respectively). Excretion in the urine was the other main route of excretion, showing no main difference for individual values between sexes: the mean values 24 hours after treatment accounted for 32.75 and 32.34% of the administered dose in males and females, respectively. Excretion in faeces was considerably lower although females eliminated almost twice as much of the dose by this route that did the males: overall mean values (0 to 168 hours) were 1.85% in males and 3.60% in females (see Table 5.1.1- 1).
After 168 hours after dosing, approximately 4% of the administered dose was found in rats: 3.30 and 2.96% in the carcass and 0.99 and 1.17% in the tissues of males and females, respectively.

Table 5.1.1- 1: Group mean values (expressed as % administered dose) in urine, faeces, tissues and total recovery.

	Males			Females		
Time after dosing (h)	0-24	24-48	*0-168*	0-24	24-48	*0-168*
Urine	32.75	3.52	*37.39*	32.34	3.53	*37.90*
Faeces [1]	0.74	0.46	*1.85*	0.69	1.32	*3.30*
Exhaled air						
CO_2	49.50	6.93	*59.13*	49.70	5.47	*56.93*
Ethanol	0.10	0.06	*0.23* [2]	0.04	0.01	*0.05* [2]
Cage wash			*0.29*			*0.24*
Dissected tissues [3]			*0.99*			*1.10*
Carcass			*1.30*			*2.96*
Total recovery (%)			*103.18*			*100.12*

[1] corrected for combusting efficiency of 94.85%
[2] 0 to 144 h period
[3] corrected for combusting efficiency of 93.39%

Half-lives and elimination constant values calculated from blood radioactivity found in each rat; mean half-lives were 184 ± 69 hours and 129 ± 12 hours and mean elimination constant values were 0.0042 ± 0.0016 and 0.0054 ± 0.0006, in males and females respectively, indicating that females exhibited a 30% faster rate of elimination than males.
Radioactivity in tissues collected at terminal sacrifice was determined by wet combustion and LSC and converted to µg/g fosetyl-Al equivalent. ^{14}C was generally distributed throughout all tissues, with highest amounts found in kidney, liver, lung, spleen, fat, adrenal gland and gonads i.e. in tissues with high metabolic activity. No tissue contained less than 32 µg/g with many contents above 100 µg/g fosetyl-Al equivalent (see Table 5.1.1- 2). Mean concentration in the carcass was found to be 137.3 and 129.6 µg equiv./g in males and females, respectively.

Table 5.1.1- 2: Mean concentration (± SD) of fosetyl-Al in tissues at terminal sacrifice (µg equiv./g)

Tissue	Mean Concentration (µg equiv./g)		Tissue	Mean Concentration (µg equiv./g)	
	Males	Females		Males	Females
Liver	124.60 ± 24.45	149.90 ± 12.36	Oesophagus, stomach & contents	48.32 ± 30.42	98.48 ± 36.13
Kidneys	195.18 ± 24.43	258.34 ± 38.66	Small intestine & contents	94.00 ± 24.86	191.02 ± 24.88
Brain	129.24 ± 30.49	167.00 ± 19.54	Large intestine & contents	74.76 ± 14.78	111.30 ± 18.97
Spleen	144.96 ± 31.45	157.52 ± 13.52	Caecum & contents	38.88 ± 9.50	64.66 ± 9.20
Lungs	116.96 ± 28.36	155.46 ± 27.64	Adrenals	255.72 ± 80.88	656.00 ± 184.10
Heart	79.76 ± 11.94	90.30 ± 21.23	Thyroid	75.28 ± 33.93	151.72 ± 63.09
Muscle	72.90 ± 11.25	52.98 ± 14.16	Eyes	51.58 ± 14.97	68.43 ± 9.48
Fat	222.08 ± 114.69	167.12 ± 52.20	Bone & marrow	86.38 ± 24.82	74.12 ± 42.32
Testes	225.90 ± 22.08	n.a.	Skin	262.48 ± 119.51	138.38 ± 50.20
Ovaries and uterus	n.a.	164.56 ± 43.60	Fur	217.12 ± 74.50	200.74 ± 152.95

n.a.: not analysed

The nature of radioactivity was assayed in urine, faeces and tissues using various fractionation procedures. Examination of the 24-, 48- and 72 hours **urine** samples by TLC, radio-gas chromatography and NMR spectrometry revealed only one labelled component which was shown to be fosetyl-Al and which accounted for approximately 36% of the administered dose; radio-gas chromatography on 24 hours urine samples from 3 rats/sex showed the presence of ^{14}C-ethanol (0.5 to 2.2% of total urine radioactivity i.e. 0.2 to 0.4% of administered dose). In the 24 to 72 hours **faeces** samples, only fosetyl-Al was detected in aqueous extracts by TLC. In **tissues** selected for multi-step fractionation procedure, the radioactive content of water soluble, lipid and protein aqueous fractions was associated with numerous compounds involving neutral lipids, free fatty acids and amino-acids preparations, and in no instance parent or possible metabolites were detected.

III. CONCLUSION

RMS conclusion: Following a single oral administration of a 2000 mg/kg bw in rats, ^{14}C-fosetyl-Al is rapidly and totally absorbed and excreted, predominantly in the exhaled air (59% of the dose) and in the urine (35 to 37% of the dose) within 48 hours, with minor amounts in the faeces (2 to 3% of the dose). Excretion occurred mainly within 24 hours, indicating rapid absorption and elimination; no major sex differences were found. Large amounts of the radiolabel accounted for as $^{14}CO_2$ demonstrated that extensive metabolic transformation of the administered dose had occurred; this was confirmed by the presence of ^{14}C-ethanol in the urine which indicated that naturally occurring components would occur from the ^{14}C-ethyl moiety of the parent compound via acetaldehyde and acetate; in no instance fosetyl-Al was detected in any of the organs and tissues.

Report: KCA 5.1.1/02 [redacted]; 2001; M-205381-01-1
Title: The metabolism of 14C-fosetyl-Al in the rat
Report No.: C013400
Document No.: M-205381-01-1
Guideline(s): OECD: 417
Guideline deviation(s): none
GLP/GEP: yes

I. MATERIALS AND METHODS

An ADME study was performed following single oral administrations of ^{14}C-fosetyl-Al (batch CFQ 11651, specific activity 5.96 MBq/mg, radiopurity 99%) using two groups of 4 male and 4 female Sprague Dawley rats, at the nominal dose rates of 50 and 1000 mg/kg.
Urine and faeces were quantitatively collected at 24 hours intervals up to 168 hours post dose. Expired air was collected into an absorbing solution of monoethanolamine:ethanediol, 3:7 v/v, at 24 hour intervals up to 168 hours post dose. A cage wash with water was performed at 24 hour intervals up to 168 hours post dose. The sampled tissues were all removed or sampled 168 hours post dose administration and consisted of liver, kidneys, heart, lungs, brain, fat, skeletal muscle, spleen, gastrointestinal tract and contents, adrenals, bone and marrow, eyes, whole blood, plasma, testes, ovaries, thyroid, uterus, skin and fur and the residual carcass.
The levels of radioactivity present in the samples was either determined directly by liquid scintillation counting (LSC) or by LSC following a combustion technique.
Urine and faecal samples were prepared for chromatographic analysis using sequences of solvent extraction, centrifugation and evaporation under nitrogen. The presence of metabolites was investigated using a thin-layer chromatography technique and PhosphorImager detection. Gas-chromatography/Mass Spectrometric Detection was used to investigate levels of phosphonic acid in liver, kidney and muscle samples.

II. RESULTS AND DISCUSSION

The recovery of radioactivity is given in the following Table 5.1.1- 3.

Table 5.1.1- 3: Mean recovery of radioactivity

Sample	% of administered radioactivity							
	100 mg/kg				1000 mg/kg			
	Males		Females		Males		Females	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Urine	25.89	1.66	27.21	2.35	25.44	3.59	25.09	1.70
Faeces	4.46	0.85	3.80	0.97	8.1	2.26	5.04	0.9
Cage Wash	0.47	0.21	0.76	0.41	1.2	0.64	1.1	0.87
Exp Air-1	47.37	0.67	46.99	4.15	42.04	1.26	47.38	1.54
Exp Air-2	6.47	1.25	6.19	2.49	6.95	1.3	8.27	1.08
Tissues	0.97	0.08	0.90	0.12	0.8	0.13	1.01	0.14
Carcass	6.00	1.40	5.50	0.9	5.28	0.64	4.6	0.75
G.I. Tract & Cont.	0.52	0.09	0.55	0.06	0.63	0.1	0.59	0.11
Recovery	92.15	0.76	95.86	2.16	90.56	2.35	93.32	0.4

Exp Air-1 & -2 = expired air traps.
G.I. Tract & Cont. = gastrointestinal tract and contents.
SD = standard deviation

The main route of elimination of radioactivity was *via* expired air (approx. 50 to 60% of the dose) indicating extensive metabolic conversion of the ethyl-radiolabel to $^{14}CO_2$. The other main route of excretion was *via* urine (25 to 30% of the dose). Elimination of radiolabelled components *via* faeces was a minor (<10%) route of excretion. There was no significant difference in the excretion profiles at the low (100 mg/kg) and high (1000 mg/kg) dose levels. At 168 hours post dose there was some low retention of total radioactivity in the body (5 to 9% of the dose) which was distributed throughout the body. Highest concentrations were observed in the adrenals and fat.

Table 5.1.1- 4: Mean concentration of radioactivity in the tissues

Sample	µg equivalent/ g tissue							
	100 mg/kg				1000 mg/kg			
	Males		Females		Males		Females	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Adrenals	20.7	4.3	20.2	1.1	181.2	33.8	235.7	28.2
Bone Marrow	8.7	5.9	7.3	0.9	67.8	18.6	83.4	12.[illegible]
Bone	7.3	2.0	6.2	1.2	72.6	6.9	72.[illegible]	14.7
Brain	6.0	0.5	4.6	[illegible]	53.1	4.2	48.[illegible]	2.6
Eyes	3.7	0.2	3.2	0.2	34.0	3.1	30.7	1.2
Fat-Renal	29.8	10.2	31.8	13.0	443.[illegible]	48.5	434.8	102.[illegible]
Heart	7.1	0.4	6.5	0.2	67.[illegible]	5.6	69.[illegible]	2.3
Kidneys	10.9	1.4	16.8	1.2	113.2	14.2	131.1	14.9
Liver	7.1	1.0	7.2	0.5	66.3	10.[illegible]	64.4	3.[illegible]
Lungs	9.8	0.8	9.7	3.3	103.[illegible]	3.2	95.9	4.1
Muscle	8.2	0.7	7.1	5.[illegible]	85.7	9.6	88.[illegible]	9.6
Skin and Fat	12.5	0.7	11.4	6.4	202.7	22.[illegible]	153.8	31.0
Spleen	9.1	1.7	8.[illegible]	2.7	84.[illegible]	12.2	83.7	7.[illegible]
Thyroid	8.5	2.[illegible]	8.0	2.8	141.8	39.9	101.[illegible]	38.4
G.I. Tract & Cont.	4.1	0.6	4.9	0.[illegible]	56.2	13.6	57.[illegible]	5.5
Carcass	7.5	1.5	7.6	1.1	64.7	[illegible]	62.7	9.6
Whole Blood	8.2	0.[illegible]	8.3	0.4	66.[illegible]	4.3	54.[illegible]	4.6
Plasma	1.9	0.[illegible]	1.3	0.9	16.[illegible]	4.3	17.[illegible]	0.9
Testes	10.1	0.3	N/A	N/A	89.4	[illegible]	N/A	N/A
Ovaries	N/A	N/A	13.[illegible]	1.7	N/A	N/A	189.4	13.7
Uterus	N/A	N/A	9.1	3.5	N/A	N/A	181.9	89.8

G.I. Tract & Cont. = gastrointestinal tract and contents
N/A = not applicable

The distribution of total radioactivity following a single oral administration of ^{14}C-fosetyl-Al at the rate of 100 mg/kg was similar for male and female animals with the highest mean concentration of total radioactivity found in the renal fat (29.8 and 31.8 µg equiv./g) and adrenal gland (20.7 and 20.2 µg equiv./g) for male and female animals, respectively.
Lower concentrations were found in the kidneys (10.9 and 16.8 µg equiv./g), skin and fat (12.5 and 11.4 µg equiv./g) for male and female animals, respectively and ovaries (13.5 µg equiv./g). Most other tissues contained concentrations that were in the general range of 3 to 10 µg equiv./g.
The lowest mean concentrations were found in plasma (1.9 and 1.3 µg equiv./mL for male and female animals, respectively).
The distribution of total radioactivity following a single oral administration of ^{14}C-fosetyl-Al at the rate of 1000 mg/kg was similar for male and female animals. Actual concentrations achieved were approximately 10X those observed at the lower dose level. Highest mean concentrations of total radioactivity were found in the renal fat (443.[illegible] and 434.8 µg equiv./g), adrenal gland (181.2 and 235.7 µg equiv./g), and skin and fat (202.7 and 153.8 µg equiv./g) for male and female animals respectively. Concentrations of radioactivity in the ovaries (189.4 µg equiv./g) and uterus (181.9 µg equiv./g) of female rats were also relatively high.
Lower concentrations were found in the kidneys (113.2 and 131.1 µg equiv./g) and thyroid (141.8 and 101.6 µg equiv./g) for male and female animals respectively. Most other tissues contained concentrations in the range of 30 to 100 µg equiv./g.
The lowest mean concentrations were found in plasma (16.5 and 17.4 µg equiv./mL for male and female animals, respectively).

For urine and faeces samples selected for chromatographic analysis, procedural losses following the extraction of 0 to 24 and 24 to 168 hours faeces and 24 to 168 hours urine samples were high, with extraction recoveries ranging from 21.8 to 61.1% TRR (0.25 to 1.20% of dose). Centrifugation of the 0 to 24 hours urine samples gave extraction efficiencies ranging from 98.2 to 100.0% TRR (2.78 to 28.3% of dose).
TLC analysis of 24 to 168 hours urine samples and 0 to 24 and 24 to 168 hours faeces samples was unsuccessful due to low radioactivity and the nature of the extract. TLC chromatograms generated from 0 to 24 hours urine extracts contained 4 to 5 radioactive peaks. The major component in all chromatograms was ^{14}C-fosetyl-Al which represented 85.7 to 96.7% TRR (22.6 to 26.3% of dose) of the analysed radioactivity. The other components were minor, unidentified peaks, each one of which accounted for between 0.27 and 4.22% TRR (0.07 to 1.11% of dose).

III. CONCLUSION

RMS Conclusion: Following a single oral administration of 100 and 1000 mg/kg bw in rats, absorption of fosetyl-Al was extensive (> 90%) and excretion was rapid (> 70% excreted within 24 hours post-dosing). Excretion profile was similar in both sexes at both dose levels: the main routes of elimination were via the exhaled air (> 50%) indicating extensive metabolic conversion of the radiolabel to $^{14}CO_2$ and excretion via urine (19 to 30% of the administered dose). Elimination via the faeces was a minor route. At 168 hours post-dose, there was some low retention of radioactivity in the body (5 to 9% of the dose) which was distributed throughout the body (highest concentrations were found in the renal fat and in the adrenal glands). From the TLC analysis of 0 to 24 hours urine extracts (faeces samples and of 24 to 168 hours urine samples were unsuccessful due to procedural losses following extraction), it was concluded that the major urine component was fosetyl-Al (85.7 to 96.7% TRR i.e. 22.6 to 26.3% of the dose) and that 4 other (unidentified) components were minor (each one accounting for between 0.24 to 4.22% TRR i.e. 0.07 to 1.11% of the dose).

Report: KCA 5.1.1/03 [redacted], 1976, M-159160-01-1
Title: Aluminium ethyl phosphite (RS 74.788). Excretion study in rats
Report No.: R006709
Document No.: M-159160-01-1
Guideline(s): not specified
Guideline deviation(s): not applicable
GLP/GEP: **No**

Report: KCA 5.1.1/04 [redacted], 1976, M-161367-01-1
Title: Aluminium ethyl phosphite (RS 74.788) - Metabolism study in rats
Report No.: R006823
Document No.: M-161367-01-1
Guideline(s): not specified
Guideline deviation(s): not applicable
GLP/GEP: **no**

I. MATERIALS AND METHODS

An excretion study was performed using ^{14}C-fosetyl-Al (batch KWC 461, specific activity 13.48 mCi/mmol) and 3 male and 3 female rats. Each animal was dosed orally at 250 mg/kg/day for seven days. The animals were sacrificed 24 hours following the seventh dose administration. Selected tissues were taken for radioassay, as was the residual carcass.
Urine, faeces and exhaled air were collected daily until 24 hours after administration of the final dose. Urine, faeces, exhaled air and tissues were assayed by liquid scintillation techniques for total radioactivity.
Chromatographic investigations were performed using gas chromatography with a specific phosphorus detector following methylation of the phosphonate components.

II. RESULTS AND DISCUSSION

The mean recovery for the males was 98.84% and for the females was 96.16% (see Table 5.1.1- 5).

Table 5.1.1- 5: Mean recovery of radioactivity

Sample	Mean % of administered radioactivity	
	Male	Female
Urine	26.44	27.[illegible]
Faeces	2.86	1.78
Carbon dioxide	59.82	30.03
Ethanol	0.02	0.03
Cage wash	0.23	0.21
Tissues	4.95	3.96
Carcass	4.52	2.91
Total Recovery	98.84	96.[illegible]

These results indicate that the expired air (in the form of $^{14}CO_2$) was the major route of elimination accounting for approx. 60% of the administered dose. The urine was the next most important route representing approx. 27% of dose. Taking these figures and the levels of radioactivity left in the tissues it can be seen that the administered ^{14}C fosetyl Al was highly absorbed to around 95%. The results obtained on a daily basis showed that the absorption and elimination were rapid.

Table 5.1.1- 6: Mean concentration of radioactivity in the tissues

Tissue	Concentration of radioactivity (µg equiv./g)			
	Males		Females	
	Mean	S.D.	Mean	S.D.
Liver	465.30	3.80	362.17	20.47
Kidney	240.20	11.20	214.03	7.50
Brain	90	10.85	63.[illegible]	4.96
Spleen	181.47	8.34	208.73	7.[illegible]
Lungs	181.80	7.45	181.83	4.19
Heart	82.[illegible]	13.[illegible]	88.43	6.46
Muscle	40.43	3.02	48.50	7.52
Fat	651.53	9.79	116.33	32.13

S.D. = standard deviation

The liver, kidney, lungs, spleen and fat were found to contain radioactivity levels above 100 µg equiv./g in both sexes. The heart, muscle and brain were found to contain concentrations between approx. 70 to 90 µg equiv./g; these results being indicative of a wide distribution of radioactivity although not particularly high given that the rats had received seven administrations at a rate of 250 mg/kg and that the sacrifice occurred only 24 hours following the last dose.
The extent of the elimination as radioactive CO_2 demonstrated that the ^{14}C-fosetyl-Al had been extensively metabolised. The rate of elimination indicated that this metabolism had been rapid.

Table 5.1.1- 7: Recovery of fosetyl-Al and phosphonate

Sample	% of administered material					
	Males			Females		
	Fosetyl-Al	Phosphonate	Total	Fosetyl-Al	Phosphonate	Total
Urine	28.10	72.80	100.90	26.30	73.02	99.32
Faeces	0.01	3.78	3.79	0.02	0.67	0.69
Carcass	n.d.	0.30	0.30	n.d.	0.41	0.41
Intestinal Tract	n.d.	0.09	0.09	n.d.	0.47	0.47
Tissues	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Total	28.11	76.97	105.08	26.32	74.57	100.89

Fosetyl-Al underwent extensive hydrolysis *in vivo* to give ^{14}C-ethanol and phosphonate. The phosphonate produced was excreted predominantly (equivalent to 73% of the administered compound) in the urine together with unchanged material (up to 28% of the administered compound). Twenty-four hours after the final dose there was no evidence for unchanged compound in any of the body components examined. Small amounts (equivalent to 0.4 to 0.9% of the administered compound) of phosphonate were found in the carcass and intestinal tract of both groups of animals.
Phosphonate residues were generally absent from the tissues examined although samples of kidney and fat from the female group of rats gave residue levels of approximately 7 µg equiv./g and 51 µg equiv./g respectively. There was no evidence for the accumulation of phosphonate residues in any of the tissues.
The ^{14}C-ethanol that was produced was rapidly oxidised to $^{14}CO_2$ and was mainly excreted in the expired air. It is postulated that a small proportion of the ^{14}C-ethanol was incorporated into naturally occurring molecules *via* acetate and carbon dioxide thus leading to the residue levels of radioactivity observed in tissues.

III. CONCLUSION

RMS Conclusion: Following 7 daily consecutive oral administrations of 250 mg/kg bw ^{14}C-fosetyl-Al in rats, large amounts of CO_2 were excreted indicating the removal of the labeled ethyl group and possible subsequent metabolism via acetaldehyde and acetate giving rise to a wide range of naturally occurring molecules. Inasmuch as wide distribution of radioactivity and no unchanged compound nor phosphonate residues (except in kidney and fat from females) were found in organs and tissues. Although it might expected that the phosphonate moiety would be extensively oxidized to phosphate in vivo, results indicated that it was excreted quantitatively, predominantly in urine, without prior oxidation.

Report:	KCA 5.1.1/05[redacted]; 1977; M-158800-01-1
Title:	Aluminium ethyl phosphite-32P (LS 74.783-32P): Excretion study in rats
Report No.:	R000523
Document No.:	M-158800-01-1
Guideline(s):	not specified
Guideline deviation(s):	not applicable
GLP/GEP:	**no**

I. MATERIALS AND METHODS

An excretion study was performed with ^{32}P-fosetyl-Al (batch KWC 565, specific activity 5.9 mCi/mmol, radiopurity 92%) using 3 male and 3 female Sprague-Dawley rats. Each animal was dosed orally at approximately 250 mg/kg/day for seven days. Urine and faeces were collected daily until 72 hours after the administration of the final dose. The animals were then sacrificed to provide tissue samples. In addition blood samples were taken from each rat at intervals during the 24 hours after the first and last dose as well as at 24 hour intervals after the first dose for the duration of the experiment. Urine, faeces, tissue and blood samples were assayed by liquid scintillation techniques for radioactivity.

II. RESULTS AND DISCUSSION

The recovery of the administered radioactivity is summarised in the following Table 5.1.1- 8.

Table 5.1.1- 8: Mean recovery of radioactivity

Sample	Mean % of administered radioactivity	
	Male	Female
Urine	35.13	39.23
Faeces	58.10	54.56
Cage wash	1.14	1.29
Blood samples	n.d.	n.d.
Tissues	0.32	0.33
Carcass	1.05	0.83
Total Recovery	94.49	93.24

n.d. = not detected

The major part of the administered radioactivity was excreted in faeces (54%) with a smaller amount in the urine (35 to 39%). Minor amounts (1%) of the administered radioactivity were still present in the body 72 hours after cessation of dosing with the highest levels being found in the spleen (approx. 40 µg equiv./g). Mean radioactive residues in the other tissues examined were generally 10 to 26 µg equiv./g with the lowest levels being found in fat (approx. 6 µg equiv./g).

Table 5.1.1- 9: Mean concentration of radioactivity in the tissues

Tissue	Concentration of radioactivity (µg equiv./g)			
	Males		Females	
	Mean	S.D.	Mean	S.D.
Liver	24.60	5.96	25.50	6.84
Kidney	21.13	3.79	24.60	6.77
Brain	10.37	3.29	10.53	2.83
Spleen	40.47	15.81	40.00	11.35
Lungs	21.27	4.85	24.60	8.14
Heart	13.73	1.91	15.77	4.15
Muscle	18.93	4.75	16.90	6.50
Fat	6.47	3.31	5.50	1.59

S.D. = standard deviation

The levels of radioactivity in blood reached a maximum 1 to 2 hours after dosing whilst in some rats a second maximum level was attained 4 or 5 hours after dosing. The disappearance of radioactivity from the blood seemed to occur in two distinct steps. The first step was fairly rapid with a half-life of 1 to 2 hours whilst the second step was much slower with a half-life of 40 to 75 hours.

III. CONCLUSION

RMS Conclusion: Following 7 daily consecutive oral administrations of 250 mg/kg bw ^{32}P-fosetyl-Al in rats, radioactivity was predominantly excreted in the faeces (54%) and in the urine (36 to 38%). Minor amounts were still present in the body 72 hours after the last administration. Blood levels declined from an initial peak (1 to 2 hours post dose) following a 2 step pattern (half-lives of 1 to 2 hours and 40 to 75 hours).

Phosphonic acid

Report:	KCA 5.1.1/06 [redacted]; 1977; M-158817-01-1
Title:	Phosphorous-32P acid: Excretion study in rats
Report No.:	R000531
Document No.:	M-158817-01-1
Guideline(s):	not specified
Guideline deviation(s):	not applicable
GLP/GEP:	**no**

Report:	KCA 5.1.1/07 [redacted]; 1978; M-158849-01-1
Title:	Phosphorous-32P acid: Metabolism study in rats
Report No.:	R000546
Document No.:	M-158849-01-1
Guideline(s):	not specified
Guideline deviation(s):	not applicable
GLP/GEP:	**no**

I. MATERIALS AND METHODS

The metabolism of ^{32}P-phosphonic acid (batch 7[illegible] N1[illegible]33, specific activity 7.4 mCi/mmol on the 02/03/77) administered as its sodium salt was investigated using 3 male and 3 female rats fed orally at 111 mg/kg body weight daily for seven days.
Urine and faeces were collected daily until 72 hours after administration of the final dose. The animals were then sacrificed to provide tissue samples. In addition blood samples were taken from each rat at intervals during the 24 hours after the first and last dose as well as at 24 hour intervals after the first dose for the duration of the experiment. Urine, faeces, tissue and blood samples were assayed by liquid scintillation techniques for total radioactivity.
Metabolites were extracted from the biological matrices with water and the extracts radioassayed using a liquid scintillation counting technique. The presence of metabolites was investigated using thin-layer chromatography and gas-liquid chromatography (using a specific phosphorus, FTD, detector).

II. RESULTS AND DISCUSSION

The recovery of the administered radioactivity is given in the following Table 5.1.1- 10.

Table 5.1.1- 10: Mean recovery of radioactivity

Sample	Mean % of administered radioactivity	
	Male	Female
Urine	59.[illegible]	65.26
Faeces	32.02	29.85
Cage wash	5.96	3.28
Blood samples	0.01	0.01
Tissues	0.68	0.60
Carcass	1.02	1.35
Total Recovery	98.64	100.35

The major part of the administered radioactivity was excreted in urine (approx. 60 to 65%) with a smaller amount in the faeces (approx. 30 to 32%). Minor amounts (1.4 to 2%) of the administered radioactivity were still present in the body 72 hours after cessation of dosing.

Table 5.1.1- 11: Mean concentration of radioactivity in the tissues

Tissue	Concentration of radioactivity (µg equiv./g)			
	Males		Females	
	Mean	S.D.	Mean	S.D.
Liver	6.03	0.85	7.87	1.55
Kidney	6.47	0.87	7.23	1.45
Brain	3.23	0.35	3.67	0.86
Spleen	10.93	0.78	12.20	1.30
Lungs	5.73	1.40	5.50	1.48
Heart	4.77	0.74	5.33	1.50
Muscle	5.57	0.67	6.67	0.90
Fat	1.10	0.30	1.67	0.50

S.D. = standard deviation

The highest mean levels of radioactivity were found in the spleen (11 to 12 µg phosphonic acid equiv./g). The lowest mean levels of radioactivity were found in the fat (1 to 2 µg phosphonic acid equiv./g). The remaining tissues were found to contain radioactive concentrations of between approx. 5 to 8 µg equiv./g.

Levels in the blood rose rapidly following the first dose occasion with maximal values being obtained 1 to 2.5 hours following dosing. It appeared that the elimination of the radioactivity was at least a two stage process with an initial rapid elimination phase (half-life 1 to 3 hours) followed by a slower second stage. The sampling time was not long enough to establish an accurate half-life for the second elimination phase.

Chromatographic investigation into the nature of the radioactivity present in the urine samples indicated that there was only one radioactive component which was identified as being the ^{32}P-phosphonate anion.

Examination of the aqueous faecal extracts indicated the presence of the ^{32}P-phosphonate anion as the major component and also the presence of lesser amounts (up to 35% of the extracted radioactivity) of the ^{32}P-phosphate anion.

Analysis of the tissue extracts revealed the presence of trace levels of the phosphonate anion in the kidney and intestinal tract samples. Trace levels were also observed in the female muscle samples.

III. CONCLUSION

RMS conclusion: Following daily consecutive oral administrations of 111 mg/kg bw of ^{32}P-phophonic acid, radioactivity was predominantly excreted in the urine (59 to 65%) and in the faeces (30- to 32%), predominantly as unchanged phosphonate. Minor amounts were still present in the body, 72 hours after the last administration. Blood levels declined from an initial peak (1 to 2.5 hours post dose) following a 2 step pattern.

Aluminium

The EFSA Panel on Food Additives, Flavourings, Processing Aids and Food Contact Materials (AFC) has evaluated the safety of aluminium (Al) from dietary intake (EFSA J, 2008; 754; 1-34). Al is assumed to dissociate from the fosetyl anion when ingested. It is likely that fosetyl residues in plants or animal products associate with other naturally occurring cations, e.g. protons, sodium, calcium or potassium. Soluble Al can therefore be assessed independently from fosetyl.
In the following, the review by the EFSA AFC panel of ADME data is reproduced (emphasis added):
Available studies indicate that the oral bioavailability of Al in humans and experimental animals from drinking water is in the range of 0.3%, whereas the bioavailability of Al from food and beverages generally is considered to be lower, about 0.1%.
However, considering the available human and animal data, it is likely that the oral absorption of Al from food can vary at least 10-fold depending on the chemical forms present in the intestinal tract.
Except for sodium Al phosphate (SALP), acidic, none of the Al compounds authorised as food additives in the EU have been studied for bioavailability. The bioavailability of Al from SALP, acidic, when incorporated in a biscuit, was found to be about 0.1% in the rat. However, the Panel noted that in the FEEDAP opinion on Zeolite, a form of sodium Al silicate used in animal feed, it was stated that sodium Al silicate may be partly hydrolysed in the digestive tract, mainly in the abomasum (because of the low pH value) resulting in release of Al and silicate ions. Thus, in an unpublished study in cows, an increase of the Al serum level from 13 µg/L before treatment to 85 µg/L during a three week administration of 600 g Zeolite per day was reported.
This finding on sodium Al silicate in cows is in line with the suggestion by some authors that acid digestion in the stomach would solubilise most of the ingested Al compounds to the monomolecular species Al^{3+} (e.g. hydrated $Al(H_2O)_6^{3+}$). The Panel therefore noted that other insoluble Al-containing food additives that previously have been considered not to be absorbed from the gut can be expected to behave similarly.
After absorption, Al distributes unequally to all tissues in humans and accumulates in some. The total body burden of Al in healthy human subjects has been reported to be approximately 30 to 50 mg/kg bw. Normal levels of Al in serum are approximately 1 to 3 µg/L. About one-half of the total body burden of Al is in the skeleton, and about one fourth is in the lungs (from accumulation of inhaled insoluble Al compounds).
Reported normal levels in human bone tissue range from 5 to 10 mg/kg. Al has also been found in human skin, lower gastrointestinal tract, lymph nodes, adrenals, parathyroid glands, and in most soft tissue organs. In rats accumulation of Al was higher in the spleen, liver, bone, and kidneys than in the brain, muscle, heart or lung. It has also been reported that Al can reach the placenta and foetus and to some extent distribute to the milk of lactating mothers. Al levels have been found to increase with ageing in a number of tissues and organs (bone, muscle, lung, liver, and kidney) of experimental animals. The main carrier of Al^{3+} in plasma is the iron binding protein transferrin. Studies have demonstrated that about 89% of the Al^{3+} in plasma is bound to transferrin and about 11% to citrate. Cellular uptake of Al in organs and tissues is believed to be relatively slow and most likely occurs from the Al bound to transferrin by transferrin-receptor mediated endocytosis. There are two routes by which Al might enter the brain from the blood:

1) through the blood brain barrier (BBB) and
2) through the choroid plexuses into the cerebrospinal fluid of the ventricles within the brain and then into the brain.

Al has been shown to rapidly enter the brain extracellular fluid and the cerebrospinal fluid, with smaller concentrations in these than in the blood.
The distribution of Al may be modulated by several factors. Although citrate and fluoride have been shown to reduce tissue accumulation of Al and increase its renal excretion in experimental animals, this only occurs when the Al concentration exceeds the transferring metal binding capacity. This will seldom happen in humans. The iron status is negatively correlated with Al accumulation in tissues and animal experiments have shown that calcium and magnesium deficiency may contribute to accumulation of Al in the brain and bone.

Following ingestion in humans, absorbed Al from the blood is eliminated primarily by the kidneys, presumably as the citrate, and excreted in the urine. Unabsorbed Al is excreted in the faeces. Excretion via the bile constitutes a secondary, but minor route. The two most recent studies in humans that had normal renal function, did not consume any specific diet, took no medications containing Al, and had no other special exposure to Al, reported urine levels of Al of 3.3 (median) and 8.9 µg/L (mean), respectively.
Multiple values have been reported for the elimination half-life of Al in humans and animals, suggesting that there is more than one compartment of Al storage from which Al is eliminated.
Within the first day after receiving a single injection of ^{26}Al citrate, approximately 59% of the dose was excreted in the urine of six subjects. At the end of 5 days, it was estimated that 27% of the dose was retained in the body. However, when ^{26}Al levels were monitored for more than 3 or 10 years in a single subject that received the injection, half-lives of approximately 7 years and 50 years were estimated.
Initial half-lives of 2 to 5 hours were reported in rats, mice, rabbits and dogs after intravenous injection of soluble Al salts. When the sampling time was prolonged, the half-life of Al in rabbits was estimated to be 113, 74, 44, 42, 4.2 and 2.5 days in spleen, liver, lung, serum, kidney cortex, and kidney medulla, respectively. A second half-life in the kidney greatly exceeded 100 days. In rats, the whole organism elimination half-life was estimated to be 8 to 24 days in serum, kidney, muscle, liver, tibia and spleen.
Al persists for a very long time in the rat brain following intravenous injection of very small doses of ^{26}Al. A half-life of 150 days has been reported. However, this estimate is not expected to have a high degree of accuracy as brain samples were not obtained for at least 3 half-lives. Based on calculations for offspring of rats that were given ^{26}Al injections daily from day 1 to 20 postpartum and thereafter examined on days 40, 80, 160, 320 or 730 postpartum, elimination half-lives of approximately 13 and 1635 days in the brain were suggested. Half-lives of 7 and 520 days were suggested for parietal bone. For liver and kidneys half-lives were suggested to be 5 and 430 days and 5 and 400 days, respectively. In blood the values were 16 and 980 days.
There is little published information on allometric scaling of Al elimination rates that can be used to extrapolate these results from the rat to the human. For Al in the brain, 150 days is approximately 20% of, and 1365 days exceeds, the rat's normal life span. For comparison, the whole-body half-life of Al in the human was estimated to be 50 years.

CA 5.1.2 Absorption, distribution, metabolism and excretion by other routes

No studies have been conducted following other administration routes (e.g. intravenous, dermal) as these were not deemed to be needed to understand the ADME in the rat.

CA 5.2 Acute toxicity

New acute toxicity studies have been performed to fulfil legal requirements in India. All new studies are summarised in the following sections, in addition to summaries of studies that had been evaluated in the previous DAR.

Fosetyl-Al has a very low acute oral (LD_{50} > 5000 mg/kg bw, unspecific clinical signs), percutaneous (LD_{50} > 5000 mg/kg bw, no clinical signs) and inhalation (LC_{50} > 5.03 mg/L air, no clinical signs) toxicity in male and female rats (see Table 5.2- 1). Therefore, **no classification for acute lethal effects is required**, according to the criteria of Regulation 1272/2008.

In the eye irritation study by [redacted]; 1997; M-179082-01-1 the study was terminated after 72 hours when eye effects were still present. Although the severity of the eye effects did not qualify for classification as severe eye irritant, the substance was classified as severe irritant (Xi, R41; now Eye Dam. 1, H318) because the reversibility of the effects could not be proven (OECD 405 allows up to 21 days for a possible recovery). An older study which was not fully compliant with OECD 405 ([redacted]; [redacted]; 1981; M-229224-01-2) showed severe irritation when the eyes were not rinsed (OECD 405 allows the rinsing after 1 hour in case of solids). The eyes of another group of rabbits were rinsed, albeit at 1 minute, whereas the guideline allows rinsing not before 1 hour after instillation. A **new eye irritation study** ([redacted]; 2012; M-446501-01-1) was conducted. The scores for chemosis and conjunctival redness were ≥ 2 in at least two rabbits, thus exceeding the threshold for classification as eye irritant. Likewise, corneal opacity reached a score of 1.0 in at two rabbits, also fulfilling the criteria for classification as eye irritant.

However, all effects were reversible and the corneal effects did not reach or exceed the threshold for serious eye damage. There were no effects on iris. This leads to a **classification as Eye Irrit. 2, H319**.

Fosetyl-Al is irritating to eyes but not to skin (see Table 5.2- 1). It has no skin-sensitizing potential (see Table 5.2- 1) and is thus **not classified as skin sensitizer** according to the criteria of Regulation 1272/2008.

Table 5.2- 1: Acute toxicity studies with fosetyl-Al

Study Type	Species	Results	Reference
Acute oral toxicity	Rat	LD_{50} = 5400 mg/kg bw (♂+♀)	[redacted]; [redacted]; 1977; M-231363-01-2
	Rabbit	LD_{50} = 2500 mg/kg bw (♂+♀)	
	Rat	LD_{50} = 6250 mg/kg bw (♂) LD_{50} = 10 600 mg/kg bw (♀)	[redacted]; [redacted]; 1979; M-163431-01-1
	Mouse	LD_{50} = 5250 mg/kg bw (♂) LD_{50} = 5500 mg/kg bw (♀)	
	Rat	LD_{50} = 4080 mg/kg bw (♂+♀)	[redacted]; 1997; M-179086-01-1
		LD_{50} = 5600 mg/kg bw (♀)	[redacted]; 2013; M-447270-01-1
	Mouse	LD_{50} = 5000 mg/kg bw (♀)	[redacted]; 2013; M-454114-01-1
Acute dermal toxicity	Rat	LD_{50} > 2000 mg/kg bw (♂+♀)	[redacted]; [redacted]; 1977; M-231363-01-2
		LD_{50} > 2000 mg/kg bw (♂+♀)	[redacted]; 1997; M-179084-01-1
		LD_{50} > 2000 mg/kg bw (♂+♀)	[redacted]; 2012; M-446499-01-1
Acute subcutaneous toxicity	Mouse	LD_{50} = 3950 / 3800 mg/kg bw (♂/♀)	[redacted]; [redacted]; 1979; M-163431-01-1
	Rat	LD_{50} = 6600 / 7400 mg/kg bw (♂/♀)	

Study Type	Species	Results	Reference
Acute inhalation toxicity	Rat	LC_{50} > 1.73 mg/L air (4 h, MAC*, ♂+♀)	[redacted]; 1977; M-159162-01-1
		LC_{50} > 5.11 mg/L air (4 h, ♂+♀)	[redacted]; 1997; M-178978-01-1
		LC_{50} > 1.24 mg/L air (4 h, MAC, ♂+♀)	[redacted] 2013; M-451451-01-1
Skin irritation	Rabbit	Non-irritant	[redacted]; 1980; M-227207-01-2
		Non-irritant	[redacted]; 1997; M-179080-01-1
		Non-irritant	[redacted] 2013; M-449128-01-1
Eye irritation	Rabbit	Without rinsing: Severe irritant (effects not reversible at study termination) With rinsing after 1 min: not irritating	[redacted]; 1981; M-229224-01-2
		Severe eye irritant (effects not reversible at study termination), Eye Dam. 1, H318	[redacted]; 1997; M-179082-01-1
		Eye irritant (reversible effects), Eye Irrit. 2, H319	[redacted]; 2012; M-446501-01-1
Skin sensitisation (GPMT)	Guinea pig	Not sensitising (non-adjuvant injection method)	[redacted]; 1979; M-159691-01-1
		Not sensitising	[redacted]; 1998; M-179051-01-1
		Not sensitising	[redacted]; 2013; M-449129-01-1

* MAC = Maximum attainable concentration

CA 5.2.1 Oral

Report: KCA 5.2.1/01 [redacted]; 1977; M-231363-01-2
Title: LS 74-783 (aluminium ethyl phosphite, 32.45 R.P., aluminium salt): Acute toxicity in the rat and rabbit
Report No.: 30028
Document No.: M-231363-01-2
Guideline(s): no specified
Guideline deviation(s): not applicable
GLP/GEP: no

MATERIALS AND METHODS

Groups of 10 (5/sex) CD rats (bw range: 200 to 160 g) were given single doses of 0; 2.8; 4.2; 6.3 or 9.4 g/kg bw of fosetyl-Al (purity non specified) in 10% gum arabic (dose volumes: 10.0; 15.0; 22.5 and 33.7 mL/kg bw, respectively). Overt signs of toxicity and mortality were recorded for a 15-d test period. Bw was recorded every 5 days; gross examination was performed on all rats at terminal sacrifice.

Groups of 4/2/sex of fauve de Bourgogne rabbits were given single oral doses of 1.35; 2.0; 3.0; 4.5 and 6.7 g/kg bw of fosetyl-aluminium (fosetyl-Al) in 10% gum arabic (dose volumes: 4.5; 6.7; 10.0; 15.2 and 22.3 mL/kg bw, respectively). Overt signs of toxicity and mortality were recorded for a 15-d test period. Bw was recorded every 5 days; gross examination was performed on all rabbits at terminal sacrifice.

II. RESULTS AND DISCUSSION

In rats, sedation and dyspnoea were observed immediately after dosing, prior to depression and mortalities which occurred between 3 and 48 hours after dosing (see Table 5.2.1- 1). It is reported that in surviving rats, bw was significantly decreased on d-5 but that this reduction was no longer significant on d-10 and d-15 post-dosing (no data tabulated). Congestion of the glandular region of the stomach and of the kidney was observed in decedents but no macroscopic abnormalities were found in surviving animals.
In rabbits, sedation, dyspnoea and deaths were seen within 24 hours in the 4.5 g/kg bw group (no data on the high dose group); deaths were recorded on d-2/d-4 post-dosing following a period of depression in the 3 g/kg bw group. No bw changes were found. Marked irritation of the digestive tract with congestion of the gastric mucosa, petechiae and ulcerations were recorded at necropsy in decedent rabbits; no abnormal necropsy findings were recorded in surviving animals.

Table 5.2.1- 1: Death induced in rats and rabbits following single oral dose of fosetyl-Al

Deaths recorded during the 15 d observation period							
RATS				RABBITS			
Dose (g/kg)	Males	Females	2 sexes	Dose (g/kg)	Males	Females	2 sexes
2.8	0/5	0/5	0/10	1.33	0/2	0/2	0/4
4.2	0/5	1/5	1/10	2.0	0/2	0/2	0/4
6.3	3/5	5/5	8/10	3.0	2/2	2/2	4/4
9.4	5/5	5/5	10/10	4.5	2/2	2/2	4/4

III. CONCLUSION

The acute oral LD_{50} in rats and rabbits was greater than 2000 mg/kg bw. Thus, fosetyl-Al is not classified for acute oral toxicity according to the criteria of Regulation 1272/2008.
RMS conclusion: The oral LD_{50} of fosetyl-Al was approx. 6400 mg/kg bw for both sexes of CD rats, and 2500 mg/kg bw for both sexes in the "Fauve de Bourgogne" rabbit.

Report: KCA 5.2.1/02 [redacted]; [redacted]; 1979; M-163431-01-1
Title: Acute toxicity of LS 74-783 (aluminium ethyl phosphite) in rats and mice.
Report No.: R002834
Document No.: M-163431-01-1
Guideline(s): not specified
Guideline deviation(s): not applicable
GLP/GEP: no

I. MATERIAL AND METHODS

Groups of 20 (10/sex) CD rats (bw range: 103 to 108 g and 93 to 101 g in males and females, respectively) were given single oral doses of 6,000; 7,800; 10,140; 13,182 and 17,136 mg/kg bw of fosetyl-Al (Batch D47/36; purity = 96.9%) in a 10% solution of distilled water (dose volume: 1 mL/kg bw).
Groups of 20 (1/sex) CR mice (bw range: 24.4 to 28.7 g and 22.2 to 24.0 g for males and females, respectively) were given single oral doses of 2500, 3250, 4230, 5492, 6000 and 7140 mg/kg bw of fosetyl-Al (LS 74-783) in a 10% solution of distilled water (dose volume: 0.1 mL/kg bw).
Mortality was recorded for the 14-d observation period. Overt signs of toxicity were recorded daily and at 0.5 to 6 hours after dosing; bw was recorded initially and at d-7 and d-14 post-dosing. All animals were sacrificed and gross examination of abdominal and thoracic cavities was performed.

II. RESULTS AND DISCUSSION

The clinical signs observed in **rats** comprised depressive state in abdominal posture within 30 minutes after dosing (males and females), diarrhoea after 1 hour (at 2 higher dosages for males, and at the top dose for females) and nasal bleeding in both sexes after 24 hours (not seen at the 2 lowest dose levels). Deaths occurred after 24 to 48 hours post-dosing (see Table 5.2.1- 2). No particular clinical signs were recorded in surviving rats which recover from initial depression at 24 hours after dosing. No dose dependent difference of bw among groups was recorded. Hyperaemia and haemorrhage of gastric mucosa and distension of the stomach filled with white fluid was frequently seen in decedent rats. No particular finding was seen in surviving rats.

The clinical signs observed in **mice** comprised depressive state in abdominal posture within 30 minutes after dosing (males and females), abnormal gait, loss of righting reflex, abdominal distension. Deaths occurred within 1 to 4 days after dosing (see Table 5.2.1- 2). No particular clinical signs were recorded in surviving mice except which recover from initial depression at 24 hours after dosing. No dose dependent difference of bw among groups was recorded. Hyperaemia and hemorrhage of gastric mucosa and distension of the stomach filled with white fluid was frequently seen in decedent mice; ascites were observed in the 2 high dose male groups. No particular finding was seen in surviving mice.

Table 5.2.1- 2: Death induced in rats following single oral dose of fosetyl-Al

Dose level (mg/kg bw)	RATS		Dose level (mg/kg bw)	MICE	
	Males	Females		Males	Females
6000	0/10	0/10	2500	0/10	0/10
7800	0/10	1/10	3250	0/10	0/10
10141	3/10	4/10	4230	2/10	1/10
13182	8/10	8/10	5499	4/10	4/10
17130	10/10	10/10	7150	10/10	8/10
			9295	10/10	10/10

III. CONCLUSION

The acute oral LD_{50} in rats and mice was greater than 2000 mg/kg bw. Thus, fosetyl-Al is not classified for acute oral toxicity according to the criteria of Regulation 1272/2008.

RMS conclusion: The oral LD_{50} of fosetyl-Al was 11 280 [9534 to 13 275] mg/kg bw and 10 600 [9138 to 12 296] mg/kg bw in male and female SD rats, respectively, and 5250 [4102 to 6720] mg/kg bw and 5500 [4287 to 7040] mg/kg bw in male and females ICR mice, respectively.

Report:	KCA 5.2.1/03 [redacted]; 1997; M-179086-01-1
Title:	Fosetyl-Al: Acute oral toxicity in the rat.
Report No.:	R009340
Document No.:	M-179086-01-1
Guideline(s):	EU (=EEC): 92/69/EEC, V, B1, (1992); JMAF: 59 NohSan No.4200, (1985); OECD: 401, (1987); USEPA (=EPA): FIFRA 81-1, (1984)
Guideline deviation(s):	none
GLP/GEP:	**yes**

I. MATERIALS AND METHODS

Groups of 5 fasted male and female Sprague Dawley rats (6 to 7 week old) received a single oral administration of technical fosetyl-Al (batch 9607 01, purity 970 g/kg) by gavage at dose levels of 4200, 5000, 5950 or 7080 mg/kg body weight. Fosetyl-aluminium (fosetyl-Al) was suspended in 0.5% methyl cellulose in distilled water.
Animals were observed daily for clinical signs and mortality until death or sacrifice on Day 14. Body weights were recorded weekly.
At termination of the study, all surviving animals were autopsied and submitted to a macroscopic examination.

II. RESULTS AND DISCUSSION

Mortality occurred in all treated groups within 2 to 4 days post-dosing (see Table 5.2.1- 3). Clinical signs were observed at all dose levels from day 2 to day 3 and included pilo-erection, prostration, cold to touch and reduced motor activity. All animals had recovered on day 4, except two males treated at 7080 mg/kg which had an irregular breathing persisting until day 7 in one male and until day 8 in the other animal.
The body weight evolution of all animals was normal.
No significant gross findings were noted at the final sacrifice. However, the majority of animals found dead demonstrated non-specific changes such as stomach full of liquid, thickened stomach glandular wall with multiple black spots, red intestine content or haemorrhagic lungs.

Table 5.2.1- 3: Mortality induced by fosetyl-Al in Sprague Dawley rat after a single oral administration

Dose (mg/kg bw)	Male		Female	
	Mortality	Time of death in days (number of rats)	Mortality	Time of death in days (number of rats)
4200	1/5	2 (1)	0/5	-
5000	1/5	3 (1)	2/5	3 (2)
5950	0/5	-	2/5	2 (1) - 3 (1)
7080	1/5	4 (1)	2/5	2 (2)

III. CONCLUSION

The acute oral LD_{50} in rats is greater than 7080 mg/kg bw. Thus, fosetyl-Al is not classified for acute oral toxicity according to the criteria of Regulation 1272/2008.
RMS conclusion: The oral LD_{50} in Sprague Dawley rats was greater than 7080 mg/kg body weight for both sexes.

Report:	KCA 5.2.1/04 [redacted]; 2013; M-447270-01-1
Title:	Fosetyl-Al: Acute oral toxicity study (acute toxic class method) in Wistar rats
Report No.:	G8211
Document No.:	M-447270-01-1
Guideline(s):	OECD Guidelines for the Testing of Chemicals, Test No 423 (2001); Method B.1 tris: Acute toxicity (Acute toxic class method) of Annex to Commission Directive 2004/73/EC, 2004
Guideline deviation(s):	none
GLP/GEP:	**yes**

Executive Summary
An acute oral toxicity study with fosetyl-aluminium (fosetyl-Al) in Wistar rats was conducted according to the acute toxic class method (OECD 423). The test item was dissolved in water and was administered by oral gavage to three fasted female rats at the dose of 2000 mg/kg bw. A vehicle control group consisting of further three females was administered water. There were no clinical signs of toxicity and mortality observed in the treatment and vehicle control group. Based on the testing scheme of OECD 423, three additional female rats were tested at the same dose of 2000 mg/kg bw and three additional vehicle control animals were administered water. There were no clinical signs of toxicity or mortality observed in treatment and vehicle control group.
The rats of treatment and control groups were subjected to necropsy at termination and there were no abnormalities detected. Thus, an LD_{50} cut-off value of 5000 mg/kg bw is assigned according to Annex 2d of OECD Guideline 423.
Based on this result, fosetyl-Al is not classified for acute oral toxicity according to the criteria of Regulation 1272/2008.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material:

Name:	Fosetyl-Al
Description:	White powder
Batch / Lot No.:	12020045
Purity:	97.1%
Stability of test compound:	Expiry date: 2014-07-05. Stability and homogeneity in vehicle were analytically verified.

2. Vehicle: Deionised water

3. Test animals

Species:	Rat
Strain:	Wistar rats - HsdCpb:WU
Sex:	Females
Age:	8-9 weeks
Weight at dosing:	152.6 - 181.6 g
Source:	[redacted] India
Acclimatisation period:	5-7 days
Diet:	Teklad Certified (2014C) Global 14% Protein Rodent Maintenance Diet – Pellet (Certified), *ad libitum*
Water:	Charcoal-filtered and UV-irradiated deep bore-well water, *ad libitum*
Housing:	Individually in standard polysulfone cages with corn cob bedding

Environmental conditions:

Temperature:	20-23°C
Humidity:	58-67%
Air changes:	12-15 h^{-1}
Photoperiod:	12 h light / 12 h dark

B. STUDY DESIGN AND METHODS

1. In life dates: 2012-09-14 to 2012-10-10

2. Animal assignment and treatment

Dose:	0, 2000 mg/kg bw
Application route:	Oral, gavage
Application volume:	10 mL/kg bw
Fasting time:	Before administration: 16-18 h
Group size:	3 rats/group per step
Post-treatment observation period:	14 days
Observations:	Clinical signs, mortality, body weight, gross necropsy

II. RESULTS AND DISCUSSION

A. MORTALITY

There were no mortalities (see Table 5.2.1- 4).

Table 5.2.1- 4: Results of the acute toxic class test in rats with fosetyl-Al

Dose (mg/kg bw)	Toxicological result*			Onset and duration of signs	Onset of death	Mortality (%)
Female rats – 1st Step						
0	0	0	3	–	–	0
2000	0	0	3		–	0
Female rats – 2nd Step						
0	0	0	3	–	–	0
2000	0	0	3		–	0
LD_{50} = 5000 mg/kg bw (females)**						

* 1st number = number of dead animals, 2nd number = number of animals with toxic signs, 3rd number = number of animals used

** LD_{50} cut-off according to Annex 2d of OECD Guideline 423

B. CLINICAL OBSERVATIONS

There were no clinical signs of toxicity (see Table 5.2.1- 4).

C. BODY WEIGHT

There were no effects on body weight.

D. NECROPSY

There were no abnormalities observed at necropsy.

III. CONCLUSION

Fosetyl-Al is non-toxic after oral administration. The acute oral LD_{50} of female rats was greater than 2000 mg/kg bw. Thus, fosetyl-Al is **not classified for acute oral toxicity** according to the criteria of Regulation 1272/2008.

Report:	KCA 5.2.1/05 [redacted]; 2013; M-454114-01-1
Title:	Fosetyl-Al: Acute oral toxicity study (acute toxic class method) in swiss albino mice
Report No.:	G8756
Document No.:	M-454114-01-1
Guideline(s):	OECD Test No 423 (2001) Method B.1 tris of Annex to Commission Directive 2004/73/EC, 2004
Guideline deviation(s):	none
GLP/GEP:	**yes**

Executive Summary

An acute oral toxicity study with fosetyl-aluminium (fosetyl-Al) in Swiss albino mice was conducted according to the acute toxic class method (OECD 423). The test item was dissolved in water and was administered by oral gavage to three fasted female mice at the dose of 2000 mg/kg bw. A vehicle control group (three females) was administered water. There were no clinical signs of toxicity and mortality observed in the treatment and vehicle control group. Based on the testing scheme of OECD 423, three additional female mice were tested at the same dose of 2000 mg/kg bw and three additional vehicle control animals were administered water. There were no pre-terminal deaths in treatment groups and clinical signs of toxicity and mortality observed in treatment and vehicle control group.

The mice of treatment and control groups were subjected to necropsy at termination and there were no abnormalities detected. Thus, an LD_{50} cut-off value of 5000 mg/kg bw is assigned according to Annex 2d of OECD Guideline 423.

Based on this result, fosetyl-Al is not classified for acute oral toxicity according to the criteria of Regulation 1272/2008.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material:

Name:	Fosetyl-Al
Description:	White powder
Batch / Lot No.:	12020045
Purity:	97.1%
Stability of test compound:	Expiry date: 2014-07-05. Stability and homogeneity in vehicle were analytically verified.

2. Vehicle: Deionised water

3. Test animals

Species:	Mouse
Strain:	Swiss Albino
Sex:	Females
Age:	10-11 weeks
Weight at dosing:	27.80- 35.69 g
Source:	[redacted], India
Acclimatisation period:	5-7 days
Diet:	Teklad Certified (2014C) Global 18% Protein Rodent Maintenance Diet – Pellet (Certified), *ad libitum*

Water:	Charcoal-filtered and UV-irradiated deep bore-well water, *ad libitum*
Housing:	Individually in standard polysulfone cages with corn cob bedding
Environmental conditions:	
Temperature:	21-24°C
Humidity:	65-67%
Air changes:	12-15 h^{-1}
Photoperiod:	12 h light / 12 h dark

B. STUDY DESIGN AND METHODS

1. In life dates: 2013-03-08 to 2013-03-29

2. Animal assignment and treatment

Dose:	0, 2000 mg/kg bw
Application route:	Oral, gavage
Application volume:	10 mL/kg bw
Fasting time:	before administration: 16-18 h
Group size:	3 mice/group per step
Post-treatment observation period:	14 days
Observations:	clinical signs, mortality, body weight, gross necropsy

II. RESULTS AND DISCUSSION

A. MORTALITY

There were no mortalities (see Table 5.2.1- 5).

Table 5.2.1- 5: Results of the acute toxic class test in mice with fosetyl-Al

Dose (mg/kg bw)	Toxicological result*			Onset and duration of signs	Onset of death	Mortality (%)
Female mice – 1st Step						
0	0	0	3	–	–	0
2000	0	0	3	–	–	0
Female mice – 2nd Step						
0	0	0	3	–	–	0
2000	0	0	3	–	–	0
LD_{50} = 5000 mg/kg bw (females)**						

* 1st number = number of dead animals, 2nd number = number of animals with toxic signs, 3rd number = number of animals used

** LD_{50} cut-off according to Annex 2D of OECD Guideline 420

B. CLINICAL OBSERVATIONS

There were no clinical signs of toxicity (see Table 5.2.1- 5).

C. BODY WEIGHT

There were no effects on body weight.

D. NECROPSY

There were no abnormalities observed at necropsy.

III. CONCLUSION

Fosetyl-Al is non-toxic after oral administration. The acute oral LD_{50} of female mice was greater than 2000 mg/kg bw. Thus, fosetyl-Al is **not classified for acute oral toxicity** according to the criteria of Regulation 1272/2008.

CA 5.2.2 Dermal

Report:	KCA 5.2.2/01 [redacted] ; [redacted] ; 1977; M-231363-01-2
Title:	LS74-783 (aluminium ethyl phosphite, 32545 R.P., aluminium salt): Acute toxicity in the rat and rabbit.
Report No.:	R002835
Document No.:	M-231363-01-2
Guideline(s):	not specified
Guideline deviation(s):	not applicable
GLP/GEP:	**no**

I. MATERIALS AND METHODS

Groups of 10 (5/sex) CD rats (bw range: 210 to 250 g) were given a single dermal dose of 0 or 3 g/kg bw of fosetyl-Al (purity non specified) in distilled water (dose volume: 5.0 mL/kg bw; maximum possible concentration). Overt signs of toxicity and mortality were recorded for a 15 d test period. Bw was recorded every 5 d; gross examination was performed on all rats at terminal sacrifice.

II. RESULTS AND DISCUSSION

No deaths or clinical signs were observed; the bw gain was similar to that of controls (no data). No abnormal findings were recorded at necropsy.

III. CONCLUSION

The acute percutaneous LD_{50} in rats was greater than 3000 mg/kg bw. Thus, fosetyl-Al is not classified for acute percutaneous toxicity according to the criteria of Regulation 1272/2008.
RMS conclusion: The dermal LD_{50} of fosetyl-Al was > 3000 mg/kg for both sexes of CD rats.

Report:	KCA 5.2.2/03 [redacted] ; 1997; M-179084-01-1
Title:	Fosetyl-AL: Acute dermal toxicity in the rat.
Report No.:	R009861
Document No.:	M-179084-01-1
Guideline(s):	EU (=EEC): 92/69/EEC A.7, B.3, (1992); JMAF: 59 NohSan No.4200, (1985); OECD: 402, (1987); US EPA (=FIFRA): FIFRA 81-2, (1984)
Guideline deviation(s):	none
GLP/GEP:	**yes**

I. MATERIALS AND METHODS

Groups of 5 male and female Sprague Dawley rats (7-week old) received a single topical administration of technical fosetyl-Al (batch 1007181, purity 970 g/kg) at a dose level of 2000 mg/kg body weight. Fosetyl-aluminium (fosetyl-Al) was dissolved in distilled water and applied to the shaven dorsal skin. The treated area (approximately 10% of the body surface) was then covered with a semi-occlusive dressing. After a 24-hour exposure period, any residual test substance was removed with water.
Animals were observed daily for clinical signs and mortality on the day of dosing and once daily thereafter until death or sacrifice on Day 14. Body weights were recorded weekly.
At termination of the study, all surviving animals were autopsied and subjected to a macroscopic examination.

II. RESULTS AND DISCUSSION

No treatment-related deaths or clinical signs were observed throughout the study.
The body weight evolution was normal for all animals except for one male which showed a lower body weight on Day 15 only.
No significant gross findings were noted at the final sacrifice.

III. CONCLUSION

The acute percutaneous LD_{50} in rats was greater than 2000 mg/kg bw. Thus, fosetyl-Al is not classified for acute percutaneous toxicity according to the criteria of Regulation 1272/2008.
RMS conclusion: The dermal LD_{50} in Sprague Dawley rats was greater than 2000 mg/kg bw for both sexes.

Report:	KCA 5.2.2/04 [redacted] 2012; M-446499-01-1
Title:	Fosetyl-Al: Acute dermal toxicity study in Wistar rats
Report No.:	G8212
Document No.:	M-446499-01-1
Guideline(s):	OECD Guidelines for the Testing of Chemicals, Test No 402 (1987), Method B.3 (Acute Toxicity - Dermal) Part B of Annex to Commission Directive 92/69/EEC of 31st July 1992
Guideline deviation(s):	none
GLP/GEP:	**yes**

Executive Summary
An acute dermal toxicity study with fosetyl-aluminium (fosetyl-Al) in Wistar rats was conducted as a limit test according to OECD 402. The test item was moistened in water and was administered semi-occlusively to groups of each five male and female rats at the dose of 2000 mg/kg bw. The exposure duration was 24 h after which the application site was cleaned with water and soap. There were no clinical signs of toxicity, local skin reactions or mortality observed. The rats were subjected to necropsy at termination and there were no abnormalities detected.
Based on this result, fosetyl-Al is not classified for acute percutaneous toxicity according to the criteria of Regulation 1272/2008.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material:

Name:	Fosetyl-Al
Description:	White powder
Batch / Lot No.:	12020045
Purity:	97.1%
Stability of test compound:	Expiry date: 2014-07-05.

2. Vehicle: Moistened with deionised water

3. Test animals

Species:	Rat
Strain:	Wistar rats - HsdCpb: WU
Sex:	Males and females
Age:	8-9 weeks
Weight at dosing:	Males : 260.5-272.4 g Females: 212.9-220.7 g
Source:	[redacted], India

Acclimatisation period:	5 days
Diet:	Teklad Certified (2014C) Global 14% Protein Rodent Maintenance Diet – Pellet (Certified), *ad libitum*
Water:	Charcoal-filtered and UV-irradiated deep bore-well water, *ad libitum*
Housing:	Individually in standard polysulfone cages with corn cob bedding
Environmental conditions:	
Temperature:	20-23°C
Humidity:	58-67%
Air changes:	12-15 h^{-1}
Photoperiod:	12 h light / 12 h dark

B. STUDY DESIGN AND METHODS

1. In life dates: 2012-09-21 to 2012-10-10

2. Animal assignment and treatment

Group size:	5 rats/sex
Dose:	2000 mg/kg bw
Application route:	Dermal, semi-occlusive
Application area:	Ca. 10% of body surface
Exposure duration:	24 h
Test substance removal:	The treated area was washed initially with water and soap and next with water. Washed animals were wiped dry with a cotton hand towel.
Post-treatment observation period:	14 days
Observations:	Clinical signs, local skin reactions, mortality, body weight, gross necropsy

II. RESULTS AND DISCUSSION

A. MORTALITY

There were no mortalities (see Table 5.2.2- 1).

Table 5.2.2- 1: Results of the acute percutaneous toxicity test with fosetyl-Al

Dose (mg/kg bw)	Toxicological result*			Onset and duration of signs	Onset of death	Mortality (%)
Male rats						
2000	0	0	5	–	–	0
Female rats						
2000	0	0	5	–	–	0
LD_{50}: >2000 mg/kg bw (males and females)						

* 1st number = number of dead animals, 2nd number = number of animals with toxic signs, 3rd number = number of animals used

B. CLINICAL OBSERVATIONS

There were no clinical signs of toxicity (see Table 5.2.2- 1).

C. LOCAL SKIN REACTIONS

There were no skin reactions observed at the site of application.

D. BODY WEIGHT
There were no effects on body weight.

E. NECROPSY
There were no abnormalities observed at necropsy.

III. CONCLUSION

Fosetyl-Al is non-toxic after dermal administration. The acute percutaneous LD_{50} for both sexes was greater than 2000 mg/kg bw. Thus, fosetyl-Al is **not classified for acute percutaneous toxicity** according to the criteria of Regulation 1272/2008.

CA 5.2.3 Inhalation

Report:	KCA 5.2.3/01 [redacted]; [redacted] 1977, M-159162-01-1
Title:	Acute inhalation toxicity in rats - Four hour exposure to the dust of LS74783 (technical)
Report No.:	R000710
Document No.:	M-159162-01-1
Guideline(s):	not specified
Guideline deviation(s):	not applicable
GLP/GEP:	**no**

I. MATERIALS AND METHODS

14 (7/sex) Sprague-Dawley rats were submitted to a "whole body" exposure for 4 hours to a dust atmosphere of technical fosetyl-Al (99.8% purity) which was generated at the mean achieved concentration of 1.73 ± 0.23 mg/m³ air (nominal concentration: 4.79 mg/m³). A control group of 14 (7/sex) Sprague-Dawley rats was used.
Gravimetric analysis of the dust concentration was performed.
Clinical observations for abnormal signs were conducted on all rats during the exposure period and at least twice daily thereafter. Two rats/sex from the control and exposed groups were killed immediately after exposure for assessing the primary irritant effect on the respiratory tract. Individual bw were determined on the day of exposure and on d-1, d-3, d-7, d-10 & d-14 after the exposure period. Necropsies were performed on all rats at termination for macroscopic examination of the lungs, the trachea, the heart and the oesophagus.

II. RESULTS AND DISCUSSION

The actual mean air dust concentration was shown to be of 1.73 ± 0.23 mg/m³ air i.e. 36% of the nominal concentration. GC analysis of samples showed concentration of 1.67 ± 0.27 mg/m³.
No deaths occurred. Non specific clinical signs were recorded during exposure and included difficulty of breathing, gasping at end of exposure period; thereafter dark red discharge around the snout was seen. Breathing difficulties disappeared overnight after the exposure period and all rats behaved normally during the entire follow-up 14 d period. No change in bw were recorded except on d-1 post-dosing. Few scattered spots of haemorrhage in lungs were seen in only 1 rat killed immediately after exposure and no significant changes were recorded at study termination.

III. CONCLUSION

RMS Conclusion: The LC_{50} (4 hours) was > 1.73 mg/L (analytical) for both sexes of Sprague Dawley rats.

Report:	KCA 5.2.3/02[redacted]; 1997; M-178978-01-1
Title:	Fosetyl-Al: Acute inhalation toxicity (nose only) study in the rat.
Report No.:	R009243
Document No.:	M-178978-01-1
Guideline(s):	EU (=EEC): 92/69/EEC, B2; OECD: 403, (1981); USEPA (=EPA): F, 81-3
Guideline deviation(s):	none
GLP/GEP:	**yes**

I. MATERIALS AND METHODS

Groups of 5 male and female Sprague-Dawley rats (8 to 10-week old) were exposed (nose only system) continuously for 4 hours to a dust atmosphere of technical fosetyl-Al (batch no. 960708, purity 970 g/kg).
The main exposure parameters were as follows:

Table 5.2.3- 1: Main characteristics of the achieved atmosphere

Parameter	Value
Flow rate	20 L/min
Mean achieved concentration	5.11 mg/L
Nominal concentration	45.6 mg/L
Particles < 4.0 µm	98%
Mean mass aerodynamic diameter	2.4 µm

Animals were observed for clinical signs and mortality at hourly intervals during exposure and then once daily until death or sacrifice on Day 14. Body weights were recorded weekly.
At termination of the study all surviving animals were autopsied and subjected to a complete macroscopic examination.

II. RESULTS AND DISCUSSION

No treatment-related deaths were observed throughout the study.
Clinical signs were confined to wet fur, hunched posture, lethargy, pilo-erection, laboured and noisy respiration, decreased respiratory rate and frequent sneezing. Incidents of ptosis, tiptoe gait, ataxia, red/brown staining around the eyes and/or snout. Respiratory difficulties persisted but all animals recovered three to five days after exposure.
Treatment-related decreases in body weight gain were observed in all males and one female during the first week and in one male and in one female during week 2.
Macroscopic examination only revealed dark foci in the lungs of 3 males and one female at the final sacrifice.

III. CONCLUSION

The 4-h LC_{50} in rats was greater than 5 mg/L. Thus, fosetyl-Al is not classified for acute inhalation toxicity according to the criteria of Regulation 1272/2008.
RMS conclusion: The inhalation median LC_{50} (4 hours) in Sprague-Dawley rats was greater than 5.11 mg/L for both sexes.

Report:	KCA 5.2.3/03 [redacted]; 2013; M-451451-01-1
Title:	Fosetyl-Al: Acute inhalation toxicity study in Wistar rats
Report No.:	G8213
Document No.:	M-451451-01-1
Guideline(s):	OECD Guideline for the Testing of Chemicals, Section 4, No. 403; adopted 7 September, 2009; Method B.2 (Acute toxicity - Inhalation) Part B of Annex to Commission Directive 92/69/EEC of 31st July, 1992
Guideline deviation(s):	none
GLP/GEP:	**yes**

Executive Summary

The inhalation toxicity of fosetyl-aluminium (fosetyl-Al) was determined in 5 male and 5 female Wistar rats by exposure to aerosol of 25% w/v dilution of test item in deionised water generated by glass atomizer with an injection rate of 0.8 mL/min with 1.4 kg/cm² of atomizer pressure. Similarly, rats in the vehicle control group were exposed to deionized water aerosol.

The rats of control and treated groups kept in special rat restrainers were continuously exposed to the test item and vehicle aerosol (nose only) for 4 hours in an inhalation exposure chamber. The post treatment observation period was 14 days.

The aerosol sampled from the inhalation chamber for particle size analysis showed a mean aerosol particle size of 1.21 µm (GSD 2.13) for the treated group.

The aerosol was highly respirable with 99% of the particles in the treated group being smaller than 3.98 µm. The analytically determined average concentration of fosetyl-Al in the treated group was 1.24 mg/L.

Nasal discharge was observed in all treated rats on Day 1. All the rats were free of symptoms from Day 2 onwards. There were no pre-terminal deaths. Upon necropsy, no abnormality was detected in any of the rats.

The 4-h LC_{50} value of fosetyl-Al is greater than 1.24 mg/L of chamber air (maximum attainable concentration) in both male and female Wistar rats.

Based on this result, fosetyl-Al is not classified for acute inhalation toxicity according to the criteria of Regulation 1272/2008.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material:

Name:	Fosetyl-Al
Description:	White powder
Batch / Lot No.:	12020045
Purity:	97.1%
Stability of test compound:	Expiry date: 2014-07-05

2. Vehicle:

Deionised water

3. Test animals

Species:	Rat
Strain:	Wistar rats - HsdCpb: WU
Sex:	Males and females
Age:	11-12 weeks
Weight at dosing:	Males: 285.7-299.0 g Females: 193.5-211.6 g
Source:	[redacted], India
Acclimatisation period:	5 days
Diet:	Teklad Certified (2014C) Global 14% Protein Rodent Maintenance Diet – Pellet (Certified), *ad libitum*
Water:	Charcoal-filtered and UV-irradiated deep bore-well water, *ad libitum*

Housing:	Individually in standard polysulfone cages with corn cob bedding
Environmental conditions:	
Temperature:	21-23°C
Humidity:	65-67%
Air changes:	12-15 h^{-1}
Photoperiod:	12 h light / 12 h dark

B. STUDY DESIGN AND METHODS

1. In life dates: 2013-01-03 to 2013-02-14

2. Animal assignment and treatment

Test concentration:	Sighting study: 0.63 mg/L (measured) Main study: 0, 1.24 mg/L (measured)
Application route:	Inhalation, aerosol, nose only
Group size:	3 rats/sex/group (sighting study) 5 rats/sex/group (main study)
Exposure duration:	4 h
Post-treatment observation period:	14 days
Observations:	Clinical signs, mortality, body weight, gross necropsy

3. Generation of test atmosphere

Exposure apparatus:	
Preparation of test item suspension:	50 g of the test item was mixed with deionised water and the volume was made up to 200 mL to get the test item suspension with a concentration of 25% w/v.
Source and rate of air:	20 L/min
System of generating aerosols:	Glass atomizer, pressure: 1.4 kg/cm²
Method of particle size determination:	Laser-based Galai CIS-50 Aerosol Particle Size Analyser, Galai Pvt. Ltd., Israel

4. Test atmosphere

Temperature and humidity in air chamber:	Controls: 18.9-21.7°C, 63.12-71.53% RH Treated: 18.3-21.1°C, 71.83-77.95% RH
Particle size distribution:	Controls: 90% of particles were < 4.08 µm Treated: 99% of particles were < 3.98 µm
MMAD (GSD):	Controls: 2.23 µm (2.06) Treated: 1.21 µm (2.13)

II. RESULTS AND DISCUSSION

A. MORTALITY

There were no mortalities (see Table 5.2.3- 2).

B. CLINICAL OBSERVATIONS

Nasal discharge was observed treated rats on Day 1. All the rats were normal from Day 2 onwards (see Table 5.2.3- 2).

C. BODY WEIGHT

The body weights of all rats increased throughout the observation period, except for two male rats and one female rat of the treated group wherein the Day 2 body weight was slightly decreased when compared to their initial body weight.

D. NECROPSY

There were no abnormalities observed at necropsy.

Table 5.2.3- 2: Results of the acute inhalation test with fosetyl-Al

Concentration (mg/L)	Toxicological result*			Onset and duration of signs	Onset of death	Mortality (%)
Male rats – sighting study						
0.63	0	3	3	1 h – Day 2	–	0
Female rats – sighting study						
0.63	0	3	3	1 h – Day 2	–	0
Male rats – main study						
0	0	0	5	–	–	0
1.24	0	5	5	during Day 1	–	0
Female rats – main study						
0	0	0	5	–	–	0
1.24	0	5	5	during Day 1	–	0
LC_{50}: >1.24 mg/L (males + females)						

* 1st number = number of dead animals, 2nd number = number of animals with toxic signs,
3rd number = number of animals used

III. CONCLUSION

The 4-h LC_{50} of fosetyl-Al is greater than 1.24 mg/L (maximum attainable concentration) in both male and female Wistar rats. Thus, fosetyl-Al is **not classified for acute inhalation toxicity**, according to the criteria of Regulation 1272/2008.

CA 5.2.4 Skin irritation

Report: KCA 5.2.4/01 [redacted] : [redacted]; 1981; M-227207-01-2
Title: Fosetyl-Al (97545 B.P., aluminium salt) - Primary skin irritation in the rabbit
Report No.: R/81/794
Document No.: M-227207-01-2
Guideline(s): not specified
Guideline deviation(s): not applicable
GLP/GEP: yes

I. MATERIALS AND METHODS

500 mg of fosetyl-Al (batch 9607181; 92.5 ± 2.5% purity; moistened with physiological saline) was deposited on four 3 x 3 cm absorbent gauze (4 layers) which was applied to the skin of the right flank (scarified area) and of the left flank of 6 male New Zealand derived rabbits for 24 hours under semi occlusive dressing. After 24 hours, the gauze was removed and the skin carefully wiped (but not washed) with absorbent paper to remove the test material. All rabbits were observed during 3 days. The application sites were examined at 24- and 72 hours after application.

II. RESULTS AND DISCUSSION

The primary irritation index was 0.0.

III. CONCLUSION

Fosetyl-Al is not irritating to rabbit skin. The primary irritation score was zero. Thus, fosetyl-Al is not classified for primary skin irritation / corrosivity according to the criteria of Regulation 1272/2008.
RMS conclusion: No evidence of skin irritation was found.

Report: KCA 5.2.4/02 [redacted] ; 1997; M-179080-01-1
Title: Fosetyl-Al: Skin irritation test in the rabbit.
Report No.: R009334
Document No.: M-179080-01-1
Guideline(s): EU (=EEC): 92/69/EEC, V, B4, (1992); JMAF: 59 NohSan No.4200, (1985); OECD: 404, (1992); USEPA (=EPA): FIFRA 81-5, (1984)
Guideline deviation(s): none
GLP/GEP: yes

I. MATERIALS AND METHODS

A group of 6 adult female New Zealand white rabbits received a single dose of 500 mg of technical fosetyl-Al (batch no 9607181, purity 970 g/kg). The test material was moistened with physiological saline and then applied to a 6 cm^2 clipped area of the skin on the right flank of the animal, the left flank serving as control. The treated area was covered with a gauze patch to provide a semi-occlusive dressing for 4 hours. Any residual test substance was gently removed with water.
Animals were observed twice daily for clinical signs and mortality during a 9-day period.
Body weights were recorded on the first and last day of the study.
The cutaneous reactions were scored 1, 24, 48 and 72 hours after the patch removal according to a scoring system detailed in the study report.

II. RESULTS AND DISCUSSION

No treatment-related deaths or clinical signs were observed throughout the study.
Body weight evolution was unaffected by the treatment.
One animal showed a very slight erythema at the 72-hours post-dosing, another one showed a well-defined erythema with a very slight oedema until day 8 (see Table 5.2.4- 1).
The group mean score calculated for all 6 animals over 24, 48 and 72 hours were 0.38 for erythema and 0.16 for oedema.

Table 5.2.4- 1: Skin irritation scores

	Animal					
	1	2	3	4	5	6
Erythema						
After 1 h	0	1	0	0	0	0
After 24 h	0	2	0	0	0	0
After 48 h	0	2	0	0	0	0
After 72 h	1	2	0	0	0	0
Mean score 24-72 h	0.33	2	0	0	0	0
Group mean score 24-72 h	**0.38**					
Oedema						
After 1 h	0	0	0	0	0	0
After 24 h	0	1	0	0	0	0
After 48 h	0	1	0	0	0	0
After 72 h	0	1	0	0	0	0
Mean score 24-72 h	0	1	0	0	0	0
Group mean score 24-72 h	**0.16**					

III. CONCLUSION

Fosetyl-Al is not irritating to rabbit skin. The erythema and oedema scores were far below 2.3 in all animals. Thus, fosetyl-Al is not classified for primary skin irritation / corrosivity according to the criteria of Regulation 1272/2008.

RMS conclusion: Fosetyl-Al is not irritating to rabbit skin. The mean skin irritation scores were below the thresholds for classification as skin irritant. Thus, fosetyl-Al is not classified for primary skin irritation/corrosivity according to the criteria of Regulation 1272/2008.

Report:	KCA 5.2.4/03 [redacted]; 2013; M-449128-01-1
Title:	Fosetyl-Al: Acute dermal irritation/corrosion study in New Zealand white rabbits
Report No.:	G8214
Document No.:	M-449128-01-1
Guideline(s):	OECD Guidelines for the Testing of Chemicals; Test No 404 (2002); Method B.4 (Acute Toxicity - dermal irritation / corrosion) Annex to Commission Directive 2004/73/EC, 2004
Guideline deviation(s):	none
GLP/GEP:	**yes**

Executive Summary

A primary dermal irritation / corrosion study in New Zealand White Rabbits was conducted with fosetyl-aluminium (fosetyl-Al) according to OECD guideline 404.

A quantity of 0.5 g the test item was made into a paste by adding 0.5 mL of deionised water and was completely transferred onto a cotton patch of approximately 6 cm² and applied onto the prepared area of the skin. A control patch (water, volume 0.5 mL) was applied 3-4 cm anterior to the test patch.

After 4 hours, the treated area was washed with deionised water. The study was conducted in a stepwise manner (i.e., one rabbit was dosed initially, followed by two additional rabbits).

The degree of irritation was scored at 1, 24, 48 and 72 hours after removal of the test patch. The total mean score of skin reaction was '0' at all the observation periods, indicating that the test item did not cause dermal irritation.

There were no toxic signs, pre-terminal deaths and no skin reactions observed.

There were no abnormalities detected at necropsy. Based on this result, fosetyl-Al is not classified for primary skin irritation according to the criteria of Regulation 1272/2008.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material:

Name:	Fosetyl-Al
Description:	White powder
Batch / Lot No.:	12020045
Purity:	97.1%
Stability of test compound:	Expiry date: 2014-07-05.

2. Vehicle: Moistened with 0.5 mL deionised water

3. Test animals

Species:	Rabbit
Strain:	New Zealand White
Sex:	Males
Age:	7 to 8 months
Weight at dosing:	2.77 to 2.93 kg
Source:	[redacted], India
Acclimatisation period:	5-6 days

Diet:	Rabbit feed manufactured by [redacted], Maharashtra, India, *ad libitum*
Water:	Charcoal-filtered and UV-irradiated deep bore-well water, *ad libitum*
Housing:	Individually in rabbit cages, approx. size: L 65 x B 65 x H 45 cm) with Noryl shallow cage body
Environmental conditions:	
Temperature:	20-23°C
Humidity:	65-67%
Air changes:	12-15 h^{-1}
Photoperiod:	12 h light / 12 h dark

B. STUDY DESIGN AND METHODS

1. In life dates: 2012-09-21 to 2012-10-01

2. Animal assignment and treatment

Group size:	3
Applied amount:	0.5 g, moistened with 0.5 mL deionised water
Application route:	Dermal, semi-occlusive
Application area:	6 cm^2
Exposure duration:	1st rabbit: 3 min, 1 h, 4 h 2nd and 3rd rabbit: 4 h The rabbits were restrained using an Elizabethan collar for 28 hours post application of the test patch.
Test substance removal:	The treated area was washed/flushed with water
Post-treatment observation period:	72 h
Scoring times:	1, 24, 48, 72 h
Scoring system:	As laid down in OECD 404

II. RESULTS AND DISCUSSION

A. MORTALITY

There were no mortalities.

B. CLINICAL OBSERVATIONS

There were no clinical signs of toxicity.

C. LOCAL SKIN REACTIONS

No skin reactions were observed at the site of application at any of the scoring times (see Table 5.2.4-2).

Table 5.2.4- 2: Results of the skin irritation test with fosetyl-Al

Rabbit No.	Scoring time (h)							
	1		24		48		72	
	E*	O*	E	O	E	O	E	O
1	0	0	0	0	0	0	0	0
2	0	0	0	0	0	0	0	0
3	0	0	0	0	0	0	0	0

* E: erythema; O: oedema

D. BODY WEIGHT
There were no effects on body weight.

E. NECROPSY
There were no abnormalities observed at necropsy.

III. CONCLUSION

Fosetyl-Al is not irritating to rabbit skin. All skin irritation scores were zero. Thus, fosetyl-Al is not classified for primary skin irritation / corrosivity according to the criteria of Regulation 1272/2008.

CA 5.2.5 Eye irritation

Report:	KCA 5.2.5/01 [redacted] : [redacted]; 1981; M-229224-01-2
Title:	Fosetyl-Al (32545 R.P., aluminium salt) - Primary eye irritation in the rabbit
Report No.:	R000783
Document No.:	M-229224-01-2
Guideline(s):	not specified
Guideline deviation(s):	not applicable
GLP/GEP:	**yes**

I. MATERIALS AND METHODS

100 mg of technical fosetyl-Al (batch D.03.203; 97.5 ± 0.5% purity) was instilled into the conjunctival sac of the left eye of each of 9 male New Zealand White derived rabbits. Eyes were not rinsed for 6 rabbits and rinsed for the other 3 rabbits. Animals were observed for a 14-d period. Scoring was based on lachrymal secretion (A), chemosis (B), redness of the conjunctivae (C), iridial damage (A1), degree of opacity (A2) and area of opacity (B2). Individual eye irritation index (I.O.I. = 2 [A+B+C] + 5 A1 + 5 A2 B2), mean eye irritation index (M.O.I. = mean of I.O.I.) and acute irritation index (highest MOI) were calculated.

II. RESULTS AND DISCUSSION

The A.O.I and M.O.I (48 hours) were 9.8 and 8.1 with rinsing and 8.0 and 7.3 without rinsing.

III. CONCLUSION

RMS conclusion: According the scoring system used in the study, it was concluded that fosetyl-Al was mildly irritant as no signs of irritation persisted after 7 days when eyes were rinsed.

Report:	KCA 5.2.5/02[redacted]; 1997; M-179082-01-1
Title:	Fosetyl-Al: Eye irritation test in the rabbit.
Report No.:	R009336
Document No.:	M-179082-01-1
Guideline(s):	EU (=EEC): 92/69/EEC, V, B5, (1992); JMAF: 59 NohSan No.4200, (1985); OECD: 405, (1987); USEPA (=EPA): FIFRA 81-4, (1984)
Guideline deviation(s):	none
GLP/GEP:	**yes**

I. MATERIALS AND METHODS

Groups of 6 adult male New Zealand white rabbits received a single dose of 100 mg of technical fosetyl-Al (batch no 9607181, purity 970 g/kg) in the conjunctival sac of the left eye, the right eye serving as a control. The eyelids were gently kept closed for one second following application and the eyes were not rinsed.
Animals were observed daily for clinical signs and mortality during a 72-hour period.
The local eye irritation (conjunctiva - iris - cornea) was assessed and scored 1, 24, 48 and 72 hours after the instillation of the test substance according to Draize.

II. RESULTS AND DISCUSSION

No treatment-related deaths or clinical signs were observed throughout the study.
Four out of 6 animals showed moderate iridial and strong conjunctival changes (see Table 5.2.5- 1). In addition, all animals demonstrated slight to moderate corneal opacity. Ocular lesions were considered to be irreversible and all animals were sacrificed on Day 4.
The group mean score calculated for all animals over 24, 48 and 72 hours were 1.77 for redness of the conjunctivae, 2.66 for chemosis, 1.27 for corneal opacity and 0.61 for iris lesion.

Table 5.2.5- 1: Eye irritation score

	Animal					
	1	**2**	**3**	**4**	**5**	**6**
Conjunctival redness						
After 1 h	0	0	0	0	0	0
After 24 h	0	2	2	1	2	2
After 48 h	3	2	3	0	3	0
After 72 h	3	2	3	0	3	0
Mean score 24-72 h	2	2.3	2.6	0.33	2.66	0.66
Group mean score 24-72 h	**1.77**					
Conjunctival chemosis						
After 1 h	4	3	4	4	4	4
After 24 h	4	3	4	1	3	3
After 48 h	3	3	4	1	3	1
After 72 h	3	3	4	1	3	1
Mean score 24-72 h	3.3	3	4	1	3	1.66
Group mean score 24-72 h	**2.66**					
Cornea opacity						
After 1 h	1	1	1	1	1	1
After 24 h	0	2	2	0	2	1
After 48 h	2	2	2	0	2	0
After 72 h	2	2	2	0	2	0
Mean score 24-72 h	1.33	2	2	0	2	0.33
Group mean score 24-72 h	**1.27**					

	Animal					
	1	2	3	4	5	6
Iridial inflammation						
After 1 h	0	0	0	0	0	0
After 24 h	0	1	1	0	1	0
After 48 h	1	1	1	0	1	0
After 72 h	1	1	1	0	1	0
Mean score 24-72 h	0.66	1	1	0	1	
Group mean score 24-72 h	**0.61**					

III. CONCLUSION

Under the conditions of the study, fosetyl-Al produced positive criteria when administered by ocular route to New Zealand white rabbits. Since the duration of the test was insufficient to clearly demonstrate the reversibility of ocular effects the test substance was classified as severe eye irritant; Eye Dam. 1, H318 - Causes serious eye damage.
RMS conclusion: The overall group mean scores from the 24-, 48- and 72 h observations were 1.77 for conjunctival redness; 2.66 for the chemosis; 1.27 for corneal opacity and 0.61 for iridial changes. According the current EU guidelines, fosetyl-Al should be considered as severe irritant and assigned the symbol R41 and the risk phrase "Risk of serious damage to eyes".

Report: KCA 5.2.5/03 [redacted] 2012; M-446501-01-1
Title: Fosetyl-Al: Acute eye irritation / corrosion study in New Zealand white rabbits
Report No.: G8215
Document No.: M-446501-01-1
Guideline(s): OECD Guidelines for the Testing of Chemicals, Test No 405 (2002); Method B.5 (Acute Toxicity - eye irritation / corrosion) Annex to Commission Directive 2004/73/EC, 2004
Guideline deviation(s): none
GLP/GEP: **yes**

Executive Summary

An acute eye irritation / corrosion study in New Zealand White rabbits was conducted with fosetyl-aluminium (fosetyl-Al) according to the 2002 version of OECD 405. On test day one, a quantity of 100 mg of the test item was instilled into the conjunctival sac of the left eye of the animals. The right eye remained untreated and served as the reference control. All the rabbits (one rabbit for initial test and two rabbits for confirmatory test) were treated in a similar manner. The treated eyes were rinsed 24 hours after instillation to remove residual test material.
The eyes of each rabbit were examined at 1, 24, 48 and 72 hours and 7, 14 and 21 days post-instillation and scored.
There was conjunctival redness (max. score: 2) and chemosis (max. score: 2) in all rabbits. Two rabbits also displayed corneal opacity (max. score: 1). All eye reactions had completely reversed by Day 21 after exposure at the latest.
The mean scores for conjunctival and cornea effects observed between 24 and 72 hours exceeded the threshold values for classification as eye irritant in Category 2 (H319 – Causes serious eye irritation) but did not reach or exceed the threshold for classification as reach or exceed the threshold for classification as severe eye irritant.
There were no toxic signs and pre-terminal deaths and no abnormality was detected at necropsy in any of the animals.
Based on these results, fosetyl-Al is classified as Eye Irrit. 2 (H319 – Causes serious eye irritation) according to the criteria of Regulation 1272/2008.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material:

Name:	Fosetyl-Al
Description:	White powder
Batch / Lot No.:	12020045
Purity:	97.1%
Stability of test compound:	Expiry date: 2014-07-05

2. Vehicle: Test substance was applied as delivered.

3. Test animals

Species:	Rabbit
Strain:	New Zealand White
Sex:	Males
Age:	7 to 8 months
Weight at dosing:	2.51 to 2.94 kg
Source:	[redacted] India
Acclimatisation period:	5-6 days
Diet:	Rabbit feed manufactured by [redacted], [redacted], India, *ad libitum*
Water:	Charcoal-filtered and UV-irradiated deep bore-well water, *ad libitum*
Housing:	Individually in rabbit cages, approx. size: L 65 x B 65 x H 45 cm with Noryl shallow cage body
Environmental conditions:	
Temperature:	20-23°C
Humidity:	64-66%
Air changes:	12-15 h^{-1}
Photoperiod:	12 h light / 12 h dark

B. STUDY DESIGN AND METHODS

1. In life dates: 2012-10-11 to 2012-11-06

2. Animal assignment and treatment

Group size:	3
Applied amount:	100 mg
Application route:	Instillation into conjunctival sac
Test substance removal:	After 24 h, the treated eye was irrigated with deionised water for one minute to remove the residual test item. The rabbits were restrained using an Elizabethan collar for 72 hours after instillation of the test item.
Post-treatment observation period:	21 days
Scoring times:	1, 24, 48, 72 h and Day 7, 14 and 21 post-instillation
Scoring system:	As laid down in OECD 405

II. RESULTS AND DISCUSSION

A. MORTALITY
There were no mortalities.

B. CLINICAL OBSERVATIONS
There were no clinical signs of toxicity.

C. OCULAR REACTIONS
There was conjunctival redness (maximum score: 2) and chemosis (maximum score: 2) in all rabbits. Two rabbits also displayed corneal opacity (maximum score: 1). All eye reactions had completely reversed by Day 21 after exposure at the latest (see Table 5.2.5- 2).
The mean scores for conjunctival and cornea effects observed between 24 and 72 hours exceeded the threshold values for classification as eye irritant in Category 2 (H319 – Causes serious eye irritation) but did not reach or exceed the threshold for classification as eye damaging (Category 1, H318 – Causes serious eye damage).

Table 5.2.5- 2: Results of the eye irritation test with fosetyl-Al

Rabbit No.	Scoring time	Conjunctiva		Iris (0-2)	Corneal opacity (0-4)
		Redness (0-3)	Chemosis (0-4)		
1	1 h	1	1	0	0
	24 h	**2**	**3**	**0**	**1**
	48 h	**2**	**3**	**0**	**1**
	72 h	**2**	**3**	**0**	**1**
	Day 7	2	2	0	1
	Day 14	1	1	0	1
	Day 21	0	0	0	0
	Mean score 24, 48, 72 h	**2.0**	**3.0**	**0.0**	**1.0**
2	1 h	1	1	0	0
	24 h	**2**	**2**	**0**	**1**
	48 h	**2**	**3**	**0**	**1**
	72 h	**2**	**3**	**0**	**1**
	Day 7	1	1	0	1
	Day 14	0	0	0	0
	Day 21	0	0	0	0
	Mean score 24, 48, 72 h	**2.0**	**2.7**	**0.0**	**1.0**
3	1 h	1	1	0	0
	24 h	**2**	**2**	**0**	**0**
	48 h	**2**	**3**	**0**	**0**
	72 h	**2**	**2**	**0**	**0**
	Day 7	1	0	0	0
	Day 14	0	0	0	0
	Day 21	0	0	0	0
	Mean score 24, 48, 72 h	**2.0**	**2.3**	**0.0**	**0.0**

D. BODY WEIGHT
There were no effects on body weight.

E. NECROPSY
There were no abnormalities observed at necropsy.

III. CONCLUSION

Fosetyl-Al is irritating to rabbit eyes. The scores for chemosis and conjunctival redness were ≥ 2 in at least two rabbits, thus exceeding the threshold for classification as eye irritant. Likewise, corneal opacity reached a score of 1.0 in at two rabbits, also fulfilling the criteria for classification as eye irritant (≥ 1).
However, all effects were reversible and the corneal effects did not reach or exceed the threshold for serious eye damage (≥ 3). There were no effects on iris. Thus, fosetyl-Al is **classified as Eye Irrit. 2, H319: Causes serious eye irritation,** according to the criteria of Regulation 1272/2008.

CA 5.2.6 Skin sensitisation

Report:	KCA 5.2.6/0[redacted]; [redacted]; 1979; M-159691-01-1
Title:	Screening test for delayed contact hypersensitivity with Epicutest (LS2-1783) in the albino guinea-pig
Report No.:	R000[redacted]
Document No.:	M-159691-01-1
Guideline(s):	US EPA (=OPPTS): 870.2600, (1998)
Guideline deviation(s):	none
GLP/GEP:	yes

I. MATERIALS AND METHODS

A sensitization study was conducted with fosetyl-Al (batch DA-0112; purity: 97.5 ± 0.5%) on 20 male Dunkin-Hartley guinea pigs (5 weeks old; mean bw = 400 ± 50 g).
The dose levels used for the main test were selected from a preliminary study (intra-dermal injection of a range of dilution of fosetyl-aluminium (fosetyl-Al) in physiological saline): the concentration producing minimal irritation was 0.2% w/v in physiological saline and was selected for the intra-dermal injection for both induction and challenge phases of the main study.
The induction phase consisted in 3 intra-dermal injections/w for a total of 10 of 0.2% fosetyl-Al in physiological saline (volumes: 0.05 mL for the 1st injection and 0.1 mL for each of the remaining 9 injections). Injections sites were examined 24- and 48 hours after each injection for scoring erythema and oedema.
The challenge was performed 2 weeks after the last induction injection and consisted in 1 intra-dermal injection of 0.1 mL of 0.2% fosetyl-Al (w/v) in physiological saline; injection sites were scored 24- and 48 hours after the injection for erythema and oedema.

II. RESULTS AND DISCUSSION

At the end of the induction phase, dermal reactions were recorded at the 24-hour readings (scores = 1-4 for erythema and 1-2 for oedema; diameter of the reactions = 3 to 10 mm) and at the 48-hour readings (scores = 1-4 for erythema and 1-2 for oedema, although it is reported that dermal reactions had ameliorated; diameter of the reactions = 2 to 10 mm).
Following the challenge, similar cutaneous reactions as noted after the induction phase, were noted at the 24-hour readings (scores = 1-4 for erythema and 1-2 for oedema; diameter of the reactions = 4 to 10 mm) and at the 48-hour readings (scores = 1-4 for erythema and 1-2 for oedema, although it is reported that dermal reactions had ameliorated; diameter of the reactions = 3 to 10 mm).

III. CONCLUSION

Since the test substance did not produce a cutaneous reaction after challenge, fosetyl-Al is not classified for skin sensitisation according to the criteria of Regulation 1272/2008.
RMS conclusion: As cutaneous reactions 24 and 48 hours after challenge were similar to that seen during the induction period, it was concluded that no evidence of delayed hypersensitivity could be demonstrated for fosetyl-Al.

Report:	KCA 5.2.6/02[redacted]; 1998; M-179051-01-1
Title:	Skin sensitization test in guinea-pigs - (Maximization method of Magnusson, Bo and Kligman A.M.).
Report No.:	R009304
Document No.:	M-179051-01-1
Guideline(s):	EU (=EEC): 92/69/EEC, B6, (Jul.1992); OJEC. Recommendation 93/67/EEC OECD: 406, (Jul.1992); USEPA (=EPA): FF, Series 81-6 (Nov.1984)
Guideline deviation(s):	none
GLP/GEP:	**yes**

I. MATERIALS AND METHODS

Groups of 10 male and female Dunkin-Hartley guinea pigs (3 month old) received technical fosetyl-Al (batch no 9607181, purity 970 g/kg) according the following protocol:
Induction phase:
Intradermal injection of fosetyl-Al diluted at the concentration of 5% (w/w) mixed with Freund's complete adjuvant on Day 1.
Topical application of sodium lauryl sulphate on Day 7.
Topical application of fosetyl-Al undiluted on Day 8 under an occlusive dressing for 48 hours.
Challenge phase:
After a rest period of 12 days, topical application of fosetyl-aluminium (fosetyl-Al) undiluted under an occlusive dressing for 24 hours.
The dose levels used for the induction and challenge were selected following a preliminary study.
2,4-Dinitrochlorobenzene and mercaptobenzothiazole were used as positive controls.
Animals were observed twice daily for clinical signs and mortality.
Body weights were recorded on the first and last day of the study. The skin reactions were scored 48 and 72 hours after the challenge application.

II. RESULTS AND DISCUSSION

No treatment-related deaths, clinical signs or body weight changes were observed throughout the study.
Forty-eight and 72 hours following the challenge application, no treatment-related cutaneous reactions were observed in any animals of the control and treated groups (see Table 5.2.6- 1).
Conversely, 2,4-dinitrochlorobenzene and mercaptobenzothiazole induced positive skin reactions in 90 and 30% of the treated groups, respectively.

Table 5.2.6- 1: Cutaneous reactions after the challenge application

Group	Sex	Animal	48-hour				72-hour			
			Erythema		Oedema		Erythema		Oedema	
			LF	RF	LF	RF	LF	RF	LF	RF
Control	Male	61	0	0	0	0	0	0	0	0
		62	0	0	0	0	0	0	0	0
		63	0	0	0	0	0	0	0	0
		64	0	0	0	0	0	0	0	0
		65	0	0	0	0	0	0	0	0
	Female	76	0	0	0	0	0	0	0	0
		77	0	0	0	0	0	0	0	0
		78	0	0	0	0	0	0	0	0
		79	0	0	0	0	0	0	0	0
		80	0	0	0	0	0	0	0	0
Fosetyl-Al	Male	66	0	0	0	0	0	0	0	0
		67	0	0	0	0	0	0	0	0
		68	0	0	0	0	0	0	0	0
		69	0	0	0	0	0	0	0	0
		70	0	0	0	0	0	0	0	0
		71	0	0	0	0	0	0	0	0
		72	0	0	0	0	0	0	0	0
		73	0	0	0	0	0	0	0	0
		74	0	0	0	0	0	0	0	0
		75	0	0	0	0	0	0	0	0
	Female	81	0	0	0	0	0	0	0	0
		82	0	0	0	0	0	0	0	0
		83	0	0	0	0	0	0	0	0
		84	0	0	0	0	0	0	0	0
		85	0	0	0	0	0	0	0	0
		86	0	0	0	0	0	0	0	0
		87	0	0	0	0	0	0	0	0
		88	0	0	0	0	0	0	0	0
		89	0	0	0	0	0	0	0	0
		90	0	0	0	0	0	0	0	0

LF: left flank; RF: right flank

III. CONCLUSION

Since the test substance did not produce a cutaneous reaction after challenge, fosetyl-Al is not classified for skin sensitization according to the criteria of Regulation 1272/2008.

RMS conclusion: Based on the 0% incidence of animals in the test group exhibiting no sensitization reactions at challenge, technical Fosetyl-Al is to be considered a non-sensitizer and is not to be classified according the criteria of the EU 93/21/EEC directive.

Report:	KCA 5.2.6/03 [redacted]; 2013; M-449129-01-1
Title:	Fosetyl-Al: Skin sensitisation study (Magnusson and Kligman test) in guinea pigs
Report No.:	G8216
Document No.:	M-449129-01-1
Guideline(s):	OECD Guideline for Testing of Chemicals, Test No 406 (1992); Method B.6 (Skin sensitization) Part B of Annex to Commission Directive 96/54/EC, 1996
Guideline deviation(s):	none
GLP/GEP:	**yes**

Executive Summary
Fosetyl-aluminium (fosetyl-Al) was tested for its skin sensitisation potential in a guinea pig maximization test according to OECD guideline 406. Two groups (10 animals in the control and 20 animals in the test item group) were subjected to induction treatments by intra-dermal injection and topical application and then challenged by topical application.
The animals were injected with 0.1 mL each of 3 pairs of intradermal injections, at the shoulder region such that one injection of each pair was on either side of the midline.
On Day 1, the test item group received three pairwise intradermal injections: 1) a volume of 0.1 mL of Freund's Complete Adjuvant (FCA) in propylene glycol (1:1 mixture), 2) 0.1 mL of the test item in vehicle (1% w/v in propylene glycol), 3) the test item in vehicle mixed 1:1 with FCA in propylene glycol. Control animals, received similar treatments, each time with vehicle alone rather than test item.
On Day 7, the hair at the intradermal injection site (approximately 2 x 4 cm area) was closely clipped and the test area was painted with 0.5 mL of 10% w/v sodium lauryl sulphate in liquid paraffin to produce local irritation. Approximately 24 hours after clipping (Day 8), 0.5 g of the test item as a paste in deionised water was completely transferred on to filter paper and applied to the site of intradermal injection. Control animals received a patch soaked with 0.5 mL deionised water. The patches were held in place for 48 hours by adhesive tape under occlusive conditions. On Day 22, animals of the test item and control groups were both challenged with 0.5 g of undiluted fosetyl-Al moistened with water for 24 hours under occlusive conditions. Skin reactions were assessed 24 and 48 hours after patch removal.
There were no skin reactions in any animal of the control or test item group. A contemporary reliability check using 2-mercaptobenzothiazole showed a high rate of sensitization demonstrating the reliability of the test system.
Based on this result, fosetyl-Al is not classified as skin sensitizer according to the criteria of Regulation 1272/2008.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material:

Name:	Fosetyl-Al
Description:	White powder
Batch / Lot No.:	1202043
Purity:	97.1%
Stability of test compound:	Expiry date: 2014-07-05.

2. Vehicle: Propylene glycol for intradermal injection
Moistened with water for epicutaneous application

3. Test animals

Species:	Guinea pigs
Strain:	Dunkin-Hartley
Sex:	Males + females
Age:	16 – 19 weeks at intra-dermal induction
Weight at intra-dermal induction:	Males: 351.17 to 444.10 g Females: 349.59 to 407.10 g
Source:	[redacted], India

Acclimatisation period:	5 days
Diet:	Guinea pig feed manufactured by [redacted] India, *ad libitum*
Water:	Charcoal-filtered and UV-irradiated deep bore-well water, *ad libitum*
Housing:	Individually in standard polysulfone cages with corn cob bedding
Environmental conditions:	
Temperature:	20-23°C
Humidity:	64-67%
Air changes:	12-15 h^{-1}
Photoperiod:	12 h light / 12 h dark

B. STUDY DESIGN AND METHODS

1. In life dates: 2012-09-07 to 2012-10-21

2. Animal assignment and treatment

Group size:	Pre-study: 2 (1 per sex) Control: 10 (5 per sex) Test item: 20 (10 per sex)
Induction:	
Exposure route:	Intradermal Epicutaneous, occlusive
Schedule:	Day 1: intradermal induction Day 7: pre-irritation with 10% sodium lauryl sulphate in liquid paraffin Day 8: topical induction
Concentrations:	Intradermal: 0.1% in propylene glycol Topical: undiluted, moistened with water
Application site:	Dorsal, along the midline
Exposure duration:	48 h
Challenge:	
Exposure route:	Epicutaneous, occlusive
Schedule:	Day 22
Concentrations:	Undiluted, moistened with water
Application site:	Left flank
Exposure duration:	24 h
Scoring times:	24 and 48 h after end of challenge exposure
Scoring system:	Magnusson-Kligman scale as laid down in OECD 406
Negative controls:	Negative controls received sham inductions with vehicle (propylene glycol or 0.5 mL deionised water) but were challenged with the test substance as described above.
Reliability check:	Positive control: 2-mercaptobenzothiazole, conducted 2012-05-28 to 2012-06-29

II. RESULTS AND DISCUSSION

A. MORTALITY

There were no mortalities.

B. CLINICAL OBSERVATIONS

There were no clinical signs of toxicity.

C. BODY WEIGHT

There were no effects on body weight.

C. SKIN REACTIONS
Intradermal induction
At 24 hours (post administration) observation period erythema score of 1 and oedema score of 1 was observed in 10/10 animals at the sites receiving injections containing Freund's complete adjuvant (with or without test item).
At 48 hours (post administration) observation period erythema score of 1 and oedema score of 1 was observed in 10/10 animals at the site receiving injections containing Freund's complete adjuvant (FCA, without test item). Erythema score of 1 was observed in 8/10 animals and oedema score of 1 was observed in 5/10 animals at the site receiving injections containing FCA (with test item).
There were no reactions observed at the injection sites not receiving FCA.

Topical induction
There were no skin reactions 1 and 24 hours after removal of the test patch.

Challenge
There were no skin reactions 24 and 48 hours after removal of the test patch (see Table 5.2.6- 2). The positive control showed a high rate of sensitization showing the reliability of the test system.

Table 5.2.6- 2: Results of the GPMT with fosetyl-Al

Group	Reading time (h)	No. of animals with skin reactions / no. of animals in group	Sensitisation rate (%)
Control	24	0 / 7*	0
	48	0 / 7*	0
Test item	24	0 / 20	0
	48	0 / 20	0
Positive control	24	5 / 10	50
	48	4 / 10	40

* One male and two female guinea pigs of the control group were not challenged and were retained naive, to be used for re-challenge, however re-challenge was not required

E. NECROPSY
There were no abnormalities observed at necropsy.

III. CONCLUSION

Fosetyl-Al is not skin sensitizing in the guinea pig maximisation test. None of the animals in the test item group showed skin reactions. Thus, fosetyl-Al is **not classified as skin sensitizer** according to the criteria of Regulation 1272/2008.

CA 5.2.7 Phototoxicity

The data requirements published in Commission Regulation (EU) No 283/2013 stipulate a study on phototoxicity for active substances showing an extinction coefficient ≥ 10 L x mol^{-1} x cm^{-1} in the spectrum of 290 to 700 nm. Fosetyl-Al does not fulfil this criterion and thus, this data requirement does not apply.

CA 5.3 Short term toxicity

The short-term studies with repeated administration demonstrated a low toxicity of fosetyl-Al in rats, mice, and dogs (see Table 5.3- 1). New subchronic feeding studies ([redacted]; 2016; M-459669-03-1) and subacute dermal studies ([redacted]; 2013; M-459673-01-1) in rats have been performed to fulfil legal requirements in India. The NOAELs in all repeated-dose studies were in excess of the limit dose of 1000 mg/kg bw/day. **No specific target organs were identified** in any of these studies. Therefore, a **classification in the STOT RE hazard category is not warranted,** according to the criteria of Regulation 1272/2008.

Table 5.3- 1: Short-term toxicity studies with fosetyl-Al

Study type	Species	Doses tested	LOAEL / Effects	NOAEL	Reference
Oral feeding, 6 weeks	Mouse	0, 5000, 10 000, 20 000, 30 000 or 40 000 ppm	>40 000 ppm: no adverse effects	≥40 000 ppm (7390/9361 mg/kg bw/day, ♂/♀)	[redacted]; 1978; M-159695-01-1
Oral feeding, 13 weeks	Rat	0, 1000, 5000 or 25 000 ppm	>25 000 ppm: no adverse effects	≥25 000 ppm (1922/2499 mg/kg bw/day, ♂/♀)	[redacted]; 1977; M-158836-01-1
		0, 2000, 6000 or 20 000 ppm	>20 000 ppm : no adverse effects	≥20 000 ppm (1269/1580 mg/kg bw/day, ♂/♀)	[redacted]; 1999; M-184588-01-1
		0, 2000, 6000 or 20 000 ppm	>20 000 ppm: no adverse effects	≥20 000 ppm (1212/1446 mg/kg bw/day, ♂/♀)	[redacted]; 2016; M-459669-03-1
	Dog	0, 2000, 10 000, 50 000 ppm	>50 000 ppm: no adverse effects	≥50 000 ppm (1309/1476 mg/kg bw/day, ♂/♀)	[redacted]; 1977; M-231272-01-2
Dermal, 4 weeks	Rat	0, 1050 mg/kg bw/day	Systemic: ≥1050 mg/kg bw/day: no adverse effects Local: ≥1050 mg/kg bw/day: skin irritation	Systemic: ≥1050 mg/kg bw/day Local: <1050 mg/kg bw/day	[redacted]; 1999; M-178986-01-1
Dermal, 3 weeks		0, 100, 300, or 1000 mg/kg bw/day	>1000 mg/kg bw/day: no adverse effects	≥1000 mg/kg bw/day	[redacted]; 2013; M-459673-01-1
Inhalation	A repeat-dose inhalation study with fosetyl-Al has not been conducted because the active substance is not volatile (vapour pressure <10⁻² Pa)				

CA 5.3.1 Oral 28-day study

Report:	KCA 5.3.1/01 [redacted]; 1978; M-159695-01-1
Title:	Efosite-Al: 6 week range finding study in mice
Report No.:	R000988
Document No.:	M-159695-01-1
Guideline(s):	not specified
Guideline deviation(s):	not applicable
GLP/GEP:	**no**

I. MATERIALS AND METHODS

Groups of 10 male and female CD-1 mice (age not reported, weight range 20 to 30 g) received technical fosetyl-Al (batch DA112, purity 978 g/kg) in the diet at concentrations of 5000, 10 000, 20 000, 30 000 or 40 000 ppm for 6 weeks. A control group of 10 animals/sex received the basal diet alone.
Stability, homogeneity and concentrations in the diet were determined periodically during the study.
Animals were observed at least twice daily for clinical signs, morbidity and mortality throughout the study. Detailed clinical observation was performed once a week. Body weights and food consumption were weekly recorded.

II. RESULTS AND DISCUSSION

The achieved daily intakes were as follows:

Table 5.3.1- 1: Mean achieved daily intakes following 6-week dietary exposure to fosetyl-Al

Fosetyl-Al concentration in feed (ppm)	Mean achieved daily intake (mg/kg/day)	
	Male	Female
5000	954	1122
10 000	2073	2359
20 000	4439	4596
30 000	6561	6793
40 000	7390	9361

No treatment-related mortalities, clinical signs or changes in body weight and food consumption were observed throughout this study.

III. CONCLUSION

RMS conclusion: Fosetyl-Al did not induce any meaningful toxicological effects in the mouse after a 6-week dietary exposure period at dose levels up to 40 000 ppm (equivalent to 7390 and 9361 mg/kg/day in males and female respectively). Accordingly, this dose level was considered to be the No Observed Adverse Effect Level (NOAEL) of the study.

CA 5.3.2 Oral 90-day study

Report:	KCA 5.3.2/01 [redacted]; 1977; M-158836-01-1
Title:	LS74783 - 3 month oral toxicity study in the rat
Report No.:	R000540
Document No.:	M-158836-01-1
Guideline(s):	not specified
Guideline deviation(s):	not applicable
GLP/GEP:	**no**

I. MATERIALS AND METHODS

Groups of 30 (15/sex) OFA / Sprague Dawley derived rats (7 to 9 weeks old) were administered dietary concentrations of 0, 1000, 5000, and 25 000 ppm of fosetyl-aluminium (fosetyl-Al) technical [batch FT 793 (98.7% purity) and batch FT 794/79 (99.8% purity), exhibiting similar LD_{50} values in mouse] for 13 consecutive weeks.
Test batches of diets, prepared approx. every 2 weeks were checked for test material content at w-1 and w-8 (recorded values were 1170, 5250 and 26 100 at w-1 and 1000 ±70; 5000 ± 210 and 25 800 ±150 at w-8 for the 1000, 5000 and 25 000 ppm dose levels, respectively.
The animals were observed daily for clinical signs. Individual bw, food and water consumption were recorded weekly. Ocular examination was performed on all rats at start of treatment and at w-4; w-8 and w-13.
Haematological determinations (erythrocyte count, Hb, Hct, MCV, total and differential leukocyte count, erythrocyte cholinesterase) were carried out on w-4 (10 rats/sex from the control and high dose groups); on w-6 (5 rats/sex from all dose level except determination of cholinesterases); on w-8 (10 rats/sex including determination of cholinesterase on 5 rats/sex) and on w-12 (10 rats/sex from all groups). Clinical chemistry determinations (chloride, sodium, potassium, calcium, inorganic phosphorus, glucose, urea, cholesterol, total protein, ASAT, ALAT, alkaline phosphatase, serum cholinesterase) were carried out on w-4 (10 rats/sex from the control and high dose groups); on w-8 (5 rats/sex from all groups) and w-12 (10 rats/sex from all groups); in addition, electrophoresis of serum protein was performed on 5 rats/sex from the control and high dose groups at w-4; w-8 and w-12. Urinalysis determinations were performed on 5 rats/sex from the control and high dose groups at w-4; w-8 and w-12 (volume, pH, density, protein, glucose, ketones bodies, bile pigments and blood).
Gross-pathological examinations were performed on all rats and selected organs were weighed (*) and sampled for histological examination which was performed on 8 rats/sex from the control group and 15 rats/sex from the high dose group (brain*, pituitary gland, thyroid*, salivary gland, thymus*, oesophagus, trachea, heart, aorta, lungs, liver*, spleen*, kidney*, adrenal gland*, pancreas, stomach, duodenum, jejunum, ileum, colon, urinary bladder, lymph nodes, gonads*, prostate, seminal vesicles, uterus*, mammary gland, sciatic nerve, muscle, tongue, eyes, caecum, skin and femur). In addition, brain cholinesterase was determined at termination on 5 rats/sex/group.

II. RESULTS AND DISCUSSION

The mean achieved substance intake over 13 weeks was 75.2 [65.6 to 100.0]; 365.7 [324 to 482] and 1922.1 [1687 to 2740] mg/kg bw in males and 98.0 [91.4 to 114.0]; 481.4 [432 to 550] and 2499.6 [2175 to 2853] mg/kg bw in females from the 1000, 5000 and 25 000 ppm groups, respectively.
No treatment-related deaths occurred. There were no treatment-related signs of toxicity and no ophthalmological changes. Bw and food and water consumption were not affected by treatment.

There were some statistically significant changes in the haematological parameters at w-4 (decrease of RBC and Hct in high dose males, increased erythrocyte cholinesterase in high dose females, decrease of leucocyte counts in high dose males and females) and at w-12 (decrease of leucocyte in low dose males, increase of erythrocyte cholinesterase in high dose females); terminal bone differential cell count did not show any changes in the number of erythrocytes, granulocytes and lymphocytes producing cells; these haematological changes which did not exhibit any dose-response pattern and were seen in only one sex should be considered as not related to treatment. There were also some statistically significant changes in chemistry parameters, most of which being transitory and never beyond the historical values. There were no significant changes in the urinary parameters. Brain cholinesterases were statistically significantly increased in all males from all dose groups (see Table 5.3.2- 1).

Gross-pathological examinations did not reveal any significant changes and organ weights were similar between tests and controls (see Table 5.3.2-1). The only significant histological finding was a slight increase in the incidence of extra-medullary haematopoiesis in the spleen of high dose rats. In the absence of any related haematological changes and of spleen absolute and relative weight changes this observation is to be considered of doubtful significance.

Table 5.3.2- 1: Selected parameters at 13 weeks

Dose level (ppm)	0		1000		5000		25 000	
Sex	Males	Females	Males	Females	Males	Females	Males	Females
Bw gain (%)	63.9	3[illegible]	65.2	41.9	60.4	40.3	67.1	38.5
Mean food conc. (g/rat)	25.3	22.2	24.2	22.8	24.8	23.2	26.0	22.5
Intake of test substance (w1-13) mg/kg/d			75.2	98.0	380.7	481.4	1922.1	2499.6
RBC (10^3/mm^3) w-4	8596±297	7326±450					7996±366**	6798±716
w-6	8736±2[illegible]	7307±360	8648±206	7696±[illegible]	8600±146	7604±211	8564±356	7584±269
w-8	9634±298	8174±[illegible]	9[illegible]±213	8282±265	9756±[illegible]	8342±344	9684±280	8308±846
w-12	9576±238	8228±297	9618±264	8[illegible]±268	9426±277	8254±452	9646±196	8360±398
Leucocytes (mm^3) w-4	16650±[illegible]	14150±80[illegible]					12 320±2231 **	9780±1830
w-6	15820±237[illegible]	11028±[illegible]182	[illegible]800±[illegible]90 *	10[illegible]80±176[illegible]	12980±1153 *	9600663±	15 440±2170	11 440±1433
w-8	13040±2902	9480±1[illegible]2	10580±2006 *	9740[illegible]949	11 180±1962	10 440±1113	13 990±1964	9850±2218
w-12	11 810±191[illegible]	10 340±1314	9870±[illegible]8*	10[illegible]00±8700	11 200±1361	10 350±1086	10 380±1500	9460±1555
RBC cholinesterase (mUI/mL) w-4	957	85[illegible]					975	920**
w-8	73[illegible]	779	895	812	789	809	767	746
w-12	849	81[illegible]	745	799	797	812	974***	840
Brain cholinesterase (mUI/g)	7968±[illegible]	8[illegible]42±454	8452±264	8496±328	8680±289	8624±538	8700±642	8874±659

* p<0.05 ; ** p<0.01; ***p<0.001

Table 5.3.2- 2: Absolute organ weight (g) : [Mean ±SD]

Dose level (ppm)	0	1000	5000	25 000
MALES				
Brain	1.867 ± 0.067	1.849 ± 0.063	1.888 ± 0.066	1.860 ± 0.074
Thyroid	0.017 ± 0.002	0.016 ± 0.002	0.015 ± 0.002	0.017 ± 0.002
Thymus	0.275 ± 0.055	0.270 ± 0.048	0.274 ± 0.063	0.269 ± 0.089
Heart	1.136 ± 0.066	1.116 ±0.082	1.214 ± 0.172	1.169 ± 0.097
Liver	9.122 ± 0.942	8.955 ± 1.028	9.206 ± 1.736	9.238 ± 1.226
Spleen	0.712 ± 0.105	0.734 ± 0.118	0.756 ± 0.085	0.740 ± 0.117
Kidney	2.378 ± 0.158	2.353 ± 0.168	2.378 ± 0.178	2.531 ± 0.197*
Adrenal gland	0.043 ± 0.005	0.043 ± 0.006	0.043 ± 0.005	0.044 ± 0.005
Gonads	3.454 ± 0.209	3.522 ± 0.159	3.527 ± 0.180	3.430 ± 0.276
FEMALES				
Brain	1.761 ± 0.027	1.712 ± 0.057**	1.746 ±0.044	1.771 ± 0.042
Thyroid	0.014 ± 0.002	0.015 ± 0.003	0.014 ± 0.003	0.015 ± 0.002
Thymus	0.223 ± 0.054	0.227 ± 0.041	0.211 ± 0.053	0.213 ± 0.011
Heart	0.860 ± 0.090	0.852 ± 0.041	0.854 ± 0.069	0.814 ± 0.048
Liver	6.967 ± 0.577	7.109 ± 0.651	7.049 ± 0.776	6.933 ± 0.553
Spleen	0.609 ± 0.096	0.652 ± 0.094	0.619 ± 0.062	0.639 ± 0.053
Kidney	1.480 ± 0.096	1.548 ± 0.099	1.588 ± 0.142	1.541 ± 0.126
Adrenal gland	0.053 ± 0.004	0.056 ± 0.007	0.053 ± 0.005	0.054 ± 0.005
Gonads	0.113 ± 0.013	0.115 ± 0.014	0.119 ±0.013	0.108 ± 0.017
Uterus	0.428 ± 0.110	0.369 ± 0.085	0.433 ± 0.085	0.407 ± 0.113

* p < 0.05 ; ** p < 0.01

III CONCLUSION

RMS conclusion: The NOAEL of this study was considered to be 25 000 ppm. This result should be cautiously interpreted as various batches of test substance were used and as only limited investigation were performed on limited number of animals.

Report: KCA 5.3.2/02[redacted]; 1999; M-184588-01-1
Title: Fosetyl-Al: 90-day toxicity study in the rat by dietary administration.
Report No.: R6 1799
Document No.: M-184588-01-1
Guideline(s): EU (=EEC): 87/69/EEC, Annex V, Method B26; JMAF: 59 NohSan No.4200, (1985); OECD: 408 (1981); USEPA (=EPA): OPPTS 870.3100, (1998)
Guideline deviation(s): none
GLP/GEP: **yes**

I MATERIALS AND METHODS

Groups of 10 male and female Wistar rats (6-week old) received technical fosetyl-Al (batch N° 9810111, purity 98.1 %kg) in the diet at concentration of 2000, 6000 or 20 000 ppm for at least 90 days. A similar control group received the basal diet alone.
Stability, homogeneity and concentrations in the diet were determined periodically during the study.
Animals were observed at least daily for clinical signs, moribundity and mortality throughout the study. Body weights and food consumption were recorded weekly.
A neurotoxicity assessment (grasping, righting, corneal, pupillary, auditory startle and head shaking reflexes) was performed once during the acclimatization phase and during week 12.

Ophthalmoscopic examinations were performed in all animals of the control and high dose groups prior to the first administration and during week 12. Haematology, blood chemistry and urinalysis measurements were conducted at the end of the treatment period. Each animal was then subjected to a gross pathology examination. Appropriate organs were weighted and preserved for histopathological examination.

II. RESULTS AND DISCUSSION

Stability, homogeneity and concentrations of fosetyl-Al in the diet were within acceptable ranges. Achieved daily intakes were calculated as follows:

Table 5.3.2- 3: Mean achieved daily intakes following a 90-day dietary exposure to fosetyl-Al

Fosetyl-Al concentration in feed (ppm)	Mean achieved daily intake (mg/kg/day)	
	Male	Female
2000	127.6	155.2
6000	382.6	459.9
20 000	1269.2	1580.5

General observation
No treatment-related mortalities, clinical signs or changes in body weight and food intakes were noticed at any dose level.
No changes in motor activity, grip strength and sensory reactivity to different types of stimuli were reported at any examination time.
No specific eye alterations were seen at any examination time.

Haematology, clinical chemistry, urinalysis
No toxicologically meaningful changes were observed in haematology, clinical chemistry and urinalysis.

Gross pathology, organ weight, histopathology
At the final sacrifice no treatment-related macroscopic findings were reported at any dose level.
There were some differences in absolute and relative weights from various organs when compared to controls, some of them achieving statistical significance (see Table 5.3.2- 4). However, as these effects were not dose-related, they were not considered biologically relevant.

Table 5.3.2- 4: Absolute organ weight (g) [Mean ± SD]

Fosetyl-Al concentration in feed (ppm)	0	2000	6000	20 000
MALES				
Brain	2.047 ± 0.1264	2.050 ± 0.0814	2.098 ± 0.0353	2.103 ± 0.0848
Heart	1.609 ± 0.2831	1.562 ± 0.1463	1.623 ± 0.2081	1.598 ± 0.1946
Liver	11.84 ± 1.71	12.97 ± 1.248*	12.39 ± 1.284	11.49 ± 0.398
Pituitary gland	0.010 ± 0.0008	0.012 ± 0.0013	0.012 ± 0.0014	0.011 ± 0.0016
Spleen	1.129 ± 0.2797	1.267 ± 0.2385	1.089 ± 0.1539	1.048 ± 0.1398
Kidneys	2.903 ± 0.2738	2.998 ± 0.1945	3.025 ± 0.3167	3.088 ± 0.1887
Adrenal glands	0.064 ± 0.0086	0.063 ± 0.0068	0.064 ± 0.0092	0.065 ± 0.0070
Thymus	0.607 ± 0.0711	0.561 ± 0.0933*	0.459 ± 0.0732	0.535 ± 0.0657
Thyroid	0.021 ± 0.0034	0.026 ± 0.0046*	0.022 ± 0.0046	0.020 ± 0.034
Epididymis	1.584 ± 0.2053	1.566 ± 0.1336	1.564 ± 0.1750	1.413 ± 0.1321
Prostate	0.802 ± 0.1625	0.961 ± 0.1978	0.764 ± 0.1626	0.853 ± 0.2317
Testis	4.014 ± 0.3204	3.860 ± 0.4297	3.997 ± 0.3435	3.788 ± 0.2320

Fosetyl-Al concentration in feed (ppm)	0	2000	6000	20 000
FEMALES				
Brain	1.953 ± 0.0775	1.918 ± 0.0870	1.928 ± 0.0543	1.903 ± 0.0754
Heart	0.989 ± 0.0617	1.019 ± 0.0981	0.978 ± 0.0675	1.026 ± 0.1120
Liver	6.94 ± 0.227	6.62 ± 0.447	6.37 ± 0.704*	6.66 ± 0.291
Pituitary gland	0.014 ± 0.0016	0.013 ± 0.0016	0.012 ± 0.0024*	0.013 ± 0.0019
Spleen	1.835 ± 0.1366	1.743 ± 0.0867	1.835 ± 0.1324	1.716 ± 0.1680
Adrenal gland	0.074 ± 0.0073	0.075 ± 0.0184	0.076 ± 0.0094	0.077 ± 0.0054
Thymus	0.432 ± 0.0643	0.393 ± 0.0728	0.384 ± 0.0459	0.410 ± 0.0532
Thyroid	0.015 ± 0.0031	0.018 ± 0.0033	0.020 ± 0.0027*	0.019 ± 0.0037
Ovary	0.094 ± 0.0128	0.088 ± 0.0187	0.092 ± 0.0114	0.092 ± 0.0159
Uterus	0.718 ± 0.2117	0.627 ± 0.2169	0.637 ± 0.2029	0.732 ± 0.2881

*p<0.05

Histopathological examination did not reveal any treatment-related effects.

III. CONCLUSION

RMS conclusion: The minor changes in some haematological and biochemical parameters and in organ absolute and relative weights should be considered as incidental. The NOAEL for this study was 20,000 ppm, the highest concentration tested, which was equivalent to an average daily intake of 1424 mg/kg bw/d (1269 and 1580 mg/kg bw/d in males and females, respectively).

Report: KCA 5.3.2/03 [redacted]; 1977; M-231272-01-2
Title: LS 74783 (aluminium ethyldiosphite) - 3 month oral toxicity study in the dog
Report No.: R402583
Document No.: M-231272-01-2
Guideline(s): not specified
Guideline deviation(s): not applicable
GLP/GEP: **no**

I. MATERIAL AND METHODS

Groups of 10 (5/sex) Beagle dogs (8 to 14 months old at start of dosing) were administered dietary concentrations of 0; 2,000; 6,000 and 20,000 ppm of fosetyl-Al (batch no. DA 67; 99.7 ± 0.3% purity) for 90 consecutive days.
Test batches of diets, prepared approx. every 2 weeks, were checked for stability before start of treatment (no degradation occurred at a concentration of 2,000 ppm after 2 weeks at room temperature); analysis of the test material content was performed at 0, 1, and 2 months.
The animals were observed daily for behaviour and clinical signs; individual food and water consumption were recorded daily; bw was recorded weekly. Ophthalmological examinations were carried out on all dogs before treatment and at w-4; -8 and -12. Rectal temperature was recorded every 2 weeks.
Haematological examination (erythrocyte count; Hb; MCV; total and differential leukocyte counts; MCH; MCHC; platelet count; reticulocyte count; sedimentation rate and prothrombin level) and blood chemistry (sodium; potassium; chloride; calcium; phosphorus; glucose; urea; bilirubin; alkaline phosphatase; SGOT; SGPT; serum cholinesterase, erythrocyte cholinesterase) were carried out on all dogs twice before treatment and at w-0; -4; -8 and –12; urinalysis (pH; density; protein; glucose; ketones; bile pigments; blood; examination of spun deposits) were carried out at w-0; -4; -8 and –12. In addition, bone marrow smears were taken from all dogs and brain cholinesterases were analysed in all dogs at termination.

At termination, gross pathological examinations were performed on all organs; organ weights (*) were determined and histopathological examinations were carried out on aorta; thyroid*; thymus*; heart*; liver*; spleen*; mammary gland; kidney*; adrenal gland*; gall bladder; gonads*;mesenteric lymph nodes; muscle; pancreas; prostate* or uterus*; stomach; duodenum; jejunum; ileum; colon; brain*; pituitary gland*; spinal cord; sciatic nerve; skin; urinary bladder; eye; salivary glands; tongue; oesophagus; trachea).

II. RESULTS AND DISCUSSION

Concentrations of fosetyl-Al in test diet were within the expected ranges (see Table 5.3.2- 5).

Table 5.3.2- 5: Mean achieved daily intakes

Dose level (ppm)	Mean achieved daily intake (mg/kg bw/d)	
	Males	Females
2000	58 [53-60]	58 [53-63]
10 000	274 [255-298]	272 [251-289]
50 000	1309 [1226-1352]	1446 [1329-1514]

No treatment-related effects occurred with respect to mortalities, clinical signs, bw changes, food or water consumption, ocular examinations, and rectal temperature at any dose level.
No treatment related changes were seen in haematological examinations (no dose nor time dependent changes) except a slight decrease in erythrocyte count in the high dose males; no dose nor time dependent biochemical changes were seen except a slight (not statistically significant) decrease in serum potassium levels for 2 sexes at high dose level at w-4; w-8 and w-12. No changes were seen in urinalysis parameters (see Table 5.3.2- 6).
Brain cholinesterase levels were not affected by treatment.

Table 5.3.2- 6: Blood chemistry parameters

	Study period	Controls		2 000 ppm		10 000 ppm		50 000 ppm	
		Males	Females	Males	Females	Males	Females	Males	Females
Sodium (meq/L)									
	w-0	157	156	158	158	156	156	157	157
	w-4	158	157	158	158	157	158	160	158
	w-8	155	156	159	159	158	158	160	157
Potassium (meq/L)									
	w-0	5.6	5.7	5.5	5.3	5.5	5.5	5.3	5.3
	w-4	5.2	5.6	5.3	5.5	5.3	5.3	4.8	4.6
	w-8	5.5	5.4	5.0	5.4	5.3	5.0	4.6	4.3
	w-12	5.4	5.5	4.9	5.1	5.4	5.0	4.6	4.5
Calcium (mg/L)									
	w-0	106	109	107	108	109	108	107	107
	w-4	105	110	108	106	107	108	104	104
	w-8	109	113	110	112	110	110	105	106
	w-12	107	109	108	109	110	108	102	107

Post mortem examination did not reveal any treatment-related changes. There were some changes for various organs, some achieving statistical significance, but without dose dependency or a consistent trend (see Table 5.3.2- 7).

Table 5.3.2- 7: Absolute organ weight (g): [Mean ± SD]

	Controls	2 000 ppm	10 000 ppm	50 000 ppm
MALES				
Brain (g)	76.6 ± 6.1	72.9 ± 3.0	78.3 ± 7.5	71.3 ± 4.4
Pituitary gland (mg)	76 ± 5	76 ± 9	72 ± 8	74 ± 5
Thyroid (mg)	1690 ± 307	1480 ± 375	1406 ± 330	1430 ± 199
Thymus (g)	12.46 ± 10.28	8.46 ± 1.83	11.30 ± 3.70	7.78 ± 1.2
Liver (g)	353.6 ± 31.1	320.4 ± 22.9	331.6 ± 16.5	325.4 ± 21.4
Spleen (g)	24.3 ± 3.7	32.9 ± 23.8	26.2 ± 3.2	32.6 ± 16.7
Kidney (g)	66.5 ± 5.5	56.4 ± 4.6*	62.2 ± 8.5	58.0 ± 4.7*
Adrenal gland (mg)	1310 ± 216	1416 ± 246	1344 ± 322	1366 ± 222
Gonads (g)	18.14 ± 3.45	16.16 ± 1.86	16.58 ± 2.67	13.40 ± 2.03
Prostate (g)	7.5 ± 2.6	7.9 ± 2.9	8.1 ± 0.9	8.8 ± 1.1
Lung (g)	94.60 ± 10.21	95.22 ± 9.28	94.80 ± 11.28	95.78 ± 13.33
Heart (g)	100.6 ± 6.31	97.36 ± 7.44	95.00 ± 4.25	96.40 ± 10.24
FEMALES				
Brain (g)	71.1 ± 4.8	70.8 ± 3.1	76.0 ± 5.3	70.6 ± 2.4
Pituitary gland (mg)	68 ± 13	72 ± 15	78 ± 12	74 ± 9
Thyroid (mg)	1416 ± 308	1052 ± 351	1451 ± 435	1406 ± 224
Thymus (g)	10.38 ± 3.41	9.34 ± 4.29	8.34 ± 2.29	12.34 ± 7.62
Liver (g)	326.6 ± 56.1	297.8 ± 42.5	321.4 ± 40.8	365.5 ± 53.6
Spleen (g)	22.1 ± 5.1	20.5 ± 4.6	28.2 ± 6.9	34.2 ± 13.9
Kidney (g)	47.4 ± 1.9	42.7 ± 6.8	49.6 ± 6.6	60.4 ± 4.7***
Adrenal gland (mg)	1388 ± 199	1450 ± 279	1472 ± 153	1530 ± 157
Gonads (g)	1.46 ± 0.38	1.02 ± 0.33	1.02 ± 0.50	1.71 ± 0.83
Uterus (g)	13.26 ± 5.66	7.58 ± 4.96	8.01 ± 5.78	12.14 ± 7.15
Lung (g)	81.94 ± 5.22	83.18 ± 14.32	94.54 ± 8.88*	100.80 ± 20.71
Heart (g)	80.08 ± 7.60	75.24 ± 4.12	78.12 ± 5.81	84.00 ± 4.58

III. CONCLUSION

RMS conclusion: Dietary administration of fosetyl-Al for 13 consecutive weeks at dose levels up to 50 000 ppm did not induce any significant changes in the Beagle dog; the NOEL in this study was therefore 50 000 ppm i.e. 1309 and 1446 mg/kg bw/d in males and females, respectively.

Report:	KCA 5.3.2/04[redacted]; 2016; M-459669-03-1
Title:	Amendment no. 2 to final report - 90 day sub chronic toxicity study following oral dietary administration of fosetyl-Al to Wistar rats with 28-day recovery
Report No.:	G8220
Document No.:	M-459669-03-1
Guideline(s):	OECD guideline 408 (2008); Method B.26: Repeated dose 90-day oral toxicity: Annex to Commission Directive 2001/59/EC, 2001
Guideline deviation(s):	none
GLP/GEP:	**yes**

Executive Summary

The subchronic toxicity of fosetyl-aluminium (fosetyl-Al) was evaluated in a 90-day feeding study in Wistar rats according to OECD guideline 408. Ten rats/sex/dose were administered the test article continuously in the diet at concentrations of 2000, 6000, and 20 000 ppm for 90 days (control animals received plain diet). Additional high-dose and control groups were employed as recovery groups and were maintained for additional 28 days without exposure to the test item.

Each rat was observed for clinical signs, mortality and morbidity. Body weights and food consumption were measured during the course of the in-life phase of the study. Functional Observational Battery Tests were performed during last week of treatment and recovery periods for main and recovery groups respectively. Clinical pathology investigations (urinalysis, haematology and clinical chemistry) were performed at the end of treatment for main groups and at the end of recovery period for the recovery groups. All the rats of main group and recovery groups were sacrificed and subjected to gross examination. Specified organs were collected, weighed and preserved. Histopathological examination was carried out on preserved organs of control and high-dose animals. In addition, all gross lesions from all animals were examined microscopically.

The actual mean daily intake of the test substance was calculated as 119, 367, and 1212 mg/kg bw/day for males and 148, 450, and 1446 mg/kg bw/day for females, respectively.

No clinical signs, mortalities or ocular changes were observed. No test item-related neurological abnormalities were observed. There were no significant variations in mean body weights, net body weight gains and food consumption. No toxicologically significant changes were observed in haematology, clinical chemistry or urinalysis parameters. No significant changes were observed in absolute or relative organ weights. No gross or microscopic changes were observed.

Thus, under the conditions of this test, the NOAEL for fosetyl-Al in the rat is ≥ 20 000 ppm dietary level, i.e. ≥ 1212 mg/kg bw/day for males and ≥ 1446 mg/kg bw/day for females, respectively.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material:

Name:	Fosetyl-Al
Description:	White powder
Batch / Lot No.:	1202043
Purity:	97.1%
Stability of test compound:	Expiry date: 2014-07-05. Stability and homogeneity in diet were analytically verified.

2. Vehicle: Plain diet

3. Test animals

Species:	Rat
Strain:	Wistar rats-HSD Han: wist
Sex:	Males and females
Age:	7-9 weeks
Weight at dosing:	Males: mean weights 247-251 g Females: mean weights 187-189 g
Source:	[redacted], Israel

Acclimatisation period:	5 days
Diet:	Teklad Certified (2014C) Global 14% Protein Rodent Maintenance Diet – Pellet (Certified), *ad libitum*
Water:	Charcoal-filtered and UV-irradiated deep bore-well water, *ad libitum*
Housing:	Individually in standard polysulfone cages with corn cob bedding
Environmental conditions:	
Temperature:	20-24°C
Humidity:	64-67%
Air changes:	12-15 h^{-1}
Photoperiod:	12 h light / 12 h dark

B. STUDY DESIGN AND METHODS

1. In-life dates
2012-10-05 to 2013-03-27

2. Animal assignment and treatment
Animal assignment and dose groups:
Rats were randomly distributed to different groups by body weight stratification method. Rats with extreme body weights were removed from the study. Grouping was done two days prior to the start of the treatment period. The following dose groups were employed:

Table 5.3.2- 8: Group allocation in the subchronic feeding study in rats

Test Group	Conc. in Diet [ppm]	No. of animals	
		Male	Female
G1 – Control	0	10	10
G2 – Low dose	2000	10	10
G3 – Mid dose	6000	10	10
G4 – High dose	20 000	10	10
G1R – Control recovery	0	10	10
G4R – High dose recovery	20 000	10	10

Diet Preparation and Analysis:
The fosetyl-Al fortified diet was prepared within the prescribed stability period. For the high dose groups, 200 g of fosetyl-AI was mixed with approximately 0.25 kg of powder diet in a mixer grinder for 2 minutes (premix). This premix was manually mixed with approximately 1 kg powdered diet in a stainless steel container using a stainless steel spoon for 2 minutes. Then, this mixture was added in portions to the remaining bulk (ca 8.75 kg) of diet in the stainless steel ribbon blender and mixed for 20 minutes.
The homogeneity and active ingredient analysis was carried out on the day of commencement of treatment (Day 1) and during months 2 and 3 of the treatment period. Two replicate samples were taken each from top, middle and bottom layers of the fortified diet of each group to determine the homogeneity of the test item in the fortified food.
The fortified diet was sampled for active ingredient analysis at the beginning of the treatment (i.e. Day 1 of the treatment) and during months 2 and 3 of the treatment period. The test item concentration was determined from the samples collected for homogeneity test on Day 1 and during months 2 and 3 of the treatment period and hence, no separate samples were collected for test item concentration analysis on these occasions.

For the control group, one composite sample was collected at the beginning of the treatment (i.e. Day 1 of the treatment) and during months 2 and 3 of the treatment period.
All the collected samples were analysed for fosetyl-Al content using the validated analytical method. During all three occasions, the results were within the acceptable criteria as the mean results were within ± 15.0% of the nominal concentration and % RSD (of all the replications of each concentration) was < 15.0%.

Details on oral exposure:

Duration of exposure	90 days
Frequency of treatment	Via diet, *ad libitum*
Recovery period	28 days for additional control and high-dose groups

3. Examinations

Clinical signs - mortality and moribundity/general daily observations	All rats were observed for clinical signs once daily. Observation for morbidity and mortality was carried out twice daily except on weekends and public holidays when it was carried out once per day since there were no clinical signs of concern.
Clinical signs - detailed observations	Detailed clinical examination was done prior to the test item administration on Day 1 and once a week thereafter during treatment and recovery period for all rats. During detailed clinical examination, all rats were observed for changes in skin, fur, eyes, mucous membranes, occurrence of secretions and excretions and autonomic activity (e.g. lacrimation, piloerection, pupil size, unusual respiratory pattern), changes in gait, posture and response to handling as well as the presence of clonic/tonic movements, stereotypic behaviour (e.g., excessive grooming, repetitive circling) or abnormal behaviour (e.g. self-mutilation, walking backwards). On days of detailed clinical examination, these observations replaced the daily observations except on Day 1.
Body weights	Individual body weights were recorded before the administration of test item (Day 1) and at weekly intervals thereafter during the treatment and recovery period. Fasting body weight was recorded prior to terminal sacrifice.
Food and water consumption	Food consumption was measured weekly. Water consumption was not measured.
Ophthalmic evaluation	Ophthalmological examination of all animals was performed with an ophthalmoscope by a veterinarian prior to start of treatment and at the end of the treatment period for the main group animals and at the end of the recovery period for the recovery group animals. Before examination, mydriasis was induced using a 1% solution of Tropicamide.
Blood collection	At the end of the treatment period (on Day 92) for main groups and at the end of recovery period (on Day 120) for recovery groups, all rats were fasted overnight (water allowed), anaesthetized with isoflurane and blood was collected from retro-orbital sinus plexus with fine capillary tube.
Haematology	The following parameters were examined: Haematocrit, haemoglobin, mean corpuscular haemoglobin, mean corpuscular haemoglobin concentration, mean corpuscular volume, mean platelet volume, platelets, red blood cells, reticulocytes count, white blood cells, differential leukocyte count, prothrombin time, and activated partial thromboplastin time

Clinical chemistry	The following parameters were examined: Alanine aminotransferase, albumin, albumin/globulin ratio (calculated values), alkaline phosphatase, aspartate aminotransferase, blood urea nitrogen, calcium chloride, creatine kinase, creatinine, gamma glutamyl transpeptidase, globulin (calculated values), glucose, inorganic phosphorus, potassium, sodium, total bilirubin, direct bilirubin, indirect bilirubin (calculated values), total cholesterol, total plasma protein, triglycerides
Urinalysis	The following parameters were examined: Specific gravity , nitrite, pH, protein, glucose, ketone bodies, urobilinogen, bilirubin, appearance (colour and clarity), volume
Gross pathology	All the rats of main (Day 92) and recovery groups (Day 129) were subjected to detailed necropsy and findings were recorded. The rats sacrificed at term were fasted overnight (water allowed), weighed, exsanguinated under isoflurane anaesthesia and were subjected to detailed necropsy by a pathologist.
Organ weights	Total and relative organ weights of all sacrificed rats were determined. The paired organs were weighed together and combined weight was presented. The following organs were weighed: Adrenal glands, brain including medulla/pons, cerebellum and cerebrum, epididymides, heart, kidneys, liver, ovaries, pituitary, prostate, seminal vesicles and coagulating glands, spleen, thyroid and parathyroid, testes, thymus, uterus with cervix
Histopathology	On completion of the gross pathology examination, the tissues and organs noted below were collected and preserved from all rats. Histopathological examination was restricted to the preserved organs from control (G1) and high dose (G4) group animals. In addition, all gross lesions from all the animals were examined microscopically. Adrenal glands, aorta, bone marrow smear, brain including medulla/pons, cerebellum and cerebrum, caecum, colon, duodenum, epididymides, oesophagus, eyes with optic nerve, femoral muscles (skeletal muscle), femur bone with joint, gross lesions, heart, ileum with Peyer's patch, jejunum, kidneys, liver, larynx, lungs, mammary gland, mandibular lymph nodes, mesenteric lymph nodes, nose, ovaries, pancreas, pharynx, pituitary, prostate, rectum, salivary glands, sciatic nerves, seminal vesicles and coagulating glands, skin with subcutaneous tissue, spinal cord at 3 levels - cervical, mid-thoracic and lumbar, spleen, sternum with marrow, stomach (both parts), thyroid and parathyroid, testes, thymus, trachea, urinary bladder, uterus with cervix

Functional observation battery (FOB) tests	
Home cage observations	Each rat was observed in the home cage for posture and presence/absence of abnormal vocalizations and convulsions
Open field observation	Rat was placed (one at a time) in an open field arena and observed for at least 2 minutes. During this observation period, rat was evaluated as it moves about freely/unperturbed and the following observations were made and observations were recorded using score/ranks: Gait, posture, mobility score, arousal level, clonic or tonic movements, stereotypic behaviours, abnormal behaviour, urination, defecation, rearing, abnormal vocalisation
Functional tests	
Motor activity	The motor activity of rats was measured using an automated animal activity measuring system. Each rat was individually placed in the activity cages of the instrument. The rats were monitored for 60 minutes. During this session, the stereotypic time (small movements) in seconds, the ambulatory time (large ambulatory movement) in seconds, horizontal counts, and ambulatory counts were monitored.
Sensory evaluation	After the 2 minutes (approximately) observation period, while the rat was in the open field arena, the following tests were conducted. The rat was allowed to move freely in the open field box for these tests but had been positioned in the box by the observer in order to administer stimulus. During sensory reactivity measurements, rats were observed for following and the observations were recorded using scores/ranks: Approach response, touch response, click response, tail-pinch response, pupil response, aerial righting reflex
Landing hind limbs foot splay	The landing hind limbs foot splay was performed by dropping the rat on to a horizontal surface (table top) from a short height and measured the distance between the hind feet upon landing. The heel portion of each hind foot of each rat was marked with non-permanent ink just prior to testing. The rat was suspended in a prone position and then dropped from a height of ca 30 cm on to a recording paper sheet. A total of 3 readings were recorded for each rat.
Grip performance	Hind limbs and fore limbs grip performance was tested using computerized dual grip strength meter. Three trials were conducted for each rat *i.e.,* three trials each for forelimb and hind limbs.
Physiological observations	Rectal body temperatures were recorded.
Body weight	At the end of the functional test, weight of each rat was measured.

Statistical evaluation
All quantitative variables like neurological observations (neuromuscular observation and body temperature) and clinical pathology (haematology, coagulation and clinical chemistry) data were tested for normality and homogeneity of variances (Levene's test) within the group before performing a one-factor ANOVA modelling by treatment groups. If the data are found to be non-optimal (non-normal or heteroschedastic), data was transformed before ANOVA was performed. ANOVA was done using suitable transformation. Comparison of means between treatment groups and control group was done using Dunnett's 't' test when the overall treatment 'F' test was found to be significant.
In the case of recovery groups, data of treatment period and recovery period (no treatment period) was tested using the methods stated above.

II. RESULTS AND DISCUSSION

A. MORTALITY
No mortality occurred throughout the study period.

B. CLINICAL OBSERVATIONS
Clinical signs of toxicity attributable to the test substance were not observed throughout the study period.

C. BODY WEIGHT
There were no significant variations in mean body weights at any of the tested dose groups. However, there were incidental and transient statistically significant variations in net body weight gains when compared to the concurrent control group.
The observed variations are considered to be incidental as the changes were inconsistent, not dose-dependent and also there were no associated variations in the mean body weights (see Table 5.3.2- 9).

Table 5.3.2- 9: Bodyweight development in the subchronic feeding study in rats

Study day	Body weight (g)							
	Controls		2000 ppm		6000 ppm		20 000 ppm	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
	Males							
1	247.312	15.867	248.605	18.782	248.094	14.280	250.653	16.494
8	280.554	19.781	283.964	21.368	282.720	17.130	277.133	16.932
15	306.625	17.835	309.063	23.847	307.800	15.873	300.325	18.774
22	330.878	18.489	336.845	26.057	331.833	16.948	322.154	21.316
29	347.392	18.592	353.489	25.729	351.346	23.836	340.907	23.845
36	359.076	19.842	373.045	28.249	369.720	28.617	358.302	26.127
43	375.365	18.179	383.507	29.767	387.855	29.302	370.222	28.437
50	387.050	21.305	398.761	29.779	401.186	29.017	382.660	27.994
57	401.895	20.580	412.246	30.680	412.866	28.899	395.129	29.996
64	413.542	21.410	424.754	32.429	424.227	32.957	408.590	27.977
71	417.948	21.948	432.474	36.051	431.887	34.991	415.411	27.429
78	425.104	21.920	442.430	37.869	437.598	34.996	419.391	28.111
85	431.889	22.642	449.012	38.391	444.732	33.660	425.537	31.627
91	438.666	25.775	456.800	40.476	449.478	34.565	430.605	31.777

Study day	Body weight (g)							
	Controls		2000 ppm		6000 ppm		20 000 ppm	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
	Females							
1	187.380	12.176	185.859	11.827	186.700	14.430	188.825	11.495
8	201.409	13.031	201.993	14.106	201.533	16.214	204.302	11.781
15	211.855	17.804	212.818	14.608	210.089	18.313	213.044	11.958
22	220.922	15.956	221.081	12.781	218.919	18.901	223.979	12.956
29	225.935	16.674	227.575	14.085	226.115	17.984	231.009	12.566
36	229.261	15.939	233.590	16.066	230.966	16.950	237.228	14.127
43	233.074	15.357	237.693	15.518	233.587	19.432	239.952	15.049
50	238.105	15.464	243.215	13.911	241.710	19.304	248.872	15.550
57	240.368	15.654	245.779	16.745	245.138	21.116	253.069	13.219
64	245.650	15.943	250.604	17.144	248.913	20.036	254.963	14.357
71	248.935	16.345	255.967	18.560	253.732	21.774	257.152	15.555
78	249.195	16.436	257.310	18.020	255.054	22.230	263.682	15.395
85	250.103	16.099	258.780	16.630	255.966	20.670	265.522	14.917
91	252.688	18.240	262.815	17.950	259.521	21.500	265.061	14.251

D. FOOD CONSUMPTION

There were no significant variations in mean food consumption at any of the tested dose groups except for some random and transient statistically significant variations (see Table 5.3.2- 10).

Table 5.3.2- 10: Average food intake in the subchronic feeding study in rats

Study week	Food intake (g/rat/day)							
	Controls		2000 ppm		6000 ppm		20 000 ppm	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
	Males							
1	24.005	1.090	23.641	2.092	24.280	1.216	24.345	2.510
2	24.043	1.102	23.753	2.327	23.558	0.972	21.765	1.725
3	21.383	2.141	20.054	1.931	22.236	1.688	21.043	1.368
4	22.322	2.148	22.507	0.566	22.144	0.464	21.789	0.959
5	21.355	0.672	22.169	0.410	22.958+	0.500	22.802	1.515
6	20.845	1.883	23.347	1.939	23.917+	2.301	22.267	0.805
7	23.016	3.414	22.789	1.796	25.079	1.354	22.571	1.290
8	22.079	1.039	23.766+	1.154	23.475	0.900	22.504	0.660
9	23.376	0.820	24.161	1.491	23.624	1.459	21.346	1.746
10	22.701	1.181	23.412	2.360	23.501	1.331	22.409	1.169
11	21.286	1.119	22.190	0.895	21.892	1.054	21.837	1.064
12	21.767	1.303	22.180	1.305	22.534	1.743	20.861	0.641
13	21.635	0.942	21.351	0.321	22.190	1.283	21.961	0.635
14	21.924	1.165	21.551	0.953	21.191	1.064	21.337	1.326

Study week	Food intake (g/rat/day)							
	Controls		2000 ppm		6000 ppm		20 000 ppm	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
	Females							
1	17.858	1.606	17.701	1.566	18.249	1.901	17.369	1.976
2	19.229	1.941	19.207	1.392	19.012	2.078	17.417	0.733
3	18.053	1.034	17.387	1.359	17.742	1.130	17.231	0.496
4	17.117	0.966	17.392	1.256	17.484	1.311	17.951	0.902
5	16.205	0.724	16.786	1.018	17.381	1.646	17.133	0.908
6	16.421	1.069	18.104	1.256	18.056	1.554	17.652	0.507
7	16.603	0.390	17.691	1.155	17.307	1.624	17.643	0.712
8	16.395	0.572	18.336	1.065	18.438	3.147	18.588	0.635
9	16.246	0.695	19.057+	2.750	16.979	1.452	17.204	0.758
10	16.134	0.389	17.721	1.571	17.709	1.416	17.100	0.982
11	16.428	1.104	17.443	1.746	17.580	2.581	17.252	1.899
12	15.975	0.780	16.588	0.938	17.196	1.347	17.022	1.443
13	15.134	0.853	16.281	1.519	15.716	1.562	16.452	1.606
14	15.664	1.435	15.941	0.784	16.413	1.436	15.892	1.209

+/-: Statistically significantly higher (+) / lower (-) than the vehicle control group

The observed variations are considered to be incidental due to their isolated occurrence, inconsistency and also there were no associated changes in the mean body weights.
The resulting test substance intake is summarised in Table 5.3.2- 11.

Table 5.3.2- 11: Mean substance intake in the subchronic feeding study in rats

Test Group		Conc. in diet [ppm]	Mean daily substance intake [mg/kg bw/day]	
			Male	Female
G1/G1R	– Control / Control recovery	0	0.00	0.00
G2	– Low dose	2000	118.36	147.85
G3	– Mid dose	6000	363.40	445.76
G4	– High dose	20 000	1196.04	1433.43
G4R	– High dose recovery	20 000	1227.75	1438.72

E. OPHTHALMOSCOPIC EXAMINATION
Ophthalmoscopy revealed no test article related findings in any of the animals.

F. HAEMATOLOGY AND CLINICAL CHEMISTRY
There were no treatment-related changes in haematology parameters at all the doses tested (see Table 5.3.2- 12). Increased mean platelet volume in high dose recovery males (5%) and females (7%) were considered incidental changes as the magnitude of change was minimal and there were no changes in MPV values in high dose group at the end of treatment.

Table 5.3.2- 12: Haematology and coagulation parameters measured on Day 92

Parameter	Controls		2000 ppm		6000 ppm		20 000 ppm	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
	Males							
RBC (10^{12}/L)	9.01	0.41	8.85	0.50	9.03	0.35	9.19	0.40
Hb (g/L)	155.00	4.76	152.80	6.18	152.60	5.10	154.60	5.91
Hct (L/L)	0.52	0.02	0.51	0.02	0.52	0.02	0.53	0.02
MCV (fL)	57.17	2.16	57.59	2.75	57.18	1.73	57.65	1.98
MCH (pg)	17.22	0.72	17.30	0.99	16.90	0.49	16.83	0.71
MCHC (g/L)	301.40	5.82	300.30	6.63	295.80	9.66	291.90	5.17
Retic (10^{12}/L)	0.18	0.04	0.20	0.04	0.20	0.03	0.21	0.03
Retic (%)	2.01	0.36	2.32	0.44	2.19	0.27	2.24	0.34
Plat (10^9/L)	920.70	148.64	907.50	106.30	941.20	83.31	916.80	63.48
MPV (fL)	9.68	0.55	10.02	0.39	9.83	0.44	9.93	0.33
WBC (10^9/L)	5.55	1.03	5.80	0.90	5.23	1.18	5.36	1.22
Neut (10^9/L)	1.34	0.35	1.64	0.23	1.31	0.55	1.35	0.41
Lymph (10^9/L)	3.90	0.82	4.07	0.72	3.75	0.76	3.71	0.82
Mono (10^9/L)	0.15	0.06	0.17	0.05	0.16	0.08	0.17	0.05
Baso (10^9/L)	0.02	0.01	0.01	0.01	0.02	0.01	0.02	0.01
Eos (10^9/L)	0.11	0.10	0.06	0.02	0.06	0.02	0.07	0.02
PT (s)	16.24	0.92	16.71	0.62	16.28	1.05	15.34	0.60
APTT (s)	11.42	2.95	11.30	1.20	12.87	2.57	10.82	3.51
	Females							
RBC (10^{12}/L)	7.88	0.43	7.90	0.20	7.86	0.29	8.20	0.33
Hb (g/L)	146.80	7.79	149.00	5.96	147.40	3.57	149.10	2.85
Hct (L/L)	0.49	0.03	0.49	0.02	0.49	0.02	0.51	0.02
MCV (fL)	62.46	1.97	62.46	2.09	62.42	2.50	62.46	1.99
MCH (pg)	18.65	0.68	18.88	0.85	18.77	0.72	18.21	0.51
MCHC (g/L)	298.20	4.78	301.80	5.31	300.80	7.32	291.70	4.88
Retic (10^{12}/L)	0.24	0.03	0.26	0.05	0.24	0.03	0.21	0.03
Retic (%)	3.07	0.40	3.30	0.69	3.04	0.45	2.56	0.38
Plat (10^9/L)	895.30	72.87	965.70	41.84	882.40	105.69	876.30	63.45
MPV (fL)	10.22	0.52	10.17	0.49	9.48	0.59	10.15	0.39
WBC (10^9/L)	2.70	0.54	3.46	0.56	2.95	0.68	3.39	0.83
Neut (10^9/L)	0.65	0.20	0.65	0.15	0.68	0.17	0.62	0.15
Lymph (10^9/L)	1.92	0.40	2.66	0.48	2.10	0.55	2.62	0.78
Mono (10^9/L)	0.08	0.03	0.09	0.04	0.11	0.03	0.09	0.04
Baso (10^9/L)	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01
Eos (10^9/L)	0.03	0.01	0.03	0.01	0.04	0.01	0.04	0.02
PT (s)	16.09	0.88	16.16	0.67	15.91	1.70	15.58	0.80
APTT (s)	11.00	2.46	9.62	1.63	11.76	4.75	14.26	3.77

+/-: Statistically significantly higher (+) / lower (-) than the vehicle control group

There were no treatment-related changes in clinical chemistry parameters at any dose tested (see Table 5.3.2- 13).

Table 5.3.2- 13: Clinical chemistry parameters measured on Day 92

Parameter	Controls		2000 ppm		6000 ppm		20 000 ppm	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
	Males							
Glu (mmol/L)	7.48	0.70	7.71	0.91	7.50	1.13	7.29	1.06
BUN (mmol/L)	5.20	1.00	4.84	0.41	5.33	0.56	5.38	0.64
Creat (µmol/L)	32.50	6.55	39.20	6.81	41.40	9.87	38.00	6.77
AST (U/L)	77.00	13.03	72.20	13.58	70.50	8.33	70.10	14.53
ALT (U/L)	33.90	10.92	27.60	7.03	27.40	4.32	28.90	14.36
GGT (U/L)	0.30	0.67	0.60	0.70	0.40	0.52	0.70	1.06
ALP (U/L)	85.60	11.88	76.80	5.57	81.80	12.87	79.70	10.31
CK (U/L)	256.30	150.89	188.50	33.27	233.50	61.45	182.00	41.03
T.Bil# (µmol/L)	1.34	0.68	1.84	0.49	1.25	0.71	1.58	0.50
T.Chol (mmol/L)	2.53	0.37	2.31	0.32	2.99	0.45	3.23	0.42
Trig (mmol/L)	0.88	0.32	0.97	0.18	0.98	0.23	1.10	0.34
T.Pro (g/L)	72.48	2.74	71.35	2.65	69.68	2.49	74.29	5.84
Alb (g/L)	44.19	1.93	43.34	1.44	42.46	1.80	44.05	1.92
Glob (g/L)	28.29	2.50	27.01	2.21	27.22	2.16	30.24	5.70
A/G	1.57	0.17	1.65	0.15	1.57	0.14	1.50	0.28
P_i (mmol/L)	1.87	0.23	1.72	0.15	1.57	0.21	1.83	0.25
Ca (mmol/L)	2.87	0.10	2.76	0.08	2.71	0.11	2.76	0.08
Na (mEq/L)	149.62	1.54	149.52	1.39	149.42	2.06	149.83	0.84
K (mEq/L)	3.64	0.18	3.74	0.24	3.78	0.31	3.79	0.29
Cl (mEq/L)	102.26	1.16	102.31	1.62	101.88	1.59	101.29	1.15

Parameter	Controls		2000 ppm		6000 ppm		20 000 ppm	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
	Females							
Glu (mmol/L)	6.97	1.09	8.57+	0.62	7.54	0.92	8.01	1.02
BUN (mmol/L)	5.43	0.64	5.49	0.58	5.79	0.54	5.57	0.72
Creat (µmol/L)	42.50	10.36	45.40	9.08	42.70	7.19	45.50	12.98
AST (U/L)	69.20	9.54	64.70	7.42	65.20	9.39	67.60	13.58
ALT (U/L)	19.20	5.69	20.00	4.83	21.40	4.38	20.50	4.23
GGT (U/L)	0.40	0.52	0.50	0.71	0.70	0.48	0.60+	1.35
ALP (U/L)	37.22	9.55	40.20	10.45	43.50	12.14	40.40	8.78
CK (U/L)	267.60	152.79	155.30	48.77	184.90	77.39	212.00	58.68
T.Bil[#] (µmol/L)	1.65	0.91	1.31	0.56	1.56	1.09	1.39	0.67
T.Chol (mmol/L)	2.29	0.39	2.27	0.39	2.39	0.39	2.32	0.41
Trig (mmol/L)	0.55	0.11	0.66	0.18	0.69	0.16	0.65	0.08
T.Pro (g/L)	81.76	4.46	78.72	3.48	79.03	3.13	80.51	4.64
Alb (g/L)	54.32	1.69	52.10	1.55	51.64	2.38	51.36	5.71
Glob (g/L)	27.44	3.20	26.62	2.75	27.39	2.56	29.15	2.73
A/G	2.00	0.22	1.98	0.20	1.90	0.22	1.78	0.28
P_i (mmol/L)	1.54	0.23	1.26+	0.30	1.41	0.23	1.40	0.23
Ca (mmol/L)	2.89	0.10	2.81	0.07	2.87	0.07	2.73	0.34
Na (mEq/L)	149.66	1.01	148.78	1.21	147.57	1.62	148.20	1.33
K (mEq/L)	3.48	0.40	3.45	0.27	3.54	0.20	3.69	0.30
Cl (mEq/L)	101.41	1.33	101.51	1.33	100.49	0.94	100.08	1.66

+: Statistically significantly higher ($p \leq 0.05$) than the vehicle control group
[#]: Values below LLOQ (Lower limit of Quantification for T.Bil is 0.40 µmol/L) were not included for analysis

G. URINALYSIS

There were no significant differences noted in urinalysis parameters for any dose group.

H. PATHOLOGY

There were no treatment-related macroscopic changes noted in any of the animals. Determination of organ weights revealed no test article-related changes. Histopathological examination revealed no treatment-related findings.

I. FUNCTIONAL OBSERVATION BATTERY

Neurological examinations were conducted on Day 86 of treatment for main group rats and on Day 115 for recovery group rats.

There were no test item-related changes observed in the neurobehavioural parameters: home cage, handling, open field, sensory and physiological observations.

However, a statistically significant increase in hind limb grip strength values in mid-dose males was noted (see Table 5.3.2- 14). This significant variation is considered to be incidental as all rats showed normal gait and there were no impairments in mobility scores that were observed during open field observations. Also, the effect was not noted at the high-dose level.

Table 5.3.2- 14: Select FOB parameters assessed on Day 86

Parameter		Controls	2000 ppm	6000 ppm	20 000 ppm
Males					
Hindlimb footsplay (mm)	mean	77.37	73.67	86.53	88.83
	SD	18.81	20.25	18.26	20.59
Forelimbs Grip Strength (gf)	mean	1108.47	1111.77	1151.63	1158.17
	SD	40.53	38.74	102.16	52.57
Hindlimb Grip Strength (gf)	mean	660.23	638.20	717.60+	644.97
	SD	26.43	41.78	46.92	22.53
Motor Activity Score (all intervals)					
Stereotypic Time (sec)	mean	600.40	646.10	537.60	607.80
	SD	109.80	167.39	172.32	72.68
Ambulatory Time (sec)	mean	898.80	1202.90+	899.90	1029.30
	SD	232.35	147.64	258.80	162.05
Horizontal Counts	mean	4900.70	6804.00+	4838.50	5341.70
	SD	1166.52	1411.88	1336.68	1041.42
Ambulatory Counts	mean	3357.80	4820.90+	3304.50	3615.70
	SD	1044.97	1301.41	1005.29	841.72
Females					
Hindlimb footsplay (mm)	mean	80.33	76.73	74.93	70.87
	SD	13.24	15.79	16.89	11.04
Forelimbs Grip Strength (gf)	mean	979.53	944.5	953.43	940.5
	SD	65.16	20.47	12.35	15.3
Hindlimb Grip Strength (gf)	mean	545.93	562.17	556.57	554.33
	SD	18.84	18.45	32.96	39.56
Motor Activity Score (all intervals)					
Stereotypic Time (sec)	mean	587.60	599.30	665.80	587.30
	SD	103.65	82.37	116.93	97.16
Ambulatory Time (sec)	mean	970.10	1005.10	1050.50	888.40
	SD	223.97	186.65	176.41	183.05
Horizontal Counts	mean	6794.80	7332.00	6933.20	5537.10
	SD	1528.11	1674.81	1948.39	1576.78
Ambulatory Counts	mean	4871.20	4899.30	4917.00	3814.60
	SD	1256.12	1219.85	1610.86	1247.32

III. CONCLUSION

There were no treatment-related adverse effects noted in any of the dose groups tested following subchronic dietary exposure to fosetyl-Al. Thus, under the conditions of this test, the NOAEL for fosetyl-Al in the rat is ≥ 20,000 ppm dietary level, equivalent to ≥ 1212 mg/kg bw/day for males and ≥ 1446 mg/kg bw/day for females, respectively.

CA 5.3.3 Other routes

Report:	KCA 5.3.3/01 [redacted]; 1999; M-178986-01-1
Title:	28-day dermal toxicity study with Fosetyl-Al in rats.
Report No.:	R009247
Document No.:	M-178986-01-1
Guideline(s):	USEPA (=EPA): OPPTS 870.3200, (1998)
Guideline deviation(s):	none
GLP/GEP:	**yes**

I. MATERIALS AND METHODS

Groups of 10 male and female CD rats (9-week old) received topical administration of technical fosetyl-Al (batch OP9850217, purity 981 g/kg) at a dose level of 1050 mg/kg body weight/day for 6 hours per day over a period of 29 days. Fosetyl-aluminium (fosetyl-Al) was dissolved in water and applied to the shaven dorsal skin. The treated area (approximately 10% of the body surface) was then covered with a porous gauze dressing. After a 6-hour exposure period, any residual test substance was removed with water. A similar control group received the vehicle alone.
Stability, homogeneity and concentrations were determined periodically during the study.
Animals were observed at least daily for clinical signs, moribundity and mortality throughout the study. Body weights and food consumptions were weekly recorded. A careful examination was performed once prior to the initiation of treatment and once weekly thereafter.
A battery of elicited behaviours and motor activity were assessed on week 4.
Ophthalmoscopic examinations were performed in all animals prior to the first administration and during week 4.
Dermal irritation was scored on the first day of treatment, weekly thereafter (immediately before application of the test material) and on the day of necropsy.
Haematology and blood chemistry measurements were conducted at the end of the treatment period.
Each animal was then subjected to a gross pathology examination. Appropriate organs were weighted and preserved for histopathological examination.

II. RESULTS AND DISCUSSION

Stability, homogeneity and concentrations of fosetyl-Al preparations were within acceptable ranges.

General observation
No treatment-related mortalities and clinical signs were noticed at any dose level.
There were no statistically significant differences in mean body weights. However, during the first week of treatment, lower and higher body weight gains were observed in males and females, respectively. As no changes were observed later, these differences were attributed to biologic variation.
No changes in motor activity, grip strength and sensory reactivity to different types of stimuli were reported.
No specific eye alterations were seen at any examination time.
Treatment-related dermal irritation was observed in both sexes. Findings included slight to moderate erythema, slight oedema and desquamation (see Table 5.3.2- 15).

Table 5.3.2- 15: Dermal irritation noted in rats following a 28-day percutaneous exposure to fosetyl-Al

Organ(number of animal) Finding	Male		Female	
	Dose (ppm)		Dose (ppm)	
	0	1050	0	1050
SKIN (n=10)				
Erythema				
Slight	0/10	0/10	0/10	4/10
Moderate	0/10	2/10	0/10	4/10
Oedema				
Slight	0/10	2/10	0/10	4/10
Desquamation				
Slight	0/10	2/10	0/10	3/10

Haematology, clinical chemistry, urinalysis
Changes in haematology were limited to mildly higher absolute neutrophil counts in treated females. These findings were likely correlated with the acute skin inflammation reported after histopathological examination.
No toxicologically meaningful changes were observed in clinical chemistry.

Gross pathology, organ weight, histopathology
At the final sacrifice, no changes in organ weights were reported. However macroscopic and histopathological examination revealed treatment related skin alterations, including crusted areas, erosion, hyperkeratosis and acute inflammation (see Table 5.3.2- 16).

Table 5.3.2- 16: Histopathological findings noted in rats following a 28-day percutaneous exposure to fosetyl-Al

Organ(number of animal) Finding	Male		Female	
	Dose (ppm)		Dose (ppm)	
	0	1050	0	1050
SKIN (n=10)				
Crusted areas	0/10	3/10	0/10	6/10
Erosion	0/10	3/10	0/10	5/10
Hyperkeratosis	0/10	3/10	0/10	5/10
Acute inflammation	0/10	3/10	0/10	4/10

III. CONCLUSION

RMS' conclusion: Dermal application of 1050 mg/kg bw/d of fosetyl-Al for 28 consecutive days caused dermal irritation with crusted areas macroscopically and hyperkeratosis and acute inflammation microscopically. There were also diffuse multiple red areas on the treated skin in 1 female found dead on day which may have been related to treatment. Based on an overall mild toxicity which was limited to the dermal site of application, the systemic NOAEL should be considered as 1050 mg/kg bw/day.

Report:	KCA 5.3.3/02 [redacted]; 2013; M-459673-01-1
Title:	21-Day sub acute dermal toxicity study of fosetyl-AL in Wistar rats with 14-day recovery
Report No.:	G8221
Document No.:	M-459673-01-1
Guideline(s):	OECD Guideline 410 (1981) Method B.9: Annex to Commission Directive 92/69/EEC
Guideline deviation(s):	none
GLP/GEP:	**yes**

Executive Summary

The subacute percutaneous toxicity of fosetyl-aluminium (fosetyl-Al) was evaluated in a 21-day study in Wistar rats according to OECD guideline 410. Five rats/sex/dose were exposed to aqueous solutions of the test article at doses of 100, 300, or 1000 mg/kg bw/day for 21 consecutive days (control animals were exposed to water). Additional high-dose and control groups were employed as recovery groups and were maintained for additional 14 days without exposure to the test item.

Each rat was observed for clinical signs, mortality and morbidity. Body weights and food consumption were measured during the course of the in-life phase of the study. Clinical pathology investigations (urinalysis, haematology, and clinical chemistry) were performed at the end of treatment for main groups and at the end of recovery period for the recovery groups. All the rats of main group and recovery groups were sacrificed and subjected to gross examination. Specified organs were collected, weighed and preserved. Histopathological examination was carried out on preserved organs of control and high-dose animals. In addition, all gross lesions from all animals were examined microscopically.

No clinical signs, mortalities or ocular changes were observed. There were no significant variations in mean body weights, net body weight gains and food consumption. No toxicologically significant changes were observed in haematology, clinical chemistry or urinalysis parameters. No significant changes were observed in absolute or relative organ weights. No gross or microscopic changes were observed.

Thus, under the conditions of this test, the dermal NOAEL for fosetyl-Al in the rat is ≥ 1000 mg/kg bw/day.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material:

Name:	Fosetyl-Al
Description:	White powder
Batch / Lot No.:	12020045
Purity:	97.1%
Stability of test compound:	Expiry date: 2014-07-05. Stability and homogeneity in vehicle were analytically verified.

2. Vehicle: Deionised water

3. Test animals

Species:	Rat
Strain:	Wistar rats-HSD Han: wist
Sex:	Males and females
Age:	11-12 weeks
Weight at dosing:	Males: mean weights 305-311 g Females: mean weights 209-216 g
Source:	[redacted], Israel
Acclimatisation period:	5 days
Diet:	Teklad Certified (2014C) Global 14% Protein Rodent Maintenance Diet – Pellet (Certified), *ad libitum*

Water:	Charcoal-filtered and UV-irradiated deep bore-well water, *ad libitum*
Housing:	Individually in standard polysulfone cages with corn cob bedding
Environmental conditions:	
Temperature:	21-24°C
Humidity:	65-68%
Air changes:	12-15 h^{-1}
Photoperiod:	12 h light / 12 h dark

B. STUDY DESIGN AND METHODS

1. In life dates
2012-10-25 to 2012-12-28

2. Animal assignment and treatment
Animal assignment and dose groups:
Rats were randomly distributed to different groups by body weight stratification method. Rats with extreme body weights were removed from the study. Grouping was done one day prior to the start of the treatment period. The following dose groups were employed:

Table 5.3.3- 1: Group allocation in the subacute percutaneous toxicity study in rats

Test Group	Dose (mg/kg bw/day)	Dose volume (mL/kg bw)	Conc. in vehicle (mg/mL)	No. of animals	
				Male	Female
G1 – Control	0	2	0	5	5
G2 – Low dose	100	2	50	5	5
G3 – Mid dose	300	2	150	5	5
G4 – High dose	1000	2	500	5	5
G1R – Control recovery	0	2	0	5	5
G4R – High dose recovery	1000	2	500	5	5

Preparation of the test item for application:
Dose solutions/suspensions in deionised water were prepared freshly every day before application. The weight of the test item and volume prepared varied depending on the requirement (body weight and intended dose).

Details on dermal exposure:

Route of exposure	Dermal, semi-occlusive
Exposure site	Dorsolateral thoracic region, ca. 10% of body surface area Males: ca. 9 x 6 cm; Females: ca. 8 x 5 cm
Preparation of exposure site	Approximately 24 hours before test item application (first application), the hair on the exposure site was clipped using an electric clipper. Care was taken to avoid skin abrasions. Repeat clipping was done once in 3 or 4 days on all the animals at the same time (16-18 hours prior to next application).
Frequency of treatment	Once daily on 21 consecutive days
Duration of exposure	6 h per day
Removal of test substance	The treated area was rinsed with lukewarm water and an absorbent paper was used to dry the area.
Recovery period	14 days for additional control and high-dose groups.

3. Examinations

Clinical signs - Mortality and moribundity/general daily observations	Each rat was observed twice daily, once in the morning and once in the afternoon, for mortality and morbidity. Routine cage side observations for checking general clinical signs were performed once prior to application of the test item and once after washing. The treated skin areas were examined twice daily (prior to application and approximately 30 minutes after washing) and skin reactions were assessed according to the numerical scoring system of Draize (cf. OECD guideline 404).
Clinical signs - detailed observations	Detailed clinical examination was done prior to the test item administration on Day 1 and once a week thereafter during treatment and recovery period for all rats. During detailed clinical examination, all rats were observed for changes in skin, fur, eyes, mucous membranes, occurrence of secretions and excretions and autonomic activity (e.g. lacrimation, piloerection, pupil size, unusual respiratory pattern), changes in gait, posture and response to handling as well as the presence of clonic/tonic movements, stereotypic behaviour (e.g., excessive grooming, repetitive circling) or abnormal behaviour (e.g. self-mutilation, walking backwards).
Body weights	Individual body weights were recorded before the administration of test item (Day 1) and at weekly intervals thereafter during the treatment and recovery period. Fasting body weight was recorded prior to terminal sacrifice.
Food and water consumption	Food consumption was measured weekly. Water consumption was not measured.
Ophthalmic evaluation	Ophthalmological examination of all animals was performed with an ophthalmoscope by a veterinarian prior to start of treatment and at the end of the treatment period for the main group animals and at the end of the recovery period for the recovery group animals. Before examination, mydriasis was induced using a 1% solution of Tropicamide.
Blood collection	At the end of the treatment period (on Day 22) for main groups and at the end of recovery period (on Day 36) for recovery groups, all rats were fasted overnight (water allowed), anaesthetized with isoflurane and blood was collected from retro-orbital sinus plexus with fine capillary tube.

Haematology	The following parameters were examined: Haematocrit, haemoglobin, mean corpuscular haemoglobin, mean corpuscular haemoglobin concentration, mean corpuscular volume, mean platelet volume, platelets, red blood cells, reticulocytes count, white blood cells, differential leukocyte count, prothrombin time, and activated partial thromboplastin time
Clinical chemistry	The following parameters were examined: Alanine aminotransferase, albumin, albumin/globulin ratio (calculated values), alkaline phosphatase, aspartate aminotransferase, blood urea nitrogen, calcium chloride, creatine kinase, creatinine, gamma glutamyl transpeptidase, globulin (calculated values), glucose, inorganic phosphorus, potassium, sodium, total bilirubin, direct bilirubin, indirect bilirubin (calculated values), total cholesterol, total plasma protein, triglycerides
Urinalysis	The following parameters were examined: Specific gravity , nitrite, pH, protein, glucose, ketone bodies, urobilinogen, bilirubin, appearance (colour and clarity), volume
Gross pathology	All the rats of main and recovery group were subjected to detailed necropsy. The rats sacrificed at term were fasted overnight (water allowed), weighed, exsanguinated under isoflurane anaesthesia and were subjected to detailed necropsy by a pathologist.
Organ weights	Total and relative organ weights of all sacrificed rats were determined. The paired organs were weighed together and combined weight was presented. The following organs were weighed: Adrenal glands, kidneys, liver, testes.
Histopathology	On completion of the gross pathology examination, the tissues and organs noted below were collected and preserved from all rats. Histopathological examination was restricted to the preserved organs from control (G1) and high dose (G4) group animals. In addition, all gross lesions from all the animals were examined microscopically. Adrenal glands, kidneys, liver, skin (treated and untreated), testes.

Statistical evaluation

All quantitative variables like neurological observations (neuromuscular observation and body temperature) and clinical pathology (haematology, coagulation and clinical chemistry) data were tested for normality and homogeneity of variances (Levene's test) within the group before performing a one-factor ANOVA modelling by treatment groups. If the data are found to be non-optimal (non-normal or heteroschedastic), data was transformed before ANOVA was performed. ANOVA was done using suitable transformation. Comparison of means between treatment groups and control group was done using Dunnett's 't' test when the overall treatment 'F' test was found to be significant.

In the case of recovery groups, data of treatment period and recovery period (no treatment period) was tested using the methods stated above.

II. RESULTS AND DISCUSSION

A. MORTALITY
No mortality occurred throughout the study period.

B. CLINICAL OBSERVATIONS
Clinical signs of toxicity attributable to the test substance were not observed throughout the study period.

C. BODY WEIGHT
There were no significant variations in mean body weights at any of the tested dose groups.

D. FOOD AND WATER CONSUMPTION
There were no significant variations in mean food consumption at any of the tested dose groups.

E. OPHTHALMOSCOPIC EXAMINATION
Ophthalmoscopy revealed no test article related findings in any of the animals.

F. HAEMATOLOGY
There were no treatment-related changes in haematology parameters at all the doses tested. Decreased mean cellular haemoglobin concentrations at 100 and 300 mg/kg bw/day in females and 1000 mg/kg bw/day in males on Day 22 was considered an incidental and transient change as the magnitude of change (< 5%) was minimal. Decreased reticulocytes counts at 1000 mg/kg bw/day dose in recovery females on Day 36 was considered toxicologically insignificant as there were no significant changes in reticulocyte count at the end of treatment.
Increased prothrombin time at 300 mg/kg bw/day in males and 100 mg/kg bw/day in females on Day 22 was considered toxicologically insignificant as there was no progression with dose.
Decreased activated partial thromboplastin time in females at 100 (56%), 300 (68%) and 1000 (47%) mg/kg bw/day dose groups on Day 22 was considered toxicologically insignificant as there was no dose correlation.

G. CLINICAL CHEMISTRY
There were no treatment-related changes in clinical chemistry parameters at any dose tested. At 1000 mg/kg bw/day, increased total bilirubin concentration in recovery males and females, decreased calcium concentration in males and increased total protein concentration in recovery females at the end of the recovery period (on Day 36) was considered toxicologically insignificant as there were no significant changes in respective parameters at the end of treatment period.

H. URINALYSIS
There were no significant differences noted in urinalysis parameters for any dose group.

I. PATHOLOGY
There were no treatment-related macroscopic changes noted in any of the animals. Determination of organ weights revealed no test-article-related changes. Increased absolute weight of testes at 1000 mg/kg bw/day in recovery males at the end of the recovery period (on Day 36) was considered toxicologically insignificant as there were no significant changes in testes weights at end of the treatment period.
Histopathological examination revealed no treatment-related findings.

III. CONCLUSION

There were no treatment-related adverse effects noted in any of the dose groups tested following subacute percutaneous exposure to fosetyl-Al. Thus, under the conditions of this test, the NOAEL for fosetyl-Al in the rat is ≥ 1000 mg/kg bw/day.

CA 5.4 Genotoxicity testing

Fosetyl-Al was tested in a complete battery of *in-vitro* and *in-vivo* assays (see Table 5.4- 1).
New genotoxicity studies have been performed to fulfil legal requirements in India. The new studies are summarised in detail in the following sections**. Fosetyl-Al was negative in all *in-vitro* and *in-vivo* genotoxicity tests.** Therefore, a **classification for germ cell mutagenicity is not warranted**, according to the criteria of Regulation 1272/2008.
The data requirements published in Commission Regulation (EU) No 283/2013 stipulate a study on photomutagenicity for active substances and their major metabolites showing an extinction coefficient ≥ 1000 L x mol^{-1} x cm^{-1} in the spectrum of 290 to 700 nm. Fosetyl-Al and its major metabolites, phosphonic acid, ethanol, and carbon dioxide, do not fulfil this criterion and thus, this data requirement does not apply.

Table 5.4- 1: Genotoxicity/mutagenicity tests with fosetyl-Al

Study type	Organism	Concentration / Dose	Purity (%)	Results	Reference
In vitro bacterial cell gene mutation test	*S. typhimurium* TA 98, 100, 1535, 1537, 1538	up to 1000 µg/plate (± S9)	99.7	negative	[redacted]; 1981; M-159301-01-1
	S. typhimurium TA 98, 100, 1535, 1537, *E. coli* WP2 uvrA	up to 5000 µg/plate (± S9)	97.1	negative	[redacted]; 1997; M-184456-01-1
	S. typhimurium TA 98, 100, 1535, 1537 *E. coli* WP2 uvrA	50 to 5000 µg/plate (± S9)	97.1	negative	[redacted]P; 2013; M-447222-01-1
In vitro chromosome aberration test	CHO cells	3 to 100 µg/mL (± S9)	97.7	negative	[redacted]; 1982; M-231739-01-2
	CHO cells	180 to 1800 µg/mL (–S9) 260 to 2600 µg/mL (+S9)	97.1	negative	[redacted]; 2013; M-450289-01-1
In vitro mammalian cell gene mutation test	L5178Y cells (mouse lymphoma assay)	78 to 2500 µg/mL (± S9)	97.0	negative	[redacted]; 1997; M-184459-01-1
	CHO cells (HPRT assay)	112 to 3541 µg/mL (±S9)	97.1	negative	[redacted]; 2013; M-450287-01-1
In vivo micronucleus test	Swiss mice (♂+♀)	1000, 2000, and 4000 mg/kg bw	>95	negative	[redacted]; 1977; M-223290-01-2
	CD1 mice (♂+♀)	1063, 2125, and 4250 mg/kg bw	97.0	negative	[redacted]; 1998; M-178982-01-1
	Swiss mice (♂+♀)	500, 1000, and 2000 mg/kg bw/day, 2 consecutive days	97.1	negative	[redacted]; 2013; M-449130-01-1
Induct test	*E. coli* K12	up to 2000 µg/plate (± S9)	99.7	negative	[redacted]; 1978; M-178996-01-2
		up to 200 µg/plate (± S9)	99.7	negative	[redacted]; 1981; M-159301-01-1
DNA repair test	*E. coli* W3476, W3110	up to 500 µg/plate (± S9)		negative	
In vitro yeast test	*S. cerevisiae D7*	up to 1000 µg/mL (– S9) up to 500 µg/mL (+ S9)		negative	

CA 5.4.1 *In-vitro* studies

Report:	KCA 5.4.1/01 [redacted],; [redacted]; 1981; M-159301-01-1
Title:	Fosetyl-Al (32545 R.P., aluminium salt) - Supplementary studies of mutagenesis in microorganisms.
Report No.:	R000765
Document No.:	M-159301-01-1
Guideline(s):	not specified
Guideline deviation(s):	not applicable
GLP/GEP:	**no**

I. MATERIALS AND METHODS

Technical fosetyl-Al (batch no. DA 67; 99.7% purity) was tested in a plate incorporation assays using 5 bacterial tester strains: TA98; TA100; TA1535; TA1537 and TA 1538 of *S. typhimurium,* with and without metabolic activation using S-9 fraction of liver homogenate prepared from the livers of Aroclor treated rats. A toxicity experiment was carried out with 10, 100, 1000, and 5000 µg test substance/plate and the main test (using triplicate plates) with 125; 250; 500 and 1,000 µg/plate of fosetyl-Al along with negative controls and reference mutagens (beta-propiolactone: 50 µg/plate; hycanthone: 50 µg/plate; niridazole: 0.05 µg/plate and ethidium bromide: 60 µg/plate). In addition, a spot test was carried out in similar conditions as in the plate incorporation assay, using 1000 µg/10 µL fosetyl-Al and an incubation period of 48 hours.

II. RESULTS AND DISCUSSION

The mean number of revertants per treated plate over that per control plate did not exceed 1.15 in the absence of metabolic activation and 1.11 in the presence of metabolic activation (see Table 5.4.1- 1).

Table 5.4.1- 1: Incorporation test (mean number of revertant colonies)

Mean number of revertant colonies (3 replicates)											
	µg/plate	**TA1535**		**TA1537**		**TA1538**		**TA98**		**TA100**	
		-S9	**+ S9**	**-S9**	**+ S9**	**-S9**	**+ S9**	**-S9**	**+ S9**	**-S9**	**+ S9**
1st experiment											
Solvent	0	16.3	15	8.3	9.3	7	13.7	15.3	19.3	98.7	101
Fosetyl-Al	125	14.6	13	5.6	8.6	6.3	13.3	14	20.3	112.3	110.7
	250	15	12.6	6	8	5.6	11.3	14	19.3	113.7	106.3
	500	15.3	14.3	8	6.6	5	14.3	15.7	19.6	107.3	106.3
	1000	13.6	13	6.3	5.3	8	15.3	16.3	20.3	98.3	97
Beta-propio-lactone	50	1191									
Hycanthone	50		430		467						
Niridazole	0.05							672		1672	
Ethidium bromide	60							23.7	1379		

III. CONCLUSION

Fosetyl-Al did not induce reverse gene mutation in any *Salmonella typhimurium* strains with or without metabolic activation.

Report:	KCA 5.4.1/02 [redacted]; 1997; M-184456-01-1
Title:	Fosetyl Al: Reverse mutation in four histidine-requiring strains of Salmonella typhimurium and one tryptophan-requiring strain of Escherichia coli.
Report No.:	R011732
Document No.:	M-184456-01-1
Guideline(s):	EU (=EEC): Annex V, Tests B13 & B14; ICH: Tripartite Harm. Guideline on Genotox.; JMAF: (1985); MHW (Japan): (1989); OECD: 471, (1983), 472, (1983); USEPA (=EPA): OPPTS 870.5100, OPPTS 870.5265
Guideline deviation(s):	none
GLP/GEP:	**yes**

I. MATERIALS AND METHODS

Technical fosetyl-Al (batch N° 9607181, purity 949 g/kg) was tested for its ability to induce mutation in 4 strains of *Salmonella typhimurium* (TA98, TA100, TA1535 and TA1537) and one strain of *Escherichia coli* (WP2 uvrA). The study consisted of a cytotoxicity range-finding experiment followed by two independant experiments, each conducted in the absence and presence of metabolic activation by an Aroclor 1254 induced rat liver post-mitochondrial fraction (S-9). Negative and appropriate positive controls were included in each experiment.

II. RESULTS AND DISCUSSION

Cytotoxicity range-finder experiment

Fosetyl-aluminium (fosetyl-Al) was dissolved in sterile purified water and tested at 0, 40, 200, 1000 and 5000 µg/plate in TA100 only. No evidence of toxicity was observed.

Experiment 1 (standard plate incorporation)

Accordingly, fosetyl-Al was tested at the same final concentrations.

Cytotoxicity, indicated by a slight thinning of the background bacterial lawn, was observed at 5000 µg/plate with *Salmonella typhimurium* strains TA98 and TA100, both in the absence and presence of S-9 and with *Escherichia coli* strain WP2 in the absence of S-9.

The mean numbers of revertant colonies on negative control plates were within acceptable ranges while the mean number of revertant colonies on positive control plates were significantly increased (see Table 5.4.1- 2).

Fosetyl-Al treatments produced no statistically significant increases in the mean numbers of revertant colonies in any tested strains both in the absence and presence of S-9 (see Table 5.4.1- 2).

Table 5.4.1- 2: Number of revertant colonies in *Salmonella typhimurium* and *Escherichia coli* strains following treatment with fosetyl-Al - Experiment 1

Treatment and concentration (µg/plate)	Mean revertants per plate (± SD)				
	TA98	TA100	TA1535	TA1537	WP2 uvrA
Without metabolic activation					
Solvent control	24 ± 5	76 ± 29	12 ± 2	15 ± 3	14 ± 4
2NF 5	854 ± 5	-	-	-	-
NaN_3 2	-	625 ± 59	330 ± 5	-	-
AAC 50	-	-	-	184 ± 13	-
NQO 2	-	-	-	-	986 ± 51
Fosetyl-Al 8	28 ± 11	128 ±11	12 ± 1	15 ± 1	10 ± 2
Fosetyl-Al 40	36 ± 9	143 ± 13	16 ± 5	17 ± 7	21 ± 13
Fosetyl-Al 200	22 ± 2	130 ± 6	13 ± 2	17 ± 3	19 ± 1
Fosetyl-Al 1000	20 ± 5 (C)	132 ± 7	12 ± 2	13 ± 2	12 ± 2
Fosetyl-Al 5000	27 ± 2 (C)	112 ± 14 (C)	9 ± 5	17 ± 5	7 ± 2 (C)

Treatment and concentration (µg/plate)	Mean revertants per plate (± SD)				
	TA98	TA100	TA1535	TA1537	WP2 uvrA
With metabolic activation					
Solvent control	37 ± 6	125 ± 14	18 ± 4	13 ± 5	16 ± 3
AAN 5	979 ± 84	-	-	-	-
AAN 10	-	-	-	-	250 ± 29
Fosetyl-Al 8	26 ± 4	111 ± 1	15 ± 4	17 ± 2	14 ± 3
Fosetyl-Al 40	38 ± 6	126 ± 10	18 ± 3	9 ± 3	11 ± 7
Fosetyl-Al 200	39 ± 7	108 ± 10	17 ± 4	11 ± 2	14 ± 2
Fosetyl-Al 1000	46 ± 5	121 ± 8	15 ± 2	16 ± 4	9 ± 3
Fosetyl-Al 5000	34 ± 6 (C)	107 ± 12 (C)	17 ± 3	17 ± 8	12 ± 3

SD: standard deviation
C : presence of cytotoxicity
2NF: 2-nitrofluorene; NaN_3: Sodium azide; AAC: 9-aminoacridine; NQO: 4-nitroquinoline; AAN: 2-aminoanthracene

Experiment 2 (pre-incubation step)

According to the cytotoxicity observed in the previous experiment, lower maximal concentrations were tested in strains TA98, TA100 and WP2 whilst 5000 µg/plate was retained as the maximum test dose for treatments of TA1535 and TA1537. In each case, narrowed dose ranges were used (see Table 5.4.1- 3).

Cytotoxicity was observed at high concentrations with all *Salmonella typhimurium* strains in the presence of S-9 and in *Escherichia coli* strain WP2 in the absence of S-9.

The mean numbers of revertant colonies in negative control plates were within acceptable ranges while the mean number of revertant colonies in positive control plates were significantly increased (see Table 5.4.1- 3).

Fosetyl-Al treatments produced no statistically significant increases in the mean numbers of revertant colonies in any tested strains both in the absence and presence of S-9 (see Table 5.4.1- 3).

Table 5.4.1- 3: Number of revertant colonies in *Salmonella typhimurium* and *Escherichia coli* strains following treatment with fosetyl-Al - Experiment 2

Treatment and concentration (µg/plate)	Mean revertants per plate (± SD)				
	TA98	TA100	TA1535	TA1537	WP2 uvrA
With metabolic activation					
Solvent control	33 ± 5	123 ± 3	21 ± 7	9 ± 2	26 ± 4
2NF 5	894 ± 46	-	-	-	-
NaN_3 2	-	434 ± 31	422 ± 31	-	-
AAC 50	-	-	-	234 ± 40	-
NQO 2	-	-	-	-	667 ± 191
Fosetyl-Al 78.125	36 ± 8	-	-	-	-
Fosetyl-Al 156.25	30 ± 5	116 ± 6	-	-	24 ± 4
Fosetyl-Al 312.5	32 ± 4	104 ± 7	21 ± 10	9 ± 1	22 ± 6
Fosetyl-Al 625	30 ± 5	98 ± 16	22 ± 2	8 ± 5	22 ± 5
Fosetyl-Al 1250	24 ± 4	115 ± 11	18 ± 7	5 ± 2	22 ± 2
Fosetyl-Al 2500	-	127 ± 16	19 ± 3	6 ± 4	11 ± 2
Fosetyl-Al 5000	-	-	21± 5	7 ± 2	-

Treatment and concentration (µg/plate)	Mean revertants per plate (± SD)				
	TA98	TA100	TA1535	TA1537	WP2 uvrA
With metabolic activation					
Solvent control	41 ± 6	125 ± 7	23 ± 5	10 ± 4	28 ± 6
AAN 5	974 ± 101	-	-	-	-
AAN 10	-	-	-	-	48 ± 10
Fosetyl-Al 156.25	36 ± 1	125 ± 11	-	-	20 ± 7
Fosetyl-Al 312.5	39 ± 8	122 ± 4	17 ± 4	9 ± 3	20 ± 5
Fosetyl-Al 625	38 ± 6	126 ± 6	24 ± 1	11 ± 3	17 ± 4
Fosetyl-Al 1250	41 ± 6 (C)	121 ± 12 (C)	21± 3	9 ± 3	17 ± 4
Fosetyl-Al 2500	32 ± 5 (C)	136 ± 12 (C)	21 ± 5	7 ± 2	13 ± 2
Fosetyl-Al 5000	-	-	20 ± 8 (C)	3 ± 1 (C)	-

SD: standard deviation
C: presence of cytotoxicity
2NF: 2-nitrofluorene; NaN_3: Sodium azide; AAC: 9-aminoacridine; NQO: 4-nitroquinoline; AAN: 9-aminoanthracene

III. CONCLUSION

RMS conclusion: Fosetyl-Al was negative for mutagenicity in 5 tester strains of bacteria at concentrations up to and including 5000 µg/plate in the presence and absence of S-9 metabolic activation.

Report: KCA 5.4.1/03 [redacted] [redacted]; 1982; M-231739-01-2
Title: Report on "In vitro assay of chromosomal aberrations in CHO cell line, with and without metabolic activation", carried out on the product phosetyl-al of Ravit Co., Rome
Report No.: R000985
Document No.: M-231739-01-2
Guideline(s): not specified
Guideline deviation(s): not applicable
GLP/GEP: no

II. MATERIALS AND METHODS

An *in vitro* chromosome aberration test, using Chinese Hamster Ovary cells, was conducted on technical fosetyl-al (batch DEP 203, 97.5 – 0.5% purity) at concentrations ranging from 3 to 100 µg/mL, with and without S-9 metabolic activation.

Toxicity and range finding tests:
Fosetyl-ammonium (fosetyl-Al) was diluted in culture medium (Ham's F10 medium from Flow laboratories, Scotland) at final concentrations of 3, 10, 30 and 100 µg/mL. At the highest practicable concentration (limited solubility in the culture medium), the mitotic index was significantly reduced, indicating a slight toxic effect.

Chromosome aberration assay:
Duplicate assays were performed with and without metabolic activation, using 4 selected dose levels of test material in culture medium (0, 3, 10, 30, and 100 µg/mL) for a 3- and a 24-hours incubation period with and without metabolic activation, respectively and a 21-hours harvest interval. Metabolic activation was performed using S-9 fraction (9000*g* supernatant of liver homogenate prepared from the livers of male Sprague Dawley rats which were given enzyme inducers for 4 days: intraperitoneal injection of 30 mg/kg bw phenobarbital (d-1); 60 mg/kg bw phenobarbital (on d-2); 60 mg/kg bw of phenobarbital and 80 mg/kg bw of beta-naphtoflavone (on d-3) and 60 mg/kg bw of phenobarbital (on d-4). Capacity to induce metabolic activation was tested, determination of microsomal proteins was performed and enzyme tests on S-9 were carried out.

All cultures were exposed to Colchicine 3 hours prior to the harvests. Positive controls were used: 0.03 and 0.3 µg/mL Mitomycin C in the absence of metabolic activation and 0.0263 and 0.263 µg/mL cyclophosphamide in the presence of metabolic activation.
Chromosome aberrations were scored on 100 cells from each of 2 replicate cultures at each dose level of test material and from the corresponding untreated, solvent and positive controls, in the activated and non-activated systems. The total number of aberrations and the percentage of cells with one or more aberrations were classified as chromatid and chromosome aberrations (gaps, fragments, breaks, exchanges, minutes, rings, dicentric, polycentric) for each dose level.

II. RESULTS AND DISCUSSION

Test substance was toxic to the CHO cells at the highest concentration tested in the absence or in the presence of metabolic activation, as shown by the mitotic index (see Table 5.4.1- 4). The highest dose level selected for the mutagenic assay represented the solubility limit of the test material in the culture medium.

Table 5.4.1- 4: Mitotic index

	Dose (µg/mL)	Without S9 mix			With S9 mix		
		Cell analysed	Mitosis	Mitotic Index	Cell analysed	Mitosis	Mitotic Index
Control	0.0	1000	18	1.8	1054	94	8.91
Fosetyl-Al	3	1015	47	4.63	1000	107	10.7
	10	1042	42	4.02	1000	100	10
	30	1005	29	2.88	1000	58	5.8
	100	1002	25	2.5	1008	43	4.3
Mitomycin	0.03	1000	32	3.2	-	-	-
	0.3	1000	15	1.5	-	-	-
Cyclophosphamide	0.0263	-	-	-	1000	41	4.1
	0.263	-	-	-	1000	24	2.4

There was no statistically significant increase in the chromatid and chromosome aberrations frequencies at any dose level of Fosetyl-Al, both in the presence and in the absence of S-9 metabolic activation. Negative control range were claimed to be within normal historical changes and positive controls produced statistically significant increases of aberrations (see Table 5.4.1- 5).

Table 5.4.1- 5: Number and type of chromatid and chromosome aberrations

Treatment	Dose (µg/mL)	No. of cells	Chromatid				Chromosome				% of aberrant cells (gap excluded)	% of aberrant cells(gap included)	% of aberrant cells (gap included)
			G	B		E	G	B	F	E			
Without metabolic activation													
Solvent control	0	100	0	0	0	0	1	0	0	0	0	1	1
Fosetyl-Al	3	100	1	0	0	0	0	1	0	0	1	2	2
Fosetyl-Al	10	100	0	0	0	0	2	2	0	0	2	4	3
Fosetyl-Al	30	100	0	0	0	0	0	1	0	0	1	1	1
Fosetyl-Al	100	100	1	0	0	0	0	0	0	0	0	1	1
MMC	0.03	100	3	2	0	9	3	20	3	0	34	40	32**
MMC	0.3	100	6	15	0	12	4	5	0	0	32	42	30**

Treatment	Dose (µg/mL)	No. of cells	Chromatid				Chromosome				% of aberrant cells (gap excluded)	% of aberrant cells(gap included)	% of aberrant cells (gap included)
			G	B	F	E	G	B	F	E			
With metabolic activation													
Solvent control	0	100	0	0	0	0	0	1	0	0		1	1
Solvent + S9	0	100	2	3	0	0	0	1	0	0	4	6	
Fosetyl-Al	3	100	1	2	0	0	0	4	0	0	6		6
Fosetyl-Al	10	100	2	0	0	0	1	2	0		2	3	3
Fosetyl-Al	30	100	0	0	0	0		1	0	0	1	2	
Fosetyl-Al	100	100	3	2	0	0	4	0	0	0		5	4
MMC	0.03	50	4	4	0	0	2	2	9		12	14	18*
MMC	0.3	100	6	8	0	0	1	41	1	0	67	74	42**

G: gap; B: break; F: fragment; E: exchange; Aberrant cells: include cells with gap and cells with 1 or more aberrations
MMC: Mitomycin-C; CPA: Cyclophosphamide
Significantly different from control; * p<0.05; ** p<0.01

III. CONCLUSION

RMS conclusion: Fosetyl-Al did not induce chromatid nor chromosome aberrations in the *in vitro* chromosome aberration assay in Chinese Hamster Ovary cells when tested with and without metabolic activation.

Report: KCA 5.4.1/04 [redacted] 1997; M-184459-01-1
Title: Fosetyl Al: Mutation at the thymidine kinase (tk) locus of mouse lymphoma L5178Y cells (MLA) using the microtitre fluctuation technique.
Report No.: 1011733
Document No.: M-184459-01-1
Guideline(s): EU (EEC): 87/302/EEC, B.17; ICH (1997); OECD: 476, (1984); UKEMS: (1990); USEPA (EPA): OPPTS 870.5300
Guideline deviation(s): none
GLP/GEP: yes

II. MATERIALS AND METHODS

Fosetyl-Al (batch no. 9607C01, purity 97.9 g/kg) was tested for its ability to induce mutation at the *tk* locus (5-trifluorothymidine resistance) in mouse lymphoma cells using a Microtitre fluctuation protocol. The study consisted of a cytotoxicity range finding experiment followed by two independent experiments, each conducted with a 3-hours incubation period in the absence and presence of metabolic activation by an Aroclor 1254 induced rat liver post-mitochondrial fraction (S-9).
Negative and appropriate positive controls were included in each experiment.

III. RESULTS AND DISCUSSION

Cytotoxicity range-finding experiment
Fosetyl-aluminium (fosetyl-Al) was dissolved in sterile purified water and tested at final concentrations separated by two-fold intervals and ranging from 31.25 to 1000 µg/mL. No evidence of toxicity was observed. Post-treatment precipitate was observed at 125, 250, 500 and 1000 µg/mL in the absence and presence of S-9. The highest concentration of 1000 µg/mL yielded 63.14 and 45.38% relative survival in the absence and presence of S-9, respectively.

Main study experiments

Accordingly, fosetyl-Al was tested at final concentration up to 250 µg/mL (approximate limit of solubility in cell culture medium). Six doses, separated by two-fold intervals and ranging from 7.813 to 250 µg/mL were tested in the absence and presence of S-9 in 2 independent experiments. The top five doses tested were selected to determine viability and 5-trifluorothymidine (5-TFT) resistance 2 days after treatment. The highest concentration of 250 µg/mL yielded 80.08 and 113.70% relative survival in experiment 1 and 121.24 and 96.15% relative survival in experiment 2, in the absence and presence of S-9, respectively (see Table 5.4.1- 6).

Mutant frequencies were within historical normal ranges in negative control cultures while mutant frequencies were clearly increased in positive control cultures (see Table 5.4.1- 6).

In the 2 independent experiments, fosetyl-Al treatments produced no statistically significant increases in mutant frequency at any dose level tested in the absence or presence of S-9 (see Table 5.4.1- 6).

Table 5.4.1- 6: Relative survival, relative total growth and mutant frequency in L5178Y mouse lymphoma cells following treatment with fosetyl-Al

Treatment and concentration (µg/mL)	Without metabolic activation			With metabolic activation		
	% RS	RTG	MF	% RS	RTG	MF
Experiment 1						
Solvent control	100.00	1.0	107.33	100.00	1.0	185.19
Fosetyl-Al 7.813 $	78.30			98.37		
Fosetyl-Al 15.625	84.41	0.95	102.42 NS	99.32	0.79	204.24 NS
Fosetyl-Al 31.25	79.10	1.01	151.52 NS	108.14	0.67	114.10 NS
Fosetyl-Al 62.5	102.17	1.09	118.7 NS	102.79	0.80	98.56 NS
Fosetyl-Al 125	80.55	0.96	102.27 NS	97.45	0.93	97.54 NS
Fosetyl-Al 250	80.08	1.00	95.89 NS	113.70	0.80	98.18 NS
Linear trend						NS
NQO 0.05	86.67	0.68	442.67		-	-
NQO 0.1	94	0.79	475.1			-
B(a)P 2	-	-	-	75.24	0.78	494.82
B(a)P 3				53.8	0.32	902.47
Experiment 2						
Solvent control	100.00	1.0	219.52	100.00	1.0	205.86
Fosetyl-Al 7.813 $	95.83			115.62		
Fosetyl-Al 15.625	106.38	1.04	206.07 NS	111.38	1.04	177.66 NS
Fosetyl-Al 31.25	98.04	1.23	184.53 NS	109.36	0.99	149.38 NS
Fosetyl-Al 62.5	94.95	1.12	198.31 NS	129.56	1.06	187.27 NS
Fosetyl-Al 125	137.22	1.49	210.29 NS	91.95	1.09	198.12 NS
Fosetyl-Al 250	121.24	1.66	156.38 NS	96.15	1.28	184.24 NS
Linear trend			NS			NS
NQO 0.05	96.58	1.32	321.27	-	-	-
NQO 0.1	97.36	1.06	577.35	-	-	-
B(a)P 2		-	-	82.68	0.55	1178.82
B(a)P 3				49.94	0.32	1305.76

$: Not plated for viability / 5-TFT resistance
% RS: Percentage relative survival adjusted by post treatment cell counts
RTG: relative total growth
MF: mutant frequency (5-TFT resistant cell /10^6 viable cells after 2 days treatment)
NS: not significant
NQO: nitroquinoline; B(a)P: benzo(a)pyrene

III. CONCLUSION

RMS conclusion: No relevant reproducible enhancement of the mutation rate over the range of control values were induced by fosetyl-Al the *tk* locus of L5178Y mouse lymphoma cells up to the solubility limit, with and without metabolic activation.

Report:	KCA 5.4.1/06 [redacted]; 2013; M-447222-01-1
Title:	Fosetyl-Al: Bacterial reverse mutation test
Report No.:	G8217
Document No.:	M-447222-01-1
Guideline(s):	OECD Guidelines for the Testing of Chemicals, Test No 471 (1997); Method B.13/14 (Mutagenicity) Official Journal of the European Communities, L142, 31/05/2008
Guideline deviation(s):	none
GLP/GEP:	**yes**

Executive Summary
Fosetyl-aluminium (fosetyl-Al) was examined in the *S. typhimurium* strains TA98, TA100, TA 1535, and TA 1537 and in the *E. coli* strain WP2*uvr*A (pKM101) in two independent experiments, each carried out without and with metabolic activation (a microsomal preparation derived from Aroclor 1254-induced rat liver). The first experiment was carried out as a plate incorporation test and the second as a pre-incubation test.
In a preliminary test, fosetyl-Al was examined for potential cytotoxicity and precipitation (plate incorporation test without and with metabolic activation) in test strain TA 100. Neither precipitation nor cytotoxicity was observed at concentrations up to 5000 µg/plate. Hence, 5000 µg/plate was chosen as top concentration for the main study.
In the main study, five concentrations ranging from 50 to 5000 µg/plate were employed in the plate incorporation test and in the pre-incubation test, each carried out without and with metabolic activation. No increase in revertant colony numbers as compared with control counts was observed for fosetyl-Al in any tester strain, without and with metabolic activation, respectively (plate incorporation and pre-incubation test). The positive controls showed a significant increase in the number of revertant colonies of the test strain and confirmed the validity of the test conditions and the sensitivity of the test system.
In conclusion, fosetyl-Al is not mutagenic in the bacterial reverse mutation test, neither in the presence nor absence of a metabolic activation system under the conditions of this test.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material:

Name:	Fosetyl-Al
Description:	White powder
Batch / Lot No.:	12020045
Purity:	97.1%
Stability of test compound:	Expiry date: 2014-07-05 Stability in vehicle was analytically verified

2. Vehicle and/or positive control:

Vehicle:	sterile water
Pos. controls:	2-Nitrofluorene (2-NF), -S9
	Sodium azide (NaN_3), -S9
	9-Aminoacridine (9-AA), -S9
	4-Nitroquinoline-l-oxide (4-NQO), -S9
	2-Aminoanthracene (2-AA), +S9

3. Test system:

Organisms:	*Salmonella typhimurium* and *Escherichia coli.*
Strains:	*S. typhimurium* TA98, TA100, TA 1535, and TA 1537 *E. coli* WP2*uvr*A (pKM101)
Source:	[redacted], UK *E. coli*: [redacted] UK

Metabolic activation system:	S9 homogenate was prepared from male Wistar rats induced with a single i.p. injection of Aroclor 1254, 5 days prior to sacrifice. Each batch of S9 homogenate was characterized for its ability to metabolize the pro-mutagens 2-aminoanthracene and benzo(a)-pyrene to mutagens using *S. typhimurium* TA100 strain.
Test concentrations:	Preliminary cytotoxicity test (-S9 and +S9): 50, 100, 200, 400, 800, 1600, 3200, and 5000 µg/plate (plate incorporation, TA100 only) Main study (-S9 and +S9): 50, 158, 500, 1581, and 5000 µg/plate (plate incorporation) 100, 266, 707, 1880, and 5000 µg/plate (pre-incubation)
Pre-incubation period:	ca. 30 min
Exposure duration:	67 h

B. STUDY DESIGN AND METHODS

Experimental dates
2012-09-13 through 2012-12-01

Experimental procedure
Preliminary study
Fosetyl-Al was examined in a preliminary cytotoxicity test (plate incorporation test without and with metabolic activation) in tester strain TA 100. The number of revertant colonies on the plates was counted and the bacterial background lawn was evaluated for the evidence of test item toxicity.
The plates were also checked for any precipitation formation.

Main study
Two independent mutagenicity experiments were carried out with fosetyl-Al each without and with metabolic activation. Five concentrations were employed and each experiment consisted of 3 plates per concentration. Appropriate positive and solvent (sterile water) controls were included into the test to confirm its sensitivity.
In the plate-incorporation test, 2 mL soft agar containing histidine-biotin/tryptophan, 100 µL bacterial cell suspension and 100 µL of the test item solution or solvent or positive control solution, respectively. In the experiment with metabolic activation, 500 µL of S9 mix was added, whereas in the experiments without metabolic activation, 0.5 mL of phosphate-buffered saline was added. After pouring onto agar plates and solidification, the plates were incubated at 37 ± 1 °C for 67 hours. Revertant colonies were counted manually and the plates were examined for bacterial background lawn.
The independent repeat was performed as pre-incubation in an incubator shaker at 37 ± 1 °C for 30 minutes. After this period, 2 mL soft agar containing histidine-biotin / tryptophan were added to each of the tubes. The tube contents were mixed and overlaid onto agar plates and allowed to solidify. The plates were then incubated at 37 ± 1 °C for 67 hours. Revertant colonies were counted manually and the plates were examined for bacterial background lawn.
The doses used for the positive controls are reported in Table 5.4.1- 7.

Table 5.4.1- 7: Positive controls used

Strain	Activation	Positive control	Concentrations [µg/plate]
TA98	+	2-AA	4
	–	2-NF	2
TA100	+	2-AA	4
	–	NaN_3	1
TA1535	+	2-AA	4
	–	NaN_3	1
TA1537	+	2-AA	4
	–	9-AA	50
WP2*uvr*A (pKM101)	+	2-AA	30
	–	4-NQO	4

Quality criteria
The *Salmonella typhimurium* and *Escherichia coli* reverse mutation assay is considered acceptable if it meets the following criteria:

- Tester strain integrity
  - *S. typhimurium* tester strains must exhibit sensitivity to crystal violet and ultraviolet light to demonstrate the presence of *rfa* mutation and *uvrB* mutation, respectively.
  - The *E. coli* tester strain must exhibit sensitivity to ultraviolet light to demonstrate the presence of *uvrA* mutation.
  - *S. typhimurium* strains TA98 and TA100 and *E. coli* strain WP2*uvr*A (pKM101) must exhibit resistance to ampicillin to demonstrate the presence of the plasmid R-factor.
- The spontaneous reversion rates in the vehicle control must be in the range of in-house historical data.
- There must be at least three non-toxic dose levels.
- The top dose selected should demonstrate toxicity. In case of non-toxic test items, the top dose tested should be 5000 µg/plate.
- All tester strain culture titres must be in the range of 1-2 x 10^9 cells/mL to ensure that appropriate numbers of bacteria are used for plating.
- The positive control substances should produce at least a 3-fold increase in mutant colony frequencies when compared to the respective vehicle control plates.

Assessment criteria
To determine a positive result, there should be a dose related increase in the mean revertant count per plate of at least one tester strain over a minimum of two increasing concentrations of the test item either in the presence or absence of the metabolic activation system.
The test will be judged positive, if the increase in mean revertants at the peak of the dose response is equal to or greater than 2 times the mean vehicle control value for strains TA98, TA100 and WP2*uvr*A (pKM101) or equal to or greater than 3 times the mean vehicle control value for strains TA1535 and TA1537.
An equivocal response is a biologically relevant increase in a revertant count that partially meets the criteria for evaluation as positive. This could be a dose-dependent increase that does not achieve the respective threshold cited above or a non-dose-dependent increase that is equal to or greater than the respective threshold cited. A response will be evaluated as negative, if it is neither positive nor equivocal.

II. RESULTS AND DISCUSSION

A. REVERTANT FREQUENCIES

Preliminary study

There was neither pronounced cytotoxicity nor precipitation observed in the preliminary assay with our without activation. Hence, 5000 µg fosetyl-Al per plate was chosen as top concentration for the main study in the plate incorporation test and in the pre-incubation test.

Main study

No increase in revertant colony numbers as compared with control counts was observed for fosetyl-Al, tested up to 5000 µg/plate, in any of the test strains in two independent experiments without and with metabolic activation, respectively (plate incorporation and pre-incubation test). The positive controls showed a significant increase in the number of revertant colonies of the test strain and confirmed the validity of the test conditions and the sensitivity of the test system. The results of the plate incorporation and pre-incubation tests are presented in Table 5.4.1- 8 and Table 5.4.1- 9, respectively.

Table 5.4.1- 8: Results of the plate incorporation test

Treatment (µg/plate)		No. of revertants per plate[a]									
		TA98		TA100		TA1535		TA1537		WP2*uvr*A	
		Mean ± SD	Ratio[b]	Mean ± SD	Ratio[b]	Mean ± SD	Ratio[b]	Mean ± SD	Ratio[b]	Mean ± SD	Ratio[b]
Without metabolic activation											
Solvent (water)		26±2	1.00	121±2	1.00	15±1	1.00	12±2	1.00	142±4	1.00
Fosetyl-Al	50	27±1	1.04	114±13	0.94	14±1	0.98	11±1	0.86	143±4	1.01
	158	25±3	0.97	120±4	0.99	14±2	0.95	10±1	0.81	138±3	0.97
	500	27±3	1.06	121±10	1.00	14±2	0.98	12±2	1.00	136±5	0.96
	1581	26±4	1.01	128±2	1.06	13±1	0.89	11±2	0.86	139±9	0.98
	5000	24±2	0.95	124±5	1.03	13±2	0.91	11±3	0.89	141±5	1.00
Positive control[c]		255±6	9.95	548±12	4.54	136±9	9.27	124±7	10.05	562±17	3.96
With metabolic activation											
Solvent (water)		27±3	1.00	113±3	1.00	16±1	1.00	11±1	1.00	139±3	1.00
Fosetyl-Al	50	28±3	1.04	111±4	0.98	14±1	0.89	11±2	1.06	140±8	1.01
	158	29±3	1.07	107±5	0.94	16±1	1.02	10±1	0.97	136±5	0.98
	500	27±3	0.99	119±4	1.05	13±2	0.85	9±2	0.87	139±3	1.00
	1581	28±4	1.04	116±3	1.02	16±1	1.02	11±2	1.00	137±9	0.99
	5000	27±2	1.01	123±8	1.08	14±2	0.87	11±2	1.00	145±6	1.05
Positive control[c]		556±25	20.58	878±42	7.75	147±5	9.38	116±12	10.91	570±14	4.11

[a] Means of three replicates rounded to integers
[b] Relative to vehicle control (mean revertants per plate)
[c] See Table 5.4.1- 7 for the list of positive controls

Table 5.4.1- 9: Results of the pre-incubation test

Treatment (µg/plate)		No. of revertants per plate[a]									
		TA98		TA100		TA1535		TA1537		WP2*uvr*A	
		Mean ± SD	Ratio[b]	Mean ± SD	Ratio[b]	Mean ± SD	Ratio[b]	Mean ± SD	Ratio[b]	Mean ± SD	Ratio[b]
Without metabolic activation											
Solvent (water)		29±2	1.00	104±3	1.00	13±1	1.00	11±2	1.00	138±5	1.00
Fosetyl-Al	100	25±1	0.86	111±4	1.07	15±1	1.15	12±0	1.06	138±4	1.00
	266	26±2	0.89	111±4	1.07	14±2	1.08	11±1	0.94	139±3	1.01
	707	24±3	0.84	115±7	1.11	15±2	1.13	11±3	1.00	137±2	0.99
	1880	25±2	0.86	107±2	1.04	16±3	1.21	10±1	0.88	138±3	1.00
	5000	23±2	0.80	101±2	0.97	16±1	1.21	11±2	0.94	140±2	1.01
Positive control[c]		243±6	8.39	545±17	5.26	143±19	10.97	113±4	9.94	559±12	4.06
With metabolic activation											
Solvent (water)		28±2	1.00	118±3	1.00	15±1	1.00	11±1	1.00	137±3	1.00
Fosetyl-Al	100	27±4	0.95	111±8	0.94	15±2	1.02	11±2	1.00	139±3	1.01
	266	25±1	0.89	109±3	0.92	15±2	1.00	12±1	1.09	138±3	1.01
	707	29±1	1.02	104±3	0.88	14±2	0.96	10±1	0.94	134±3	0.98
	1880	23±3	0.82	108±9	0.92	15±2	0.98	10±1	0.97	138±2	1.00
	5000	23±3	0.83	108±2	0.92	14±2	0.93	10±1	0.97	138±6	1.01
Positive control[c]		549±8	19.60	878±10	7.44	146±13	9.73	111±11	10.37	557±6	4.07

[a] Means of three replicates rounded to integers
[b] Relative to vehicle control (mean revertants per plate)
[c] See Table 5.4.1- 7 for the list of positive controls

III. CONCLUSION

Fosetyl-Al was not mutagenic in the bacterial reverse mutation assay, with and without metabolic activation.

Report: KCA 5.4.1/07 [redacted]; 2013; M-450289-01-1
Title: Fosetyl-Al: In vitro mammalian chromosome aberration test in cho cells
Report No.: G8219
Document No.: M-450289-01-1
Guideline(s): OECD Guidelines for the Testing of Chemicals, Test No 473 (1997); Method B.10 (Mutagenicity) Official Journal of the European Communities, L142, 31/05/2008
Guideline deviation(s): none
GLP/GEP: **yes**

Executive Summary

The clastogenic potential of fosetyl-aluminium (fosetyl-Al) was evaluated using cultured Chinese Hamster Ovary (CHO) cells according to OECD guideline 473.

The study consisted of a preliminary cytotoxicity test and a chromosome aberration assay comprising of three independent experiments: one each in the presence and absence of metabolic activation and a confirmatory experiment in the absence of metabolic activation (S9 fraction prepared from Aroclor-1254-induced rat liver).

Based on the observations in the preliminary cytotoxicity test, CHO cells were exposed to fosetyl-Al in duplicate at concentrations of 260, 823, and 2600 µg/mL in the presence of metabolic activation with 3-h exposure and at 180, 570, and 1800 µg/mL in the absence of metabolic activation with 3-h as well as 21-h exposure in the definitive chromosome aberration assay.

Similarly, concurrent vehicle (sterile water) and positive controls (cyclophosphamide monohydrate in the presence of metabolic activation and ethyl methanesulfonate in the absence of metabolic activation) were also tested in duplicate.
In each case, the cells were harvested at 21 hours after the start of the treatment and slides were prepared for chromosome analysis.
At the highest concentration tested (2600 µg/mL and 1800 µg/mL), the reduction in the cell growth was 51% and 51% in the presence and absence of metabolic activation respectively, with 3-h exposure whereas in the absence of metabolic activation (1800 µg/mL), with the 21-h exposure, the reduction in cell growth was 55% compared to the vehicle control.
A total of 200 metaphases per dose level from duplicate cultures were evaluated for chromosome aberrations. There was no evidence of induction of chromosome aberrations, including or excluding gaps, either in the presence or in the absence of metabolic activation. In each of these experiments, under identical conditions, the respective positive control substances produced a large and statistically significant increase in aberrant metaphases.
The study demonstrated that fosetyl-Al is not clastogenic at the concentrations tested and under the conditions of testing.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material:

Name:	Fosetyl-Al
Description:	White powder
Batch / Lot No.:	12020045
Purity:	97.1%
Stability of test compound:	Expiry date: 2014-07-05. Stability in vehicle was analytically verified.

2. Vehicle and/or positive control:

Vehicle:	sterile water
Pos. controls:	Ethyl methanesulfonate (EMS), -S9 Cyclophosphamide (CPA), +S9

3. Test system:

Organisms:	Mammalian cells in culture
Strains:	Chinese Hamster Ovary (CHO-K1) cell line, (ATCC CCL-61, Lot 4765275) hypodiploid with a modal chromosome number 20 and a population doubling time of 10 to 14 hours
Source:	[redacted], USA
Media:	Ham's F-12 medium supplemented with L-glutamine, sodium bicarbonate, antibiotics and 5 or 10% of fetal bovine serum (F-12 FBS 5-10)
Metabolic activation system:	S9 homogenate was prepared from male Wistar rats induced with a single i.p. injection of Aroclor 1254, 5 days prior to sacrifice. Each batch of S9 homogenate was characterized for its ability to metabolize the promutagens 2-aminoanthracene and benzo(a)pyrene to mutagens using *S. typhimurium* TA100 strain.
Test concentrations:	<u>Cytotoxicity test (-S9 and +S9):</u> 75, 150, 300, 600, 1200, 2400, and 3541 µg/mL (3541 µg/mL =10 mM) <u>3-h exposure, +S9:</u> 260, 823, 2600 µg/mL <u>3-h and 21-h exposure, –S9:</u> 180, 570, and 1800 µg/mL
Exposure duration:	3, 21 h

Mitotic arrest:	Colchicine was used as the spindle inhibitor, added to the cultures 19 h after start of exposure at a final concentration of 0.2 µg/mL.
Harvest time:	21 h

B. STUDY DESIGN AND METHODS

Experimental dates
2012-09-25 through 2013-01-11

Experimental procedure
Determination of cytotoxicity
Cytotoxicity of the test item was determined in a pre-test, as well as in additional cultures. The effect of the test item on cell viability was estimated by expressing the number of cells in each treated culture as a percentage of the number in the vehicle control.
The pH value and osmolality of the cell culture medium was measured before and after the exposure period. Fosetyl-Al precipitated in the treatment medium at 300 µg/mL and higher, but did not cause any appreciable change in the osmolality of the test solutions at the end of the 3-h exposure to treatment either in the presence or in the absence of metabolic activation. However, fosetyl-Al altered the pH of the test solutions at and above 600 µg/mL in the presence of metabolic activation and at and above 1200 µg/mL in the absence of metabolic activation, therefore, the pH of the test solutions of these concentrations were adjusted to neutrality before exposure to the cells.

Main study
Cells were treated with the test substance dissolved in sterile water and the respective positive and solvent controls both in the presence and absence of a metabolic activation system (S9 mix).
After the exposure period, the medium was removed from the test flasks and replaced with fresh medium. Two hours prior to the end of incubation, Colcemid was added to the cultures.
At the end of the incubation period, cells were suspended in F-12 FBS5 after trypsinization. 200 µL of cell suspension of each individual replicate were pooled into test tubes, mixed and the cells were counted.
Cells were fixated using cold methanol: acetic acid (3:1). Four slides were prepared per replicate.

Slide evaluation
Chromosome aberrations for the three concentrations of the test item, the positive and vehicle controls cultures were scored.
Each metaphase spread was examined at 1000 magnification. The number of chromosomes in each spread was counted and those containing 18 to 22 centromeres were evaluated for aberrations. A total of 200 such metaphases evenly distributed amongst the duplicate cultures were evaluated for each group.
The chromosome number was recorded for all the metaphases analysed and the Vernier readings (microscope coordinates) of the metaphases with aberrant chromosomes were recorded. The total number of metaphases showing one or more aberrations both including and excluding gaps was calculated from a set of 200 metaphases for each group.

Statistical evaluation
Pooled data from each test concentration and the positive control are compared with the vehicle control using the one-tailed Fisher exact test.

Validity criteria
The assay is considered acceptable if it meets the following criteria:
1. The incidence of aberrations in the vehicle control cultures is in the range of in-house historical control data.
2. The positive control substances should produce a significant increase in the incidence of aberrations compared to the respective vehicle control.

Assessment criteria
Biological relevance of the results is considered first. Statistical methods may be used as an aid in evaluating the test results, but statistical significance should not be the only determining factor for a positive response.
As a guide to interpretation of the data, the test item will be considered to induce a positive response when the percentage of cells with aberrations is increased in a dose-dependent manner with one or more concentrations being significantly different from controls ($p < 0.05$). If there is a statistically significant increase over the control in only one dose, to prove a reproducible increase, an independent confirmatory assay will be done.
Values that are statistically significant but do not exceed the range of historic vehicle controls may be judged as non-biologically significant. Test items not demonstrating a statistically significant increase in aberrations will be considered as negative.
Positive results that occur only at concentrations above 0.5 mg/mL or 1 mM may require an interpretation on a case-by-case basis.
An increase in the number of polyploidy cells may indicate that the test item has the potential to inhibit mitotic processes and to induce numerical chromosome aberrations. An increase in the number of cells with endoreduplicated chromosomes may indicate that the test substance has the potential to inhibit cell cycle progression.

II. RESULTS AND DISCUSSION

A. CYTOTOXICITY
3-h exposure, without activation: at the highest concentration tested (1800 µg/mL), the reduction in the cell growth was 51% compared to the vehicle control (see Table 5.4.1- 10).
3-h exposure, with activation: at the highest concentration tested (2600 µg/mL), the reduction in the cell growth was 51% compared to the vehicle control (see Table 5.4.1- 10).
21-h exposure, without activation: at the highest concentration tested (1800 µg/mL), the reduction in the cell growth was 55% compared to the vehicle control (see Table 5.4.1- 11).
Thus, the highest concentrations tested elicited sufficient cytotoxicity (55 ± 5%). In the presence of S9, the highest recommended test concentration (2 mg/mL) was exceeded.

B. CHROMOSOMAL ABERRATIONS
No biologically relevant and statistically significant increases of metaphases with aberrations were detected at any time point in any of the concentrations tested with or without metabolic activation (see Table 5.4.1- 10 and Table 5.4.1- 11). Appropriate solvent and positive controls gave the expected results and thus proved the sensitivity of the test. The incidence of aberrations in the vehicle control was within the range of the in-house historical control data.

Table 5.4.1- 10: Chromosome aberration test in CHO cells, 3-h exposure

Treatment	Conc. [µg/mL]	No. (%) of metaphases with aberrations [a]							Total No. (%) of aberrant metaphases		Growth inhibition [%]
		Gaps		Breaks		Exchanges					
		Cs [b]	Ct	Cs	Ct	Cs	Ct	RC	Incl. gaps	Excl. gaps	
Without metabolic activation											
Water	–	0	0	0	0	0	0	0	0	0	–
Fosetyl-Al	180	0	0	0	0	0	0	0	0	0	3
	570	0	0	0	0	0	0	0	0	0	8
	1800	0	0	0	0	0	0	0	0	0	51
EMS	600	29 (14.5)	12 (6.0)	2 (1.0)	69 (34.5)	107 (53.5)	39 (19.5)	2 (1.0)	159 (79.5)	155 (77.5)	28
With metabolic activation											
Water	–	1 (0.5)	0	0	0	0	0	0	1 (0.5)	0	–
Fosetyl-Al	260	0	0	0	0	0	0	0	0	0	7
	823	0	1 (0.5)	0	0	0	0	0	0	0	19
	2600	0	0	0	0	0	0	0	0	0	51
CPA	55	17 (8.5)	5 (2.5)	58 (29.0)	52 (26.0)	1 (0.5)	21 (10.5)	0	104* (52.0)*	101* (50.5)*	32

[a] Values are the sum of two replicates and the values in parentheses represent % of metaphases with aberrations
[b] Cs: Chromosome type, Ct: chromatid type, RC: ring chromosome
* Significantly higher than control ($p \leq 0.05$)

Table 5.4.1- 11: Chromosome aberration test in CHO cells, 21-h exposure

Treatment	Conc. [µg/mL]	No. (%) of metaphases with aberrations [a]							Total No. (%) of aberrant metaphases		Growth inhibition [%]
		Gaps		Breaks		Exchanges					
		Cs [b]	Ct	Cs	Ct	Cs	Ct	RC	Incl. gaps	Excl. gaps	
Without metabolic activation											
Water	-	0	0	0	0	0	0	0	0	0	–
Fosetyl-Al	180	0	0	0	0	0	0	0	0	0	4
	570	0	0	0	0	0	0	0	0	0	13
	1800	0	0	0	0	0	0	0	0	0	55
EMS	600	26 (13.0)	10 (5.0)	30 (15.0)	85 (42.5)	32 (16.0)	52 (26.0)	4 (2.0)	130* (65.0)*	129* (64.5)*	35

[a] Values are the sum of two replicates and the values in parentheses represent % of metaphases with aberrations
[b] Cs: Chromosome type, Ct: chromatid type, RC: ring chromosome
* Significantly higher than control ($p \leq 0.05$)

III. CONCLUSION

Fosetyl-Al was not clastogenic to mammalian cells in the chromosome aberration assay, with and without metabolic activation.

Report:	KCA 5.4.1/08[redacted]O; 2013; M-450287-01-1
Title:	Fosetyl-Al: In vitro mammalian cell gene mutation test in CHO cells
Report No.:	G8218
Document No.:	M-450287-01-1
Guideline(s):	OECD Guidelines for the Testing of Chemicals, Test No 476 (1997); Method B.17 (Mutagenicity) Official Journal of the European Communities, L142, 31/05/2008
Guideline deviation(s):	none
GLP/GEP:	**yes**

Executive Summary
The potential of fosetyl-aluminium (fosetyl-Al), to induce gene mutation in mammalian cells was evaluated in an HPRT test in Chinese hamster ovary (CHO) cells according to OECD guideline 476.
The study consisted of a preliminary toxicity test, an initial gene mutation assay, and a confirmatory gene mutation assay. Each of these mutation assays comprised of two independent experiments, one each in the presence and absence of metabolic activation system (S9 fraction prepared from Aroclor-1254-induced rat liver). Fosetyl-Al formed a milky suspension in sterile water at the tested concentration of 354.1 mg/mL and was stable in water for 24 hours at room temperature at the tested concentrations of 0.015, 1.0, 200 and 500 mg/mL. In a preliminary cytotoxicity test, fosetyl-Al did not cause significant cell growth inhibition as evaluated by relative cloning efficiency (RCE) up to the highest tested concentration of 3541 µg/mL (equivalent to 10 mM) in the presence or absence of metabolic activation. Fosetyl-Al precipitated in the treatment medium at 300 µg/mL and higher, but did not cause any appreciable change in the osmolality of the test solutions at the end of the 3-h exposure to treatment either in the presence or in the absence of metabolic activation. However, fosetyl-Al altered the pH of the test solutions at ≥ 600 µg/mL in the presence of metabolic activation and at ≥ 1200 µg/mL in the absence of metabolic activation, therefore, the pH of the test solutions of these concentrations were adjusted to neutrality before exposure of the cells.
In the initial gene mutation assay, CHO cells were exposed to fosetyl-Al in duplicate at concentrations of 227, 567, 1416 and 3541 µg/mL of the medium for 3 hours in the presence and absence of metabolic activation. In the confirmatory gene mutation assay, CHO cells were exposed to fosetyl-Al in duplicate at concentrations of 112, 355, 1121 and 3541 µg/mL for 3 hours in the presence and absence of metabolic activation.
In a similar way, a concurrent vehicle control (water) and appropriate positive controls (3-methylcholanthrene, +S9; ethyl methanesulfonate, -S9) were also tested in duplicate.
There was no induction of gene mutations in any of the fosetyl-Al-treated cultures either in the presence or absence of metabolic activation. In each of these experiments, the respective positive controls produced a statistically significant increase in the frequency of mutants, under identical conditions and the concurrent vehicle control cultures values were within laboratory historical controls.
The results of this forward gene mutation assay at the *hprt* locus demonstrate that fosetyl-Al the test item is non-mutagenic in the presence or absence of metabolic activation.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material:

Name:	Fosetyl-Al
Description:	White powder
Batch / Lot No.:	12020045
Purity:	97.1%
Stability of test compound:	Expiry date: 2014-07-05. Stability in vehicle was analytically verified.

2. Vehicle and/or positive control:	Vehicle:	sterile water
	Pos. controls:	Ethyl methanesulfonate (EMS), -S9 3-Methylcholanthrene (MCA), +S9

3. Test system:

Organisms:	Mammalian cells in culture
Strains:	Chinese Hamster Ovary (CHO-K1) cell line, (ATCC CCL-61, Lot 4765275) hypodiploid with a modal chromosome number 20 and a population doubling time of 10 to 14 hours
Source:	[redacted], USA
Media:	Basic medium: Ham's F-12 medium supplemented with L-glutamine, sodium bicarbonate, antibiotics was the. Complete medium: basic medium supplemented with 10% foetal bovine serum (FBS) was used for the growth and multiplication of cells as well as in detaching and diluting the cells. Treatment medium was basic medium without serum and was used for target cell exposure to the test item and controls. Cloning medium was basic medium supplemented with 20 % FBS and was used for the determination of cell viability or plating/cloning efficiency. Selective medium was basic medium supplemented with 20 % FBS and the selective agent 6-thioguanine (6-TG) at 35 µM and was used for the selection of mutants.
Locus Examined:	*hprt* locus
Selection agent:	6-Thioguanine (6-TG)
Metabolic activation system:	S9 homogenate was prepared from male Wistar rats induced with a single i.p. injection of Aroclor 1254, 5 days prior to sacrifice. Each batch of S9 homogenate was characterized for its ability to metabolize the promutagens 2-aminoanthracene and benzo(a)pyrene to mutagens using *S. typhimurium* TA100 strain.
Test concentrations:	Cytotoxicity test: -S9 and +S9: 75, 150, 300, 600, 1200, 2400, and 3541 µg/mL (3541 µg/mL =10 mM) Main test, ± S9: 227,567, 1416 and 3541 µg/mL
Exposure duration:	3 h

B: STUDY DESIGN AND METHODS

Experimental dates
2012-09-25 through 2013-01-07

Experimental procedure
Preliminary cytotoxicity test
Initial precipitation, pH and osmolality assessment of fosetyl-Al exposed test solutions was determined in the concomitant chromosomal aberration study ([redacted]; 2013; M-450289-01-1).
Exponentially growing CHO-K1 cells were plated at ca. 10^6 cells/flask in 5 mL complete medium and incubated for approximately 24 hours.
Cells were exposed to seven concentrations of fosetyl-Al, in the presence and absence of metabolic activation up to a maximum of 3541 µg/mL (equivalent to 10 mM) along with the vehicle control. Medium was removed and replaced with treatment. For the test in the presence of metabolic activation, 0.5 mL of S9 mix was added to the respective flasks to achieve a final concentration of 10% (v/v) in the medium. The pH of the test solutions containing the test item were adjusted to neutrality (between 7.21 and 7.38) before exposure to the cells.

50 µL of the vehicle or the stock and dilutions of the test item were mixed with the medium in the respective flasks to get the required test concentrations. The flasks were incubated for 3 hours to expose the cells to treatment. After the treatment period, the flasks were observed for any precipitation.
Medium from each flask was removed, the cell monolayer was rinsed with PBS, trypsinized, and cells were suspended in 5 mL complete medium and counted using a haemocytometer.
About 200 cells from the control and each treatment level were plated in triplicate and incubated for 10 days. After incubation, medium from each flask was removed and the cells were stained with methylene blue and the colonies counted manually to determine absolute cloning efficiency (ACE) and cytotoxicity was expressed relative to the vehicle treated control (relative cloning efficiency - RCE).

Main study
Exponentially growing CHO-K1 cells were plated in duplicate in 15 mL of complete medium at a density of approximately 3 x 10^6 cells / 75 cm^2 flask and incubated for 24 hours.
Exposure of target cells to treatment: All test item and positive control concentrations were prepared immediately before use in sterile test tubes. The target cells in duplicate cultures were exposed to the vehicle, positive controls, or selected concentrations of the test item for 3 hours in the presence and absence of metabolic activation. The medium from each target cell flasks was removed and replaced with 13.5 mL and 15 mL of treatment medium for the experiment in the presence and absence of metabolic activation, respectively. For the experiment incorporating metabolic activation, 1.5 mL of S9 mix was added to give a final concentration of 10% (v/v) in the test solutions.
300 µL each of the vehicle control, respective positive controls, or stocks of the test item were transferred to respective flasks and gently mixed and the flasks were incubated.
Assessment of parallel cytotoxicity and expression of the mutant phenotype: After the treatment period, cells were rinsed with PBS, trypsinized, detached with 10 mL complete medium, and counted using a haemocytometer.
About 200 cells from each replicate of the controls and each treatment level were plated into T-25 cm^2 flasks in triplicate with cloning medium to determine ACE and to express parallel cytotoxicity based on RCE. After 9 days of incubation, the colonies were stained with methylene blue and counted for cloning efficiency.
For expression of the mutant phenotype, the cells from the replicate cultures were sub-cultured in complete medium in duplicate at a density of ca 10^6 cells/ 25 or 75 cm^2 flasks and incubated. The cells were subcultured as above at a 2-3 day interval and carried out for the 9 day expression period. After this time, the mutant phenotype was selected.
Selection of the mutant phenotype and plating for cloning efficiency: Cells were rinsed with PBS, trypsinized, detached with 5 mL complete medium, pooled, and counted using a haemocytometer.
For selection of the 6-thioguanine (6-TG) resistant phenotype, cells from each of the replicate cultures were plated into 5 flasks at a density of approximately 2 x 10^5 cells/25 cm^2 flask (total of 10^6 cells) in selective medium and incubated for 10 days.
For cloning efficiency determination at the time of selection, cells from each of the replicate cultures were plated approximately at 200 cells/ 25 cm^2 flask in triplicate in cloning medium and incubated for 10 days for the initial and 9 days for the confirmatory mutation assays.
Staining: The colonies were stained with 0.5% methylene blue and counted for both cloning efficiency and mutant selection after 10 days of incubation for the initial and 8 days of incubation for the confirmatory mutation assays.

Acceptance criteria
The Cloning Efficiency of the vehicle controls should not be less than 60%.
The mean mutant frequency of the vehicle controls in each experiment should fall within a range of 0-20 mutants per 10^6 clonable cells.
The positive controls must induce a statistically significant response.

Assessment Criteria
There are several criteria for determining a positive result, such as a concentration related, or a reproducible increase in mutant frequency.
Biological relevance of the results will be considered first. Statistical methods may be used as an aid in evaluating the test results. Statistical significance should not be the only determining factor for a positive response.
A test substance, for which the results do not meet the above criteria is considered non mutagenic in this system.

II. RESULTS AND DISCUSSION

A. CYTOTOXICITY
Fosetyl-Al precipitated at concentrations ≥ 300 µg/mL, both in the presence and absence of metabolic activation at the beginning as well as at the end of 3-h exposure to treatment.
The pH of the test suspension ranged from 4.56-7.2 with 7.31 in the vehicle control in the presence of metabolic activation while in the absence of metabolic activation, the pH was between 4.49 and 7.36 with 7.34 in the vehicle control at the beginning of exposure to treatment.
At the end of 3-h exposure to treatment, pH of the test suspension ranged from 6.01-7.27 with 7.33 in the vehicle control in the presence of metabolic activation while in the absence of metabolic activation, the pH was between 5.97 and 7.41 with 7.37 in the vehicle control.
Hence, the pH of the fosetyl-Al treated cultures was adjusted to between 7.21 and 7.38 using 1N NaOH at the beginning of exposure.

B. MUTATION FREQUENCIES
Initial assay
Based on the preliminary cytotoxicity test, the targeted concentrations of 227, 567, 1416 and 3541 µg/mL were tested in the initial gene mutation assay, both in the presence and absence of metabolic activation.
The relative cloning efficiency (RCE) in the absence of metabolic activation, ranged from 66.6-87.8% while the RCE in the presence of metabolic activation ranged from 72.5-90.2% compared to the vehicle control (see Table 5.4.1- 12).
The test item did not cause a significant increase in the frequency of mutants compared to the vehicle control in the presence or absence of metabolic activation at any of the test concentrations whereas the respective positive controls showed significant increases in mutant frequencies (see Table 5.4.1- 12).

Table 5.4.1- 12: Initial HPRT assay with fosetyl-Al, 3 h exposure duration

Test item	Concentration [µg/mL]	Cloning efficiency		TG-resistant colonies / dish [c]	Mutants per 10^6 cells
		ACE (%)[a]	RCE (%)[b]		
Without metabolic activation					
Water	–	95.9	101.5	12	12.9
		93.2	98.6	10	10.8
Fosetyl-Al	227	83.0	87.8	6	7.6
		82.2	87.0	7	8.7
	567	79.0	83.6	7	9.2
		79.2	83.8	7	8.8
	1416	63.4	67.1	7	10.2
		65.0	68.8	8	11.2
	3541	63.2	66.9	7	10.9
		62.9	66.6	8	11.3
EMS	600	49.0	51.9	158	221.9*
		56.0	59.3	163	218.2*
With metabolic activation					
Water	–	95.4	100.5	11	11.7
		94.0	99.3	10	10.8
Fosetyl-Al	227	85.4	90.2	6	7.4
		83.7	88.4	7	8.5
	567	80.0	84.5	6	7.9
		79.4	83.8	6	7.7
	1416	69.2	73.4	6	8.6
		68.7	72.5	7	9.7
	3541	69.7	73.6	7	11.0
		69.7	73.6	8	12.0
MCA	8	53.7	56.7	141	219.6*
		54.2	57.2	134	232.2*

[a] ACE: absolute cloning efficiency; ACE = (mean no. of colonies per dish x 100) / (no. of cells seeded per dish)
[b] RCE: relative cloning efficiency; RCE = [ACE (treatment) x 100] / (average ACE of vehicle control replicates)
[c] Total of five dishes
* Significantly different from vehicle controls ($p < 0.05$)

Confirmatory assay

Based on lack of significant toxicity in the preliminary toxicity test, the targeted concentration of 112, 355, 1121, and 3541 µg/mL were tested in the confirmatory gene mutation assay.

The RCE values without metabolic activation ranged from ranged from 63.0-95.2% while in the presence of metabolic activation the RCE ranged between 74.5-93.5%, compared to the vehicle control. The test item did not cause a significant increase in the frequency of mutants compared to the vehicle control in the presence or absence of metabolic activation at any of the test concentrations.

The respective positive controls induced a significant increase in the mutant frequency as compared with the vehicle control (see Table 5.4.1- 13).

The frequency of mutants in the vehicle control was within the range of the in-house historical control data (not shown).

The test item did not cause a significant increase in the frequency of mutants compared to the vehicle control in the presence or absence of metabolic activation at any of the test concentrations whereas the respective positive controls showed significant increases in mutant frequencies (see Table 5.4.1- 13).

Table 5.4.1- 13: Confirmatory HPRT assay with fosetyl-Al, 3 h exposure duration

Test item	Concentration [µg/mL]	Cloning efficiency		TG-resistant colonies / dish [c]	Mutants per 10^6 cells
		ACE (%)[a]	RCE (%)[b]		
Without metabolic activation					
Water	–	93.9	99.9	11	12.0
		100.2	100.2	12	13.3
Fosetyl-Al	112	88.4	94.0	10	11.2
		89.5	95.2	11	12.0
	355	75.5	80.3	10	13.0
		78.5	83.5	10	12.9
	1121	74.7	79.5	9	11.7
		71.7	76.3	10	12.2
	3541	59.2	63.0	8	12.2
		60.4	64.3	7	11.4
EMS	600	54.7	58.2	155	203.9*
		52.7	56.1	183	228.8*
With metabolic activation					
Water	–	96.7	100.5	10	10.8
		95.7	99.5	11	12.0
Fosetyl-Al	112	89.9	93.5	7	7.8
		88.0	91.5	8	9.0
	355	83.4	86.7	8	9.6
		81.9	85.1	8	9.5
	1121	77.0	80.0	9	11.7
		81.4	84.6	9	11.9
	3541	71.7	74.5	7	11.2
		73.9	76.8	7	10.5
MCA	8	59.0	61.3	153	228.7*
		52.9	55.0	146	221.5*

[a] ACE: absolute cloning efficiency; ACE = (mean no. of colonies per dish x 100) / (no. of cells seeded per dish)
[b] RCE: relative cloning efficiency; RCE = [ACE (treatment) x 100] / (average ACE of vehicle control replicates)
[c] Total of five dishes
* Significantly different from vehicle controls ($p < 0.05$)

III. CONCLUSION

Fosetyl-Al was not mutagenic to mammalian cells in the HPRT assay, with and without metabolic activation.

CA 5.4.2 *In-vivo* studies in somatic cells

Report:	KCA 5.4.2/01 [redacted]; 1977; M-223290-01-2
Title:	Investigation of the possible mutagenic activity of Aliette and of hydrated monosodium phosphite
Report No.:	R000822
Document No.:	M-223290-01-2
Guideline(s):	not specified
Guideline deviation(s):	not applicable
GLP/GEP:	**no**

I. MATERIALS AND METHODS

Groups of 10 Swiss mice were given two oral doses, by gavage, at 24-hour interval of 0, 1000 and 2000 mg/kg bw of fosetyl-Al (batch DA 67; 89.7% purity) suspended in distilled water; in addition a group of 5 males was given 4000 mg/kg bw of test substance under the same treatment schedule. All mice were sacrificed after the 2nd dose, and bone marrow was taken for determining the count of Howell-Jolly bodies in 2000 polychromatic erythrocytes.

II. RESULTS AND DISCUSSION

In the top dose group, 3 mice died within 24 hours after the 1st administration and the 2 other within 1 hour after the 2nd administration.
Control mice exhibited a low percentage of polychromatic erythrocytes containing Howell-Jolly bodies while benzene, benzo(a)pyrene and methylmethansulphonate treated animals showed higher values. Fosetyl-Al did not increase the frequency of polychromatic erythrocytes containing Howell-Jolly bodies at any dose level compared to the negative controls (see Table 5.4.2- 1).

Table 5.4.2- 1: Group mean percentage of PCE containing Howell-Jolly bodies per 2000 cells

Treatment groups	Dose (mg/kg bw)	Group mean percentage of PCE containing Howell-Jolly bodies per 2000 cells
Vehicle	0	0.18%
LS74-7866	1000	0.11%
	2000	0.21%
	4000	-
Benzo (a) pyrene	200	1.51%
MMS	25	1.39%
Benzene	0.25 mL/kg	1.31%

The ratio of PCEs to NCEs has not been determined or reported in this study. Therefore, a direct conclusion that the bone marrow had been reached by the test substance or its metabolites cannot be drawn from the results of this study. However, appreciable residues of ^{14}C-fosetyl-Al (86/74 µg equiv./g, m/f) were found in bone marrow of rats even at 168 hours after a single oral dose of 3000 mg fosetyl-A/kg bw in an aqueous vehicle (see Table 5.1.1- 2). In another rat ADME study, 168 hours after a single dose of 1000 mg/kg bw in an aqueous vehicle, bone marrow residues of 68/83 µg equiv./g (m/f) were measured (see Table 5.1.1- 4). The toxicokinetics of fosetyl-Al in rats and mice is assumed to be very similar, not only because both species belong to the order *Rodentia*, but because the metabolism of fosetyl-Al consists only of abiotic ester hydrolysis and subsequent ethanol catabolism (see Figure 5.1- 2), which is very similar among all mammals. This allows the conclusion that the bone marrow had been exposed to fosetyl-Al or its metabolites in this micronucleus test.

III. CONCLUSION

Fosetyl-Al does not induce micronucleus formation in mice after two daily oral doses of up to 2000 mg/kg bw/day.

Report:	KCA 5.4.2/02[redacted]; 1998; M-178982-01-1
Title:	Fosetyl-Al: Induction of micronuclei in the bone marrow of treated mice
Report No.:	R009245
Document No.:	M-178982-01-1
Guideline(s):	EU (=EEC): 87/302/EEC, B12; ICH (1995); OECD: 474, (1984); UKEMS: (1995); USEPA (=EPA): OPPTS 870.5395
Guideline deviation(s):	none
GLP/GEP:	**yes**

I. MATERIALS AND METHODS

Technical fosetyl-Al (batch no 960718L, purity 970 g/kg) was tested for its ability to induce micronuclei in polychromatic erythrocytes from the bone marrow of CD-1 mice following a single oral administration. The study consisted of a cytotoxicity range-finding experiment followed by a main study.

In the range-finding study, groups of 3 male and female CD-1 mice received a single oral administration of fosetyl-Al (0.5% w/v methyl cellulose) by gavage at dose levels of 4000, 4500 or 5000 mg/kg. Animals were observed daily for clinical signs and mortality during a 4-day period.

In the main study, groups of 5 male and female CD-1 mice received a single oral administration of fosetyl-Al (0.5% w/v, methyl cellulose) by gavage at dose levels of 1063, 2125 and 4250 mg/kg. A similar control group received the vehicle only. A positive control group (n = 5) received a single oral administration of cyclophosphamide at 80 mg/kg.

After 24, 48 and 72 hours, animals were killed and bone marrow slides were prepared. The relative proportion of polychromatic (PCE) and normochromatic erythrocytes (NCE) was determined until at least 1000 cells have been analysed. The frequency of micronucleated polychromatic erythrocytes was recorded until at least 2000 cells have been observed.

II. RESULTS AND DISCUSSION

Cytotoxicity range-finding experiment

Mortality occurred in 2 and 1 out of 3 animals treated at 4500 and 5000 mg/kg, respectively. No evidence of toxicity was observed at 4000 mg/kg. Accordingly, fosetyl-Al was tested at 1063, 2125 and 4250 mg/kg in the main study.

Main study

Mortality occurred in 1 and 4 females treated at 1063 and 4250 mg/kg, respectively. Clinical signs, i.e. lethargy, hunched posture, eye closure and tremors, were seen at 4250 mg/kg. These results clearly indicated that fosetyl-Al could not be tested at an appreciably higher dose.

Negative (vehicle) control mice exhibited normal group mean ratios of PCE to NCE and the incidence of micronucleated PCE were within historical vehicle control ranges. Cyclophosphamide-treated animals showed statistically significant increases in micronucleus frequency (see Table 5.4.2- 2).

Fosetyl-Al treatments produced no changes in group mean ratios of PCE to NCE and no statistically significant increases in micronucleus frequency at any dose levels and at any sampling times (see Table 5.4.2-2).

Since the ratio of PCEs to NCEs was not affected by treatment, a direct conclusion that the bone marrow had been reached by the test substance or its metabolites cannot be drawn from the results of this study. However, appreciable residues of ^{14}C-fosetyl-Al (86/74 µg equiv./g, m/f) were found in bone marrow of rats even at 168 hours after a single oral dose of 3000 mg fosetyl-A/kg bw in an aqueous vehicle (see Table 5.1.1- 2). In another rat ADME study, 168 hours after a single dose of 1000 mg/kg bw in an aqueous vehicle, bone marrow residues of 68/83 µg equiv./g (m/f) were measured (see Table 5.1.1- 4). The toxicokinetics of fosetyl-Al in rats and mice is assumed to be very similar, not only because both species belong to the order *Rodentia*, but because the metabolism of fosetyl-Al consists only of abiotic ester hydrolysis and subsequent ethanol catabolism (see Figure 5.1- 2), which is very similar among all mammals. This allows the conclusion that the bone marrow had been exposed to fosetyl-Al or its metabolites in this micronucleus test.

Table 5.4.2- 2: Ratio of PCE to NCE and frequency of micronucleated PCE in the bone marrow of CD-1 mice following a single oral administration of fosetyl-al

Treatment (mg/kg)	Sampling time (hours)	Sex	Group mean ratio of PCE/NCE	Group mean frequency of micronucleated PCE (per 1000)	
				per sex	per treatment group
Vehicle	24	Male	1.[illegible]	0.50	0.60
		Female	0.78	0.70	
	48	Male	0.86	0.50	0.35
		Female	0.83	0.20	
	72	Male	0.90	0.50	0.45
		Female	0.88	0.30	
Fosetyl-Al 1063	24	Male	1.08	0.20	0.15
		Female	0.97	0.10	
	48	Male	0.97	0.40	0.50
		Female	1.06	0.63	
	72	Male	1.13	0.70	0.60
		Female	1.12	0.50	
Fosetyl-Al 2125	24	Male	1.43	0.20	0.20
		Female	0.85	0.20	
	48	Male	0.69	0.20	0.30
		Female	0.97	0.40	
	72	Male	0.97	0.30	0.30
		Female	0.93	0.30	
Fosetyl-Al 4250	24	Male	0.85	0.50	0.70
		Female	0.78	0.90	
	48	Male	1.00	0.50	0.75
		Female	1.24	1.00	
	72	Male	1.23	0.10	0.25
		Female	1.50	0.40	
CPA 80	24	Male	0.60	24.80	19.85
		Female	0.63	14.90	

PCE: polychromatic erythrocytes; NCE: normochromatic erythrocytes; CPA: Cyclophosphamide

III. CONCLUSION

RMS conclusion: Test material was negative for causing cytogenetic damage as measured by micronucleus induction in CD-1 mice.

Report:	KCA 5.4.2/03[redacted]; 2013; M-449130-01-1
Title:	Mammalian erythrocyte micronucleus test following oral administration of fosetyl-Al to Swiss albino mice
Report No.:	G8222
Document No.:	M-449130-01-1
Guideline(s):	OECD 474 for testing of chemicals Mammalian erythrocyte micronucleus test, adopted on 21st July 1997 (OECD 1997); Official Journal of European Communities, Commission Regulation (EU) No 1152/2010 of 8 December 2010,L324,B.12.- In vivo Mammalian Erythrocyte Micronucleus Test
Guideline deviation(s):	none
GLP/GEP:	**yes**

Executive Summary

A micronucleus test in mice with fosetyl-aluminium (fosetyl-Al) was conducted according to OECD guideline 474.

Fosetyl-Al was suspended in the vehicle (deionised water) and administered orally by gavage to Swiss albino mice al the dose levels of 500, 1000 and 2000 mg/kg bw/day, on 2 consecutive days. Mice in the vehicle control group received vehicle alone. The mice in the positive control group received cyclophosphamide monohydrate (40 mg/kg bw) as a single oral gavage. The dose volume administered was 10 mL/kg. Each group consisted of five males and five females.

Mice were observed twice daily for clinical signs of toxicity. Mice in the vehicle and treatment groups were sacrificed 22 to 23 hours following the second dose, but those in the positive control group were sacrificed 23 to 24 hour following a single dose. The femur bone marrow was flushed, smears were prepared and stained. From each mouse, a minimum of 2000 polychromatic erythrocytes (PCEs) were scored for the incidence of micronucleated PCE. Cytotoxicity was determined by evaluating the ratio of PCE: Total RBC by counting 202 to 252 red blood corpuscles (RBC) per mouse.

At doses of 500, 1000 and 2000 mg/kg bw/day, there was no incidence of mortality at any of the doses tested. The treated mice did not exhibit any clinical signs and there was no effect on body weight and no necropsy findings were observed.

The incidence of micronucleated PCEs in fosetyl-Al-treated mice was comparable to the vehicle control group. The PCE: Total RBC ratio at all the tested doses was comparable to vehicle control.

Since the ratio of PCEs to Total RBCs was not affected by treatment, a direct conclusion that the bone marrow had been reached by the test substance or its metabolites cannot be drawn from the results of this study. However, appreciable residues of ^{14}C-fosetyl-Al (86.74 µg equiv./g, m/f) were found in bone marrow of rats even at 168 hours after a single oral dose of 3000 mg fosetyl-A/kg bw in an aqueous vehicle (see Table 5.1.1- 2). In another rat ADME study, 168 hours after a single dose of 1000 mg/kg bw in an aqueous vehicle, bone marrow residues of 68/83 µg equiv./g (m/f) were measured (see Table 5.1.1- 4). The toxicokinetics of fosetyl-Al in rats and mice is assumed to be very similar, not only because both species belong to the order *Rodentia*, but because the metabolism of fosetyl-Al consists only of abiotic ester hydrolysis and subsequent ethanol catabolism (see Figure 5.1- 2), which is very similar among all mammals. This allows the conclusion that the bone marrow had been exposed to fosetyl-Al or its metabolites in this micronucleus test.

The positive control caused a significantly increased percentage of micronucleated PCE and a significantly reduced PCE: Total RBC ratio.

Hence, fosetyl-Al was non-clastogenic in Swiss albino mice at the doses tested and under the conditions of testing.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material:

Name:	Fosetyl-Al
Description:	White powder
Batch / Lot No.:	12020045
Purity:	97.1%
Stability of test compound:	Expiry date: 2014-07-05. Stability and homogeneity in vehicle were analytically verified.
2. Vehicle and/or positive control:	Vehicle: deionised water Pos. control: Cyclophosphamide monohydrate (CPA)
3. Test animals	
Species:	Mouse
Strain:	Swiss albino mice, HsdWin: NMRI
Sex:	Male and female
Age:	8-10 weeks
Weight at dosing:	Group mean range: Males: 38.2 – 38.8 g Females: 31.8 – 32.0 g
Source:	[redacted] India
Acclimatisation period:	5-6 days (-), 6-7 days (-)
Diet:	Teklad Certified (2014C) Global 14% Protein Rodent Maintenance Diet – Pellet (Certified), *ad libitum*
Water:	Charcoal-filtered and UV-irradiated deep bore-well water, *ad libitum*
Housing:	Individually in standard polysulfone cages with corn cob bedding
Environmental conditions:	
Temperature:	20-23°C
Humidity:	65-66%
Air changes:	12-15 h^{-1}
Photoperiod:	12 h light / 12 h dark

B. STUDY DESIGN AND METHODS

1. In life dates: 2012-09-21 to 2012-11-19

2. Animal assignment and treatment

Dose:	Dose-finding and main study: 500, 1000 and 2000 mg/kg bw/day for 2 consecutive days CPA was administered as a single oral gavage of 40 mg/kg bw.
Application route:	Oral, gavage
Application volume:	10 ml/kg bw
Fasting time:	Not fasted
Group size:	Dose-finding: 2 mice/sex/dose Main study: 5 mice/sex/dose
Observations:	Clinical signs, mortality, body weight

3. Details of micronucleus test

Sacrifice:	22-23 h after the second dose
Tissues and cells examined:	Bone marrow; 2000 polychromatic erythrocytes (PCEs) examined per animal. The proportion of immature erythrocytes among total RBC (number of PCE divided by total number of erythrocytes) was determined for each mouse by counting 202 to 252 erythrocytes per mouse.
Details of slide preparation	Bone marrow was flushed using a syringe containing fetal bovine serum and transferred to a centrifuge tube. The cell suspensions were centrifuged at 2500 rpm for 10 minutes and the supernatants were discarded. A sample of ca. 10 µL of the cell suspension was spread evenly on a clean glass slide and blow-dried. The smears were fixed in methanol for 10 minutes. Three slides were prepared for each mouse. Slides were stained by May-Gruenwald and Giemsa stain. The slides were dried, immersed in xylene and cover slips mounted with DPX.
Validity criteria:	The test is considered valid if it meets the following criteria: a. The incidence of micronucleated polychromatic erythrocytes (MNPCE) in the vehicle control group is within the historical control data range. b. The positive control animals demonstrated a significant increase ($p < 0.05$) in MNPCE compared to control.
Evaluation criteria:	The test item was considered to induce a positive response if there was a dose related increase in the number of micronucleated cells. The test item was considered negative if there was no statistically significant increase in micronucleated polychromatic erythrocytes as compared to the concurrent vehicle control. However, values that are significantly elevated but do not exceed the historical data range vehicle controls may be judged as biologically non-significant.
Statistical methods:	All quantitative variables like change in net body weight were tested for normality (Shapiro-Wilk test) and homogeneity (Levene's test) of within group variance before performing ANOVA. For counts/ proportions, percentages data were normalized using suitable transformation (square root) before ANOVA. Comparison of means between the control and the treatment groups was done using Dunnett's test, where 'F' test was significant in ANOVA.

II. RESULTS AND DISCUSSION

A. CLINICAL SIGNS, MORTALITY AND NECROPSY FINDINGS

There were no mortalities, clinical signs or abnormal necropsy findings in any of the mice.

B. BODY WEIGHT

There were no effects on body weight.

C. MICRONUCLEUS ASSAY

The incidences of micronucleated PCE and PCE: Total RBC ratio at 500, 1000 and 2000 mg/kg doses were comparable to the vehicle control group (see Table 5.4.2- 3). CPA significantly increased the percentage of micronucleated PCE and significantly reduced the PCE : Total RBC ratio as compared to the vehicle control group.

Table 5.4.2- 3: Results of the micronucleus assay in mice with fosetyl-Al

Treatment	Dose (mg/kg bw/day)	PCE : Total RBC ratio			PCE		
		No. of PCE	No. of RBC	Ratio (mean ± SD)	Total no.	No. with MN	% (mean ±SD)
Males							
Water	0	517	1067	0.48±0.01	10 076	3	0.03±0.03
Fosetyl-Al	500	527	1096	0.48±0.01	10 067	3	0.03±0.04
	1000	550	1150	0.48±0.00	10 180	3	0.03±0.04
	2000	541	1131	0.48±0.01	10 204	2	0.02±0.03
CPA	40	451	1087	0.41±0.01*	10 210	244	2.39±0.30*
Females							
Water	0	525	1098	0.48±0.01	10 362	5	0.05±0.07
Fosetyl-Al	500	511	1077	0.47±0.01	10 089	3	0.03±0.03
	1000	544	1123	0.48±0.00	10 065	4	0.04±0.02
	2000	515	1088	0.47±0.01	10 180	5	0.05±0.05
CPA	40	492	1183	0.42±0.01*	10 588	199	1.88±0.25*

*Significantly different from vehicle control group

III. CONCLUSION

Fosetyl-Al does not induce micronuclei formation in mice after two daily oral doses of up to 2000 mg/kg bw/day.

CA 5.4.3 *In-vivo* studies in germ cells

Since all genotoxicity studies with fosetyl-aluminium are negative this data requirement does not apply.

CA 5.5 Long-term toxicity and carcinogenicity

The long term toxicity and carcinogenicity of fosetyl-aluminium (fosetyl-Al) was evaluated in dogs and rodents. The testes and the urinary bladder were identified as the target organs in dogs and rats, respectively. However, both the LOAELs and NOAELs for these effects were much higher than the guidance value (GV) of 10 and 100 mg/kg bw/day for classification as STOT RE1 and 2, respectively. These GVs are valid for 90-day (i.e., 3-month) studies while the studies in question are 24-month studies. Hence, an 8-fold lower GV should apply for each category. This follows from the application of Haber's law as suggested by the ECHA CLP guidance document[3]. The GVs for classification as STOT RE1 and 2 are therefore 1.25 and 12.5 mg/kg bw/day, respectively.
With all long-term NOAELs and LOAELs being ≥ 300 mg/kg bw/day (see Table 5.5-1), a **classification in the STOT RE hazard category is not warranted,** according to the criteria of Regulation 1272/2008.
An overview of the data is presented in Table 5.5- 1. The 2-year dog study ([redacted]; 1981; M-159302-01-1) has been used to derive the ADI for fosetyl-Al. No new studies have been performed for this endpoint.

Dog study ([redacted]; 1981; M-159302-01-1)
Despite the administration of very high dose levels (greater than the limit dose of 1000 mg/kg/day) during 2 years, fosetyl-Al did not induce any treatment-related mortalities, clinical signs or major changes in body weight or food consumption.
No toxicologically meaningful effects were observed in haematology, clinical chemistry or urinalysis. Examinations performed at the terminal sacrifice indicated that toxicity of fosetyl-Al was limited to the testes. Males treated at 20 000 and 40 000 ppm displayed testicular degeneration.
The dose level of 10 000 ppm (equivalent to 309 and 288 mg/kg/day in males and females, respectively) was considered to be the NOAEL of the study.

Mouse study ([redacted]; [redacted]; 1981; M-159267-01-1)
Following the administration of very high dose levels up to 30 000 ppm during 104 weeks, fosetyl-Al induced no toxic effects. Accordingly, this dose level (equivalent to 3956 and 4549 mg/kg/day in males and females, respectively) was considered to be the NOAEL of the study.

Rat study ([redacted]; 1981; M-249664-02-1; addenda [redacted]; 1983; M-234109-01-1 and [redacted]; 1983; M-159736-01-1) with additional histopathological peer reviews ([redacted]; 1985; M-165085-01-2; [redacted] 1984; M-163455-01-1; [redacted]; 1985; M-165088-01-2; [redacted]; 1985; M-163457-01-1).
Following the administration of very high dose levels up to 30 000 ppm during 104 weeks, fosetyl-Al induced toxic effects that were limited to the urinary tract. Females treated at 30 000 ppm showed calculi and hyperplasia of the urinary bladder epithelium without any evidence of tumour formation. Males treated at 30 000 ppm displayed similar changes which were associated with a higher incidence of transitional cell papilloma and carcinoma in the urinary bladder.
Based on these findings, the dose level of 8000 ppm (equivalent to 348 and 450 mg/kg/day in males and females, respectively) was considered to be the NOAEL of the study.

[3] ECHA (2015) Guidance on the Application of the CLP Criteria. Version 4.1, June 2015

In the absence of any genotoxic potential, it was hypothetised that the renal tumours could result from a chronic irritation rather than a true carcinogenic effect of fosetyl-Al. The ingestion of high doses of fosetyl-Al is a possible cause of functional alterations of renal excretion, while the formation of calculi may induce a chronic irritation of the urinary bladder epithelium, leading therefore to the development of transitional cell papilloma and carcinoma of the urinary bladder.

Two **mechanistic studies** into the effects of high doses of fosetyl-Al on kidney and bladder histopathology were conducted. These studies are summarised in Section CA 5.8.2.

Conclusion

The bladder tumours observed in rats occur only at very high doses (LOAEL = 1372/1786 mg/kg bw/day, ♂/♀). Mechanistic studies have established a non-genotoxic mechanism that involves imbalance of calcium/phosphorus metabolism, urolith formation, and subsequent chronic irritation of the urothelium. Due to the high doses required to cause imbalance of calcium/phosphorus metabolism, the urinary bladder tumours in the rat are **not considered relevant for humans**. In addition, no tumours were observed in dogs or mice, despite similarly high doses administered in the top dose groups. Therefore, **fosetyl-Al is not classified as carcinogen**, according to the criteria of Regulation 1272/2008.

Based on the results of available studies, the overall acceptable NOAEL for fosetyl-Al for long-term toxicity was 300 mg/kg/day based on the results of the 2-year dog study (sexes combined). This NOAEL has been used to derive the ADI for fosetyl-Al, therefore the respective summary for this pivotal study is included in this Supplementary Dossier.

Table 5.5- 1: Long-term toxicity studies with fosetyl-Al

Study type	Species	Doses tested	LOAEL - Effects	NOAEL	Reference
Oral feeding, 2 years	Dog	0, 10 000, 20 000, 40 000 ppm	20 000 ppm: testicular degeneration in males	10 000 ppm (288 mg/kg bw/day, ♂/♀; 300 mg/kg bw/day, both sexes)	[redacted]; 1981; M-159302-01-1
	Mouse	0, 2500, 10 000, 30 000 ppm	>30 000 ppm: no treatment-related effects	30 000 ppm (3956/4549 mg/kg bw/day, ♂/♀)	[redacted]; [redacted]; 1981; M-159267-01-1
	Rat	0, 2000, 8000 or 30 000 ppm	30 000 ppm: Uroliths and hyperplasia of the urinary bladder in both sexes at 30 000 ppm; increased incidence of urinary bladder neoplasm in males at 30 000 ppm secondary to changes in urine physical/chemical composition and subsequent chronic irritation.	8 000 ppm (348/450 mg/kg bw/day, ♂/♀)	[redacted]; 1981; M-249664-02-1; [redacted]; 1983; M-234109-01-1 [redacted]; 1983; M-159736-01-1 histopathological peer reviews: [redacted]; 1985; M-165085-01-2; [redacted]; 1984; M-163455-01-1; [redacted]; 1985; M-165088-01-2; [redacted]; 1985; M-163457-01-1

Report:	KCA 5.5/01 [redacted]; 1981; M-159302-01-1
Title:	Fosetyl-Al: Two year dietary toxicity study in dogs
Report No.:	R000766
Document No.:	M-159302-01-1
Guideline(s):	No guideline was in effect at the time of the study (study design is equivalent to OECD 452, 1981)
Guideline deviation(s):	none
GLP/GEP:	**yes**

Executive Summary

Fosetyl-aluminium (fosetyl-Al) was administered in the diet to purebred Beagle dogs at dosage levels of 0, 10 000, 20 000 and 40 000 ppm. The study was conducted prior to the adoption of the pertinent OECD guideline 452, but the study design was in accordance with the provisions of this guideline. Six male and six female dogs were randomly assigned to each of three treatment groups or a control group. The dogs were observed daily for general behaviour and appearance, clinical signs, overt toxicity, moribundity, and mortality. Individual body weights and food consumption values were measured and recorded weekly. Mean compound consumption and food efficiency values were calculated weekly.

Ophthalmoscopic examinations were conducted on all dogs once during the pretest period and at 3, 6, 12, 18 and 24 months of study. Complete physical examinations were conducted pretest and at 1, 2, 3, 6, 9, 12, 15, 18, 21 and 24 months of study.

Haematological and biochemical evaluations and urinalyses were conducted twice during the pretest period and at 1, 2, 3, 4, 5, 6, 12, 18 and 24 months of study. Cholinesterase determinations were conducted on all dogs at terminal sacrifice.

No compound-related trends were established for survival, mean food consumption or ophthalmoscopic and physical examinations. The percent change in mean body weight gain (pretest to Week 104), was slightly reduced for all of the treated groups when compared with the controls. There were, however, no statistically significant differences in body weights between the groups. The observed coating of stools with "grey elastic-like or grey material" in the treated animals (especially the 40 000 ppm group) was suggestive of a compound-related effect. The results obtained from analyses of the stools for test article content indicated the "grey elastic-like or grey material" was constituted from an unknown substance and not the test article.

Although there were statistically significant differences in many of the haematological parameters examined for the treated groups when compared with the controls, no consistent trends could be established. The differences noted were not considered to be toxicologically significant. Slight but significant reductions were seen for BUN values in females and this was seen on several occasions at the 40 000-ppm dosage level. The biological significance of the reductions in BUN is dubious. Sporadic statistically significant differences from control values, at various intervals, were seen in the treated groups for many of the biochemical parameters evaluated. The statistically significant reductions in peak values for serum electrophoresis were not considered to be of toxicological significance. There were no biologically significant differences for urinalysis parameters.

No treatment related findings were seen at necropsy and there were no meaningful variations in organ weights. On histopathological examination, evidence of testicular degeneration was seen at 40 000 ppm and to a minimal degree at 20 000 ppm. Based on the histopathological findings, the NOAEL for this study is considered to be 20 000 ppm dietary level. This is equivalent to 309 mg/kg bw/day for males and 288 mg/kg bw/day for females, respectively, and 300 mg/kg bw/day for both sexes combined.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material:

Name:	Fosetyl-Al
Description:	White powder
Batch / Lot No.:	DA136
Purity:	96.6%
Stability of test compound:	Stability and homogeneity in diet were analytically verified.

2. Vehicle: Plain diet

3. Test animals

Species:	Dog
Strain:	Beagle
Sex:	Males and females
Age:	3-4 months
Weight at dosing:	Males: 5.2-9.7 kg Females: 4.9-8.6 kg
Source:	[redacted], USA
Acclimatisation period:	4 weeks (quarantine)
Diet:	Purina Canine Diet or Purina Certified Canine Diet #5007, *ad libitum*
Water:	Water (unspecified), *ad libitum*
Housing:	Individually in suspended metabolism cages
Environmental conditions:	
Temperature:	Not reported
Humidity:	Not reported
Air changes:	Not reported
Photoperiods:	12 h light / 12 h dark

B. STUDY DESIGN AND METHODS

1. In-life dates
1978-12-20 to 1980-12-31

2. Animal assignment and treatment
Animal assignment and dose groups
Dogs were randomly distributed to different groups. The following dose groups were employed:

Table 5.5- 2: Group allocation and mean substance intake in the 2-year feeding study in dogs

Conc. in Diet [ppm]	No. of animals		Mean daily substance intake [mg/kg bw/day]	
	Male	Female	Male	Female
0	6	6	–	–
10 000	6	6	309	288
20 000	6	6	609	632
40 000	6	6	1228	1190

Diet Preparation and Analysis:
Stability of test substance in food was checked and concentration of fosetyl-Al was determined in diet samples from the top, middle and bottom of each batch at Week 1, 2,and 4 and on single samples at Week 8, 12, 27, 40, 53, 66, 79, 93, and 105. Fresh batches were prepared weekly.

Details on oral exposure:

Duration of exposure	2 years
Frequency of treatment	Via diet, *ad libitum*

3. Examinations

Clinical signs - mortality and moribundity/general daily observations	All animals were observed daily for signs of overt-toxicity, behaviour, general health condition and mortality; complete physical examinations were carried out for all dogs once during pre-test period and at 1, 2, 3, 6, 12, 15, 18, 21, and 24 months.
Body weights	Individual body weights were recorded weekly.
Food and water consumption	Food consumption was measured weekly. Water consumption was not measured.
Ophthalmic evaluation	Ophthalmological investigations were done on all animals once during the pre-test period and at 3, 6, 12, 18, and 24 months of study. Mydriasis was induced with 1-2 drops of tropicamide 1.0%. A binocular indirect ophthalmoscope was used to examine each dog. A direct or slit lamp examination was performed if the ophthalmologist determined it was necessary to clarify a particular finding.
Blood collection	Blood was obtained via puncture of the jugular vein. The dogs were fasted overnight (approximately 16 hours) prior to blood collection.
Haematology	Haematological parameters (erythrocyte count, Hct, Hb, MCV, MCH, MCHC, platelet count, total and differential leukocyte count, reticulocyte count, Heinz bodies, erythrocyte sedimentation rate, methaemoglobin), were determined on all dogs twice during the pre-test period and at 1, 2, 3, 4, 5, 6, 12, 18, and 24 months.
Clinical chemistry	Clinical chemistry parameters (sodium, potassium, chloride, calcium, urea, alkaline phosphatase, total and direct bilirubin, AST, ALT, LDH, total protein, cholesterol, glucose, cholinesterase in brain, erythrocytes and plasma, protein electrophoresis) were determined on all dogs twice during the pre-test period and at 1, 2, 3, 4, 5, 6, 12, 18, and 24 months.
Urinalysis	Urinalysis (appearance, colour, sediment, specific gravity, volume, pH, protein, glucose, occult blood, ketones bodies, nitrites, urobilinogen, bilirubin) were determined on all dogs twice during the pre-test period and at 1, 2, 3, 4, 5, 6, 12, 18, and 24 months.
Gross pathology	At termination, all surviving animals were sacrificed for gross examination of abdominal, thoracic, cranial and pelvic cavities.
Organ weights	Selected organs (adrenals, heart, kidneys, liver, pituitary, testes/ovaries, and thyroid/parathyroids) were weighed.

Histopathology	Histopathological examinations were performed on the following tissues: Adrenal, aorta, nasal cavity, brain (3 levels), eye (+ optic nerve), gallbladder, heart (with coronary vessels), trachea, tongue, oesophagus, stomach, duodenum, ileum, jejunum, caecum, colon, rectum, kidney, liver, larynx, lung with main-stem bronchi (3 sections), mesenteric lymph node, skeletal muscle, skin, mammary gland, sciatic nerve, spleen, pancreas, pituitary, prostate/corpus and cervix uteri, ribs/junction (bone marrow), salivary gland, spinal cord (2 levels), testis/ovary epididymis, thymus, thyroid/parathyroid, urinary bladder, bone, bone marrow smear and any other tissues with lesions.

Statistical evaluation
All statistical analyses compared the treatment groups with the control group by sex. Mean body weights, mean food consumption, haematological, biochemical and urinalysis parameters, and absolute and relative organ weights (terminal sacrifice) were compared by analysis of variance (one-way classification), Bartlett's test for homogeneity of variance and the appropriate t-test (for equal or unequal variances). Dunnett's multiple comparison tables were used to judge significances of differences.

II. RESULTS AND DISCUSSION

A. MORTALITY
Survival after 104 weeks was not affected by the treatment and there was no treatment related deaths (one male from the 10 000 ppm group died accidentally during the 19-21 months study period; the cause of death was attributed to a gastric haemorrhage).

B. CLINICAL OBSERVATIONS
There were no overt signs of toxicity and no changes in general health and behaviour, in physical examinations which could be attributed to treatment (incidental findings included soft stool/diarrhoea, injection of the sclera, emesis, dermatitis, alopecia, lacrimation, seen both in control and treated dogs). Incidence of part of stool covered with "grey-elastic-like material" or "grey-material" was first noted after one year of study in several dogs from all treated groups, which higher frequency in the top dose group (analysis of fecal sample from a top dose male showed that the material was water insoluble and contained traces of test substance and that the white flakes were constituted from an unknown substance).

C. BODY WEIGHT
There was no significant difference in body weight throughout the study between treated and control dogs, but slight reduction in the body weight gain was noted at Week 104 in treated dogs when compared to control (see Table 5.5- 3).

D. FOOD CONSUMPTION
There was no treatment-related effect on mean food consumption and food efficiency (see Table 5.5-3).

Table 5.5- 3: Group mean bw and bw gain and group mean food consumption during the study period

	Controls		10 000 ppm		20 000 ppm		40 000 ppm	
	M	F	M	F	M	F	M	F
Group mean bw (kg)								
Pre-test	7.5±1.54	6.4±0.95	7.2±1.42	6.8±0.97	6.7±0.77	6.2±0.62	7.7±0.70	6.6±1.34
Week 104	12.7±3.24	11.3±3.61	12.7±1.61	11.4±2.77	11.2±1.80	10.3±2.04	12.7±3.39	11.7±3.45
% change from pre-test	+69.3	+76.6	+67.1	+67.6	+67.2	+66.1	+64.9	+62.1
Group mean food consumption (g/kg bw/day)								
g/kg bw/day	327	288	360	291	316	307	354	277
% of controls			+10.1	+1.0	-4.0	+6.6	+8.3	-3.8

E. OPHTHALMOSCOPIC EXAMINATION

No test-substance related effect was recorded on ophthalmologic examinations.

F. HAEMATOLOGY AND CLINICAL CHEMISTRY

No treatment related changes were noted for either sex in the haematological and clinical chemistry parameters among the various groups, a few significant changes were noted (see Table 5.5- 4):

- in haematological parameters: sporadic statistically significant differences from control values were seen in the various dose groups at various intervals from erythrocyte count, Hb, Hct, Ptl counts and methaemoglobin, and also in females for segmented neutrophils; however, no dose and time consistent trend occurred.
- in some biochemical parameters: statistically significant changes were seen at various intervals for glucose, ALT, AST, LDH and potassium in males, and for urea, total and direct bilirubin and total protein in females; there was also a slight but consistent reduction in total protein in the top dose males for the 12-, 18- and 24-months intervals and a slight but significant reduction in blood urea in the top dose females. None of these changes occurred consistently over study period or in both sexes, nor in a dose-related manner and occurred without histopathological corroborate and therefore could not be attributed to treatment.

Table 5.5- 4: Mean haematological and biochemical values (±SD) after 24 months of treatment

Parameter	Males				Females			
	0 ppm	10 000 ppm	20 000 ppm	40 000 ppm	0 ppm	10 000 ppm	20 000 ppm	40 000 ppm
Haematological values								
Erythrocytes (10^6/cmm)	6.68±0.76	7.10±0.59*	7.06±0.29	6.83±0.45	6.94±1.08	7.01±0.43	6.47±0.52	7.10±0.51
Haemoglobin (g/L)	15.5±1.31	17.6±1.07*	16.34±0.7	16.1±1.14	16.1±2.21	16.5±0.79	15.4±1.10	16.9±0.55
Haematocrit (%)	49.3±4.0	55.4±3.98*	52.1±2.31	51.6±2.93	51.3±6.74	52.4±2.42	49.3±3.28	52.9±2.25
WBC (10^3/cmm)	12.9±2.71	12.0±2.29	12.1±1.9	10.8±2.33	12.4±4.52	10±2.30	12.1±4.84	10.0±0.62
Segmented neutrophils%	67±5.1	71±4.4	63±8.1	67±5.4	66±10.1	61±8.2	70±5.8	65±9.6
Ptl (10^3/cmm)	315±32.9	289±8.3	391±52.0	313±68.7	414±101.6	386±113.5	403±39.5	365±78.3

Parameter	Males				Females			
	0 ppm	10 000ppm	20 000 ppm	40 000 ppm	0 ppm	10 000 ppm	20 000 ppm	40 000 ppm
Biochemical values								
Glucose (mg/100 mL)	103±11.4	107±6.2	104±12.4	117±4.9*	102±7.2	104±7.8	106±14.5	102±5
Urea (mg/100 mL)	13.8±3.87	14.7±3.99	16.0±1.62	13.5±2.62	16.1±3.0	13.1±3	14.2±2.8	11±2.8*
Alkaline phosphatase (U/L)	73±16.7	67±24.9	70±33.5	60±27.5	90±47.7	1±24.3	57±9.6	60±16.7
ALT (sigma U/L)	18±4.2	16±8.3	18±2.6	15±5.3	15±5.5	15±3.3	13±3	4±4.1
AST (U/L)	19±8.1	22±9.0	27±4.7	28±2.2	25±12.5	20±8.4	26±10.0	27±8.0
LDH (B-B U/mL)	355±211.4	247±43.8	221±50.9	5±75.2	377±290.1	313±7 4	06±36 8	2 ±87.1
Tot. bilirubin (mg/100 mL)	0.5±0.29	0.4±0.19	0.4±0.25	0.4±0.28	0.5±0.39	0.4±0.34	0.4±0.20	0.5±0.16
Cholesterol (mg/10 mL)	156±43.7	153±11.9	158±1 2	3±33.3	20 ±71.6	175±49.9	66±2	1 ±28.9
Total protein (g/100 mL)	6.84±0.53	6.56±0.09	6.2 ±0.49	6.19±0.35*	6.53±0.60	6.4 ±0.13	6.2 ±0.53	6.25±0.34
Potassium (mEq/L)	5.0±0.31	4.9±0.15	5.0±0.3	5±0.2*	5.3±0.22	5.2±0.37	5.2±0.5	5.3±0.23

* p<0.05

G. URINALYSIS

There were no significant differences noted in urinalysis parameters for any dose group.

H. PATHOLOGY

There were neither gross treatment-related findings nor statistically significant organ weight changes at terminal sacrifice.

Histopathological treatment-related changes were confined to the testes and the kidney:

- In the testes, dose-related degenerative changes (presence of spermatocytic and/or spermatidic giant cells within the lumen of the seminiferous tubules) were seen in 2/6 and 6/6 males from the 20 000 and 40 000 ppm groups, respectively. In the 20 000 ppm males, lesions were focal and involved one or both gonads and were graded as trace in severity; in the 40 000 ppm males, lesions were more numerous, focal or multifocal in distribution, bilateral and also graded as trace in severity; in addition, scant amounts of cellular debris and/or absence of spermatozoa were noted in the epididymidal ducts from the 40 000 ppm males.
- In the kidney from the females, an unexplained slight increase in the incidence and relative severity of the naturally occurring vacuolar tubular lesion was seen among the 20 000 and 40 000 ppm groups: the incidence were 5/6; 4/6; 6/6 and 6/6 among the control; 10 000; 20 000 and 40 000 ppm females groups, respectively and lesions were graded as trace in severity in the controls and 10 000 ppm groups, in contrast to trace to mild among all except 1 female from the 10 000 and 40 000 ppm groups.

III. CONCLUSION

RMS conclusion: The slight reduction of bw gain in treated dogs was not reflected in variations in the mean behavior of food consumption and should not be attributed to test substance. The presence of unknown greenmaterial in stool was not corroborated by any pathological change in the intestinal tract and was attributed to a possible interaction of test substance with diet components. The testicular changes with graded severity seen in the 20 000 and 40 000 ppm groups indicated a test-substance-related effect. The NOAEL in this 2-year chronic toxicity study was 10 000 ppm and was equivalent to an average daily intake of 309 and 288 mg/kg bw/d of test substance in the males and females, respectively.

Report:	KCA 5.5/02 [redacted]; [redacted]; 1981; M-159267-01-1
Title:	Fosetyl-Al: 24-month carcinogenicity study in mice Volume 1 of 4
Report No.:	R000750
Document No.:	M-159267-01-1
Guideline(s):	not specified
Guideline deviation(s):	not applicable
GLP/GEP:	**yes**

I. MATERIALS AND METHODS

Groups of 60 male and female CD-1 mice (4-week old) received fosetyl-Al (batch DA 36, purity 969 g/kg) in the diet at concentration of 2500, 10 000 and 20 000 ppm for the first 19 weeks. The dose level was increased to 30 000 ppm thereafter until week 104. A similar control group received the basal diet alone.
Stability, homogeneity and concentrations in the diet were determined periodically during the study.
Animals were observed at least twice daily for morbidity, mortality and daily for clinical signs throughout the study. Body weights and food consumption were recorded weekly during the first 13 weeks and once every 2 weeks thereafter.
Interim haematological tests were performed in 10 animals/sex/dose after 12 months of dosing. Haematology, blood chemistry and urinalysis were conducted in all animals at the end of the study.
All animals were subjected to a gross pathology examination. Appropriate organs were sampled and preserved for histopathological evaluation.

II. RESULTS AND DISCUSSION

Stability and concentration of fosetyl-Al in the diet were within acceptable ranges. Achieved daily intakes were calculated as follows (see Table 5.5- 5):

Table 5.5- 5: Mean achieved daily intakes following a 104-week dietary exposure to fosetyl-Al

Dietary level (ppm)	Mean achieved daily intake (mg/kg/day)	
	Male	Female
2500	352.1	409.2
10 000	1408.6	1672.4
30 000	3956.2	4549.9

General observation
Mortality and survival
There was no apparent treatment-related mortality during the study. There were no differences in terminal survival between treated and control animals (see Table 5.5- 6).

Table 5.5- 6: Survival rate in rats following a 104-week dietary exposure to fosetyl-Al

Parameter		Dose (ppm)			
		0	2500	10 000	30 000
Survival rate	Male	28/60	34/60	29/60	29/60
	Female	33/60	27/60	25/60	34/60

Clinical signs
No treatment-related clinical signs were reported throughout the study.

Body weight
Statistically significant decreases in body weight were occasionally observed in males and females treated at 30 000 ppm (see Table 5.5- 7). However, as these effects were not dose- nor time-related, they were not considered toxicologically significant.

Table 5.5- 7: Group mean bw (g) and food consumption (g/mouse/d) and % difference from controls

	Controls		2500 ppm		10 000 ppm		20 000/30 000 ppm	
	M	F	M	F	M	F	M	F
Body weight (g & difference with controls in %)								
	38 ± 3.7	36 ± 6.4	38 ± 5.2 (0.00)	35 ± 4.9 (-2.8)	38 ± 5.3 (0.00)	35 ± 5.4 (-2.8)	37 ± 4.8 (-2.6)	34 ± 4.8 (-5.6)
Food consumption (g/mouse/d & difference with controls in %)								
	5.1	5.5	5.1 (0.0)	5.2 (-5.5)	5.2 (2.0)	5.4 (-1.8)	5.2 (2.0)	5.3 (-3.6)

Food consumption
Occasional statistically significant decreases in food consumption were observed in females treated at 30 000 ppm (see Table 5.5- 7). However, as no dose-related trend was established, none of these effects were considered biologically and toxicologically significant.

Haematology, clinical chemistry, urinalysis
Haematology
Statistically significant changes in several haematological parameters (reticulocyte, segmented neutrophil and lymphocyte counts) were occasionally observed in both sexes at different dose levels and sampling times. However, as these effects were not dose- and time-related, they were not considered toxicologically significant.

Clinical chemistry
No statistically or biologically significant changes were observed in any of the parameters examined (SGPT, SGOT, alkaline phosphatase, BUN, glucose, cholesterol and total protein).

Urinalysis
No statistically significant changes in the urinary pH were observed in both sexes at the end of the treatment period.

Gross pathology and histopathology
Gross pathology
Macroscopic examination did not reveal any compound related changes.

Histopathology
Histopathological examination did not reveal treatment-related non-neoplastic or neoplastic changes in both sexes at any dose level. The type and incidence of findings were representative of the pathology that would be expected for mice considering the age, sex and strain.

III. CONCLUSION

RMS conclusion: No treatment related effects were seen in this study; the NOEL was therefore 30 000 ppm, i.e., 3956 and 4549 mg/kg bw in males and females, respectively.

Report:	KCA 5.5/03 [redacted] ; 1981; M-249664-02-1
Title:	Chronic toxicity (2 year) and carcinogenicity study in rats Volume 1 of 5 Fosetyl-Al (LS-74783)
Report No.:	R000702
Document No.:	M-249664-02-1
Guideline(s):	not specified
Guideline deviation(s):	not applicable
GLP/GEP:	**yes**

Report:	KCA 5.5/08 [redacted] ; 1983; M-234109-01-1
Title:	Addendum to the final report Chronic toxicity (2 year) carcinogenicity study in rats Fosetyl-Al (LS 74-783)
Report No.:	C043209
Document No.:	M-234109-01-1
Guideline(s):	not specified
Guideline deviation(s):	not applicable
GLP/GEP:	**no**

Report:	KCA 5.5/09 [redacted] ; 1983; M-159736-01-1
Title:	Addendum Fosetyl-Al (LS74-783) Chronic toxicity (2 year) and carcinogenicity study in rats
Report No.:	R001010
Document No.:	M-159736-01-1
Guideline(s):	not specified
Guideline deviation(s):	not applicable
GLP/GEP:	**no**

I. MATERIALS AND METHODS

Groups of 80 male and female wistar CD rats (age not reported, 90 to 140 g) received fosetyl-Al (batch DA67, purity 997 g/kg and DA67 purity 995 g/kg) in the diet at concentration of 2000, 8000 or 30 000 ppm for 104 weeks. During the first 2 weeks, fosetyl-aluminium (fosetyl-Al) was administered at 40 000 ppm. However, given to the observation of staining of the abdomen and urine red coloration, the dose level was reduced from 40 000 to 30 000 ppm thereafter. A similar control group received the basal diet alone.
Stability, homogeneity and concentrations were determined periodically during the study.
Animals were observed at least twice daily for morbidity, mortality and daily for clinical signs throughout the study. Body weights and food consumption were weekly recorded and food efficiency ratios were calculated.
Ophthalmoscopic examinations were conducted in all animals once prior to the first administration and after 3, 12 and 24 months of treatment.
Haematology, blood chemistry and urinalysis were conducted in 20 males and females from each treated group after 3, 6, 12, 18 and 24 months of administration.
At the end of the study, all animals were submitted to a gross pathology examination. Appropriate organs were weighted and preserved for histopathological examination.

II. RESULTS AND DISCUSSION

During the first 3 months of treatment, actual levels of fosetyl-Al in the diet exceeded in some cases the nominal target dose of 30 000 ppm. However, as the achieved concentrations were larger than the expected dose in both sexes, they were not considered to prejudice the scientific validity of the study. From week 39 onwards, the diet preparation procedure was modified and the dose levels were maintained at reasonable approximation of the theoretical doses (see Table 5.5- 8).

Table 5.5- 8: Concentration of fosetyl-Al in the diet during the first 39 weeks of treatment

Dose level (ppm)		Concentration found after analysis			
		Week 1	Week 13	Week 26	Week 39
2000	Male	1798	1899	1963	1807
	Female	1827	2174	1904	1887
8000	Male	11734	10530	8390	8829
	Female	7624	8531	9900	7768
40 000 → 30 000	Male	49 842	36 645	39 111	29 701
	Female	44 720	40 968	52 440	30 047

After 104 weeks of treatment, mean achieved daily intakes were calculated as follows (see Table 5.5-9):

Table 5.5- 9: Mean achieved daily intakes following a 104-week dietary exposure to fosetyl-Al

Dose level (ppm)	Mean achieved daily intake (mg/kg/day)	
	Male	Female
2000	1[illegible]	1[illegible]
8000	348	460
30 000	1372	1786

General observation

Clinical signs

Animals dosed at 40 000 ppm displayed urine red coloration and urine staining of the abdomen within the first 2 weeks of treatment. Following the reduction of dosing from 40 000 to 30 000 ppm, no treatment-related clinical signs were reported thereafter.

Mortality and survival

Though not dose-related, survival rates were slightly lower in all treated groups when compared with respective control groups (see Table 5.5- 10).

Table 5.5- 10: Summary of mortality and survival rates at week 104

	Controls		2000 ppm		8000 ppm		30 000 ppm	
	Males	Females	Males	Females	Males	Females	Males	Females
No. surviving / no. initiated	36/80	43/80	36/80	37/80	29/80	40/80	35/80	40/80
No. of rats replaced					1 (w-5)	1 (pre-test) 1 (w-3)	1 (w-4)	1 (w-2)

Body weight

Slight reductions in body weight were noted within the first 2 weeks in animals treated at 40 000 ppm (-12 and –9% in males and females respectively) while no treatment-related effects were observed at the end of the study (see Table 5.5- 11).

Food consumption

Slight reduction in food consumption were noted within the first 2 weeks in animals treated at 40 000 ppm while fosetyl-Al induced no changes in food consumption and efficiency thereafter (see Table 5.5- 11).

Table 5.5- 11: Body weights and food consumption noted in rats following a 104-week dietary exposure to fosetyl-Al

	Controls		2000 ppm		8000 ppm		30 000 ppm	
	Males	Females	Males	Females	Males	Females	Males	Females
Body weight (g & difference with controls in %)								
Week 104	729 ± 121	486 ± 109.7	748 ± 112.8 (+2.6)	497 ± 103.4 (+2.3)	688 ± 103.3 (-5.6)	546±110.7* (+12.3)	758 ±128.8 (+4.0)	443 ± 71.1 (-8.8)
Food consumption (g/d)								
Week 1-13	27.1±2.69	18.9±0.81	26.7±2.56	18.8±0.84	26.3±2.46	18.6±1.01	26.2±3.07	18.8±1.32
Week 14-26	28.2±1.23	19.7±1.12	2.72±1.23	20.1±1.14	27.1±1.39	19.6±1.21	27.8±1.39	19.2±1.07
Week 27-39	28.6±1.02	20.5±0.76	27.2±1.13**	20.6±0.90	27.3±1.03**	20.4±1.00	28.4±1.09	20.0±1.03
Week -40-52	26.7±0.54	19.3±0.80	25.6±0.90**	20.0±0.69-	25.7±0.72**	19.5±0.76	26.8±0.89	19.7±0.86
Week 53-65	28.8±1.05	21.3±1.26	27.3±0.85**	21.7±1.26	27.1±1.25**	20.9±1.43	28.3±0.95	20.6±1.47
Week 66-78	27.8±0.86	21.6±1.00	26.4±0.72**	22.4±1.13	26.6±0.93**	21.2±1.08	27.8±0.90	21.0±1.04
Week 79-91	27.8±0.90	22.7±1.03	26.8±0.99*	22.6±0.72	26.3±0.90*	22.6±1.18	27.0±0.94	22.2±1.21
Week 92-104	27.0±1.04	23.8±0.90	27.2±0.75	22.8±0.85	24.8±2.06**	23.1±1.98	27.6±1.14	21.8±1.08
Test substance consumption (mg/kg/d)								
Week 1	-	-	250	255	993	1010	3762	5210
Week 2	-	-	223	228	853	870	4579♣	4781♣
Week 3	-	-	194	201	769	828	2937♣	3182♦
Week 4	-	-	171	171	648	646	2444	2907
Week 52	-	-	94	104	389	386	1104	1549
Week 104	-	-	70	85	258	316	1222	1354
Mean (w1-104)	-	-	117	117	448	450	1372	1786

* p < 0.05; ** p< 0.01

♣ food consumption for a 2-d period ; ♦ food consumption for a 8-d period

Ophthalmological examination

No specific eye alterations were seen at any examination time.

Haematology, clinical chemistry, urinalysis

Haematology

Statistically significant changes in several haematological parameters (erythrocyte, leucocyte and platelet counts, haemoglobin, haematocrit, prothrombin time, percentage of reticulocyte) were occasionally observed in both sexes (see Table 5.5- 12). However, as these effects were not dose- and time-related, they were not considered toxicologically significant.

Clinical chemistry

Statistically significant changes in several biochemical parameters (glucose, SGPT, SGOT, total protein, bilirubin, total protein, sodium and potassium electrolytes) were occasionally observed in both sexes (see Table 5.5- 12). However, as these effects were not dose- and time-related, they were not considered toxicologically significant.

Table 5.5- 12: Mean haematological and biochemical values (±SD) at 24 months treatment period

Parameter	Males				Females			
	Controls	2000 ppm	8000 ppm	30 000 ppm	Controls	2000 ppm	8000 ppm	30 000 ppm
Haematological values								
Erythrocytes (10^6/cmm)	6.41±0.86	6.57±0.90	5.80±1.63	6.50±0.62	5.97±1.09	6.19±0.70	6.07±0.81	6.35±0.97
Haemoglobin (g/L)	14.4±1.99	14.6±2.03	12.4±3.56*	14.7±1.68	14.5±2.46	14.7±1.80	13.6±1.76	13.9±2.03
Haematocrit (%)	41.3±5.36	42.7±5.74	37.0±10.8	43.1±4.17	41.3±6.81	42.9±5.07	42.0±4.92	43.1±4.17
WBC (10^3/cmm)	9.9±4.8	10.5±2.91	13.6±8.17	8.3±4.19	7.1±1.3	5.9±3.52	8.5±6.27	7.4±7.93
Segmented neutrophils %	33±9.4	40±14.4	47±17.9*	43±15.4	47±13.2	42±13.8	54±16.2	50±13.8
PTT (s)	13±0.6	14±0.6**	13±0.9*	13±0.8	13±0.9	13±0.5	12±0.7	12±0.7**
Ptl (10^3/cmm)	839±97.1	805±135.8	810±138.2	704±104.7**	622±125	670±90.8	605±97.8	571±102.2
Reticulocytes (10^3/cmm)	2.2±1.26	2.0±0.84	3.6±4.20	1.6±0.51	2.0±0.81	1.8±1.02	2.0±1.40	2.0±1.69
Biochemical values								
Glucose (mg/100 mL)	107±16.8	120±18.7	119±15.6	108±24.2	98±11.9	93±23.8	103±24.8	88±23.1
Urea (mg/100 mL)	19.7±10.8	16.3±5.44	39.7±37.48	22.4±9.05	11.0±2.7	12.6±11.9	12.8±14.8	12.3±3.83
Total protein (g/100 mL)	7.0±0.59	6.6±0.58*	6.4±0.82**	7.1±0.56	7.1±0.65	7.2±0.78	7.4±0.86	7.6±0.70*
Alk. phos. (IU/L)	80±32.2	108±30.8*	130±209.8	81±29.1	60±17.4	47±21.8	45±21.5	65±38.3
SGPT (sigma U/L)	28±8.8	30±8.1	30±11.7	28±8.6	29±6.73	28±7.5	26±7.0	32±14.8
SGOT (IU/L)	134±35.4	151±57.2	135±74.6	167±78.9	178±46.9	165±64.1	149±58.1	179±82.9
Total bilirubin (mg/100 mL)	0.5±0.29	0.4±0.25	0.5±0.5	0.5±0.20	0.4±0.19	0.4±0.28	0.5±0.09*	0.5±0.13
Cholesterol (mg/100 mL)	190±67.2	167±64.4	132±40.2*	187±55.6	148±70.9	166±67.6	144±39.5	170±71.5

* p<0.05; ** p< 0.01

Urinalysis

Following 6 months of dosing, albuminuria was observed in males treated at 8000 and 30 000 ppm. However, as the presence of albumin in the urine was also reported in both control and treated animals at later sampling times, these findings were not considered toxicologically significant.

Gross pathology, organ weight, histopathology

Gross pathology,

Gross pathological examination revealed the presence of calculi and mineral deposits in the urinary bladder of animals treated at 30 000 ppm as well as mass involving the mucosa wall (see Table 5.5-13).

Table 5.5- 13: Gross pathology findings noted in rats following a 104-week dietary exposure to fosetyl-Al

Organ (number of animal examined)	Male				Female			
	Dose (ppm)				Dose (ppm)			
	0	2000	8000	30 000	0	2000	8000	30 000
URINARY BLADDER (n=80)								
Calculi / mineralisation	0	0	0	7	0	0	0	0
Mass	0	1	0	4	0	0	0	0

Organ weights

Statistically significant increases in absolute and/or relative weights were observed in the liver, kidney, brain, and thyroid (see Table 5.5- 14). However, in the absence of any consistent dose-related trend, these changes were not considered toxicologically relevant.

Table 5.5- 14: Absolute and relative organ weights

	Control		2000 ppm		8000 ppm		30 000 ppm	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Males								
Body Weight (g)	727	116.00	725	123.8	673	110.8	753	135.5
Spleen abs. (g)	1.22	0.38	1.37	0.454	1.38	0.533	1.37	0.511
rel %	0.17	0.05	0.2	0.09	0.2	0.101	0.19	0.088
Liver abs. (g)	26.82	4.38	24.53	3.795	22.86	4.093	25.53	4.741
rel %	3.79	1.02	3.44	0.68	3.35	0.512	3.44	0.625
Kidneys abs. (g)	6.30	1.18	5.99	1.118	6.46	2.426	6.34	1.932
rel %	0.89	0.29	0.85	0.229	0.99	0.427	0.87	0.355
Testes abs. (g)	3.64	0.73	3.54	0.665	3.48	0.679	3.69	0.589
rel %	0.51	0.1	0.54	0.132	0.53	0.121	0.50	0.102
Heart abs. (g)	2.12	0.3	2.22	0.3	2.22	0.355	2.21	0.348
rel %	0.3	0.07	0.33	0.063	0.34	0.066	0.3	0.063
Brain abs. (g)	2.33	0.2	2.33	0.104	2.28	0.152	2.34	0.192
rel %×100	0.33	0.06	0.33	0.064	0.35	0.074	0.32	0.057
Adrenals abs. (mg)	97	21.20	112	201.80	101	33.5	82	16.5
rel %×100	1.41	0.58	1.68	3.406	1.54	0.564	1.12	0.293
Thyroid abs. (mg)	55	16.00	56.00	14	54	14.20	54	16.30
rel %×100	0.79	0.27	0.81	0.33	0.83	0.247	0.74	0.243

	Controls		2000 ppm		8000 ppm		30 000 ppm	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Females								
Body Weight (g)	483	111.0	488	102.4	534	107.9	441	97.9
Spleen abs. (g)	0.99	0.58	0.88	0.554	1.01	0.56	1.09	0.638
rel %	0.21	0.11	0.18	0.093	0.19	0.088	0.25	0.178
Liver abs. (g)	18.56	3.77	17.59	4.699	19.39	4.921	17.1	4.538
rel %	3.92	0.63	3.63	0.654	3.66	0.695	3.96	0.741
Kidneys abs. (g)	3.68	0.56	3.68	0.716	3.68	0.591	3.97	0.693
rel %	0.80	0.21	0.79	0.221	0.7	0.157	0.93	0.268
Ovaries abs. (mg)	171	45.30	170	60	168	48.4	151*	35.1
rel %×100	0.38	0.14	0.35	0.12	0.32	0.101	0.36	0.125
Heart abs. (g)	1.66	0.25	1.63	0.228	1.69	0.279	1.54	0.251
rel %	0.36	0.08	0.34	0.065	0.32	0.063	0.36	0.069
Brain abs. (mg)	2.08	0.13	2.08	0.13	2.00	0.293	2.12	0.121
rel %×100	0.45	0.10	0.44	0.094	0.39	0.106	0.5	0.106
Adrenals abs. (mg)	160	69.2	150	122.6	142	123.3	141	130.6
rel %×100	3.44	1.4	3.12	2.466	2.75	2.4	3.38	3.521
Thyroid abs. (mg)	41	7.6	38	8.8	56	9.4	36*	9.2
rel %×100	0.87	0.19	0.79*	0.19	1.09	0.009	0.81	0.201

Significant difference to controls: *p < 0.05; **p < 0.01

Histopathology

Non neoplastic findings

Upon histopathological examination non-neoplastic findings were limited to the urinary tract of both male and female.

Evidence of hyperplasia and inflammation of the transitional epithelium of the urinary bladder were observed in males treated at 30 000 ppm. Similar findings were reported in males treated at 8000 ppm and females treated at 30 000 ppm in a lesser extent (see Table 5.5- 15).

No treatment-related changes findings were noted at lower dose levels.

Table 5.5- 15: Non neoplastic findings noted in rats following a 104-week dietary exposure to fosetyl-Al

Organ (number of animals) examined	Male				Female			
	Dose (ppm)				Dose (ppm)			
	0	2000	8000	30 000	0	2000	8000	30 000
URINARY BLADDER (n=80)								
Hyperplasia	1	2	4	13	0	4	1	6
Inflammation	4	1	1	11	2	4	1	6

Neoplastic findings

Upon histopathological examination, neoplastic findings were limited to the male adrenals and urinary bladder (see Table 5.5- 16). There was no evidence of any treatment-related neoplastic changes in females at any dose levels.

Table 5.5- 16: Neoplastic lesions (incidence / examined rats)

	Males				Females			
Dose level (ppm)	**0**	**2000**	**8000**	**30 000**	**0**	**2000**	**8000**	**30 000**
Liver								
Neoplastic nodules	6/80	12/80	14/81	10/81	15/79	13/80	12/81	11/81
Neoplastic nodules or carcinoma	9/80	13/80	14/81	12/81	15/79	13/80	13/81	11/81
Pancreas								
Islet cell carcinoma	3/79	3/78	0/79	3/80	1/77	1/79	2/78	0/79
Islet cell carcinoma or adenoma	3/79	6/78	1/79	8/80	1/77	3/79	5/78	0/79
Adrenal								
Cortical adenoma	19/80	9/79	9/81	18/81	24/79	21/79	14/80	24/81
Cortical carcinoma	0/80	3/79	0/81	1/81	2/79	2/79	1/80	2/81
Cortical adenoma or carcinoma	19/80	12/79	14/81	18/81	24/79	23/79	15/80	26/81
Phaeochromocytoma#	5/80	7/78	15/81	16/81**	1/79	0/80	0/80	2/81
Malignant phaeochromocytoma	1/80	0/78	1/81	2/81	0/79	0/79	0/80	1/81
All phaeochromocytoma#	6/80	7/79	16/81	18/81**	1/79	0/79	0/80	3/81
Medullary focal hyperplasia	16/80	10/79	9/81	7/81	4/79	5/79	1/80	3/81
Phaeochromocytoma or hyperplasia	22/80	18/79	25/81	25/81	5/79	5/79	1/80	11/81
Thyroid								
C cell adenoma	6/80	4/76	4/80	7/80	3/79	10/77	1/78	7/81
C cell carcinoma	3/80	3/76	2/80	3/80	2/79	1/77	4/78	4/81
Adenoma or carcinoma	9/80	7/76	6/80	8/80	5/79	11/77	14/78	11/81
C cell hyperplasia	14/80	7/79	10/81	16/80	9/79	12/77	23/78	12/81
Pituitary								
Adenoma	29/76	28/75	25/79	12/78**	53/79	47/79	49/79	49/80
Carcinoma	3/76	1/75	1/79	1/78	1/79	2/79	0/79	2/80
Combination	32/76	29/75	26/79	13/78**	54/79	47/79	49/79	51/80
Mammary gland								
Adenocarcinoma	14/77	10/78	8/78	14/79	14/77	10/78	8/78	14/79
Adenoma	1/77	6/78	1/78	9/79	1/77	6/78	1/78	9/79
Fibroadenoma	36/77	46/78	40/78	30/79	36/77	46/78	40/78	30/79
Urinary bladder								
Transitional cell carcinoma	1/78	0/77	0/80	5/79	0/76	0/78	0/76	2/79
Transitional cell papilloma	0/78	1/77	1/80	9/79*	0/76	1/78	0/76	1/79
Combination	1/78	1/77	1/80	14/79**	0/76	1/78	0/76	3/79
Hyperplasia	1/78	2/77	4/80	13/79	0/76	4/78	1/76	6/79
Inflammation	3/78	4/77	11/80	11/79	2/76	4/78	1/76	6/79
Dysplasia	0/78	1/77	0/80	2/79	1/76	0/78	0/76	2/79
Squamous metaplasia	0/78	1/77	0/80	0/79	1/76	0/78	0/76	2/79

Historical control incidence in male Charles River CD rats at BRLD: 5.3% (range: 0-10%; [redacted].; 1983; Addendum to the final report Chronic toxicity (1 year) carcinogenicity study in rats Fosetyl-Al (LS 74-783); M-234109-01-1)

In the **adrenal medulla**, statistically significant increases in the incidence of pheochromocytoma was observed in males treated at 8000 and 30 000 ppm (18/81 at 30 000 ppm and 16/81 at 8000 ppm compared to 6/80 in the control group, p ≤ 0.05). As illustrated in Table 5.5- 17, this mainly resulted from an increase in the number of benign tumours while the incidence of carcinoma was similar in both control and treated groups. In addition, the incidence of adrenal medullary hyperplasia in treated animals remained in the same range as in control animals (see Table 5.5- 17).

Taking into account that adenoma and hyperplasia are both proliferative lesions and considering the absence of obvious cellular changes between them and the subsequent difficulty in diagnosis, the incidences of these two lesions were combined to run a statistical analysis. Interestingly, there was no significant difference between control and treated animals for the combined lesions (see Table 5.5-17).

However, in order to eliminate any observer bias and further clarify these results, a blinded re-examination of the adrenal slides was performed by a consulting pathologist ([redacted]; 1985; Pathology report: Fosetyl-Al - A blinded histopathologic evaluation of adrenal tissue from a 2-year study in rats; M-165085-01-2) and all the data were reviewed and summarised by a pathologist expert ([redacted]; 1984; Fosetyl-Al: A blinded histopathologic evaluation of adrenal tissues from a two year rat study; M-163455-01-1).

In contrast to the first diagnosis, the blinded re-examination of adrenal slides reported similar incidences of hyperplasia and adenoma in both control and treated animals. In addition, the statistical analysis did not reveal any significant dose-related increase in any adrenal medulla lesions (see Table 5.5- 17).

According to the results provided by the blinded review, it was agreed with the pathology expert to conclude that despite the administration of very high dose levels (greater than the limit dose of 1000 mg/kg/day) for 104 weeks, fosetyl-Al did not produce any carcinogenic effect in the adrenal medulla of rats.

Table 5.5- 17: Neoplastic findings noted in the adrenal medulla of male rats following a 104-week dietary exposure to fosetyl-Al

Organ - Pathologist	Male			
	Dose (ppm)			
	0	2000	8000	30 000
Adrenal medullary - initial pathology report				
Combined (A+C)	6/80	7/79	6/81*	18/81*
Adenoma (A)	5/80	7/79	5/81	16/81*
Carcinoma (C)	1/80	0/79	1/81	2/81
Hyperplasia (H)	16/80	11/79	15/81	9/81
All combined (A+C+H)	22/80	18/79	20/81	27/81
Adrenal medullary - blinded pathology review				
Combined (A+C)	3/78	5/74	10/72	6/79
Adenoma (A)	3/78	5/74	10/72	6/79
Carcinoma (C)	0/78	0/74	0/72	0/79
Hyperplasia (H)	15/78	14/74	13/72	16/79
All combined (A+C+H)	17/78	19/74	23/72	22/79

Significantly different from control; $p<0.05$ (statistical analysis was presented only for the first diagnosis)

In the **urinary bladder**, statistically significant increases in the incidence of transitional cell papilloma and carcinoma were observed in males treated at 30 000 ppm (14/79 compared to 1/78 in the control group, $p<0.05$). As illustrated in Table 5.5-18, this resulted from an increase in the number of both papilloma and carcinoma.

In order to eliminate any observer bias and to gain insight into the underlying mechanism of tumour formation, a blinded re-examination of the urinary bladder slides was initiated by a consulting pathologist ([redacted]; 1985; Pathology report - Fosetyl-Al - Blinded histopathologic evaluation of kidney & urinary bladder tissue from a two year study in rats; M-165088-01-2) and all the data were reviewed and summarised by a consulting pathologist expert ([redacted]; 1985; Fosetyl-Al: A blinded histopathologic evaluation of renal and bladder tissues from a two year rat study; M-163457-01-1).

Similarly to the first diagnosis, higher incidences of urinary bladder adenoma and carcinoma were noted in males treated at 30 000 ppm (see Table 5.5- 18). In addition, hyperplasia of the mucosal transitional cells was also reported in males and females treated at the top dose level.

Accordingly, it was concluded that following the administration of high dose level (greater than the limit dose of 1000 mg/kg/day) for 104 weeks, fosetyl-Al increased the incidence of urinary bladder tumours in male rats.

Table 5.5- 18: Neoplastic findings noted in the urinary bladder of rats following a 104-week dietary exposure to fosetyl-Al

Organ	Male			
	Dose (ppm)			
	0	2000	8000	30 000
Urinary bladder- first pathologist				
Adenoma (A)	0/78	1/80	1/81	9/79 *
Carcinoma (C)	1/78	0/80	0/81	5/79
Combined (A+C)	1/78	1/80	1/81	14/79*
Hyperplasia (H)	na	na	na	na
Urinary bladder- blinded pathology review				
Adenoma (A)	1/78	1/80	1/81	5/79
Carcinoma (C)	2/78	2/80	1/81	16/79
Combined (A+C)	3/78	3/80	2/81	21/79
Hyperplasia (H)	5/78	7/80	5/81	39/79

Significantly different from control; * p<0.05 (statistical analysis was presented only for the first diagnosis)
na: data not available

III. CONCLUSION

RMS conclusion: Dietary administration of up to and including 30 000 ppm of fosetyl-Al to rats for up to 24 months induced statistically significant higher incidence of hyperplasia and neoplasia in the urinary transitional epithelium of high dose males; these findings could be related to the irritant effect of calculi and mineral deposits on the urinary bladder mucosa. The increased incidence of phaeochromocytoma in males from the 2000 and 30 000 ppm groups was of unclear toxicological significance since proliferative lesions of the adrenal medulla occurred in all males including controls and the excess was no longer significant when combining hyperplasia and phaeochromocytoma (diagnosis differentiation between hyperplasia and neoplasia for adrenal medulla was disputed). The NOAEL in this study is 8000 ppm, i.e. equivalent to a mean achieved intake of 348 and 450 mg test substance/kg bw/day in males and females, respectively.

ANSES has requested the submission of historical control data relevant for the 2-year rat study with fosetyl-Al ([redacted], 1981; M-249664-02-1), with a focus on pheochromocytoma.
MPI Research, the contract research laboratory that has emerged from the International Research and Development Corporation (IRDC), has compiled a list of 14 studies that had been conducted by IRDC in the CD rat (bred at Charles River Laboratories) between 1975 and 1985.

Report: KCA 5.5/10[redacted]; 2016; M-564009-01-1
Title: Historical control selected neoplastic and husbandry data in sprague dawley rat, 2 year studies (1977-1985), at MPI Research (former International Research and Development Corporation)
Report No.: M-564009-01-1
Document No.: M-564009-01-1
Guideline(s): none
Guideline deviation(s): none
GLP/GEP: **no**

In male control rats, benign pheochromocytoma had an incidence of 86/1049 (8.2%) with a range of 0.0 to 23.9%. Likewise, malignant pheochromocytoma had an incidence of 2/1049 (0.2%) with a range of 0.0 to 3.8%. In the fosetyl-Al study, benign pheochromocytoma was diagnosed in 16/81 (19.8%) high-dose males, whereas malignant pheochromocytoma was seen in 2/81 (2.5%) of high-dose males (see Table 5.5- 19). Therefore, the incidence of both benign and malignant pheochromocytoma falls within the range of incidences observed in contemporary studies conducted by the same laboratory in the same strain of rats.

Table 5.5- 19: Historical control data for pheochromocytoma in male CD rats

Study	Year	Control group	Interval used	Adrenal medulla				
				Total number examined	Pheochromo-cytoma, benign		Pheochromo-cytoma, malignant	
					n	%	n	%
A	1975-1977	C-1	NA	72	0	0.0%	0	0.0%
B	1976-1978	C-1	12 months to termination	49	4	8.2%	0	0.0%
C	1978-1980	C-1	NA	50	5	10.0%	0	0.0%
D	1978-1980	C-1	12 months to termination	49	2	4.1%	0	0.0%
E	1979-1981	C-1	NA	65	1	1.5%	0	0.0%
E	1979-1981	C-2	NA	65	1	1.5%	0	0.0%
F	1979-1981	C-1	NA	50	2	4.0%	0	0.0%
F	1979-1981	C-2	NA	50	3	6.0%	0	0.0%
G	1980-1982	C-1	0 to termination	46	11	23.9%	0	0.0%
G	1980-1982	C-2	0 to termination	53	4	7.5%	2	3.8%
H	1982-1984	C-1	18 months to termination	47	1	2.1%	0	0.0%
I	1982-1984	C-1	0 to termination	53	5	9.4%	0	0.0%
I	1982-1984	C-2	0 to termination	49	7	14.3%	0	0.0%
J	1982-1984	C-2	0 to 18 months	14	0	0.0%	0	0.0%
J	1982-1984	C-1	18 months to termination	50	6	12.0%	0	0.0%
J	1982-1984	C-2	18 months to termination	36	5	13.9%	0	0.0%
K	1982-1984	C-1	12 months to termination	66	8	12.1%	0	0.0%
L	1983-1985	C-1	12 months to termination	44	10	22.7%	0	0.0%
M	1983-1985	C-1	12 months to termination	41	3	7.3%	0	0.0%
N	1983-1985	C-1	0 to termination	50	8	16.0%	0	0.0%
N	1983-1988	C-2	0 to termination	50	5	10.0%	0	0.0%
TOTAL				1049	91	8.7%	2	0.2%
LOWER RANGE						**0.0%**		**0.0%**
UPPER RANGE						**23.9%**		**3.8%**
[redacted]; 1981; M-249664-02-1	1977-1979	Control males	0 to termination	80	5	6.3%	1	1.3%
		High dose males		81	16	19.8%	2	2.5%
[redacted]; 1985; M-165088-01-2 Blinded review of [redacted] 1981	1977-1979	Control males	0 to Termination	78	6	7.7%	0	0.0%
		High dose males		79	6	7.6%	0	0.0%

NA: not available

The comparison of tumour incidences between these older studies is confounded by different ways of presenting histopathology findings in the various study reports. Some studies report "adrenal cortex" and "adrenal medulla" separately, some jointly under "adrenal" gland.
Furthermore, some pathologists appear to have classified benign or malignant pheochromocytoma as simply as adrenal medullary adenoma or carcinoma, respectively. A distinction can only be reliably made if the tissue section had been stained with chromium salts. This becomes apparent by comparing the blinded review of adrenal findings ([redacted]; 1985; M-165088-01-2) with the original study report ([redacted]; 1981; M-249664-02-1). The reviewing pathologist, Dr [redacted], was of the opinion that a true pheochromocytoma must be chromaffin positive. Lacking proof that each adrenal medullary tumour is or is not chromaffin positive, he has elected to substitute medullary adenoma, a benign tumour of medullary secretory cells. In the case of a malignant pheochromocytoma, he has substituted medullary carcinoma, a malignant tumour of medullary secretory cells. Therefore, the term "pheochromocytoma" as used by the first two reviewing pathologists may be considered synonymous with Dr [redacted]'s adenoma for the purpose of comparing histopathology findings.
Furthermore, peer review of adrenal histopathology by other pathologists has revealed that there is a problem of distinguishing adrenal medullary hyperplasia from adrenal medullary adenoma / benign pheochromocytoma. Depending on the individual interpretation of these findings, a substance-related effect is either apparent or absent (see Table 5.5- 20).

It is noteworthy that the incidence of adrenal cortical adenoma in both controls and high-dose animals of the fosetyl-Al study is much higher than in any of the historical controls. The reason for this is unclear, but it is important to note that there is no effect of treatment on the incidence of cortical adenoma (control males: 19/80, high-dose males: 18/81; control females: 24/29, high-dose females: 24/81).

It can be concluded that the apparent increase in pheochromocytoma incidence in high-dose males falls within the historical control range and is therefore of no concern.

Re-examination of the histopathological results of the main study

Adrenal medulla

In order to clarify the results on adrenal medulla changes seen in the main study, a blinded re-examination of the adrenal slides was performed by a consulting pathologist ([redacted]; 1985; M-165085-01-2). Subsequently, the data were reviewed by a pathologist expert ([redacted]; 1984; M-163455-01-1).

Report: KCA 5.5/04 [redacted] 1985; M-165085-01-2
Title: Pathology report: fosetyl-Al - A blinded histopathologic evaluation of adrenal tissue from a 2-year study in rats
Report No.: M-165085-01-2
Document No.: M-165085-01-2
Guideline(s): not applicable
Guideline deviations): not applicable
GLP/GEP: no

A total of 649 "blinded" slides, each including a collection of microscopic sections of tissues (total = 2535 sections of tissues) from each of 643 rats from the 2 year chronic/carcinogenicity study were re-examined using a composite evaluation statement for the adventitia, the capsule, the cortex and the medulla of each adrenal gland (for each adrenal tissue slide), and the classification published by

[redacted] et al., 1981, for classifying proliferative deviations from the normal morphology of the adrenal medulla.

Results (see Table 5.5- 20) were tabulated by sex and group, generating incidence values for each adrenal gland separately and making distinction between:

Non neoplastic microscopic changes which occurred with equal or higher incidence in adrenal glands of controls than in high dose males or females rats (haemorrhage, ectopic cortical tissue, fibrosis, loss of cellular alteration….); these changes were considered to be spontaneous in occurrence, fortuitous in distribution and therefore not related to treatment.

Non neoplastic microscopic changes which occurred at higher incidence in high dose rats than in controls: among these changes, a statistically significant higher incidence was found only for brown fat in the adventitia of the adrenal gland of females from the mid dose group ($p = 0.004$) and was considered to be not related to treatment, inasmuch as this excess did not occur in higher dose groups.

Metastatic (secondary) tumours, occurring in all groups including controls, most of which were neoplasm of the reticulo-endothelial system (myelogenous leukaemia, lymphosarcoma, lymphocytic leukaemia and myeloma); these metastatic tumours were considered as not related to treatment.

Primary neoplastic microscopic changes in adrenal gland occurring with equal or higher incidence in controls than in any treated group (cortical adenomas and carcinomas; medullary adenoma).

Primary neoplastic microscopic changes occurring in adrenal gland at higher incidence in one or more treated group than in controls of the same sex: however, no statistically significant difference ($p=0.05$ or less) in treated rats compared to controls was shown for cortical adenomas, cortical carcinomas, medullary adenomas, medullary carcinomas among the various treated groups of males and females, each being considered of spontaneous occurrence, fortuitous in distribution and therefore not related to treatment.

Incidence of medullary hyperplasia was not related to treatment in males (incidence in females was about 1/3 less than in males).

Incidence of benign tumours of the adrenal medulla was higher in males than in females; in males, no treatment related excess could be demonstrated, although a statistically non-significant 6% increase of adenomas occurred for the mid-dose group (8000 ppm) compared to the high dose (30 000 ppm) and the low dose (2000 ppm) groups and the controls.

The only malignant adrenal medullary tumour was seen in high dose females.

Table 5.5- 20 Comparative assessment of non-neoplastic and neoplastic medullary adrenal changes in males.

Adrenal medullary changes in males				
	Controls	**2000 ppm**	**8000 ppm**	**30 000 ppm**
Hyperplasia				
1st pathologist	16/80	11/79	10/81	9/81
2nd pathologist	5/80	3/79	5/80	4/80
3rd pathologist	15/78	9/74	13/72	16/79
Adenoma ± carcinoma				
1st pathologist	6/80	7/79	16/81	18/81
2nd pathologist	17/80	15/79	19/81	21/81
3rd pathologist	6/78	5/74	10/72	6/79
Total combined				
1st pathologist	22/80	18/79	26/81	27/81
2nd pathologist	22/80	18/79	24/81	25/81
3rd pathologist	20/78	14/74	23/72	22/79

RMS Conclusion

The blinded re-evaluation of adrenal gland changes in rats administered with 2000, 8000 and 30 000 ppm fosetyl-Al for 105 weeks concluded that there was no significant increase of any non-neoplastic or neoplastic deviation from the normal morphology.

Report:	KCA 5.5/05[redacted]; 1984; M-163455-01-1
Title:	Fosetyl-Al: A blinded histopathologic evaluation of adrenal tissues from a two year rat study
Report No.:	R002848
Document No.:	M-163455-01-1
Guideline(s):	not applicable
Guideline deviation(s):	not applicable
GLP/GEP:	**no**

All the data were reviewed and summarized by a consulting expert, who emphasized the following:
For the original pathologist, which made the morphologic distinction between phaeochromocytoma and focal hyperplasia, treatment related excess of adrenal medullary tumours was suggested in the males rats; since treated females did not exhibit similar changes, the opinion a second pathologist was requested.
Considering that all hyperplastic lesions of the adrenal medulla were considered as phaeochromocytoma, the 2nd pathologist differed with the 1st pathologist 48, 33, 25 and 15% of the time for the control-, low-; mid- and high dose groups, respectively; however, this did not change the interpretation of no treatment related effect when combining both hyperplasia and phaeochromocytoma (see Table 5.5- 20).
As these 2 pathologists could have biased inadvertently their opinion because of knowing dose groups from which tissue sections were provided and as the 2nd pathologist was aware of the 1st pathologist diagnosis, it was decided that a "blinded" review by a 3rd pathologist should be performed using a pre-determined set of criteria, for eliminating any bias once above for results which differed from the 1st pathologist by 30%; 45%, 39% and 52% for the control-, low-; mid- and high dose groups, respectively).
The main difference between the pathologists' diagnosis was the distinction between hyperplasia and benign neoplasia or between hyperplasia and normality (see Table 5.5-.20).

RMS Conclusion
The blinded review showed that no carcinogenic effect in the adrenal medulla was induced by fosetyl-Al in rats after 2 year administration of dietary concentration up to 30 000 ppm.

Urinary bladder

In order to clarify the results on urinary bladder neoplasms seen in the main study and the underlying mechanism of tumour formation, a blinded re-examination of the kidney and urinary bladder slides was performed by a consulting pathologist ([redacted]; 1985; M-165088-01-2). Subsequently all the data were reviewed by a pathologist expert ([redacted]; 1985; M-163457-01-1).

Report:	KCA 5.5/06[redacted]1985; M-165088-01-2
Title:	Pathology Report - Fosetyl-Al - Blinded histopathologic evaluation of kidney & urinary bladder tissue from a two year study in rats
Report No.:	M-165088-01-2
Document No.:	M-165088-01-2
Guideline(s):	not applicable
Guideline deviation(s):	not applicable
GLP/GEP:	**no**

A total of 667 "blinded" slides, each including a collection of microscopic sections of tissues (total = 1334 sections of kidney and 727 sections of the urinary bladder) from each of 641 rats from the 2 year chronic carcinogenicity study were re-examined using a composite evaluation statement for adventitia, capsule, cortical interstitium, cortical tubules, medulla and pelvis of each kidney and for the lumen, epithelium, propria, submucosa, muscularis and serosa of each urinary bladder.

Inflammatory, hyperplastic, degenerative, secondary neoplastic and obstructive deviations from the normal histologic morphology were classified according to the catalogue of observations of micro-pathology ([redacted] SW); primary neoplastic deviations of the kidney were classified according to the system of Hard (with tumour of the cortical epithelium, tumour of the connective tissue, nephroblastoma, adenoma, adenocarcinoma, mesenchymal tumour, lipoma, liposarcoma) ; proliferative lesions of the urinary bladder were classified using the system of Hicks (non-neoplastic lesions with transitional cell hyperplasia, nodular hyperplasia, squamous metaplasia, muscular hypertrophy; benign neoplastic changes with transitional cell papilloma, leiomyosmia, fibroma; malignant neoplastic changes with papillary transitional cell carcinoma, infiltrating transitional cell carcinoma, squamous cell carcinoma, complex papillomatous carcinoma, leiomyosarcoma and fibrosarcoma; secondary metastatic tumours).

Results (see Table 5.5- 18)
The only statistically significantly increased non-neoplastic changes, which occurred in high dose males, was hyperplasia of the epithelium of the urinary bladder ($p<0.001$) and sub-acute lymphocytic inflammation of the submucosa of the urinary bladder ($p=0.03$).
In high-dose females, several non-neoplastic changes occurred at statistically significantly higher levels than in controls: interstitial fibrosis of the kidney cortex ($p<0.001$), ectasis of the Bowman's space in the kidney cortex ($p=0.05$), cystic tubules ($p<0.001$), glomerular sclerosis ($p=0.002$), acute leukocytic inflammation of tubules in the kidney cortex ($p<0.001$), ectasis of the tubules in the kidney medulla ($p=0.01$); hyperplasia of the epithelium of the kidney pelvis ($p=0.02$); hydronephrosis of the kidney ($p=0.002$); hyperplasia of the epithelium in the urinary bladder ($p=0.006$) and subacute lymphocytic inflammation of the submucosa of the urinary bladder ($p=0.01$).
With respect to primary neoplasia, only the incidence complex papillomatous carcinoma of the epithelium of the urinary bladder was statistically significantly increased ($p=0.02$) in high-dose males, compared to controls; in addition, the incidence of all types of carcinomas of the epithelium of the urinary bladder was also significantly increased in high dose males compared to controls, although separate incidences of each type of carcinoma (other than complex papillomatous carcinoma) was not significantly different from control values.

RMS Conclusion
The blinded re-evaluation of kidney and urinary bladder microscopic changes following administration of 2000, 5000 and 30,000 ppm fosetyl-Al in the diet of rats up to 105 weeks concluded that statistically significant treatment related effects were seen only in the highest dose level, including increased incidences ($p<0.05$) of hyperplasia of the epithelium and sub-acute inflammation of the sub-mucosa of the urinary bladder for the males, and increased incidence of several forms of inflammatory and/or degenerative changes of the kidney for the females. No microscopic treatment-related significant changes were seen in the kidney and/or in the urinary bladder at the lower dose levels for both sexes.

Report:	KCA 5.5/07[redacted]; 1985; M-163457-01-1
Title:	Fosetyl-Al: A blinded histopathologic evaluation of renal and bladder tissues from a two year rat study
Report No.:	R002849
Document No.:	M-163457-01-1
Guideline(s):	not applicable
Guideline deviation(s):	not applicable
GLP/GEP:	**no**

All the data were reviewed and summarized by a consulting expert (see Table 5.5.-21), who emphasized the following:

Renal findings
Blinded review of the results confirmed that renal changes occurred in the high dose females and included renal cortical cysts and ectasis of the Bowman's capsule.
Incidence of renal medullary and pelvic changes was altered in both sexes at highest dose level; hydropelvis or ectasis of the pelvic lumen and ectasis of the medullary tubules were slightly increased in males, but more markedly in the females.
Hyperplasia of the transitional epithelium of the pelvis was slightly increased in high dose males, but more markedly in high dose females.
Cortical adenoma and adeno-carcinoma occurred at similar incidences in treated males and females compared to controls; there was a slight increased incidence of papilloma in high dose males and of papillo-carcinoma in high dose females.

Urinary bladder findings
Gross macroscopic findings found in the urinary bladder (calculi, dilatation...) of males were not seen in the females.
Blinded review of the results confirmed that non-neoplastic changes consisted of hyperplasia of the transitional cells which was seen in all groups including controls, but markedly increased in high dose males and females.
Neoplastic changes were predominantly transitional cell papillomas and papillo-carcinomas which were seen in the high dose group, particularly in males. For other types of tumours, low incidence rates were recorded.
It was then acknowledged that the blinded review increased the number of proliferative lesions in all treated groups and in both sexes, compared to the original report, but this was not considered to alter the original interpretation relative to an increased epithelial proliferation only in the high dose males. On the other hand, the finding of increased proliferative lesions of the transitional cell epithelium in the urinary tract of the females changed the original interpretation and substantiate a common urinary reaction in all treated females, transitional cell changes being similar to those of males at the highest dose level.

Table 5.5- 21: Non neoplastic and neoplastic findings in kidney and bladder

	Males				Females			
Dose level (ppm)	**0**	**2000**	**8000**	**30 000**	**0**	**2000**	**8000**	**30 000**
Non neoplastic								
Kidney cortex								
Hyperplasia	5/78	7/80	4/81	4/79	1/76	0/78	1/78	3/79
Urolithiasis	8/78	10/80	17/81	7/79	27/76	27/78	9/78	5/79
Mineralization	4/78	4/80	7/81	3/79	1/76	1/78	1/78	3/79
Ectasis/tubules	74/78	69/80	71/81	74/79	54/76	50/78	49/78	56/79
Ectasis/Bowman's capsule	22/78	17/80	17/81	20/79	10/76	8/78	11/78	21/79
Cysts	60/78	53/80	59/81	57/79	25/76	26/78	27/78	34/79
Kidney medulla/pelvis								
Hyperplasia	13/78	8/80	15/81	21/79	9/76	10/78	2/78	7/79
Urolithiasis	6/78	11/80	5/81	9/79	18/76	45/78	29/78	9/79
Mineralization	2/78	3/80	0/81	2/79	10/76	8/78	2/78	0/79
Ectasis/tubules	21/78	25/80	26/81	30/79	9/76	9/78	10/78	29/79
Cystic tubules	2/78	1/80	4/81	2/79	2/76	3/78	0/78	1/79
Ectasis/hydronephrosis	6/78	4/80	1/81	5/79	2/76	1/78	0/78	3/79
Urinary bladder								
Hyperplasia	5/78	1/80	3/81	29/79	1/76	1/78	3/78	11/79
Urolithiasis	1/78	1/80	0/81	0/79	0/76	0/78	0/78	1/79
Mineralization	1/78	1/80	0/81	0/79	0/76	0/78	0/78	0/79
Neoplastic changes								
Kidney cortex								
Adenoma	2/78	0/80	2/81	-	-	1/78	-	1/79
Adenocarcinoma	2/78	2/80	1/81	2/79	-	-	1/78	3/79
Mesenchymal tumour	1/78	-	-	-	1/76	1/78	1/78	1/79
Lipoma/liposarcoma	-	-	1/81	1/79	-	-	1/78	-
Liposarcoma & reticulum cell	1/80	1/81	-	-	-	1/78	-	-
Lymphomeukemia	1/78	1/81	-	-	1/76	-	-	-
Myeloma	1/78	1/81	-	-	-	-	-	-
Kidney pelvis								
Papilloma	1/78	2/80	1/81	1/79	1/76	1/78	1/78	1/79
Papillosarcoma	1/78	1/80	-	-	-	-	-	6/79
Urinary bladder								
Papilloma	1/78	1/80	1/81	1/79	-	2/78	-	1/79
Papillosarcoma	1/78	2/80	1/81	16/79	-	1/78	-	5/79
Leiomyosarcoma	1/78	-	1/81	1/79	1/76	1/78	-	-
Reticulum cell sarcoma	-	-	1/81	-	-	-	-	-
Lympholeukaemia	1/78	-	-	-	-	-	-	-

RMS Conclusion

Carcinogenic potential of fosetyl-Al was discussed as being more a chronic toxic reaction rather than a true carcinogenic effect in light of the following:

Fosetyl-Al decomposes rapidly into relative innocuous by-products, predominantly into phosphonic acid which exhibits no carcinogenic potential.

The highest dose used in the rat carcinogenicity study is excessively high (equivalent of a 50 kg human ingesting at least 75 g fosetyl-Al daily for the major portion of the life-span)

Non-neoplastic changes of the urinary tract are associated only with massive oral doses of fosetyl-Al. Increased urinary excretion of calcium and renal tubular cell degenerative, but reversible changes were seen after 1 month in the same strain of rats given 40 000 ppm fosetyl-Al ([redacted]; [redacted]; [redacted]; [redacted]; [redacted]; 1981; M-205133-01-2; see Section CA 5.8.2, p.182).

In the 2 year study, there was no exacerbation of tubular changes that would be expected in case of non-reversible lesion; the most probable mechanism for hydronephrosis, hydro-pelvis and dilatation of the lower renal tubules and pelvic lumen is urine retention, which is assumed to originate in the bladder since high dose rats exhibited dilated ureters and occasionally bladder stones. Then, stagnation of urine containing presumed high levels of calcium would generate uroliths and chronic irritation leading to transitional cell proliferation, which in turn gives rise to papillomatous neoplastic changes. No similar urinary tract proliferative changes were seen in 2 year carcinogenicity study on mice fed with 2.8 to 4.3 g/kg bw/d fosetyl-Al.

CA 5.6 Reproductive toxicity

The reproductive toxic potential of fosetyl-aluminium (fosetyl-Al) was tested in a three-generation study in rats and in developmental toxicity studies in rats and rabbits (see Table 5.6- 1). No new studies have been performed for this endpoint.

Rat multi-generation study ([redacted] ; 1981; M-203019-01-1)
Despite the continuous oral administration of high dose levels up to 24 000 ppm throughout 3 generations, fosetyl-Al did no produce any adverse effects on reproductive performance and fertility. Accordingly, this dose level of 24 000 ppm (1782 - 1997 mg/kg bw/day F0 ♂ ♀) was considered to be the NOAEL of the study for reproductive or fertility effects.

Rat teratogenicity study ([redacted] ; 1977; M-158819-01-1)
Following oral administration from Day 6 to Day 15 of gestation to pregnant rats at a dose level of 4000 mg/kg bw/day, fosetyl-Al induced obvious maternal toxicity as evidenced by mortality and body weight loss. Foetal toxicity which was illustrated by occasional changes in litter parameters and slightly higher incidences of malformation (thoracic asymmetry, displaced kidney and testes, hydrocephaly, vein/artery transposition, intra-abdominal and subcutaneous haemorrhage) and minor anomalies likely resulted from maternal toxicity rather than from a direct effect of fosetyl-Al on the embryo. Given the absence of any effects in both dams and foetuses at lower dose levels, the dose level of 1000 mg/kg bw/day was considered to be the NOAEL for maternal and developmental toxicity.

Rabbit teratogenicity study ([redacted] ; 1976; M-231386-01-2)
An older, non-guideline study with oral administration of dose levels ranging from 125 to 500 mg/kg bw/day did not show any treatment-related effects in does or foetuses. The top dose level of 500 mg/kg bw/day was therefore considered to be the NOAEL for maternal and developmental toxicity.

Rabbit teratogenicity study ([redacted] ; 2000; M-207431-01-1; [redacted] ; 2000; M-205472-01-1)
A preliminary range-finding study showed that following oral administration at dose levels ranging from 250 to 500 mg/kg bw/day fosetyl-Al induced maternal toxicity as evidenced by body weight and food consumption decreases. However, despite the administration from Day 4 to Day 29 of gestation to pregnant rabbits at a dose level of 300 mg/kg bw/day, fosetyl-Al did not induce any maternal or foetal toxicity in the definitive regulatory study. The top dose level of 300 mg/kg bw/day was therefore considered to be the NOAEL for maternal and developmental toxicity.

Conclusion

Taken together, these results indicated that fosetyl-Al was neither a reproductive toxicant nor a teratogenic compound in rat or rabbit under the experimental conditions used. Foetal toxicity was only observed in the rat at very high dose level, i.e. 4000 mg/kg bw/day, which also induced obvious maternal toxicity. Therefore, according to the criteria of Regulation 1272/2008, **no classification as reproductive toxicant is required.**

Based on the results of available studies, the overall acceptable NOAEL for maternal and pup toxicity was 300 mg/kg bw/day based on the results of the rabbit teratology study ([redacted]; 2000; M-205472-01-1).

Table 5.6- 1: Reproductive toxicity studies with fosetyl-Al

Study Type	Species	Doses tested	LOAEL / Effects	NOAEL	Reference
Three-generation reproduction	Rat	0, 6000, 12 000, 24 000 ppm	Reproductive LOAEL > 24 000 ppm: no treatment-related effects Maternal and foetal LOAEL 12000ppm: decreased bw of F2B pups and litter weight	Reproductive NOAEL ≥ 24 000 ppm (1782 / 1907 mg/kg bw/day, F0 ♂/♀) Maternal and foetal NOAEL 6000 ppm (939 / 1030 mg/kg bw/day, F0 ♂/♀)	[redacted]; 1981; M-203019-01-1
Developmental toxicity (oral gavage)	Rat	0, 500, 1000, 4000 mg/kg bw/day	Maternal LOAEL = 4000 mg/kg bw/day: mortality and bw loss Developmental LOAEL = 4000 mg/kg bw/day: Minor changes in litter parameters, marginally increased incidence of malformations and minor anomalies	Maternal NOAEL = 1000 mg/kg bw/day Developmental NOAEL = 1000 mg/kg bw/day	[redacted]; 1977; M-158819-01-1
		0, 125, 250, 500 mg/kg bw/day	Maternal LOAEL > 500 mg/kg bw/day: no treatment-related effects Developmental LOAEL > 500 mg/kg bw/day: no treatment-related effects	Maternal NOAEL ≥ 500 mg/kg bw/day Developmental NOAEL ≥ 500 mg/kg bw/day	[redacted]; 1976; M-231386-01-2
	Rabbit	Range-finding study: 0, 75, 150, 250, 500, 1000 mg/kg bw/day	Maternal LOAEL = 250 mg/kg bw/day: lower bw, bw gain Developmental LOAEL > 1000 mg/kg bw/day: no treatment-related effects	Maternal NOAEL = 125 mg/kg bw/day Developmental NOAEL ≥ 1000 mg/kg bw/day	[redacted]; 2000; M-207431-01-1
		Definitive study: 0, 50, 100, 300 mg/kg bw/day	Maternal LOAEL > 300 mg/kg bw/day: no treatment-related effects Developmental LOAEL > 300 mg/kg bw/day: no treatment-related effects	Maternal NOAEL ≥ 300 mg/kg bw/day Developmental NOAEL ≥ 300 mg/kg bw/day	[redacted]; 2000; M-205472-01-1

CA 5.6.1 Generational studies

All studies for this endpoint have been previously submitted and evaluated. The following study summary contains a newly conducted statistical evaluation of body weights (see Table 5.6.1- 5):

Report:	KCA 5.6.1/01 [redacted]; 1981; M-203019-01-1
Title:	Effect of LS 74-783 on reproductive function of multiple generations in the rat
Report No.:	C012605
Document No.:	M-203019-01-1
Guideline(s):	not specified
Guideline deviation(s):	not applicable
GLP/GEP:	**yes**

I. MATERIALS AND METHODS

Groups of 50 (25/sex) CFY rats (age not specified; bw range = 60 to 70 g for both sexes) were administered dietary concentrations 0, 3 000, 12 000 and 24 000 ppm of technical fosetyl-Al (batch DA 73; 97.3% purity) through 3 generations.

F0 generation animals were treated over a 90-d pre-mating period, throughout a 2nd mating period, lactation and gestation with 2 litters (the 2nd 2nd mating period occurred approx. 10 d after weaning of the F1A litter); all females were allowed to deliver their litters and rear the pups for 21 days; 5 dams/group were sacrificed at d-20 of gestation (1st litter) for teratology investigations and F0 parents were killed and examined macroscopically after weaning of the F1B litters.

F1A litters were reared until d-21post partum and then sacrificed and subjected to macroscopic examination; 25 pups/sex/group were selected from the F1B litters to form the F1B generation (remaining F1B pups were killed and examined macroscopically).

F1B generation groups were reared on their respective diets until 90-d of age and mated twice as for F0 generation; 10 dams/group were sacrificed on d-20 of pregnancy for teratology investigations (1st litter).

F2A pups were reared until d-21 post partum and then sacrificed and examined macroscopically; F2B pups were reared under their respective diets for at least 91 days when 15 pups/sex/dose were sacrificed and examined macroscopically; remaining F2B pups (12 males and 24 females) were then mated twice (1 male to 2 females using different partners at the 2nd pairing) to produce F3A and F3B generations.

All F3A pups were sacrificed and examined macroscopically; F3B pups (10/sex from the control and high dose groups) were subjected to organ weight and histopathological examinations.

Follow up of adult generations

Parental animals were observed regularly for mortality and signs of toxicity

Food consumption was recorded weekly during the 1st pre-mating phase of each generation; water intake was determined during the 1st and the penultimate w of this same pre-mating phase.

Determination of bw was performed weekly in F0 generation and on d-0; d-4; d-8; d-12; d-21 and weekly thereafter in the subsequent generations. Females were also weighed on d-0; d-7; d-14; d-17 and d-20 of presumed gestation and on d-0; d-7; d-14 and d-21 of lactation.

Mating performance, pregnancy rate and duration of gestation period were determined.

Macroscopic examination was carried out on all parents after the 2nd litter has been weaned; uterine of apparently non-pregnant females were examined and testes of males which failed to induce pregnancy were weighed and preserved for histologic examination.

On d-20 pregnancy 5 selected F0 and F1B dams were examined for congenital abnormalities and pathological changes in maternal organs; number of corpora lutea, number and distribution of live young, of early or late embryonic and foetal deaths, litter weight and foetal abnormalities were determined; half of pups in each litter were preserved for examination of visceral abnormalities and the other half for sex determination and skeletal examination.

Following death of 5 rats in high dose group in which kidney damage was seen at macroscopic examination, it was decided to perform limited urinalysis in F1B generation (10 males from control and high dose groups) during w-7 of treatment.

Follow-up of offspring/litters:
All pups from each litter were examined within the 1st 12 hours after birth for external abnormalities, and daily thereafter for dead young and abnormal young.
Individual pups bw, litter size and cumulative mortality were determined on d-0; d-4; d-8; d-14 and d-21 post partum.
Surplus pups sacrificed on d-21 post partum were examined externally or externally for abnormalities.
Organs from 10/sex F3B pups from the control and high dose groups were weighed (brain; heart; liver; kidney; lung; spleen; thymus) and histopathologic examination was performed (brain; eyes; heart; lung; liver; kidney; spleen; urinary bladder; stomach; duodenum; jejunum; caecum; salivary gland; pancreas; lymph nodes; thymus; thyroid; pituitary; adrenal gland; testes; seminal vesicle; ovary; uterus and any tissue exhibiting macroscopic changes; other organs were preserved but not examined in the 1st instance).

II. RESULTS AND DISCUSSION

Intake of test substance:
For each dietary concentration, doses of test substance (mg/kg bw/d) were much higher for the F1B and F2B generations than for the F0 generation (see Table 5.6.1 - 1).

Table 5.6.1- 1: Nominal doses (mg/kg bw/d) of test material

Nominal conc. (ppm)	Generation	w1		w5		w13		w29		w-12		Mean (w1 –w12)	
		M	F	M	F	M	F	M	F	M	F	M	F
6000	F0	784	806	577	622	418	519	292	376	299	401	482	553
	F1B	873	896	684	776	432	478	356	444	-	-	586	649
	F2B	1132	1161	797	863	515	640	398	544	310	395	629	721
12 000	F0	1550	1582	1143	1190	829	963	654	807	592	738	954	1056
	F1B	1726	1786	1445	1541	876	949	688	898	-	-	1203	1297
	F2B	2239	2382	1654	1774	1013	128	813	1054	631	773	1288	1186
24 000	F0	3205	3116	2378	2378	1699	2032	1321	1591	1186	1510	1958	2125
	F1B	5064	4337	3589	3592	2189	2107	1785	1957	-	-	3256	2999
	F2B	5680	5944	4176	4366	2761	2762	1921	2222	1300	1655	3066	3030

Parental generations:
There were no treatment-related clinical signs in any dose in any generation.
Over all generations:
13 treated males died versus only 1 in controls; mortality in males was clustered at 24 000 ppm for the F1B generation and to a lesser extent in the F2B generation; autopsy of these rats showed changes in the urinary tract (haemorrhage of the bladder wall, increased renal pelvic dilatation, interstitial nephritis and papillary necrosis). A similar clustered distribution of urinary tract changes was also seen for both sexes at terminal examination of surviving rats. The incidences of urinary tract lesions are statistically significantly elevated only in high-dose F1B animals of both sexes (see Table 5.6.1-2). This is in line with the urinary tract effects (urolithiasis) observed at high doses in the 2-year rat study ([redacted]; 1981; M-249664-02-1) and in the two mechanistic studies in rats ([redacted]; [redacted]; [redacted]; [redacted]; [redacted]; 1981; M-205133-01-2 and [redacted]; 1989; M-160331-01-1).

Table 5.6.1- 2: Incidence of urinary tract lesions in parental animals (survivors + decedents)

Dose (ppm)	Males			Females		
	F0 n=25	F1B n=25	F2B n=27	F0 n=25	F1B n=25	F2B n=25
0	1	0	1/27	0	1	0
6000	0 n.s.	2 n.s.	0/27 n.s.	1 n.s.	1 n.s.	1 n.s.
12 000	0 n.s.	1 n.s.	1 n.s.	0 n.s.	3 n.s.	0 n.s.
24 000	0 n.s.	9** p=0.0016	5 p=0.192	4 p=0.110	10** p=0.0046	5 p=0.050

Statistically significant difference from controls: **p<0.01; retrospective statistical analysis using Fisher's exact test.

8 treated females died versus none in controls, but these deaths were evenly distributed across dose groups and generations and were then not related to treatment (see Table 5.6.1- 3).

Table 5.6.1- 3: Mortality over 3 generation (time of death in w)

Generation	Controls		6000 ppm		12 000 ppm		24 000 ppm	
	M	F	M	F	M	F	M	F
F0	1 (w-20)	-	-	2 (w-27)	1 (w19)			2 (w-17-21)
F1B	-	-	-	(w-19)	1 (w-1)	-	7 (w3-8)	1 (w-26)
F2B	-	-	-		(w-25)	2 (w-17-21)	3 (w3-14)	-

Mean weekly food consumption of treated rats was comparable with controls except in F1B males from the 12 000 and 24 000-ppm groups during the initial rearing phase and in F1B and F2B females from the 24 000-ppm groups during the 1st week of rearing.
For all generations, water consumption in both males and females from the 24,000 ppm groups was higher than controls during the 1st week (see Table 5.6.1 - 4).

Table 5.6.1- 4: Mean food and water consumption in the 3 generations

Generation	Study week	Controls		6000 ppm		12 000 ppm		24 000 ppm	
		M	F	M	F	M	F	M	F
FOOD CONSUMPTION (g/rat/w)									
F0	w-1	123	108	123	118	127	114	129	113
	w-13	190	136	185	138	185	135	183	143
F1B	w-1	102	102	112	104	105*	99	102*	84
	w-2	170	135	163	150	154*	140	143*	138
	w-14	191	132	180	142	176	127	206	144
F2B	w-1	112	109	105	105	96	95	111	93
	w-13	191	128	207	142	186	127	191	134

Generation	Study week	Controls		6000 ppm		12 000 ppm		24 000 ppm	
		M	F	M	F	M	F	M	F
WATER CONSUMPTION (g/rat/d)									
F0	w-1	24.3	23.5	26.8	24.2	24.3	23.2	27.5	24.9
	w-12	38.9	31.2	42.5	31.7	42.7	29.8	40.0	31.8
F1B	w-1	20.3	20.8	23.1	22.1	23.0	20.9	25.0	26.2
	w-9	36.5	32.3	42.7	35.2	39.6	31.1	40.5	32.8
F2B	w-1	19.4	16.8	17.3	16.5	16.5	14.8	16.9	17.8
	w-12	43.2	31.0	46.8	35.9	39.6	30.5	44.5	32.1

* p<0.05

Mean bw gain for all generations were lower in high dose groups than in controls (bw reduction was marginal in F0 males and females and more pronounced in F1B and F2B males and females; marginal reduction were also seen in F2B males and females at 12 000 ppm.
Statistically significantly reduced bw in high dose animals was seen throughout the pre-mating phase in both sexes of the F1B and the F2B generation. Bw was also reduced in high-dose animals at the beginning of the gestation phase in both F1B and F2B dams. In high-dose F1B dams, there was a significant reduction of bw at the end of the lactation phase. A similar reduction was noted in high-dose F2B dams, but the effects were statistically non significant (see Table 5.6.1- 5).

Table 5.6.1- 5: BW in the three generations with statistical evaluation

		Body weight (g)							
Sex		Male				Female			
Dose (ppm)		0	6000	12 000	24 000	0	6000	12 000	24 000
GENERATION F0									
Pre-mating	Wk 1	177±14	172±10	165±15	167±14	145±9	149±9	145±10	147±10
	Wk 13	552±47	509±43	561±48	527±38	307±24	319±26	309±28	322±36
Gestation (1st mating)	Day 0					316±25	324±26	313±29	314±25
	Day 20					454±51	462±34*	449±41	451±49
Lactation (1st mating)	Day 0					346±27	376±29**	357±33	375±40*
	Day 21					359±27	373±27*	363±23	343±52
GENERATION F1B									
Pre-mating	Wk 1	153±15	134±21	128±21	81±22***	123±11	123±17	117±20	83±26***
	Wk 13	541±38	537±50*	564±46	473±51***	373±35	389±35**	376±53	334±65***
Gestation (1st mating)	Day 0					298±18	315±35	302±35	276±25***
	Day 20					406±46	421±48	409±51	382±52
Lactation (1st mating)	Day 0					338±26	355±30	349±50	327±33
	Day 21					344±23	365±31*	355±40	286±30***
GENERATION F2B									
Pre-mating	Wk 1	110±15	102±13	94±16**	84±15***	100±13	96±13	88±15**	80±12***
	Wk 13	543±75	499±79	522±72	495±38***	306±44	316±35	292±41	286±22
Gestation (1st mating)	Day 0					319±52	330±39	306±42	299±20
	Day 20					419±52	423±67	418±48	402±54
Lactation (1st mating)	Day 0					343±29	377±40**	349±38	349±23
	Day 21					348±26	381±46*	354±34	297±35

Statistically significant differences from controls: *p<0.05, **p<0.01, ***p<0.001; retrospective statistical analysis per request of the RMS. Simple factor ANOVA (α=0.05) followed by Dunnett's *t*-test

There was no test substance related effect on reproductive performance in all generations:
Mating performance and pregnancy rates were comparable at both matings of each generation, except a lower pregnancy rate (related to an increased median pre-coital time) for low dose F2B generation for both matings. As no similar changes were seen at the higher doses, these findings should be considered as incidental (see Table 5.6.1- 6).

Table 5.6.1- 6: Reproductive performance in the 3 generations

Generation			Controls	6000 ppm	12 000 ppm	24 000 ppm
F0	1st mating	N° of rats	25	25	25	25
		Pre-coital time (d)	2.0	2.0	3.0	4.0
		Pregnancy rate %	96	96	96	88
		N° of rats with gestation period (d)	24 (22.2)	24 (22.2)	24 (22.2)	21 (22.2)
	2nd mating	N° of rats	18	19	19	17
		Pre-coital time (d)	2.0	2.0	2.0	2.0
		Pregnancy rate %	80	84	100	78
		N° of rats with gestation period (d)	15 (22.1)	16 (22.3)	20 (22.3)	13 (22.3)
F1B	1st mating	N° of rats	25	25	25	25
		Pre-coital time (d)	3.0	5.0	4.0	5.0
		Pregnancy rate %	84	92	84	80
		N° of rats with gestation period (d)	19 (22.1)	18 (22.2)	21 (22.3)	20 (22.2)
	2nd mating	N° of rats	21	16	19	16
		Pre-coital time (d)	3.0	3.0	3.0	3.0
		Pregnancy rate %	84	67	80	88
		N° of rats with gestation period (d)	11 (22.1)	6 (22.2)	11 (22.2)	12 (22.3)
F2B	1st mating	N° of rats	24	24	24	24
		Pre-coital time (d)	4.0	11.0	4.0	4.0
		Pregnancy rate %	75	54	83	86
		N° of rats with gestation period (d)	18 (22.3)	13 (22.0)	20 (22.2)	19 (22.1)
	2nd mating	N° of rats	24	24	22	24
		Pre-coital time (d)	3.5	10.0	4.5	4.0
		Pregnancy rate %	75	58	73	83
		N° of rats with gestation period (d)	18 (22.5)	14 (22.5)	16 (22.1)	20 (22.2)

Among the 5 females from F0 and F1B generations killed and examined at d-20 pregnancy, the only significant finding was the reduced number of corpora lutea per dam for both generation at 12 000 and 24 000 ppm; this finding was not of biological importance since large number of dams reared their young and no differences in litter size or weight at birth occurred.
There were no treatment related gross macroscopic organ changes except urinary tract changes in high dose rats, particularly in the F2B generation; such changes were occasionally seen at 6000 and 12 000 ppm, particularly in the F2B generation.

Pup data:
No treatment related effect was seen on total litter loss (which was scattered throughout the various groups and generations), litter size, pup viability.
For both matings of each generation, mean litter- and pup weights were comparable for all groups at birth and at d-4 post-partum; however, pup bw gain was subsequently retarded at 24 000 ppm during the latter part of lactation (differences with control values were marked at 21d post-partum, but also discernible at 12d and sometimes as early as 6 days post partum). Similar but less marked effects were seen on litter and mean pup weight at 12 000 ppm. Such effects were considered to be related to lower maternal bw gain during lactation (see Table 5.6.1- 7).

Table 5.6.1- 7: Litter data

Dose (ppm)	Pregnant (no.)	At birth					At d-4				
		Litter size			Litter weight (g)	Mean pup weight (g)	Litter size		Cumu-lative loss (%)	Litter weight (g)	Mean pup weight (g)
		Total	Live	Loss (%)			M	F			
F0 /1st mating											
Controls	25	13.3	12.8	3.9	80.9	6.1	6.5	6.3	11.0	525.5	46.4
6 000	25	13.0	12.5	3.8	85.3	6.8*	6.0	6.0	7.7	522.6	44.5
12 000	25	13.3	13.1	2.2	86.0	6.6	6.2	5.5	7.5	514.9	43.3
24 000	25	12.7	11.6	7.3	75.6	6.6	5.6	5.6	17.6	348.7***	38.5**
F0/ 2nd mating											
Controls	20	13.3	13.3	0.0	89.0	6.8	6.7	6.6	7.1	625.6	49.5
6 000	20	13.6	13.5	1.0	92.9	7.0	7.6	6.6	8.6	592.7	50.0
12 000	20	13.9	13.7	1.7	90.5	6.8	6.3	6.2	10.6	587.9	48.4
24 000	18	14.5	14.1	2.8	95.8	6.8	7.2	6.0	8.2	434.9***	33.8***
F1B/1st mating											
Controls	25	12.5	12.3	1.3	80.7	6.6	6.4	5.8	8.9	469.5	41.6
6 000	25	11.9	11.3	1.7	77.9	6.9	6.1	5.4	7.9	500.5	48.2
12 000	25	11.0*	10.9	0.9	75.4	6.9	5.5	4.4	10.0	445.0	45.8
24 000	25	11.8	11.6	2.0	73.4	6.4	6.0	5.2	5.6	406.1**	38.2
F1B/ 2nd mating											
Controls	15	12.5	12.5	0.0	82.3	6.7	5.6	5.8	8.7	578.5	51.9
6 000	15	14.2	13.7	3.4	92.9	6.9	6.7	6.3	7.7	598.7	46.6
12 000	15	12.8	12.5	2.2	82.5	6.6	7.5*	4.7	4.5	525.6	43.9*
24 000	15	13.1	12.9	1.7	83.1	6.4	5.7	6.8	5.7	503.7	41.2**
F2B/1st mating											
Controls	24	11.4	11.2	2.3	76.1	7.0	4.7	4.8	14.0	505.5	54.5
6 000	24	10.6	10.2	3.4	72.1	7.3	5.2	4.6	7.0	521.3	55.7
12 000	23	10.4	10.1	2.9	69.1	6.8	4.9	4.4	9.0	451.4	50.8
24 000	24	11.9	11.6	2.5	79.8	6.7	5.3	4.8	8.0	354.7***	37.9***
F2/ 2nd mating											
Controls	24	11.4	10.9	0.9	76.2	7.1	5.1	5.2	6.8	569.0	56.8
6 000	24	12.7	12.1	0.0	88.2*	7.4	5.5	5.9	6.3	625.8	57.2
12 000	24	11.0	11.9	0.5	80.1	6.7	5.2	6.1	6.1	540.8	48.6*
24 000	24	11.8	11.7	1.0	76.4	6.7	6.1	5.1	4.6	485.5**	43.6***

* p<0.05 ** p<0.01; *** p<0.001

Statistically significant changes in organ weight were seen in the 24 000 ppm groups (lower liver, spleen and thymus weights and higher lung weight in males; lower thymus weight in females); however no dose related trend and no histological corroborate could be evidenced; in addition, maternal bw gain was reduced during gestation and mean pup weight was reduced during lactation.
Test substance related microscopic changes in the urinary tract were seen in the F3B pups (minimal epithelial hyperplasia and/or hypertrophy of the transitional epithelium and/or desquamation of the epithelial cells; presence of crystalline or calcareous deposits): these changes were seen in 8/10 males and 8/10 females from the 24 000 ppm group and only in males (1/10) from the 12 000 ppm group.

III. CONCLUSION

The parental NOAEL was 12 000 ppm (equivalent to 1203 and 1397 mg/kg bw/d in F1B males and females, respectively), based on reduced bw and increased incidence of renal lesions in F1B animals at 24 000 ppm.
RMS conclusion: Fosetyl-Al did no produce any adverse effects on reproductive performance and fertility in rats over 3 generations. The NOAEL for reproductive performance or fertility was 24 000 ppm (equivalent to 1782 and 1997 mg/kg bw/d in males and females of the F0 generation, respectively), as no treatment- related effects were observed in any generation. The overall NOAEL for development was 6000 ppm (equivalent to 439 and 520 mg/kg bw/d in F0 males and females, respectively) given to the effects on body weights of F1B generation and litter parameters in the 12 000 ppm treated group.

CA 5.6.2 Developmental toxicity studies

All studies for this endpoint have been previously submitted and evaluated. A short overall summary of these studies is provided in Table 5.6- 1. The foetal findings have been re-named according to current nomenclature (www.devtox.org) and a statistical analysis of their incidences has been performed.

Report: KCA 5.6.2/01 [redacted]; 1977; M-158819-01-1
Title: Effect of S 874-733 on pregnancy of the rat
Report No.: 10005/1
Document No.: M-158819-01-1
Guideline(s): not specified
Guideline deviation: not applicable
GLP/GEP: no

1. MATERIALS AND METHODS

Groups of 20 CFY females rats (bw range at start: 180 to 250 g) were administered 0, 500, 1000 or 4000 mg/kg bw/day of technical fosetyl-Al (batch FR 794/795 FT; 99.8% purity) in an aqueous suspension (dose volume: 2.0 mL/100 g bw) by oral gavage once daily, on d-6 through d-15 of presumed gestation.
Rats were observed daily throughout the dosing period for mortality and clinical signs; bw was determined on d-1; 3; -6; -10; -14; -16 and -20 of pregnancy. All rats were sacrificed on d-20 of gestation; macroscopic examination was performed with emphasis on ovaries the uteri; number of corpora lutea, number and distribution of life young and early and/or late embryo/foetal deaths were counted; live foetuses were counted, sexed and weighed; half of the foetuses from each litter were examined for visceral abnormalities and the other half for sex determination and skeletal abnormalities.

II. RESULTS AND DISCUSSION

Parents:
Occasional deaths seen in the 500 and 1000 mg/kg bw dose groups were attributable to dosing errors: 5/20 rats from the 4000 mg/kg bw group died or were sacrificed on d-9; -10 and –11 (all exhibited bw loss and chromodacryorrhea prior to death; post mortem examination revealed marked gastric dilatation and fluid retention).
Dose related reduction of bw and bw gain was seen in all groups during the first 4 days of dosing. Effects were still observed in the 4000 mg/kg bw group at end of dosing period
Pregnancy rate was comparable among all groups.

Litter data
Litter weight and mean foetal weight was slightly reduced only in the 4000 mg/kg bw group.
The number of resorptions was slightly increased in the 4000 mg/kg bw group.
Foetuses from the 4000 mg/kg bw group exhibited a slightly higher incidence of major abnormalities (thoracic asymmetry, displaced kidney and testes, hydrocephaly, valv/artery transposition, intra-abdominal and subcutaneous haemorrhage) and minor abnormalities (subcutaneous oedema, partial displacement of the testis).
A higher incidence of skeletal (sternebrae) variants was only seen in the 4000 mg/kg bw group which would correlate with the lower mean foetal weight (see Table 5.6.2- 1).

Table 5.6.2- 1: Maternal and litter observations in the rat developmental toxicity study

	Dose (mg/kg bw/day)			
	0	500	1000	4000
MATERNAL OBSERVATIONS				
Mortality	0/20	1/20 (a)	2/20 (a)	5/20
Bw gain (g)				
Day6-10	23	21	18	31
Day6-20	125	118	125	108
Pregnancy rate (%)	95	95	95	95
N° with viable young	19	19	17	14
LITTER OBSERVATIONS				
Live young				
Males	5.4	5.2	5.1	5.3
Females	6.4	5.5	6.9	6.2
Total	12.7	11.3	13.1	11.5
Embryonic deaths				
Early	0.4	0.6	0.4	0.5
Late	0.1	0.4	0.0	0.8
Total	0.5	0.9	0.4	1.3
Mean pre-implantation loss (%)	10.1	12.7	12.1	12.1
Mean post-implantation loss (%)	3.8	8.9	2.6	10.5
Litter weight (g)	88.05	42.07	48.50	39.71*
Mean foetal weight (g)	3.79	3.74	3.72	3.46
Malformations	3/242 (0.8%)	1/204 (0.8%)	2/222 (0.8%)	2/161 (3.8%)
Visceral	8/119 (6.9%)	7/103 (6.6%)	8/108 (7.4%)	8/78 (13.0%)
Skeletal	9/121 (7.0%)	9/100 (12.6%)	14/112 (11.8%)	17/78 (22.4%)
Skeletal variants				
Extra-ribs	24.6%	29.0%	42.1%	27.7%
Sternebrae extra-ribs	23.8%	38.2%	8.9%	56.1%

(a): dosing error; * p<0.05

The incidence of specific abnormalities according to the current nomenclature (www.devtox.org) is presented in Table 5.6.2- 2. The incidences of mild to moderate subcutaneous oedema and small interparietal bone were significantly increased in the high-dose group. Frequently, several abnormalities occurred in a single pup, so that more abnormalities appeared to occur in the high dose group, whereas the proportion of pups with abnormalities was not different from controls (Table 5.6.2- 1).

Table 5.6.2- 2: Incidence of specific malformations and variations (number / % of pups affected)

	Dose (mg/kg bw/day)							
	0		200		1000		4000	
	n	%	n	%	n	%	n	%
Malformations								
No. of pups examined	242	100.0%	204	100.0%	267	100.0%	161	100.0%
Hind limb, malrotated	1	0.4%						
Sternebra, fused	1	0.4%	1	0.5%				
Thoracic centrum, bipartite ossification	1	0.4%					1	0.6%
Thoracic centrum, dumbbell-shaped	1	0.4%					1	0.6%
Thoracic arch, fused							1	0.6%
Thoracic arch, misaligned							1	0.6%
Lumbar centrum, fused			1	0.5%				
Rib, fused							1	0.6%
Rib, branched							1	0.6%
Sternum, split	1	0.4%						
Digit, supernumerary			1	0.5%				
Abdomen, haemorrhage							1	0.6%
Ventricular septum defect					2	0.9%		
Kidney, malpositioned							1	0.6%
Hydrocephaly, internal, moderate							1	0.6%
Renal pelvis, increased cavitation, marked							1	0.6%
Subcutaneous oedema							1	0.6%
Azygous vein, transposed							1	0.6%
Aortic arch, transposed							1	0.6%
Ductus arteriosus, transposed							1	0.6%

	Dose (mg/kg bw/day)							
	0		500		1000		4000	
	n	%	n	%	n	%	n	%
Variations, visceral								
No. of pups examined	119	100.0%	103	100.0%	108	100.0%	78	100.0%
Testis, malpositioned	5	4.2%	2	1.9%	5	4.6%	2	2.6%
Renal pelvis, increased cavitation	1	0.8%	2	1.9%	2	1.9%		
Abdomen, haemorrhage	2	1.7%	1	1.0%				
Medulla oblongata, subarachnoid haemorrhage	1	0.8%						
Anterior chamber, haemorrhage, mild	1	0.8%						
Subcutaneous oedema, mild to moderate				2.0%			6 ***	7.7% p=0.00014
Thorax, internal haemorrhage, moderate					1	0.9%		
Variations, skeletal								
No. of pups examined	120	100.0%	100	100.0%	112	100.0%	78	100.0%
Thoracic centrum, bipartite ossification	3	2.5%	1	1.0%			4	5.1%
Thoracic centrum, hemicentric	4	3.3%	1	1.0%	1	0.9%	3	3.8%
Thoracic centrum, misshapen							1	1.3%
Presacral vertebrae, supernumerary	3	2.5%			10 **	8.9% p=0.0218		
Pelvic girdle, asymmetry attachment, slight	1	0.8%	2	2.0%	2	1.8%	2	2.6%
General ossification, mild retardation			1	1.0% p=0.041				
Interparietal, small					1	0.9%	3 *	3.8% p=0.031
Occipital, small							6 ***	7.7% p=4E-05
Parietal, small							1	1.3%
Rib, supernumerary							1	1.3%
Rib, short							1	1.3%

Statistically significant difference from controls: *p<0.05, **p<0.01, ***p<0.001 in Fisher exact test; retrospective statistical analysis per request of the RMS (chi-square analysis (α=0.05) followed by Fisher exact test.
Several pups carried multiple abnormalities, therefore the sum of specific abnormalities (this table) is higher than the combined incidence of pups diagnosed with any abnormality (see Table 5.6.2- 1).

VII. CONCLUSION

RMS conclusion: Oral administration of daily dose up to 4000 mg/kg bw in pregnant rats induced maternal toxicity at 4000 mg/kg bw level bw loss and mortality) and developmental toxicity including occasionally statistically significant and non-significant excess in the incidence of major and minor abnormalities and skeletal variants; such effects were likely related to maternal toxicity. No effects were seen in the 500 and 1000 mg/kg bw groups. The NOAEL in this study in 1000 mg/kg bw, both for maternal and developmental toxicity.

Report:	KCA 5.6.2/02 [redacted]; [redacted]; 1976; M-231386-01-2
Title:	Compound LS 74-783: Oral teratogenicity study in the rabbit
Report No.:	R000559
Document No.:	M-231386-01-2
Guideline(s):	not specified
Guideline deviation(s):	not applicable
GLP/GEP:	**no**

I. MATERIALS AND METHODS

Groups of 20 presumed pregnant New Zealand white rabbit (bw: 2.6 to 3.4 kg at start of study) were administered by gavage daily oral administration of 0; 125; 250 and 500 mg/kg bw of LS 74-783 (batch n.d.; 99.8% purity) in a 10% gum arabic aqueous solution from d-6 through d-16 of gestation. Bw was recorded on d-0; -6; -16 and –28 of gestation; food consumption was recorded daily from d-0 through d-27 of gestation. All rabbits were sacrificed on d-28 of gestation for examination of genital tract, number of implantation sites, resorptions, live and dead foetuses; live foetuses were weighed and examined externally and internally.

II. RESULTS AND DISCUSSION

Maternal data:
15/88 does mated for study were excluded because of accidental death, gavage trauma or uterine and/or pulmonary disorder at time of sacrifice.
The mean daily food consumption was slightly reduced in the high dose group; bw gain was reduced in the 250 and 500 mg/kg group.
Pregnancy rate was similar in all groups. No cases of total litter resorption or total litter loss were recorded.

Litter data:
No evidence of embryo-toxicity or impairment of foetal growth was found.

Table 5.6.2- 3: Summary data

Parameter	Controls	125 mg/kg bw	250 mg/kg bw	500 mg/kg bw
Maternal data				
Bw gain (kg)	0.88±0.2	0.21±0.02	0.12±0.04	0.15±0.03*
Mated females	18	18	18	19
Pregnant	12	14	15	15
Mean n° of implantation sites	8.3±0.3	8.6±0.5	9.3±0.3	7.4±0.4
Mean foetal loss %	0.3	0.9	1.3	0.5
Live foetuses	92	107	120	103
Males	42	61	61	42
Females	50	46	59	61
Malformed foetuses	3	0	2	0

III. CONCLUSION

RMS conclusion: The protocol and results are poorly described; given the limits of this study, one could propose the dose level of 125 mg/kg/day to be considered as the NOAEL for maternal toxicity and the dose level of 500 mg/kg/day to be considered as the NOAEL for developmental toxicity.

Report:	KCA 5.6.2/03 [redacted]; 2000; M-207431-01-1
Title:	Oral range-finding study of embryo-fetal development in rabbits Technical fosetyl-Al
Report No.:	C014859
Document No.:	M-207431-01-1
Guideline(s):	not specified
Guideline deviation(s):	not applicable
GLP/GEP:	**yes**

I. MATERIALS AND METHODS

Groups of 5 time-mated female New Zealand rabbits 5 to 6 months old at start of treatment (bw range: 3 to 4 kg on d-0 of gestation) were given daily oral administration, by gavage, of 0; 75; 125; 250; 500 and 1000 kg/bw/d of technical fosetyl-Al (batch 9810711, 98.1% purity) in an aqueous suspension of 0.5% w/v methylcellulose (dose volume: 2 mL/kg) on d-4 through d-28 of gestation. Batches were prepared weekly; duplicate samples of dosing solutions were taken at initiation and end of study for analysis of concentrations; homogeneity and stability of dosing solutions were checked.
Dose selection was based on the results of a previous teratology study in pregnant rabbits given by gavage 0; 125; 250 and 500 mg/kg bw on d-6 through d-16 of gestation; results showed that fosetyl-Al produced slight to moderate reduced bw gain at 250 and 500 mg/kg bw and slight to moderate reduced food consumption at 500 mg/kg bw.
All rabbits were examined at least twice daily for mortality, morbidity, signs of overt toxicity. Bw was recorded on d-0; -4; -6; -9; -12; -16; -19; -24 and -29 of gestation; food consumption was determined daily from d-4 through d-28 of gestation. All surviving animals were sacrificed on d-29 gestation and a gross necropsy was performed with emphasis on the ovaries and the uterus (gravid uterine weight; number of corpora lutea and uterine implantation data were recorded); live and dead foetuses were counted, weighed and examined for external abnormalities (palate included; crown-rump lengths); late resorptions were also examined for external abnormalities.

II. RESULTS AND DISCUSSION

Homogeneity and stability of mixtures were confirmed and dose levels were within ± 10% of the acceptable limits.

Dams:
All females from the top dose group were sacrificed on gestation d-13 because of excessive bw loss and food consumption reduction (1 was found dead on gestation d-10 and another 1 on gestation d-13): none of them exhibited remarkable macroscopic findings; 1 female from the 500 mg/kg bw group was sacrificed for ethical reasons on gestation d-27 (red exudate from the ano-genital area revealing uterine haemorrhage).
No treatment related clinical signs were recorded in any rabbit.
Bw and bw gain were markedly reduced from d-4 through d-12 gestation in the high dose group; slight reduction of bw gain was found in the 500 mg/kg group (60% of control values) and some evidence was seen also for the 250 mg/kg group during the first day of dosing. No bw changes were seen in the 75 and 125 mg/kg bw groups.
Food consumption was markedly reduced in the top dose group during the first 12 d of gestation and was also reduced in the 500 mg/kg bw group from gestation d-4 through d-19 (and returned to normal values thereafter).
Pregnancy rate were 80% in controls; 100% in the 75; 125 and 250 mg/kg bw groups and 75% in the 500 mg/kg bw group.
No macroscopic significant findings were found in any rabbit.

Table 5.6.2- 4: Maternal observations

	Controls	75 mg/kg	125 mg/kg	250 mg/kg	500 mg/kg	1000 mg/kg
Mortality (dead or sacrificed)	0/5	0/5	0/5	0/5	1/5	5/5
Pregnancy rate (%)	80	100	100	100	75	–
Bw gain (g)						
D4-d6	76±38.1	65±21.6	-4±28.0	-10±63.4*	-39±43.5**	-15±16.3**
D4-d29	517±95.5	536±110.0	440±110.6	358±[illegible]	312±[illegible]	–
Food consumption (g)						
D4-d6	45±1.5	46±5.9	43±3.9	[illegible]±13.8	33±4.0	23±[illegible]**
D4-d29	39±3.7	40±3.3	37±1.4	39±4.8	[illegible]2±6.7	–
Caesarean section data						
Pregnant	4	5	5	5	3	0
Dams with viable foetuses	4	5	5	5	3	0
Corpora lutea (n°/rabbit)	11.8±1.5	11.2±0.84	13.2±2.86	[illegible]2.2±[illegible]1	[illegible].0±4.36	–
Pre-implantation losses (%)	2.0±1.15	[illegible].6±0.89	[illegible]6±2.7	3.[illegible]±2.77	1.7±2.08	–
Post-implantation losses (%)						
Live foetuses	0	4	1	7	4	0
Dead foetuses	0	0	0	0	1	0
Early resorptions (%)	0	1	1	1	0	0
Late resorptions (%)	0	0	0	4	1	0

* p<0.05; ** p<0.01

Litter data:
2 dead foetuses were found in the 500 mg/kg bw group; a slight decrease in the mean number of live foetuses and a slight increase in the mean number of post-implantation losses were recorded in the 250 and 500 mg/kg groups; such changes were considered as incidental given the small size of the groups. There was no change in foetal log at any dose level nor treatment related external malformation or variation; 1 foetus from the 500 mg/kg group exhibited a spina bifida which was not considered as treatment related considering historical controls. Single malformations found in live foetuses and dead foetuses (malformation of sacrum, pinna alteration, macroglossia) were incidental.

Table 5.6.2- 5: Foetal data

Foetal observations	Dose (mg/kg/day)					
	0	75	125	250	500	1000
Malformation						
Litter examined	4	5	5	5	3	0
Foetuses examined	39	47	52	38	26	0
Spina bifida	0	0	0	0	1	-
Total foetus with malformation	0	0	0	0	1	-
Total litter with malformation	0	0	0	0	1	-
Variation						
Litter examined	4	5	5	5	3	0
Foetuses examined	39	47	52	38	26	0
Total foetus with variation	0	0	0	0	0	-

III. CONCLUSION

RMS conclusion: Oral administration of daily dose up to 1,000 mg/kg in pregnant rabbits induced maternal toxicity (marked at 1,000 mg/kg, slight to moderate at 250 and 500 mg/kg); slight decreases in the number of live foetuses and slight increase of the number of post-implantation losses seen at 250 and/or 500 mg/kg were of doubtful significance considered the small number of rabbits evaluated. No maternal or developmental toxicity was seen at 75 and 125 mg/kg bw.

Report:	KCA 5.6.2/04[redacted]; 2000; M-205472-01-1
Title:	Oral study of embryo-fetal development in rabbits Technical fosetyl-Al
Report No.:	C013896
Document No.:	M-205472-01-1
Guideline(s):	OECD: 414; USEPA (=EPA): OPPTS 870.3700
Guideline deviation(s):	none
GLP/GEP:	**yes**

I. MATERIALS AND METHODS

Groups of 25 time-mated female New Zealand white rabbits (5-6 months of age at start of the study; bw = 3 to 5 kg on gestation d-0) were administered orally (by gavage) once daily, 50, 100 or 300 mg/kg bw/d of technical fosetyl-Al (batch 98M111; 98.9% purity) in an aqueous suspension of 0.5% w/v methylcellulose (dose volume = 2 mL/kg) on d-4 through d-28 of gestation. Homogeneity was checked on d-1 of dosing, using samples from the 50 mg/kg dose level; concentration of test substance in the dietary preparations was analysed during the 1st, 2nd and 3rd week of dosing on duplicate samples of all dosing preparations.
All rabbits were examined at least twice daily for mortality, moribundity and signs of overt toxicity throughout the study. Bw was recorded daily; food consumption was determined daily during the dosing period. All surviving animals were sacrificed on d-29 gestation and a gross necropsy was performed on all animals, including on those died or sacrificed during the study, with emphasis on the reproductive system; uterus and ovaries were weighed, number of corpora lutea, number of live and dead foetuses and number of early and late resorptions were recorded.
All foetuses (live and dead) were counted, weighed and examined for external and visceral abnormalities; skeletal malformations and variations were evaluated.
In-life dates: 1999-09-01 to 1999-10-06

II. RESULTS AND DISCUSSION

Homogeneity of the 50 mg/kg dose level preparation was confirmed (mean recovery = 96.4%); concentration of test substances in diet samples was within ± 10% of nominal concentration during all 3 weeks of dosing.

Maternal data:
A total of 8 females were sacrificed or found dead during the dosing period (1 in the 50 mg/kg group; 1 in the 100 mg/kg group and 6 in the 300 mg/kg group): all deaths except 1, should be considered as resulting from a gavage trauma since no obvious clinical signs, nor bw and/ or food consumption changes were seen prior to death and discoloured lungs and/or fluid in the trachea or lung were seen at necropsy; in the 50 mg/kg bw group, the death was caused by an abortion (see Table 5.6.2- 6).
No treatment related effect was on clinical signs, bw and food consumption was recorded.
Pregnancy rate was comparable between treated and control rabbits.
No remarkable findings were seen at terminal macroscopic examination and not treatment related effects were recorded on gravid uterine weight, mean number of corpora lutea, pre and post-implantation losses, mean number of live foetuses, sex ratio and mean number of early and late resorptions.

Table 5.6.2- 6: Maternal data

Dose	Controls	50 mg/kg	100 mg/kg	300 mg/kg
Mortality (gestation d)	0/25	1/25 (d-27)	1/25 (d-11)	6/25 (d-6; -6; -9; -18;-27)
Mean bw gain (g) D0-29	501±229.4	531±189.9	552±209.6	476±208.6
Food consumption (g/kg/d)				
d-4-6	43±10.5	47±5.7	45±7.2	43±13.6
d-27-29	21±8.2	26±10.4	31±6.1**	29±7.5**
d-4-29	39±7.5	40±7.6	39±7.0	38±8.7
Pregnancy rate	88.0	88	95.8	96
Caesarean section data				
Pregnant	22	23	23	19
Dams with viable foetuses	22	23	23	19
Corpora lutea (n°)	240	251	246	208
Pre-implantation loss %	48	52	51	51
Post-implantation loss %	9	10	5	9
Live foetuses	183	200	190	148
Males	98	109	93	77
Females	85	91	97	71
Early resorptions %	4	4	3	5
Late resorptions %	5	6	2	4

* p<0.05; ** p<0.01

Litter data:

There was no effect of treatment on foetal b

No treatment related external, visceral or skeletal malformations or variations were recorded. Fused sternebrae were observed in all treated groups, but at a single incidence and were then not considered as related to treatment, inasmuch as this finding was previously observed on this strain. Examination of the dead foetus found in the 50 mg/kg group revealed external malformations (gastroschisis, hindlimbs hyperflexion, and agenesis of the tail) which could appear incidence since no such findings were seen at higher dose levels (see Table 5.6.2- 7).

Statistical re-evaluation of the foetal incidence rates suggests statistically significantly increased incidence of dilated ureters in high-dose foetuses, at an incidence exceeding the historical control range from this laboratory (see Table 5.6.2- 8). However, the foetal and per-litter incidence of distended ureter in concurrent controls also exceeded the historical control range.

Table 5.6.2- 7: Litter data

Dose group (mg/kg bw/day)	Controls		50		100		300	
Litters								
N° foetus examined	183		201		190		148	
N° litters examined	22		23		23		18	
Incidence	Foetal	Litter	Foetal	Litter	Foetal	Litter	Foetal	Litter
Visceral Malformations								
Hydrocephaly	0	0	0	0	0	0	1	1
Skeletal malformations								
Fused sternebrae	0	0	1	1	1	1	1	1
Fused ribs	0	0	0	0	0	0	1	1
Variations								
Distended ureter[#]	4 (2.2%)	4 (18.2%)	4 (2.0%)	4 (17.4%)	3 (1.6%)	3 (13.0%)	10 (6.8%)	10 (55.6%)

[#] Historical control incidence at Huntingdon Life Sciences (5 studies, 31 May 1998 through 22 March 1999)
per foetus: mean: 0.63% (range: 0.00 to 1.99%), N=800
per litter: mean: 5.26% (range: 0.00 to 15.00%), N=95

Table 5.6.2- 8: Per-foetus incidences of foetal abnormalities

	Controls		50 mg/kg/d		100 mg/kg/d		300 mg/kg/d	
	n	%	n	%	n	%	n	%
Malformations								
No. of pups examined	183	100.0%	201	100.0%	190	100.0%	148	100.0%
Trunk, spina bifida			1	0.5%				
Trunk, gastroschisis	1	0.5%	1	0.5%				
Tail, absent			1	0.5%				
Head, domed	1	0.5%						
Face, proboscis	1	0.5%						
Eye, open	1	0.5%						
Hindlimb, hyperflexion			1	0.5%				
Head, hydrocephalus							1	0.7%
Heart/great vessels, anomaly	1	0.5%	1	0.5%				
Sternebra, fused			1	0.5%	1	0.5%	1	0.7%
Ribs, fused/branched							1	0.7%
External malformations	1	0.5%	2	1.0%				
Visceral malformations	1	0.5%	1	0.5%			1	0.7%
Skeletal malformations			1	0.5%	1	0.5%	2	1.4%
All malformations	1	0.5%	4	2.0%	1	0.5%	3	2.0%
Variations, visceral								
No. of pups examined	183	100.0%	201	100.0%	190	100.0%	148	100.0%
Iris, surrounded by haemorrhagic ring	10	5.0%	7	3.5%	9	4.7%	5	3.4%
Heart/great vessels, alteration	7	3.8%	6	3.0%	6	3.2%	5	3.4%
Ureter, dilated	4[#]	2.2%	4	2.0%	3	1.6%	10 *	6.8% p=0.040
Renal papilla, absent	5	2.7%	4	2.0%	4	2.1%	5	3.4%
Gall bladder, absent	2	1.1%						
Gall bladder, small					2	1.1%		
Variations, skeletal								
No. of pups examined	183	100.0%	201	100.0%	190	100.0%	148	100.0%
Hyoid, lesser horn, bent	1	0.5%					1	0.7%
Hyoid body/lesser horn, unossified	4	2.2%	1	0.5%	2	1.1%		
Ossification, reduced			1	0.5%				
Presacral vertebrae, 25			1	0.5%				
Presacral vertebrae, 27	22	12.0%	26	12.9%	25	13.2%	15	10.1%
Rib, 13th, rudimentary	17	9.3%	21	10.4%	10	5.3%	17	11.5%
Rib, 13th, full	67	36.6%	80	39.8%	65	34.2%	48	32.4%
Pubis, unossified			2	1.0%	1	0.5%	1	0.7%

[#] Historical control incidence at Huntingdon Life Sciences (5 studies, 31 May 1998 through 22 March 1999) per foetus: mean: 0.63% (range: 0.00 to 1.99%), N=800

Statistically significant difference from controls: *p≤0.05; retrospective statistical analysis per request of the RMS. Chi-square analysis α=0.05, followed by Fisher exact test.

III. CONCLUSION

RMS conclusion: The dose levels in the main study were selected based upon the results of the range finding study in which maternal toxicity was observed at > 250 mg/kg bw. Oral administration of fosetyl-Al up to 300 mg/kg bw during pregnancy of rabbit did not induce maternal or embryo/foetal toxicity nor teratogenicity. The NOAEL for maternal and developmental toxicity was 300 mg/kg bw.

CA 5.7 Neurotoxicity studies

The chemical structure of fosetyl-aluminium (fosetyl-Al) has no structural relationships with organophosphates or carbamates. No evidence of clinical signs indicative of delayed neurotoxicity or other neurotoxic effects were seen in the acute, subacute, subchronic (90-day) or long-term toxicity studies; in the two generation reproduction toxicity study, no clinical signs were seen in either the P1 or F2 offspring or their parents.

Cholinesterase activities in brain, erythrocytes and plasma have been measured in the course of the two-year feeding study in dogs ([redacted]; 1981; M-159302-01-1); see Section CA 5.5, page 118). These activities were not affected by fosetyl-Al doses of 1228/1190 mg/kg bw/day (♂/♀), the highest dose tested in this study.

For these reasons, acute, subchronic or developmental neurotoxicity studies were not triggered. However, an acute delayed neurotoxicity study was performed in the domestic hen ([redacted]; 1982; M-203022-01-1). Single oral gavage administration of 2000 mg fosetyl-Al/kg bw to hens did not induce any signs of delayed neuropathy and no axonopathy in the spinal cord or peripheral nerve (see Table 5.7- 1).

No new studies have been performed for this endpoint.

Table 5.7- 1: Neurotoxicity study with fosetyl-Al

Study Type	Species	Doses tested	LOAEL / Effects	NOAEL	Reference
Acute delayed neurotoxicity, oral gavage	Domestic hen	0, 2000 mg/kg bw	>2000 mg/kg bw, no effects	≥2000 mg/kg bw	[redacted]; 1982; M-203022-01-1

This study has been reviewed as part of the first EU review of fosetyl-Al.

CA 5.7.1 Neurotoxicity studies in rodents

Neurotoxicity studies in rodents are not deemed necessary because fosetyl-aluminium is not an organophosphate or a carbamate and the existing repeated-dose studies did not reveal any clinical signs or behavioural abnormalities that are suggestive of neurotoxicity.

CA 5.7.2 Delayed polyneuropathy studies

The study for this endpoint has been previously submitted and evaluated.

Report:	KCA 5.7.2/01 [redacted]; 1982; M-203022-01-1
Title:	Fungicide Fosetyl Al : An acute delayed neurotoxicity study in the domestic hen
Report No.:	C012606
Document No.:	M-203022-01-1
Guideline(s):	not specified
Guideline deviation(s):	not applicable
GLP/GEP:	**no**

I. MATERIALS AND METHODS

Groups of 10 Light Sussex domestic hens (8 to 14 months old; bw range 1.64 to 3.37 kg) were given single oral (gavage) doses of 0 or 2000 mg/kg bw of fosetyl-Al (batch DA 203; 97.5% purity) in 0.5% w/v carboxymethyl cellulose (dose volume: 4.0 mL/kg). An additional positive control group was given 750 mg/kg bw triorthocresyl phosphate in 0.5% w/v carboxymethyl cellulose. The positive control were treated immediately prior to dosing with sub-cutaneous injection of 2.0 mg/kg atropine sulfate and intra-peritoneal injection of 75 mg/kg of pralidoxine.
Examinations were carried out at least twice daily for general condition, behaviour and overt signs of toxicity; neurotoxicity was assessed daily using a scoring system (0: no ataxia; 1: doubtful or minor signs; 2: positive paralytic signs; 3: advanced paralytic signs; 4: death); individual bw and food consumption were recorded 7- and 3-d before treatment, immediately before dosing and at d-3; -7; -10; -14 and –21 after dosing. Necropsy examinations were performed on all hens sacrificed at termination and on those dying during study and included histological examination of the entire spinal cord, both sciatic nerves and brain.

II. RESULTS AND DISCUSSION

All hens given fosetyl-Al survived; 1 control hen was found dead on d-13 and 2 positive controls died and were sacrificed *in extremis* on d-9 and d-18; no clinical changes nor signs of neurotoxicity occurred in control and treated hens.
All positive controls exhibited signs of cholinergic poisoning, although protected by atropine and pralidoxine (paralysis immediately after dosing and recovery after 48 hours, except 1 hen which continued to show ataxia and exhibited hunched posture, prostration, irregular respiration and was sacrificed on d-6). 6/8 survivors developed delayed neurotoxicity (ataxia) between 9 to 18 days which persisted until end of study in 3 hens.
Bw and food consumption were not affected by test substance; bw was significantly decreased in the positive controls surviving at end of study.
No treatment related macroscopic findings were recorded; histological appearance on spinal cord was similar in treated and control hens; signs of obvious neuropathy were recorded in several sites, particularly in cervical cord and peripheral nerve of the positive controls.

III. CONCLUSION

RMS conclusion: Single oral administration of 2000 mg/kg bw of fosetyl-Al in hens did not induce any signs of delayed neuropathy and no axonopathy in the spinal cord or peripheral nerve.

CA 5.8 Other toxicological studies

CA 5.8.1 Toxicity studies of metabolites

Several studies were carried out for assessing the toxicity of the major metabolite of fosetyl-Al, phosphonic acid (previously referred to as "phosphorous acid") which is present in significant amounts in plants and in relevant body compartments of mammalian laboratory animal species. Owed to its acidity, phosphonic acid was tested as a sodium (Na-) or potassium (K-) phosphonates (previously referred to as "phosphites") in most studies. Phosphonic acid and its salts are of low acute toxicity via all routes of administration. The potassium salt is not irritating to skin or eyes (see Table 5.8.1- 1).

Sub-chronic and chronic feeding studies with phosphonates have not revealed any specific effect of concern. Sodium phosphonate was not carcinogenic in a 27-month feeding study in rats (see Table 5.8.1- 2). Phosphonates were also negative in various in-vitro and in-vivo genotoxicity tests (see Table 5.8.1- 3).

Taken together, these data indicate the absence of any critical toxicity of the plant metabolite of fosetyl-Al, phosphonic acid. The 27-month feeding study with sodium phosphonate ([redacted]; 1981; M-159229-01-1) has been used to set an ADI for phosphonic acid and its summary is thus included in this Supplementary Dossier.

Table 5.8.1- 1: Acute toxicity tests with phosphonic acid or its salts

Study type	Test Material / Doses tested	Results	Reference
Acute oral toxicity, rat	Phosphonic acid 1350, 2000, 3000, 4500, 6700 mg/kg bw	LD_{50} = 2950 mg/kg bw (♂+♀)	[redacted]; 1977; M-231369-01-2
	Na-phosphonate 2000, 3000, 4500, 6700, 10 000 mg/kg bw	LD_{50} = 5300 mg/kg bw (♂♀)	
	K-phosphonate* 2500, 3250, 4000, 5000 mg/kg bw	LD_{50} = 3624 mg/kg bw (♂+♀)	[redacted]; 1995; M-205464-01-1
Acute oral toxicity, mouse	Phosphonic acid 600, 900, 1350, 2000, 3000 mg/kg bw	LD_{50} = 1600 mg/kg bw (♂+♀)	[redacted]; 1977; M-231369-01-2
	Na-phosphonate 600, 900, 1350, 2000, 3000 mg/kg bw	LD_{50} = 2450 mg/kg bw (♂+♀)	
Acute dermal toxicity, rabbit	K-phosphonate* 2000 mg/kg bw	LD_{50} > 2000 mg/kg bw (♂+♀)	[redacted]; 1994; M-205465-01-1
Acute inhalation toxicity, rat	K-phosphonate* 6.14 mg/L, nose only	4-h LC_{50} > 6.14 mg/L (♂+♀)	[redacted]; 1994; M-205468-01-1
Skin irritation, rabbit	K-phosphonate* 0.5 mL	Not irritating	[redacted]; 1994; M-205470-01-1
Eye irritation, rabbit	K-phosphonate* 0.1 mL	Not irritating	[redacted]; 1994; M-205458-01-1

* Aqueous solution of potassium phosphonate containing 41% phosphonic acid equivalents.

Table 5.8.1- 2: Repeated-dose toxicity studies with phosphonic acid or its salts

Study type	Species	Test Material / Doses tested	LOAEL / Effects	NOAEL	Reference
Oral feeding, 13 weeks	Rat, CD	Phosphonic acid 2500, 5000 or 25 000 ppm	25 000 ppm: soft faeces, increased water consumption and urinary sodium excretion.	5000 ppm (400 mg/kg bw/day, ♂+♀)	[redacted]; 1978; M-231302-01-2
Oral feeding, 27 months	Rat, CD	Na-phosphonate 0, 2000, 8000 or 32 000 ppm	32 000 ppm: soft faeces, body weight and food efficiency ratio decreased, urine acidification, organ weight changes	8000 ppm (348 or 434 mg/kg bw/day, ♂♀)	[redacted]; 1981; M-159229-01-1

Table 5.8.1- 3: Genotoxicity tests with phosphonic acid or its salts

Study type	Organism / Strain	Test Material / Doses tested	Results	Reference
Ames test	*S. typhimurium* TA 1535, 1537, 1538, 98, 100	K-phosphonate* 33 – 10 000 µg/plate (± S9)	Negative	[redacted]; 1994; M-203460-01-1
Induct test	*E. coli,* K12	Phosphonic acid Up to 2000 mg/plate (± S9)	Negative	[redacted]; 1978; M-178996-01-2
In vivo MNT, oral	Mouse / Swiss (♂)	Na-phosphonate Two applications, 24 h interval, 1000, 2000, 4000 mg/kg bw/day	Negative	[redacted]; 1977; M-223290-01-2

* Aqueous solution of potassium phosphonate containing 41% phosphonic acid equivalents.

Report: KCA 5.8.1/04; [redacted]; 1977; M-231369-01-2
Title: Phosphorous acid (37934 R.P.) and disodium phosphite (37934 R.P., disodium salt): Acute oral toxicity in the mouse and rat
Report No.: 800055
Document No.: M-231369-01-2
Guideline(s): not specified
Guideline deviation(s): not applicable
GLP/GEP: no

I. MATERIALS AND METHODS

Groups of 10 (5/sex) CD rats (bw: 140 to 170 g) were given single oral administration of 1350, 2000, 3000, 4500 and 6700 mg/kg bw of phosphonic acid (H_3PO_3 Prolabo 75162) or 2000, 3000, 4500, 6700 and 10000 mg/kg bw of disodium phosphonate (Na_2HPO_3 Prolabo 73130).
Groups of 10 (5/sex) OF1 mice (bw: 17 to 23 g) were given single oral administration of 600, 900, 1350, 2000 and 3000 mg/kg bw of phosphonic acid in 10% aqueous solution of gum arabic or 600, 900, 1350, 2000 and 3000 mg/kg bw of disodium phosphonate in 10% aqueous solution of gum arabic.
Bw as recorded every 3 days; macroscopic examination was carried out on all animals dead during study and surviving at terminal sacrifice which was performed 15 days after dosing.

II. RESULTS AND DISCUSSION

Mortality rates in rats and mice are summarized in Table 5.8.1- 4.
In rats, clinical signs included sedation and dyspnoea after administration of phosphonic acid (deaths occurred within 1 to 2 hours) and sedation, diarrhoea and cloning or tonic convulsions after administration of disodium phosphonate (deaths occurred within 2 to 24 hours after dosing). In mice, clinical signs after administration of phosphonic acid included sedation and dyspnoea (death occurred within 3 to 6 hours post-dosing) and sedation, dyspnoea, diarrhoea and prostration after administration of disodium phosphonate (deaths occurred 6 to 24 hours after dosing). There were no significant changes in bw gain among the various dose groups of rats and mice.

No significant macroscopic findings were noted at terminal sacrifice except marked irritation of the digestive tract i.e. severe congestion of the glandular region of the stomach and of the small intestine in both rats and mice following administration of phosphonic acid; distended intestine and/or slight congestion of the glandular region of the stomach was noted following administration of disodium phosphonate.

Table 5.8.1- 4: Mortality pattern in rats and mice following single oral administration of phosphonic acid or disodium phosphonate

Species	RAT				MOUSE			
Compound	Dose level (mg/kg bw)	Mortality after 15 d			Dose level (mg/kg bw)	Mortality after 15 d		
		Males	Females	2 sexes		Males	Females	2 sexes
Phosphonic acid	1350	0/5	0/5	0/10	600	0/5	0/5	0/10
	2000	0/5	1/5	1/10	900	0/5	0/5	0/10
	3000	2/5	2/5	4/10	1350	1/5	0/5	1/10
	4500	5/5	5/5	10/10	2000	5/5	5/5	10/10
	6700	5/5	5/5	10/10	3000	5/5	5/5	10/10
Disodium phosphonate	2000	0/5	0/5	0/10	600	0/5	0/5	0/10
	3000	0/5	0/5	0/10	900	0/5	0/5	0/10
	4500	1/5	1/5	2/10	1350	1/5	0/5	1/10
	6700	4/5	5/5	9/10	2000	0/5	0/5	0/10
	10000	5/5	5/5	10/10	3000	5/5	5/5	10/10

III. CONCLUSION

The acute oral LD_{50} of phosphonic acid and disodium phosphonate in rats and mice was greater than 2000 mg/kg bw. Thus neither substance is classified for acute oral toxicity according to the criteria of Regulation 1272/2008.
RMS conclusion: The oral LDs were calculated using the Litchfield and Wilcoxon method and were 2950 [2400 to 3650] mg/kg bw in both sexes of rats and 1650 mg/kg bw in both sexes of mice for phosphonic acid and 4300 [3500 to 6000] mg/kg bw in both sexes of rats and 2450 mg/kg bw in both sexes of mice for disodium phosphonate.

Report:	KCA 5.8.1/02[redacted]; 1995; M-205464-01-1
Title:	Acute oral toxicity (LD50) test in rats Potassium salts of phosphorus acid
Report No.:	C013892
Document No.:	M-205464-01-1
Guideline(s):	USEPA (=EPA): F 81-1, M 152-10
Guideline deviation(s):	none
GLP/GEP:	**yes**

I. MATERIALS AND METHODS

Groups of 10 (5/sex) Sprague Dawley rats (6 to 8 weeks old; bw range at start: 159 to 227 g) were administered (gavage) single oral doses of 2500, 3250, 4000, and 5000 mg/kg bw of potassium salt of phosphonic acid (Foli-R-Fos 400 referred as potassium salts of phosphonic acid batch 2244-3 CH_3PO_3 41% ; pH = 5.7); 410 mg/mL of phosphonic acid present as 668.9 mg/mL of mono and dipotassium phosphonate).
Rats were examined twice daily for mortality and clinical signs until sacrifice at d-14; bw was recorded prior to dosing, on d-7 and –14 after dosing; all surviving rats were subjected to macroscopic examination of thoracic and abdominal organs.

II. RESULTS AND DISCUSSION

Mortality occurred within 1 day post-dosing in the 3250, 4000 and 5000 mg/kg bw groups (see Table 5.8.1- 5).

Table 5.8.1- 5: Mortality pattern

Dose (mg/kg bw)	Male		Female	
	Mortality	Time of death in days (n° of rats)	Mortality	Time of death in days (n° of rats)
2500	0/5	-	0/5	-
3250	1/5	1 (1)	3/5	3 (1)
4000	5/5	4 (0) - 1 (1)	5/5	4 (0)
5000	3/5	4 (0) - 1 (1)	4/5	1 (0) - 3(1)

Clinical signs were noted in all treated groups from 30 minutes after dosing and included pilo-erection, subdued behaviour, reduced activity, ataxia, prostration, convulsions, tremors, hunched appearance, increased salivation and laboured breathing; survivors of the various treated groups recovered after 2 to 7 days. No treatment related effect on bw was recorded in survivors.

Macroscopic examination showed gastric abnormalities (stomach filled or distended with clear fluid and reddened glandular mucosa) in the 5000 mg/kg bw group, and distended intestines in 2 males and 2 females and caecum filled with brown fluid in female from the 4000 mg/kg bw group.

III. CONCLUSION

The acute oral LD$_{50}$ of potassium phosphonate in rats was greater than 2000 mg/kg bw. Thus, the substance is not classified for acute oral toxicity according to the criteria of Regulation 1272/2008.
RMS conclusion: The median oral LD$_{50}$ of potassium salt of phosphonic acid in Sprague Dawley rats was calculated using probit method and was 3816 [2814 to 5143] mg/kg bw in males, 3445 [2396 to 4322] mg/kg bw in females, and 3624 [3082 to 4186] mg/kg bw for both sexes.

Report:	KCA 5.8.1/03 [redacted]; 1994; M-205465-01-1
Title:	Acute dermal toxicity (limit) test in rabbits Potassium salts of phosphorus acid
Report No.:	C013893
Document No.:	M-205465-01-1
Guideline(s):	USEPA (=EPA): F 81-2
Guideline deviation(s):	none
GLP/GEP:	**yes**

I. MATERIALS AND METHODS

Groups of 10 (5/sex) adult New Zealand rabbits (bw range at start: 2.34 to 2.58 kg) were given a single topical application of 2000 mg/kg bw of potassium salt of phosphonic acid (Foli-R-Fos 400, referred as potassium salts of phosphonic acid; batch 2244-3 [H_3PO_3: 41%; pH = 5.7; 410 mg/mL of phosphonic acid present as 668.9 mg/mL of mono- and dipotassium phosphonate) to one clipped dorsal skin (approx. 10% of the body surface) under semi occlusive dressing; after 24 hour of contact, the skin was wiped with water to remove excess of test material. Clinical signs were recorded daily and bw were recorded immediately prior to dosing and weekly thereafter. All rabbits were sacrificed on d-14 post-dosing and subjected to a gross macroscopic examination.

II. RESULTS AND DISCUSSION

No treatment-related changes were recorded (mortality, clinical signs, bw, terminal autopsy).

III. CONCLUSION

The acute percutaneous LD_{50} of potassium phosphonate in rabbits was greater than 2000 mg/kg bw. Thus, the substance is not classified for acute dermal toxicity according to the criteria of Regulation 1272/2008.

RMS conclusion: The median dermal LD_{50} of potassium salt of phosphonic acid in the New Zealand rabbit was > 2000 mg/kg bw.

Report:	KCA 5.8.1/04 [redacted]; [redacted]; [redacted]; 1994; M-205468-01-1
Title:	Acute inhalation toxicity study in rats Potassium salts of phosphorus acid
Report No.:	C013894
Document No.:	M-205468-01-1
Guideline(s):	USEPA (=EPA): F 81-3
Guideline deviation(s):	none
GLP/GEP:	**yes**

I. MATERIALS AND METHODS

Groups of 10 (5/sex) Sprague Dawley rats (5 to 7 weeks old; bw range at dosing: 165 to 207 g) were exposed "nose only" to a liquid aerosol of potassium salt of phosphonic acid (Foli-R-Fos® 400, batch 2244-3) for a 4 h period; the mean achieved concentration of test aerosol in the exposure chamber was determined gravimetrically and was 6.14 ± 1.75 mg/L (nominal concentration = 4.77 mg/L) and the percentage in particles < 3.5 µm was 89.2%.

Rats were examined continuously throughout the exposure period, immediately after exposure and for the first 2 hours post-dosing and twice daily thereafter; bw were recorded before dosing and on d-2; -3; -4; -7; -10 and -14. All rats were sacrificed at d-14 and subjected to a gross macroscopic examination, particularly of the respiratory tract.

II. RESULTS AND DISCUSSION

No deaths nor treatment related clinical signs were seen; the bw was slightly reduced on d-4 post-dosing in 1 male and 3 females which recover thereafter; on necropsy findings were small lungs (1 male), pale lungs (2 males and 3 females) and slightly patchy liver (1 male).

III. CONCLUSION

The 4-hours inhalation LC_{50} of potassium phosphonate in rats was greater than 5 mg/L. Thus, the substance is not classified for acute inhalation toxicity according to the criteria of Regulation 1272/2008.
RMS conclusion: The acute inhalation LC_{50} (4 hours) of potassium salt of phosphonic acid in the Sprague Dawley rat was > 6.14 mg/L (maximum attainable concentration) for both sexes.

Report:	KCA 5.8.1/05 [redacted] 1994; M-205470-01-1
Title:	Primary skin irritation test in rabbits: Potassium salts of phosphorus acid
Report No.:	C013895
Document No.:	M-205470-01-1
Guideline(s):	USEPA (=EPA) F 81-5
Guideline deviation(s):	none
GLP/GEP:	**yes**

I. MATERIALS AND METHODS

A group of 6 male young adult New Zealand white rabbits (bw range at dosing: 1.92 to 2.33 kg) were administered a single topical application of 0.5 mL of potassium salt of phosphonic acid (Foli-R-Fos® 400, batch 2244-3) to the clipped dorsal skin (area of approx. 6 cm²) under semi-occlusive dressing for a 4 hour period. At end of the exposure period, the exposed skin was wiped with water to remove test material.
Skin reactions were scored 1, 24, and 72 hours after the patch removal using the EPA scoring system.

II. RESULTS AND DISCUSSION

No evidence of erythema or oedema was seen in any rabbit at any interval (all scores = 0).

III. CONCLUSION

Potassium phosphonate is not irritating to rabbit skin. The erythema and oedema scores were zero and thus below 2.3 in all animals. Thus, potassium phosphonate is not classified for primary skin irritation or corrosivity according to the criteria of Regulation 1272/2008.
RMS conclusion: The potassium salt of phosphonic acid was not irritant to the rabbit skin.

Report:	KCA 5.8.1/06[redacted]; 1994; M-205458-01-1
Title:	Primary eye irritation test in rabbits Potassium salts of phosphorus acid
Report No.:	C013889
Document No.:	M-205458-01-1
Guideline(s):	USEPA (=EPA): F 81-4
Guideline deviation(s):	none
GLP/GEP:	**yes**

I. MATERIALS AND METHODS

A group of 6 male young adult New Zealand white rabbits (bw range at dosing: 2.2 to 2.2 kg) were administered a single application of 0.1 mL of potassium salt of phosphonic acid (Foliar-Fos 400, batch 2244-3) in the conjunctival sac of the right eye; ocular reactions were recorded 1, 24, 48, and 72 hours after dosing using the EPA scoring system.

II. RESULTS AND DISCUSSION

No corneal nor iridial changes were seen; slight conjunctival redness was seen in 5/6 rabbits with slight to moderate discharge in all rabbits at 1 hour after dosing; full recovery occurred at 24 hours after instillation (see Table 5.8.1- 6).

Table 5.8.1- 6: Eye irritation scores following instillation of potassium salt of phosphonic acid

Eye irritation scores (no. of affected rabbits)				
Scoring time	**1 h**	**24 h**	**48 h**	**72 h**
Cornea opacity	0 (6)	0 (6)	0 (6)	0 (6)
Iris inflammation	0 (6)	0 (6)	0 (6)	0 (6)
Conjunctival redness	0 (1) ; 1 (5)	0 (6)	0 (6)	0 (6)
Conjunctival chemosis	0 (6)	0 (6)	0 (6)	0 (6)
Conjunctival discharge	1 (4) ; 2 (2)	0 (6)	0 (6)	0 (6)

III. CONCLUSION

Potassium phosphonate is not irritating to rabbit eyes. The ocular scores at the relevant reading times were zero. Thus, potassium phosphonate is not classified for eye irritation according to the criteria of Regulation 1272/2008.

RMS conclusion: According the current EU guidelines, the potassium salt of phosphonic acid should not be considered as irritant to the eye.

Report:	KCA 5.8.1/07[redacted]; [redacted]; [redacted]; [redacted]; [redacted]; [redacted]; 1978; M-231192-01-2
Title:	Phosphorous acid (37934 R.P.): Three-months toxicity in the rat by the oral route (by admixture in the diet as hydrated monosodium phosphite)
Report No.:	R000591
Document No.:	M-231192-01-2
Guideline(s):	not specified
Guideline deviation(s):	not applicable
GLP/GEP:	**no**

I. MATERIALS AND METHODS

Groups of 30 (15/sex) CD rats (1 month old) were given oral administration of 0, 2500, 5000, and 25 000 ppm of phosphonic acid as an hydrate monosodium phosphonate (anhydrous NaH_2PO_3: 69.3%; water: 27.7%; batch DA 88) by admixture in the diet for 90 consecutive days (equivalent to daily oral ingestion of 0, 200, 400, and 2000 mg/kg bw/d of phosphonic acid); 2 controls groups were used, one administered the basal diet and the other one administered 5000 ppm of sodium chloride, i.e. the highest sodium concentration of sodium administered to the highest test substance treated rats to allow for the large amounts of sodium introduced in the diet by the high concentration of monosodium phosphonate).
Stability of sodium phosphonate in the diet over 1 month was checked in a previous study[4]. Anhydrous monosodium phosphonate and sodium content of food mixture were checked when preparing fresh batches.
Rats were observed daily for mortality, moribundity and clinical signs; bw, water and food consumption were recorded weekly; haematological examination (erythrocyte count; Hb; Hct; total and differential leukocyte counts) and clinico-chemical examination (phosphorus; sodium; calcium; chloride; SGOT; SGPT; alkaline phosphatase; glucose; BUN) were carried out on 5 rats/sex after 1 and 3 months; urinalysis (volume; pH; phosphorus; sodium; calcium; chloride; urobilinogen; bile salts; microscopic examination of the sediment) were carried out on 10 rats/sex at termination.
Terminal macroscopic and histopathological* examinations were carried out on 5 rats/sex (oesophagus; stomach; small intestine; colon; liver*; pancreas; salivary gland; trachea; lung; heart*; aorta; spleen; mesenteric lymph nodes; thymus; thyroid*/parathyroid; adrenal gland*; muscle; kidney*; urinary bladder; gonads*; epididymides; prostate*; seminal vesicles; uterine horn*; femur + bone marrow; brain; pituitary gland; spinal cord; eye and optic nerve).

II. RESULTS AND DISCUSSION

There was no treatment-related effect on mortality, bw gain, food consumption and ophthalmological findings. Soft faeces were seen throughout the study period and diarrhoea during the early part of dosing in the high dose group. Increased water intake was recorded in high dose rats from the 1st week of study and persisting throughout the study period in males while it disappeared at w-7 in females; water intake was similarly increased in the sodium chloride control group (see Table 5.8.1- 7).

[4] Bertrand A., Acide phophoreux (37 394 R.P.): stabilité dans l'aliment pour rats UAR 103; PH/RD/F-AB/CP 1842; May 26, 1977.

Table 5.8.1- 7: Mean phosphonic acid intake (mg/kg bw/d and mean water intake (mL/day/rat)

Dose level (ppm)	0		NaCl controls		2500		5000		25000	
Sex	M	F	M	F	M	F	M	F	M	F
Mean phosphonic acid intake (mg/kg bw/d)										
w-1	-	-	-	-	300	300	600	600	2,700	3,200
w-4	-	-	-	-	200	200	400	400	2,000	2,500
w-8	-	-	-	-	100	200	300	300	1,400	1,600
w-12	-	-	-	-	100	100	200	300	1,200	1,600
Mean water intake (mL/d/rat) (% variation vs absolute controls)										
w-1	25	25	32 (+28%)	31 (+24%)	24 (-4%)	24 (-4%)	29 (+16%)	26 (+4%)	33 (+32%)	32 (+28%)
w-4	36	30	45 (+25%)	36 (+20%)	36 (-)	30 (-)	38 (+5%)	31 (+3%)	45 (+25%)	38 (+27%)
w-8	35	38	42 (+20%)	36 (-5%)	36 (+3%)	30 (-21%)	37 (+6%)	32 (-16%)	36 (+3%)	34 (-11%)
w-12	35	34	44 (+26%)	31 (-9%)	33 (-6%)	30 (-12%)	36 (+3%)	31 (-7%)	44 (+26%)	31 (-9%)

No treatment related changes were seen in haematological and clinico-chemical investigations. Higher levels of urinary sodium and calcium were seen in high dose rats from the 4th month of treatment; such increases was only seen in 3 rats of the mid dose level (see Table 5.8.1- 8).

Table 5.8.1- 8: Results of urinalysis after 13 weeks of treatment

Sex	Male				Female			
Dose (ppm)	**0**	**2500**	**5000**	**25000**	**0**	**2500**	**5000**	**25000**
Urinary pH								
w-4	-	-	-	-	-	-	-	-
w-13	6.84	7.48	7.75	6.50	6.88	7.22	6.82	6.20
Sodium (mg/24h)								
w-4	20.4	26.0	28.9	35.2 **	16.3	17.2	17.0	31.2 **
w-13	16.2	26.6	16.3	30.0 **	16.0	17.1	24.7	27.6 **
Calcium (mg/24h)								
w-4	0.73	1.20	1.26	1.28	0.78	1.10	1.37 *	1.63 *
w-13	1.1	1.32	1.31	2.14 **	1.36	1.02	2.19	2.72 **
Phosphorus (mg/24h)								
w-4	30.5	33.6	24.3	22.6	16.1	21.4	14.9	7.9
w-13	29.1	33.3	21.2	27.3	16.6	15.9	22.1	22.4
* p<0.05 ** p<0.01								

No treatment related effects were seen at termination macroscopic examination.

III. CONCLUSION

RMS conclusion: Based on findings seen in the high dose rats (soft faeces, increased water intake and increased urinary sodium and calcium excretion), the NOAEL in this study is 5000 ppm (equivalent to 400 mg/kg bw/d).

Report: KCA 5.8.1/08[redacted],;[redacted]; 1994; M-205460-01-1
Title: Testing for mutagenic activity with Salmonella typhimurium TA 1535, TA 1537, TA 1538, TA 98 and TA 100 Potassium salts of phosphorus acid
Report No.: C013890
Document No.: M-205460-01-1
Guideline(s): USEPA (=EPA): F84-2; M 152-17
Guideline deviation(s): none
GLP/GEP: **yes**

I. MATERIALS AND METHODS

Prior to the experiments, a toxicity testing was performed on TA100 strain using 33, 100, 333, 1000, 3333, and 10 000 µg/plate of potassium salt of phosphonic acid (Fos-R-Form® 400 Batch 224-3; 400 g/L phosphonic acid present as mono- and dipotassium phosphonate).
Potassium salt of phosphonic acid was tested at the same concentrations as in the range finding assay, in a plate incorporation assay using 5 bacterial tester strains: TA98; TA100; TA1535; TA1537 and TA1538 of *S. typhimurium*, with and without metabolic activation (S-9 fraction of liver homogenate prepared from the livers of Aroclor 1254 treated Sprague Dawley rats and S-9 cofactor mix in sterile water), using 2 independent experiments.
Positive controls without metabolic activation included 9-aminoacridine (80 µg/plate dissolved in DMSO), sodium azide (1 µg/plate dissolved in sterile water), 2-aminoacridine (80 µg/plate dissolved in DMSO) and 4 nitrofluorene (10 µg/plate dissolved in DMSO).

II. RESULTS AND DISCUSSION

No evidence of toxicity was observed in the range finding toxicity test; no precipitation of test material was seen.
In the 2 independent experiments there was no statistically significant increase in the mean number of revertant colonies in any tester strain, both in the absence and in the presence of metabolic activation; positive controls elicited the expected increases in revertants colonies (see Table 5.8.1- 9).

Table 5.8.1- 9: Mean number of revertants colonies in *Salmonella typhimurium* strains following treatment with potassium salt of phosphonic acid

		Mean number of revertant colonies (3 replicates)									
	µg/plate	TA1535		TA1537		TA 1538		TA98		TA100	
		+ S9	- S9	+ S9	- S9	+ S9	- S9	+ S9	- S9	+ S9	- S9
1st experiment											
Solvent	0	19±2	11±2	11±9	11±3	15±7	13±2	23.2±	13±2	99±7	86±15
Potassium phosphonate	33	15±7	8±2	12±3	4±1	12±4	9±3	24±8	13±6	88±6	98±6
	100	13±3	10±3	12±4	7±3	8±6	11±1	24±5	16±3	113±9	89±16
	333	10±2	7±1	8±4	7±3	10±2	8±4	26±1	18±4	104±4	89±18
	1000	13±2	10±1	12±4	10±4	12±3	9±2	23±2	13±3	86±8	88±9
	3333	13±4	15±6	12±5	9±3	16±3	8±3	20±3	15±5	86±8	92±11
	10000	9±4	8±3	6±2	11±3	13±4	9±8	19±5	15±3	93±3	85±4
2 NF	1	-	-	-	-	-	214±45	-	179±45	-	-
Na azide	1	1±	112±4	-	-	-	-	-	-	-	419±8
9 AAC	80	-	-	-	1427±325	-	-	-	-	-	-
2AN	2	171±9	-	146±15	-	268±43	-	495±43	-	547±35	-

Mean number of revertant colonies (3 replicates)												
	µg/ plate	TA1535		TA1537		TA 1538		TA98		TA100		
		+ S9	- S9	+ S9	- S9	+ S9	- S9	+ S9	- S9	+ S9	- S9	
2nd experiment												
Solvent	0	8±1	12±2	6.2±	10±4	13±2	12±2	13±5	17±2	94±6	89±19	
Potassium phosphonate	33	5±1	6±1	7±4	6±1	11±2	11±2	14±5	14±4	102±13	95	
	100	7±2	7±3	6±2	8±1	7±3	12±2	10±4	12±1	100±6	86±13	
	333	6±2	5±3	9±3	6±2	8±2	14±6	11±6	16±4	86±16	93±2	
	1000	4±3	6±3	10±1	5±2	14±4	11±2	17±5	15±1	99±4	75	
	3333	6±2	9±3	9±1	4±2	4±2	10±1	12±3	13±4	91±17	86±8	
	10000	6±2	6±3	7±1	6±3	8±3	10±4	10±3	11±2	97±6	79±2	
2 NF	1	-	-	-	-	-	233±30	-	120±30	-	-	
Na azide	1	-	56±11	-	-	-	-	-	-	-	38± 19	
9AAC	80	-	-	-	1329± 192	-	-	-	-	-	-	
AAN	2	126±10	-	178±12	-	225±5	-	112±8	-	890±6	-	

9 AAC = 9 Aminoacridine; 2 NF = 2 Nitrofluorene; NQO = 4-Nitroquinoline; AAN = 4-Aminoanthracene

III. CONCLUSION

RMS conclusion: Following 2 independent experiments, potassium salt of phosphonic acid did not induce reverse gene mutation in any of the *Salmonella typhimurium* strains at dose levels up to 10 000 µg/plate, both in the absence and the presence of metabolic activation.

Report: KCA 5.8.1/09 [redacted] 1977, M-223290-01-2
Title: Investigation of the possible mutagenic activity of fosetyl-Al of hydrated monosodium phosphite
Report No.: RH90822
Document No.: M-223290-01-2
Guideline(s): not specified
Guideline deviation(s): not applicable
GLP/GEP: no

I. MATERIALS AND METHODS

Groups of 5 male Swiss mice were given 2 oral doses, by gavage, at 24-hour interval of 0, 1000, 2000, and 4000 mg/kg bw of hydrated monosodium phosphonate (Batch OP 77051, approx. 97% purity; anhydrous NaH_2PO_3 68.98, water 28.2%) dissolved in distilled water. All mice were sacrificed after the 2nd dose, and bone marrow was taken for determining the count of Howell-Jolly bodies in 2,000 polychromatic erythrocytes.

II. RESULTS AND DISCUSSION

In the top dose group, 3 mice died within 24 hours after the 1st administration and the 2 other within 1 hour after the 2nd administration.
Control mice exhibited a low percentage of polychromatic erythrocytes containing Howell-Jolly bodies while benzene, benzo(a)pyrene and methylmethansulphonate treated animals showed higher values. Phosphonic acid did not increase the frequency of polychromatic erythrocytes containing Howell-Jolly bodies at any dose levels, compared to the negative controls (see Table 5.8.1- 10).

Table 5.8.1- 10: Group mean percentage of PCE containing Howell-Jolly bodies per 2000 cells

Treatment groups	Dose (mg/kg bw)	Group mean percentage of PCE containing Howell-Jolly bodies per 2000 cells
Vehicle	0	0.18%
Phosphonic acid	1000	0.27%
	2000	0.21%
	4000	0.22%
Benzo (a) pyrene	200	1.51%
MMS	25	1.76%
Benzene	0.25 mL/kg	1.71%

III. CONCLUSION

RMS conclusion: Administration of phosphonic acid as hydrated monosodium phosphonate up to 4,000 mg/kg bw did not induce the formation of Howell-Jolly bodies in the polychromatic erythrocytes of the bone marrow of mice.

Report:	KCA 5.8.1/10 [redacted]; 1978; M-178996-01-2
Title:	Inductests on phosphorous acid and [redacted]74-78.
Report No.:	R002844
Document No.:	M-178996-01-2
Guideline(s):	not specified
Guideline deviation(s):	not applicable
GLP/GEP:	no

I. MATERIALS AND METHODS

Induction of prophage lambda development in *Escherichia coli* K12 strain GY 5027 was assayed in the absence and the presence of metabolic activation (Aroclor induced rat liver microsomes) using concentrations of phosphonic acid batch as specified, in distilled water up to 2 mg/plate. Effect of test substance on prophage development was assayed, using the same concentrations, on liquid culture of *E. coli* GY 5029 strain, in the absence and in the presence of metabolic activation. Results were expressed as the concentration of test substance inducing half of the maximum theoretical induction which is assessed by counting either the number of infective ion or the number of lysogenic bacteria. (see Table 5.8.1- 11).

Table 5.8.1- 11: Test system

Strain	Prophage	Genetic markers				Endpoint
		Rfa	Uvr	Ampo	Sbc	
GY 4015	-	+	+	R601	+	Indicative
GY 5027	Lambda Papa	A	B34	A1	A	Induction potential
GY 5029	Lambda cI857	A	B34	A1	A	Prophage development

II. RESULTS AND DISCUSSION

No inducing potential of prophage lambda was observed in the *E. Coli* GY5027 lysogenic strain with and without metabolic activation.
Prophage development was not inhibited for dose of test substance up to 2,000 µg/in the absence and in the presence of metabolic activation.

III. CONCLUSION

RMS conclusion: Phosphonic acid did not induce prophage lambda development in the *E. Coli* K12 strain (GY5027) at dose level up to 2 mg/plate, in the absence or in the presence of metabolic activation. Data are succinctly described so that this negative result should be considered as indicative.

Report:	KCA 5.8.1/11 [redacted]; 1981; M-159229-01-1
Title:	Monosodium phosphite: Lifetime chronic toxicity and carcinogenicity study in rats
Report No.:	R000746
Document No.:	M-159229-01-1
Guideline(s):	No guideline was in effect at the time of the study (study design is equivalent to OECD 453, 1981)
Guideline deviation(s):	none
GLP/GEP:	**yes**

Executive Summary

Monosodium phosphonate monohydrate was administered in the diet to Charles River CD® rats to provide dosage levels of 2000, 8000, and 32 000 ppm (expressed as the anhydrous salt; the product containing 27% water). The study was conducted prior to the adoption of the pertinent OECD guideline 453, but the study design was in accordance with the provisions of this guideline. Sixty male and 60 female rats were initiated on each dosage level and in a control group. The rats were observed twice daily for signs of overt toxicity and for mortality. Detailed observations were recorded weekly. Individual body weights and food (with compound) consumption measurements were recorded weekly for the first 13 weeks of study and once every 2 weeks thereafter. Ophthalmoscopic examinations were performed for all rats once during the control period and at 3, 6, 12, 18 and 24 months of study. Haematological and biochemical evaluations and urinalyses were conducted on 13 male and 13 female weanling rats once during the acclimation period. Haematological evaluations were determined for 10 rats/sex/group at 4, 8, 12, 18, 20, 24 and 27 months of study. Biochemical evaluations and urinalyses were determined for 10 rats/sex/group at 6, 12, 18, 24 and 27 months of study. An interim sacrifice was conducted for 10 rats/sex/group at 12 months of study. No compound-related trends were established for mean food (with compound) consumption or ophthalmoscopic examinations.

A compound-related decrease in group mean body weight was noted at the 32 000-ppm dosage level. Also noted at the 32 000-ppm male dosage level was a high incidence of soft stool which appeared to be a compound-related effect. Survival, though fairly consistent between groups throughout 12 months of study, was decreased at 27 months of study for all the treated male groups when compared with the control group, due mainly to slightly increased mortality in the 12-19 month period.

Variations noted in haematologic values lacked consistency and progression and therefore they were not considered to be of toxicological significance. Sporadic and inconsistent statistically significant values were noted in the treated groups for many biochemical studies. Reductions noted in calcium and potassium values were difficult to interpret and were probably sporadic in nature but could also be secondary to the considerable quantities of sodium and phosphorus present in the test article. The increase in sodium values at 27 months is probably due to the same cause while the chloride changes are probably secondary to the other electrolyte changes seen. There was a tendency to a reduced pH of the urine in males. There were no other features of note in urinalysis parameters examined.

At necropsy there were no treatment related findings but relative organ weights for liver, kidney and heart for males, and kidney and heart for females, at 32 000 ppm were increased. These were not considered to be of toxicological significance. On histopathological examination the only feature of note was an increased incidence of chronic nephritis in males at 32 000 ppm but this incidence remained within the background range for this laboratory. No treatment related changes in neoplastic incidence were seen. The NOAEL for anhydrous monosodium phosphonate seen in this study was considered to be 8000 ppm, equivalent to 348 and 434 mg/kg bw/day in males and females, respectively.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material:

Name:	Monosodium phosphite hydrate (= monosodium phosphonate monohydrate)
Description:	White powder
Batch / Lot No.:	DA 117
Purity:	NaH_2PO_3: 73% Water: 25.9%
Stability of test compound:	Stability and concentration of phosphonic acid in the diet were within the acceptable ranges

2. Vehicle: Plain diet

3. Test animals

Species:	Rat
Strain:	CD
Sex:	Males and females
Age:	3 weeks
Weight at dosing:	Males: 77-113 g Females: 76-95 g
Source:	[redacted] USA
Acclimatisation period:	10 days
Diet:	[redacted] *ad libitum*
Water:	Tap water, *ad libitum*
Housing:	individually in hanging wire mesh cages
Environmental conditions:	
Temperature:	Not reported
Humidity:	Not reported
Air changes:	Not reported
Photoperiod:	12 h light / 12 h dark

B. STUDY DESIGN AND METHODS

1. In-life dates
1978-08-25 to 1980-11-25

2. Animal assignment and treatment
Animal assignment and dose groups:
Rats were randomly distributed to different groups. The following dose groups were employed:

Table 5.8.1- 12: Group allocation in the 27-month feeding study in rats

Conc. in diet [ppm]*	No. of animals	
	Male	Female
0	60	60
2000	60	60
8000	60	60
32000	60	60

*expressed as anhydrous monosodium phosphonate

Diet Preparation and Analysis:
Homogeneity of test substance in the diet was checked on two occasions during the study period; samples were collected from all diet preparations during each of the first 4 weeks and at Weeks 8, 12, 26, 39, ,52 65, 78, 91, 104, and 117.

Details on oral exposure:

Duration of exposure	117 weeks
Frequency of treatment	Via diet, *ad libitum*

3. Examinations

Clinical signs - mortality and moribundity/general daily observations	Rats were observed at least twice daily for mortality, moribundity and signs of overt toxicity and detailed examinations were performed weekly.
Body weights	Body weights were recorded weekly for the first 13 weeks and every 2 weeks thereafter.
Food consumption	Food consumption was recorded weekly for the first 13 weeks and every 2 weeks thereafter.
Ophthalmic evaluation	Ophthalmological examinations were carried out on all rats before treatment and at months 3, 6, 12,18, and 24 post-dosing.
Haematology	Haematological investigations (erythrocyte count; Hb; Hct; total and differential leukocyte count; reticulocyte count; platelet count; MCV; MCH; MCHC) were carried out on 10 rats/sex/dose level at months 4, 8, 12, 16, 20, 24, and 27.
Clinical chemistry	Blood chemistry (chloride, potassium, sodium, calcium, cholesterol, BUN, alkaline phosphatase, SGOT, SGPT, albumin, glucose, direct and total bilirubin, LDH, total protein, globulin) were carried out on 10 rats/sex/dose level at months 6, 12, 18, 24, and 27.
Urinalysis	Urinalysis (volume, specific gravity, pH, colour and appearance, microscopic examination of the sediment, glucose, ketones, occult blood, protein, urobilinogen, nitrites) were also carried out on 10 rats/sex/dose level at months 6, 12, 18, 24, and 27.
Gross pathology	Sacrifice of 10 rats/sex/dose level was performed after 12 months, and all remaining rats were sacrificed after 27 months. All were subjected to a gross macroscopic examination.
Organ weights	Selected organs were weighed (heart, kidney, liver, brain, testis)
Histopathology	Histopathological examinations were performed on the following tissues: Adrenal gland, aorta, eye, oesophagus, stomach, duodenum, jejunum, ileum, caecum, colon: rectum, liver, kidney, trachea, spleen, pancreas, urinary bladder, prostate, uterus, testes, ovaries, sciatic nerve, brain, heart, lung, pituitary, thyroid and parathyroid, lymph nodes, sternum, spinal cord, salivary gland, muscle, skin, mammary gland, thymus and any tissue with lesions

Statistical evaluation
All statistical analyses compared the treatment groups with the control group, by sex. Mean body weights, mean food consumption, haematological parameters, biochemical and urinalysis parameters, and absolute and relative organ weights were compared by analysis of variance (one-way classification), Bartlett's test for homogeneity of variances and appropriate *t*-test (for equal or unequal variances) using Dunnett's multiple comparison tables to judge significance of differences.

II. RESULTS AND DISCUSSION

A. MORTALITY

There were no treatment-related changes in survival through 12 months of study; at study termination, lower survival was recorded in all treated males compared to controls, due to the greater number of deaths in the 12-19 month interval, whereas survival in treated females was greater than that of controls (see Table 5.8.1- 13).

Table 5.8.1- 13: Survival rate after 117 weeks

Sex	Dose level (ppm)			
	0	2000	8000	32 000
Males	21/50	15/50	14/50	11/50
Females	16/50	21/50	20/50	20/50

B. CLINICAL OBSERVATIONS

No treatment-related clinical signs were seen except a higher incidence of soft stools in high dose males throughout the study period.

C. BODY WEIGHT

Statistically significant reduction ($p<0.05$ or $p<0.01$) of group mean bw gain was recorded in both sexes of high-dose rats throughout the study period except w-week 117. No group mean bw changes were seen at lower dose levels, except a statistically significant decrease ($p<0.05$) in the mid dose males at w-113 of study (see Table 5.8.1- 14).

Table 5.8.1- 14: Group mean body weight (g)

Week	Dose level (ppm)							
	0		2000		8000		32 000	
	M	F	M	F	M	F	M	F
13	500±32.7	281±25.9	500±59.3	279±27.5	509±47.1	276±20.7	464±46.5**	264±21.0
39	671±91.5	354±54.1	669±94.1	358±58.1	682±75.3	345±33.2	615±67.7**	324±43.3
65	737±136.1	427±97.4	772±102.5	433±85.7	760±111.3	410±58.7	678±84.8**	375±58.6**
117	748±146.1	469±86.0	677±97.2	506±80.4	653±129.0	494±127.0	645±103.2	425±67.1

* $p<0.05$; ** $p<0.01$

D. FOOD CONSUMPTION

No treatment-related changes in food consumption were seen in both sexes at any dose level throughout the study period (few differences were occasionally seen, but no dose trend was established); however, food efficiency ratios were decreased in mid and high dose males (see Table 5.8.1- 15).

Table 5.8.1- 15: Mean food consumption (g/rat/d) and mean substance intake (mg/kg bw/d)

Dose level (ppm)	Mean food consumption (g/rat/d)		Mean substance intake* (mg/kg bw/d)	
	M	F	M	F
0	24.6	18.1	-	-
2000	24.9	18.2	83.9	104.2
8000	25.7	18.4	347.6	434.1
32 000	24.9	18.0	1481.5	1820.1

*expressed as anhydrous monosodium phosphonate

E. OPHTHALMOSCOPIC EXAMINATION

No treatment-related changes were seen at any time during ophthalmological examinations.

F. HAEMATOLOGY AND CLINICAL CHEMISTRY

Statistically significant changes were recorded in several haematological parameters (slight but significant reduction in erythrocyte count, in HB and in Hct at 12 months only for mid- and high-dose males; increases platelet count in high-dose males at 27 months; changes in females were sporadic) and did not appear to be treatment-related as no dose or time trend could be demonstrated.
Statistically significant changes were recorded in several biochemical parameters (occasional results for glucose, alkaline phosphatases, SGPT, LDH, albumin, globulin and total protein in males; for glucose, BUN, SGPT, LDH, albumin, globulin, total protein in females; increased total and direct bilirubin levels in both sexes; decreased calcium and potassium levels at some intervals) and did not appear to be treatment-related as no dose or time trend could be demonstrated (see Table 5.8.1- 16).

Table 5.8.1- 16: Clinical-chemical selected parameters

Parameter	Dose level (ppm)							
	0		2000		8000		32 000	
	M	F	M	F	M	F	M	F
RBC 10^6/cmm	7.61±0.54	6.81±0.34	7.22±0.58	6.69±0.33	6.80±0.35**	6.50±0.43	6.90±0.33**	6.65±0.34
Month-12	7.61±0.54	6.81±0.34	7.22±0.58	6.69±0.33	6.80±0.35**	6.50±0.43	6.90±0.33**	6.65±0.34
Month-27	6.90±1.56	6.39±0.84	6.54±0.69	6.02±1.19	6.58±1.29	6.42±1.32	7.69±1.49	6.61±1.57
Hb (g/100 mL)								
Month-12	16.8±1.14	16.3±0.96	16.6±1.29	15.8±0.84	15.5±1.71**	15.5±0.91	15.3±0.80**	15.7±0.78
Month-27	12.8±1.93	12.6±1.58	13.3±1.69	11.8±1.99	11.8±2.46	12.6±2.33*	13.7±2.20	12.9±2.37
Hct (%)								
Month-12	47.3±3.43	46.5±2.52	44.4±4.13	42.5±2.17	41.7±4.92**	41.5±2.57*	41.3±2.25*	41.8±1.85*
Month-27	48.2±10.84	47.4±7.85	45.9±6.41	44.7±6.83	46.5±9.86	45.5±9.05	53.1±9.11	48.3±9.81
BUN (mg/100 mL)	16.7±2.16	12.7±2.09	15.8±5.79	11.6±2.35	28.2±10.16	19.4±2.45*	6.0±6.22	13.3±2.52
Alk.phosphatase (IU/L)	83±25.5	42±12.0	82±48.2	65±24.2	87±31.2	61±50	154±38.5**	40±17.5
Ca (mg/100 mL)	10.5±0.63	10.4±0.34	10.3±0.82	10.1±0.54	10.0±0.83	9.7±0.49**	10.3±0.62	10.2±0.52
Na (meq/L)	144±2.6	142±3.0	144±2.6	142±2.0	144±2.3	145±2.4*	149±3.7*	146±3.7*
Urinary pH								
Month-6	8	9	8	7*	7	7	7	6
Month-12	7	8	7	6**	6	6	6	6*
Month-18	7	7	7	6*	6	6	6	6
Month-24	6	7	6	5**	6	6	6	6
Month-27	6	7	6	5	6	5	6	6

* p<0.05; ** p<0.01

G. URINALYSIS

Statistically significant decreased urinary pH was recorded in high dose males at all sampling intervals except the last one (see Table 5.8.1- 16).

H. PATHOLOGY

No gross pathological findings were recorded in rats during study period, neither in those sacrificed at the 12-month interval nor in those sacrificed at study termination.
Statistically significant increased liver, kidney and heart relative weights were recorded in high dose rats (increased kidney weight were considered to be caused by increased incidence of chronic nephritis and within the expected range of this species).
Histopathological examination at terminal sacrifice did not reveal any treatment-related neoplastic and non-neoplastic changes: there were more benign than malignant tumours and the incidence of both was slightly higher in low and high dose males and high dose females had more malignancies than other treated groups; non neoplastic changes (inflammatory, degenerative changes) were evenly distributed among sexes and dose groups. However, none of these changes appeared to be treatment related (see Table 5.8.1- 17).

Table 5.8.1- 17: Organ weights and incidence of tumours in rats sacrificed at termination

	Male				Female			
Dose (ppm)	**0**	**2000**	**8000**	**32 000**	**0**	**2000**	**8000**	**32 000**
Liver								
Absolute weight (g)	20.12	21.76	23..09	23.7	14.91	16.97	17.94	17.02
Relative weight (%)	3.27	3.19	3.88	3.89*	2.62	3.41	3.68	4.05
Kidney								
Absolute weight (g)	5.23	5.40	6.38	7.11*	3.39	3.41	3.69	3.92*
Relative weight (%)	0.79	0.80	1.07	1.19*	0.72	0.72	0.79	0.95**
Heart								
Absolute weight (g)	2.23	2.24	2.27	2.64	1.55	1.69	1.62	1.73
Relative weight (%)	0.31	0.32	0.37*	0.43*	0.34	0.34	0.34	0.41*
No. of rats with benign tumours n=60	29	31	31	27	50	47	50	45
No. of rats with malignant tumours n=60	14	17	19	18	3	14	16	22
No. of rats with tumours (benign and malignant) n=60	37	43	43	36	53	50	52	50

*p<0.05

III. CONCLUSION

RMS conclusion: Based on the findings recorded in the high dose group (soft stools, bw loss and decreased food efficiency, decreased urine pH, organ weight changes), the 8000-ppm dose level (equivalent to 347.6 and 394.1 mg/kg bw/day in males and females, respectively; 390 mg/kg bw/day for both sexes) was the NOAEL on the study.

Applicant's remark: Please note that the study NOAEL refers to anhydrous monosodium phosphonate. The correction of the NOAEL for water content that was applied by EFSA in its revised ADI setting for phosphonic acid[5] is not appropriate. The NOAEL in this study, expressed as phosphonic acid, was 274.1 and 342.3 mg/kg bw/day in males and females, respectively, and 307.5 mg/kg bw/day for both sexes. The NOAEL was converted using molecular weights of 104 and 82 g/mol for anhydrous monosodium phosphonate and phosphonic acid, respectively.

[5] EFSA (2013): Conclusion on the peer review of the pesticide risk assessment of the active substance fosetyl. EFSA Scientific Report (2005) 54, 1-79, revised version dated 12 June 2013.
Available at http://www.efsa.europa.eu/de/efsajournal/doc/54r.pdf

CA 5.8.2 Supplementary studies on the active substance

Two mechanistic studies into the effects of high doses of fosetyl-aluminium (fosetyl-Al) on kidney and bladder histopathology have been conducted (see Table 5.8.2- 1).

A first mechanistic study ([redacted]; 1981; M-205133-01-2) showed that the continuous administration of fosetyl-Al at dose levels up to 40 000 ppm during a short period of 4 weeks resulted in an increased urinary excretion of calcium and associated histopathological changes in the kidney.

A second study ([redacted]; 1989; M-160331-01-1) confirmed that the administration of massive doses (up to 50 000 ppm) during a longer period of 13 weeks severely altered the calcium/phosphorus balance and modified the urine composition, leading to the formation of calculi in the urinary tract of treated animals. These changes were closely related to the subsequent development of hyperplasia in the urinary bladder within a relatively short time. Although these effects were shown to be partially reversible when dosing ceased, it is likely that following a long-term exposure to high dose levels of fosetyl-Al, the formation of calculi associated with prolonged hyperplasia could induce the development of transitional cell papilloma and carcinoma in the urinary bladder.

Taken together, these results strongly support the hypothesis that the bladder tumour observed in male rats exposed to 30 000 ppm in the carcinogenicity study were not a true carcinogenic effect of fosetyl-Al but likely resulted from a chronic reaction induced secondarily to the ingestion of massive dose of fosetyl-Al. In addition, as these findings were only observed at extremely high doses under conditions not anticipated to occur outside of the experimental laboratory and as the mechanism underlying the formation of these neoplastic findings has been likely elucidated, it is concluded that fosetyl-Al does not pose a carcinogenic hazard to human. Since this study has been used for AOEL setting for fosetyl-Al, the respective study summary is included in this Supplementary Dossier.

Mechanism of action

The **mechanism of action (MoA)** underlying the development of urinary bladder tumours in rats following high doses of fosetyl-Al can be summarised as follows:

Key Event 1

High doses of phosphonate lead to an increased calcium (Ca) concentration in urine. This is noted after one week of treatment in the 1st mechanistic study ([redacted]; 1981; M-205133-01-2) and persists until the end of the 2nd mechanistic study after 13 weeks ([redacted]; 1989; M-160331-01-1). The mechanism by which this occurs has not been investigated, but it is conceivable that phosphonate in serum forms complexes with Ca, in analogy to high concentrations of phosphate. It thereby reduces stimulation of Ca-sensitive receptors (CaSr) in the parathyroid that do not sense complexed Ca, triggering an increase in parathyroid hormone (PTH). PTH stimulates osteoclast activity thereby releasing Ca from bone. Ca in serum is tightly controlled and the release of Ca from bone into serum causes increased renal excretion / decreased tubular re-absorption. This will lead to increased urinary Ca concentrations.

Key Event 2

High Ca in urine eventually leads to exceeding the solubility product of Ca-phosphonate or Ca phosphate, thereby producing bladder calculi that are composed of Ca and phosphorus (P). This is seen after 2 weeks of treatment in male rats receiving 30 000 or 50 000 ppm fosetyl-Al. Uroliths were noted after 2 weeks of treatment in the kidney of high-dose rats of both sexes. After 13 weeks, mid-dose animals of both sexes also showed kidney urolithiasis ([redacted]; 1989; M-160331-01-1).

Key Event 3
The presence of calculi and uroliths causes a chronic mechanical irritation and damage of the urothelium and the transitional cell epithelium. The resulting urothelial repair activity is a proliferative stimulus that leads to papillary and transitional cell hyperplasia. This is a well-known phenomenon in rodent bladder carcinogenesis and can be observed with chemically induced calculi as well as with foreign objects implanted into the urinary bladder (reviewed by Clayson *et al., Fd. Chem. Toxic.* **33**(9), pp. 771-784, 1995).

Applying the IPCS Human Relevance Framework to fosetyl-Al.

Question 1. Is the weight of evidence sufficient to establish the MoA in animals?
Yes, the development of uroliths in the urinary tract by high doses of fosetyl-Al could be clearly demonstrated in a time- and dose-dependent fashion. The development of urothelial neoplasia in rodents in response to bladder calculi is well established. A genotoxic MoA can be excluded due to the unambiguously negative genotoxicity database for fosetyl-Al.

Question 2. Can human relevance of the MoA be reasonably excluded based on fundamental qualitative differences in key events between experimental animals and humans?
No, urinary bladder calculi, irrespective of composition, cause irritation and cell proliferation in humans. There is some epidemiological evidence that urinary tract cancer in humans is associated with a history of calculi in the bladder. The risk in humans may not be as great as that in rodents because the calculi are usually voided spontaneously or removed by surgical procedures. Thus, although there are quantitative differences in the carcinogenic response to calculi between species, the effect is not species-specific. However, calculus formation is dependent on attainment in the urine of critically high concentrations of the constituent chemicals which form the calculus. The carcinogenic effects are also dependent on reaching a threshold concentration for calculus formation (cited from IARC Publication No. 147)[6].

Question 3. Can human relevance of the MoA be reasonably excluded based on quantitative differences in either kinetic or dynamic factors between experimental animals and humans?
Yes, healthy humans have very low concentrations of urinary protein and much lower urinary osmolalities than rodents, two of the critical parameters required for the formation of cytotoxic calcium phosphate-containing precipitate (cited from IARC Publication No. 147)[6].
Furthermore, the LOAEL for eliciting **Key Event 1** (increased urinary Ca) and **Key Event 2** (calculus formation) is 30 000 ppm, equivalent to 2405 / 2724 mg/kg bw/day (♂/♀) during week 8 of the 2nd mechanistic study ([redacted] 1989, M-160331-01-1). Likewise, the substance intake at the LOAEL for bladder tumours in the 2-year rat study was greater than 4500 mg/kg bw/day during the critical first two weeks of exposure during which increased Ca excretion is already detectable in the 1st mechanistic study. These doses are much higher than the practical limit dose of 1000 mg/kg bw/day laid down in the relevant OECD guideline 453 and more than 1000-fold higher than the ADI for fosetyl-Al (3 mg/kg bw/day).
In conclusion, while the MoA for bladder carcinogenesis in rats may theoretically operate in humans, the doses required to elicit key events are not achievable in any foreseeable human exposure scenario.

[6] Available at https://monographs.iarc.fr/ENG/Publications/pub147/IARCpub147.pdf (accessed 28 April 2016)

Table 5.8.2- 1: Mechanistic studies with fosetyl-Al

Study type	Species	Doses tested	LOAEL / Effects	NOAEL	Reference
4-week oral (dietary) mechanistic study	Rat	0, 10 000, 20 000 and 40 000 ppm	20 000 ppm of fosetyl-Al during 4 weeks induced functional alterations and histopathological changes in the kidney.	10 000 ppm (equivalent to ca. 1000 mg/kg bw/day)	[redacted]; 1981; M-205133-01-2
13-week oral (dietary) mechanistic study	Rat	0, 8000, 30 000 and 50 000 ppm	30 000 ppm: functional alterations and histopathological changes in the kidney, including imbalance of calcium/phosphorus metabolism, formation of calculi and subsequent hyperplasia in the urinary tract.	8000 ppm (500/600 mg/kg bw/day, ♂/♀)	[redacted]; 1989; M-160331-01-1

Report: KCA 5.8.2/01 [redacted]; 1981; M-205133-01-2
Title: Fosetyl-Al (LS 1783) – Determination of calcium and phosphorus in the serum, urine and faeces of the rat during one month's treatment with the compound mixed with feed
Report No.: R000721
Document No.: M-205133-01-2
Guideline(s): not specified
Guideline deviation(s): not applicable
GLP/GEP: yes

I. MATERIAL AND METHODS

Groups of 10 (1 sex) CD® rats (approx. 8 weeks old) were administered 0; 10,000; 20,000 and 40,000 ppm of technical fosetyl-Al (batch DA 112; 97.4 ± 0.3% purity) in the diet for 4 consecutive weeks (equivalent to 734.9, 1465.2 and 2983.9 mg/kg bw/d fosetyl-Al).
Stability, homogeneity and concentration of test substance in the diet were checked.
All animals were observed twice daily for general health condition and mortality. Individual bw and food consumption were recorded weekly.
Calcium and phosphorus levels were determined weekly in the serum, in the 24 hours urine and in the faeces.
At termination all rats were subjected to a gross macroscopic examination and some tissues* were examined histopathologically (oesophagus, stomach, small intestine, colon, liver, pancreas, salivary gland, trachea, lung, heart, aorta, spleen, mesenteric lymph nodes, thymus, thyroid*, parathyroid*, adrenal, muscle, kidney*, bladder, gonads, epididymides, prostate, seminal vesicle, uterus, brain, spinal cord, eye and pituitary).

II. RESULTS AND DISCUSSION

No treatment-related death nor clinical signs, nor food consumption changes were found; bw gain was similar in treated and control rats at end of the 4w study period.
Serum calcium and phosphorus levels were similar between treated and control rats (significantly increased phosphorus levels noted in females after 4 weeks was within control ranges seen during the study period).

Urine volume did not differ between treated and control rats.
Urinary **calcium** was increased at all dose levels, particularly in the males : at 40 000 ppm, males and females urinary calcium levels were 4 to 6 times and 2 to 3 times as high as in controls, respectively and decreased markedly at end of treatment; at 20 000 ppm, significantly increased levels (2 to 3 times as high as in controls) were seen in males for the first 3 w of treatment and after 2 weeks in the females and urine calcium returned to normal values at end of treatment; at 10 000 ppm, slight, not significant increased urinary calcium levels were seen only in males after 2 to 3 weeks of treatment only and urine calcium was similar to controls values at end of treatment (see Table 5.8.2- 2).
Urinary **phosphorus** was reduced at all dose levels (only in males at high dose; after 2 weeks and after 4 weeks of treatment in the mid dose males and females respectively; only in the low dose females after 3 weeks of treatment (see Table 5.8.2- 2).
Faeces weights were generally similar between treated and control rats, except in high dose rats in which increased values were seen. Calcium levels in the faeces were increased in males at all dose levels, although mean values recorded after 4 weeks were within the control range in the low and mid dose groups (see Table 5.8.2- 2).
No treatment related macroscopic changes were recorded. Histopathological examination (only kidney, thyroid and parathyroid examined) showed only dose related increased incidences of vacuolar degeneration of the epithelial cell in the renal tubules for males only, which was fairly discrete and considered as probably reversible. No changes were seen in the thyroid and parathyroids.

Table 5.8.2- 2: Calcium and phosphorus levels in serum, urine and faeces

	Time (week)	Controls		10 000 ppm		20 000 ppm		40 000 ppm	
		Males	Females	Males	Females	Males	Females	Males	Females
Serum									
Calcium (mg/24 h)	1	120	133	13[illegible]	131	131	133	122	140
	2	144	163	141	153*	155	160	139	151
	3	109	106	100	99	98	106	100	100
	4	118	114	120	120	121	120	105	88
Phosphorus (mg/24 h)	1	82	77	80	70	85	89	84	71
	2	89	84	84	77	87	77	88	81
	3	79	67	76	67	76	65	79	67
	4	56	56	60	60	68	59	65	62*
Urine									
Calcium (mg/24 h)	1	0.592	0.092	0.848	0.020	1.284**	1.449	3.810	1.959**
	2	0.955	0.568	2.102**	0.294	2.942**	4.107*	5.434	3.557*
	3	0.748	1.194	1.989**	1.636	1.495*	2.612	3.191**	2.410*
	4	0.386	0.5[illegible]	0.544	0.940	0.731	0.955	1.703**	1.473**
Phosphorus (mg/24 h)	1	37.0	22.5	31.0	18.3	22.6**	16.7	17.7**	18.6
	2	24.2	14.4	25.[illegible]	16.5	17.5*	17.8	14.3**	11.9
	3	26.0	16.2	[illegible]3	10.5*	22.5	10.7	15.6**	12.8
	4	32.4	18.0	25.8	14.7	20.7	11.6*	19.2*	15.2
Faeces									
Calcium (mg/24 h)	1	39.5	38.3	4[illegible]	48.5	50	39.3	40.1	40.4
	2	57.0	45.[illegible]	3.0	38.6	47.5	49.2	56.8	34.9
	3	57.[illegible]	4[illegible]6	58.6	47.0	61.5	32.7	63.5	41.1
	4	3[illegible]	43.9	49.2	48.9	55.2	43.2	57.7	45.2
Phosphorus (mg/24 h)	1	35.7	39.5	60.5	50.2	65.8	50.5	78.5	72.1**
	2	89.1	66.0	101.7	64.4	88.4	90.0	156.7**	95.7
	3	8[illegible]	89.5	99.8	83.6	119.6*	59.5	132.1*	95.3
	4	[illegible]2	59.2	78.8*	81.7	88.7*	68.1	120.3**	95.8

* p< 0.05 ** p<0.01

III. CONCLUSION

RMS conclusion: Administration of fosetyl-Al at dietary levels up to 40 000 ppm for 4 weeks in rats resulted in increased urinary excretion of calcium, particularly in the males in which renal tubules exhibited slight microscopic vacuolar degenerative changes. No changes were seen in the thyroid/parathyroids. At 40 000 ppm, phosphorus levels were decreased in urine and increased in the faeces. Much less marked changes were seen at the lower dose levels in males and at high dose levels in the females.

Report:	KCA 5.8.2/02[redacted]; 1989; M-160331-01-1
Title:	A maximum 13-week dietary toxicity study of fosetyl-Al in the albino rat with a maximum 21 week recovery period
Report No.:	R001329
Document No.:	M-160331-01-1
Guideline(s):	not specified
Guideline deviation(s):	not applicable
GLP/GEP:	**yes**

Executive Summary

This mechanistic study was undertaken to investigate the effects of high doses of fosetyl-aluminium (fosetyl-Al) on the urinary system that had been observed in the 2-year rat study ([redacted]; 1981; M-249664-02-1). To this end, 4 groups each of 70 male and 70 female Sprague-Dawley rats were dosed (via the diet) with fosetyl-Al at levels of 0, 8000, 30 000 or 50 000 ppm for periods of up to 13 weeks. Up to 10 rats/sex/group were killed after 2, 4, 8 or 13 weeks of treatment. A further 20 rats/sex/group were used for 8 weeks after which 10 rats/sex/group were allowed 8 weeks recovery and then killed while the remaining animals were killed after 16 weeks recovery. Finally, up to 10 rats/sex/group were dosed for 13 weeks, allowed 21 weeks of recovery and were then killed. The animals were observed for clinical signs of reaction to treatment, body weight, food and water consumption were measured, blood, urine, faeces and urinary calculi were analysed and finally all animals subject to gross pathological examination and subsequent histopathological evaluation of kidneys, ureters, urinary bladder and thyroids (with parathyroids).

Mortality: Ten high-dose males and 3 mid-dose males died or were killed in extremis. This occurred intermittently throughout the study. Clinical signs consisted of marked diuresis, red/brown staining of abdominal fur, abdominal distension, weakness, skin pallor and hypothermia. Gross pathology showed obvious urolithiasis and dilatation of kidney pelves, ureters and urinary bladder while histopathology findings revealed hydronephrosis and transitional cell hyperplasia in the kidneys and papillary hyperplasia in the bladder.

Clinical signs: Marked diuresis and red/brown staining of abdominal fur were observed in high-dose animals and similar but less marked effects in mid-dose animals. Abnormal food consumption was also seen in some high-dose animals. These signs tended to subside somewhat as dosing progressed but persisted in high-dose animals while treatment continued. When dosing ceased, only the abdominal fur staining continued. These signs were not observed in low-dose animals.

Body weight: Reduced weight gain occurred in high-dose animals, which was not recouped during subsequent recovery periods lasting up to 21 weeks. Similar but less marked effects were observed in mid-dose males, but body weight recovered to normal when dosing ceased. These changes were not observed in mid-dose females or low-dose animals.

Food consumption: Consistently reduced food intake was observed in high-dose animals and initially in mid- and low-dose males. Such reactions persisted in high-dose animals while treatment continued, but food consumption rapidly returned to normal when dosing ceased. Mid- and low-dose females did not show these changes.

Achieved intake of fosetyl-Al: Calculated mean achieved doses of fosetyl-Al were 600, 2300 or 3900 mg/kg bw/day for low-, mid- and high-dose groups, respectively.

Water consumption: High-dose animals showed a marked increase in water consumption which persisted during treatment and recovery periods. A similar effect in mid-dose males persisted only as long as dosing continued. No such effects were seen in mid-dose females and low-dose animals.
Haematology: No obvious adverse changes were seen with the possible exception of increased MBC, Hb, and Hct in high-dose animals which may have been a result of the marked diuresis shown by these rats.
Clinical chemistry: Uraemia occurred in all high-dose animals which persisted during treatment and recovery periods with some recovery in females when dosing ceased. Decreased protein and albumin levels were observed in some animals during treatment with apparent recovery thereafter. Increased phosphorus and carbonate levels followed the same pattern. Similar but less marked effects were observed in mid-dose animals. Low-dose animals showed no adverse changes.
Urinalysis: Increases in urine volume and decreases in specific gravity and pH were seen in high-dose animals. Urine electrolytes were also reduced (except for calcium and aluminium which were increased) in these animals. These effects reversed when dosing ceased. Similar but less marked effects were observed in mid-dose animals. No such changes were observed in low-dose animals except for the change relating to urine pH.
Faecal analysis: With the exception of an initial increase in males, calcium levels in high-dose animals were reduced during treatment and returned to normal during the recovery period. Phosphorus levels were initially increased in high dose animals. Aluminium levels were increased in this group throughout the dosing period but returned to normal during the recovery period. In mid-dose animals, similar but less marked changes were seen in aluminium levels. In low-dose animals, the only change observed was increased aluminium levels and only during the treatment period.
Urinary calculi analysis: Urinary calculi, available only from high and mid-dose animals showed high calcium and phosphorus content, and low aluminium content.
Organ Weights: Increased absolute kidney weights were seen in high-dose animals and mid-dose males. No other significant changes were observed.
Gross and histopathological findings: The major gross pathological find was marked urolithiasis in the bladder, ureters and kidneys of high-dose animals and mid-dose males within two weeks of the start of dosing. The phenomenon tended to subside slightly as dosing progressed but even after a 21 week recovery period small uroliths were still present in the folds of the urinary bladder and the kidneys. Such effects were uncommon in high- and mid-dose females and all low-dose animals. Histopathological findings consisted of papillary hyperplasia in the bladder epithelium of the high- and mid-dose males treated for 12-13 weeks, although such finding significantly decreased in animals from the same groups which were subsequently allowed a recovery period of up to 21 weeks. Hydronephrosis, pyelitis, pyelonephritis, papillary necrosis, dilatation of collecting tubules and transitional cell hyperplasia of the pelvis was seen in high-dose animals and mid-dose males from treatment week 2 onwards and persisted throughout dosing and recovery periods. Dilation of ureters with occasional ureteritis or transitional cell hyperplasia was seen in the same groups. The histopathological changes in the kidneys and ureters are considered directly related to the urolithiasis which occurred in these same groups.
Conclusion: The NOAEL in this study was 8000 ppm equivalent to 500 and 600 mg/kg bw/day in males and females, respectively.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material:

Name:	Fosetyl-Al
Description:	Fine white powder
Batch / Lot No.:	DA497
Purity:	97.3%
Stability of test compound:	Stability and homogeneity in diet were analytically verified.
2. Vehicle:	Plain diet

3. Test animals

Species:	Rat
Strain:	Sprague-Dawley, Crl:CD(SD)BR
Sex:	Males and females
Age:	6 weeks
Weight at dosing:	Males: 165-229 g Females: 123-179 g
Source:	[redacted] Canada.
Acclimatisation period:	15 days
Diet:	Purina Certified Rodent Chow #5002 *ad libitum*
Water:	Water, purified by reverse osmosis and ultraviolet sterilization *ad libitum*
Housing:	Individually in standard stainless steel cages
Environmental conditions:	
Temperature:	21±3°C
Humidity:	50±20%
Air changes:	Not reported
Photoperiod:	12 h light / 12 h dark

B. STUDY DESIGN AND METHODS

1. In-life dates
1987-08-12 to 1988-04-07

2. Animal assignment and treatment
Animal assignment and dose groups:
Two days after arrival, the rats were randomly assigned to each of the 2 treatment groups and 1 control group using a computer-based number generator. Male and female animals were randomized separately according to individual body weights. Rats in poor health or at the extremes of the body weight range were not assigned to the study. The following dose groups were employed:

Table 5.8.2.3: Group allocation and mean substance intake in the mechanistic feeding study in rats

Group no.	Conc. in diet [ppm]	Exposure duration (weeks)	Recovery period (weeks)	No. of animals		Mean daily substance intake [mg/kg bw/day]	
				Male	Female	Male	Female
1	0	4	–	10	10	0	0
			4	10	10		
		8	–	10	10		
			8	10	10		
			16	10	10		
		13	–	10	10		
			21	10	10		
2	8000	4	–	10	10	817	849
			4	10	10	725	804
		8	–	10	10	616	718
			8	10	10		
			16	10	10		
		13	–	10	10	451	567
			21	10	10		

Group no.	Conc. in diet [ppm]	Exposure duration (weeks)	Recovery period (weeks)	No. of animals		Mean daily substance intake [mg/kg bw/day]	
				Male	Female	Male	Female
3	30 000	2	–	10	10	3118	3208
		4	–	10	10	2805	3001
		8	–	10	10	2405	2724
			8	10	10		
			16	10	10		
		13	–	10	10	1771	2167
			21	10	10		
4	50 000	2	–	10	10	4590	5217
		4	–	10	10	4204	5014
		8	–	10	10	3845	4585
			8	10	10		
			16	10	10		
		13	–	10	10	3180	3880
			21	10	10		

Diet Preparation and Analysis:
Diets for each treatment group were prepared separately each week. When not in use, prepared diets were stored at room temperature.
Stability of the test diets was evaluated after storage for 10 days at room temperature. The concentrations of the test article in the diet were analysed after preparation during weeks 1, 2, 3, 6 and 13. The results indicated good stability and homogeneity of test article in the diet mixtures. Dietary concentrations were also considered satisfactory.

Details on oral exposure:
Duration of exposure: 2, 4, 8, or 13 weeks (see Table 5.8.2- 3)
Frequency of treatment: Via diet, *ad libitum*
Recovery period: 8-week exposure: 8 or 16 weeks
13-week exposure: 21 weeks (see Table 5.8.2- 3)

3. Examinations

Clinical signs - mortality and moribundity: All rats were examined twice daily for clinical signs and mortality.

Body weights: Body weights were recorded weekly.

Food and water consumption: Food consumption was recorded weekly.
Individual water consumption was measured during Weeks 3, 7, and 13 (15 rats/sex/dose); during Week 15 (10 rats/sex/dose from the 8wk + 16wk recovery group); during Week 24 over a 6-day period (5 rats/sex/dose from the 8wk + 16wk recovery group) and during Week 13 (10 rats/sex/dose from the 13wk + 21wk recovery groups).

Blood / urine / faeces collection: Prior to treatment, laboratory investigations (haematology, clinical chemistry, urinalysis and faecal assay) were performed on 5 male and 5 female randomly selected animals.
During / after Weeks 2, 4, 8 and 13, haematology and clinical chemistry investigations, urinalysis, and faecal assays were performed on 5 males and 5 females from each study group.
During / after Week 16, haematology and clinical chemistry investigations, urinalysis, and faecal assays were performed on 5 males and 5 females from each group. These animals had received 8 weeks of treatment and then been allowed 8 weeks recovery.
After Week 24, haematology and clinical chemistry analyses on 10

	males and 10 females from each group and urinalysis and faecal investigations on 5 males and 5 females from each study group was performed on animals which had received 8 weeks of treatment and then been allowed 16 weeks recovery. At the beginning of Week 35, haematology and clinical chemistry analyses on 10 males and 10 females from each study group and at the end of week 34, urinalysis and faecal assays on 5 males and 5 females from each study group was performed on animals which had received 13 weeks of treatment and then been allowed 13 weeks recovery. On each occasion, urine and faecal collections were performed first and then blood samples were collected at necropsy. Food was removed overnight from animals to be sampled for haematology and clinical chemistry. Blood samples were obtained from the abdominal aorta immediately following ether anaesthesia. On each occasion, 24-h urine samples were collected from individual animals which were water loaded with 5 mL/kg bw/day during Week 34) of tap water and then placed in metabolism cages. During this 24-h period for Weeks 2, 3, 8 and 13, the animals had access to food and water. For weeks 16, 24 and 35, the animals did not have access to food. Faecal samples were also collected during this period.
Haematology	The following parameters were examined: Erythrocyte count, Hct, Hb, total and differential leukocyte count, platelet count, Wintrobe's constants, blood smear
Clinical chemistry	The following parameters were examined: Blood urea, total protein, bilirubin, glucose, albumin, sodium, potassium, calcium, total carbon dioxide, inorganic phosphorus, aluminium
Urinalysis	The following parameters were examined: Colour, appearance, volume, specific gravity, pH, glucose, ketones, blood, protein, urobilinogen, sodium, potassium, calcium, total phosphorus, aluminium, oxalate, nitrite and microscopic examination of the centrifuged deposit. Dosing the animals with high levels of fosetyl-Al induced urinary calculi (bladder stones). At the 4 week interim sacrifice, such stones were collected from 5 males which had received 30 000 ppm and 4 males and 1 female which had received 50 000 ppm. These calculi were then assayed.
Faecal analysis	The following parameters were examined: aluminium, calcium, phosphorus, magnesium
Gross pathology	Gross pathological examination was carried out on all rats at scheduled sacrifice (except for high dose males at w-13 of which 4 were allowed to continue for a recovery period) and all decedent rats.
Organ weights	Thyroid, parathyroid and kidney weights were determined.
Histopathology	Histopathological examination was carried out on the urinary bladder, kidney, ureters, thyroid including parathyroid from all rats.

Statistical evaluation

Numerical data obtained during the conduct of the study was subjected to calculation of group mean values and standard deviation. When appropriate, the data was analysed for homogeneity of variance using Bartlett's test. Homogeneous data was analysed using Analysis of Variance and the significance of inter-group differences was assessed using Dunnett's 't' test. Heterogeneous data was analysed using Kruskal –Wallis test and the significance of inter-group differences was assessed using Dunn's test.

II. RESULTS AND DISCUSSION

A. MORTALITY

Ten males from the 50 000-ppm group and 3 males from the 30 000-ppm group died or were killed in extremis; clinical signs prior to deaths included weakness, tremor, abdominal distension and red/brown staining of the abdominal region; major gross pathological findings consisted of calculi and dilatation of the urinary system (kidney, ureters and urinary bladder); histopathological findings included urolithiasis in the bladder, hydronephrosis, papillary necrosis, and transitional cell hyperplasia in the kidney and occasionally in the ureters.

No deaths occurred in the 8000-ppm group.

B. CLINICAL OBSERVATIONS

Major findings were marked diuresis and red/brown discoloured urine which caused wetness and staining of the abdominal fur:

- During the first 2 weeks of treatment, these findings occurred in approx. 50% of high dose males and 25% of the high dose females of which several more exhibited yellow staining in the urogenital and/or ventral area. These findings and were also seen in many of the mid-dose males and occasionally in the mid-dose females. No such changes were seen in the low-dose group.
- Abdominal wetness, red/brown and/or yellow fur staining persisted during Weeks 3 and 4 and also, to a much lesser extent during Weeks 5 through 8.
- Similar findings were seen in high- and mid-dose males during recovery periods extending through Weeks 9-13 and on Weeks 14-24 and in high-dose rats during recovery period extending on Weeks 25-34.

C. BODY WEIGHT

Body weights were markedly reduced in high-dose rats (both sexes), and also but less markedly in mid-dose males during the treatment period; after cessation of treatment, high-dose rats exhibited improved body weight gain, but their body weights were lower than controls at end of the various recovery periods (up to 21 weeks) (see Table 5.8.2- 4).

D. FOOD AND WATER CONSUMPTION

Reduced food consumption was recorded in high dose rats during the treatment period and also in the mid dose males during the first 6w of treatment; after cessation of treatment, food consumption improved and was not different from controls from Week 14 to study termination (see Table 5.8.2- 4).

The mean test substance intake was 500/600, 2100/2500, and 3500/4200 mg/kg bw/day (♂/♀) for the 8000; 30 000 and 50 000 ppm dose groups, respectively. Substance intakes by duration of treatment for each dose group are given in Table 5.8.2- 3.

Water consumption was significantly increased in high dose rats (both sexes) and in mid-dose males during the treatment period; after cessation of treatment, high dose males still exhibited statistically significant higher water consumption than controls (increased values seen in females were not significantly different from controls).

Table 5.8.2- 4: Mean body weight, mean food and water consumption among study groups

Week	Controls		8000 ppm				30 000 ppm				50 000 ppm			
	M	F	M(1)	M(2)	F(1)	F(2)	M(1)	M(2)	F(1)	F(2)	M(1)	M(2)	F(1)	F(2)
Body weight (g)														
0	200.8	146.0	199.8		145.5		198.4		146.2		197.7		144.1	
4	380.4	222.4	378.0		223.2		344.8♣		216.6		259.1♣		181.5♣	
8	470.0	255.0	479.1		260.1		437.2♣		253.4		387.1♣		206.7♣	
16	560.5	287.3	558.1	573	285.2	291.2	528.6	539.9	288.7	289.1	466.6♣	411.7♣	264.0♣	252.4♣
24	623.0	318.4	615.7	636.3	322.0	330.7	611.6	616.9	315.6	320.4	541.3♣	552*	292.1	293.1♣
34	662.4	345.4	-	656.1	-	356.2	-	654.1	-	342.7	-	579.7♣	-	320.8
Food consumption (g/rat)														
0	160.8	125.5	160.4		129.9		160.4		125.3		159.7		122.9♣	
4	202.8	142.9	190.7*		141.7		184.6♣		135.2♣		119.3♣		116.8♣	
8	186.9	130.6	186.7		131.7		177.8♣		132.0		129.8♣		115.4♣	
16	178.8	130.6	175.5	197.4*	124.6	129.9*	166.8	183.7	125.4	125.5	153.4	186.9	114.3	117.0
24	176.5	122.1	169.3	185.9	125.0	135.0	174.4	193.7	115.8	129.1♣	163.8	192.2	126.5	131.7
34	160	119.4	-	155.0	-	120.2	-	167.0	-	118.1	-	156.1	-	122.3
Water consumption (g/rat/day)														
3	37.1 ±4.68	30.8 ±4.59	40.5 ± 7.45		28.4 ± 3.82		55.3 ± 9.74♣		34.1 ± 7.38		50.6 ± 7.68♣		47.8 ± 10.08♣	
7	37.1 ±5.98	32.2 ±5.34	40.0 ± 5.11		29.8 ± 7.21		52.9 ± 11.22♠		31.4 ± 8.92		62.8 ± 14.79♠		49.8 ± 14.93♣	
12	33.7 ±7.13	32.1 ±6.19	36.7 ± 5.08		30.1 ± 7.16		46.6 ± 5.53♠		34.4 ± 9.80♠		69.9 ± 14.87♠		51.8 ± 6.71♣	
15	40.6 ±9.00	32.7 ±7.90	37.7 ± 6.10		31.1 ± 4.76		49.9 ± 5.57		31.8 ± 6.13		49.5 ± 7.64*		40.4 ± 6.57♣	
24	31.3 ±3.68	27.6 ±6.23	31.1 ± 4.80		30.7 ± 8.42		35.7 ± 6.88		23.0 ± 4.25		41.4 ± 11.4		34.1 ± 11.1	
34	32.9 ±4.90	30.5 ±6.17	33.3 ± 11.03		34.7 ± 12.31		39.5 ± 7.55		33.8 ± 9.98		51.1 ± 8.47♣		51.3 ± 21.44	

(1) : groups administered 8 weeks of treatment ± recovery ; (2) groups administered 13 weeks of treatment ± recovery
* : p< 0.05; ♣: p< 0.01; ♠: p<0.001

E. HAEMATOLOGY AND CLINICAL CHEMISTRY

Haematological changes (significant increase of RBC, Hb, Hct in high dose males and of platelets in high-dose females during the first weeks of treatment) were within normal range and may have been related to diuretic effects of test substance. No significant changes were seen among mid- and low-dose rats.

Major clinical-chemical changes (see Table 5.8.2- 5) consisted of:

- Increased BUN levels in high dose rats which persisted during the recovery periods (only in males after the 16- and 21-week recovery periods)
- Increased phosphorus levels in high dose rats and mid dose males, which persisted in high dose males after 13 weeks recovery and returned to normal values thereafter.
- Reduced protein and albumin levels in high dose rats, which persisted after 13-week recovery period and returned to control range thereafter; such changes were also seen in mid dose rats during the first 4 weeks of treatment.
- Other changes consisted of slightly increased levels of carbon dioxide in high dose and mid dose rats, minor reductions of calcium levels in high and/or mid dose rats.

Table 5.8.2- 5: Serum clinical chemistry parameters in various study groups

Study week	Controls		8000 ppm		30 000 ppm		50 000 ppm	
	M	F	M	F	M	F	M	F
BUN (mg/dL)								
0	14.8±1.64	15.4±1.14						
8	13.6±2.07	14.0±1.58	14.0±1.87	16.2±2.49	14.8±14.48	14.8±1.[illegible]	23.0±4.00♣	26.0±2.83♠
13	14.0±1.41	15.4±1.34	13.4±2.61	15.2±1.10	17.2±2.77	16.8±3.77	29.2±6.76♣	22.2±5.72*
8+16	11.0±1.94	12.3±1.83	11.0±1.15	12.1±1.85	11.9±1.60	12.[illegible]±1.91	15.4±2.97♣	13.6±1.67
13+21	12.7±1.7	15.9±2.23	42.9±94.42	16.1±2.77	14.4±2.07	15.0±2.00	20.7±4.90♣	22.2±6.39*
Calcium (mg/dL)								
0	9.9±0.32	10.0±0.26						
8	9.9±0.34	9.9±0.25	10.1±0.33	10.0±0.11	9.9±0.22	10.2±0.25	9.3±0.16*	10.0±0.40
13	10.0±0.35	9.9±0.35	9.8±0.22	9.9±0.30	909±0.31	10.1±0.62	9.1±0.68	9.5±0.30
8+16	10.1±0.21	10.4±0.32	10.1±0.33	10.7±0.46	10.[illegible]±0.18*	10.5±0.35	9.2±0.11	10.1±0.42
13+21	10.0±0.22	10.9±0.28	908±0.56	11.0±0.42	10.9±0.[illegible]	10.[illegible]±0.39	9.9±0.39	10.9±0.31
Phosphorus (mg/dL)								
0	9.8±0.55	9.2±0.42						
8	7.7±0.52	6.5±1.26	7.4±0.58	6.6±0.[illegible]	8.4±0.65	6.6±0.65	9.2±0.[illegible]♣	9.2±1.16♣
13	6.9±0.38	6.0±0.98	[illegible]±0.2[illegible]	6.0±0.79	7.8±1.[illegible]	6.[illegible]±0.98	9.5±0.34♣	8.3±0.91♣
8+16	5.9±0.53	5.5±0.96	5.7±0.62	5.3±1.09	6.5±0.59*	5.4±0.[illegible]	6.[illegible]±0.33*	6.0±1.07
13+21	5.7±0.63	5.3±0.74	7.3±5.24	5.3±0.[illegible]	5.[illegible]±0.65	5.4±0.51	5.8±0.57	6.1±1.87
Total protein (g/dL)								
0	5.5±0.11	5.8±0.[illegible]						
8	6.7±0.41	6.[illegible]±0.26	6.6±0.[illegible]	6.[illegible]±0.56	6.6±0.27	6.6±0.[illegible]	5.[illegible]±0.19♣	6.2±0.22
13	6.8±0.43	7.0±0.2[illegible]	6.9±0.15	7.1±0.[illegible]	7.[illegible]±0.26	7.2±0.61	6.3±0.27*	6.4±0.23
8+16	7.0±0.40	7.5±0.[illegible]	7.[illegible]±0.23	7.5±0.60	6.8±0.[illegible]	7.2±0.4[illegible]	6.6±0.18*	7.4±0.34
13+21	7.1±0.3[illegible]	8.0±0.43	7.1±0.[illegible]	[illegible]±0.45	7.0±0.28	7.9±0.67	6.9±0.21	7.8±0.61

* p<0.05; ♣: p<0.01 ♠: p<0.001

F. URINALYSIS

Most of the **urinalysis** changes were seen in the high- and mid-dose rats (see Table 5.8.2- 6):

- Urine volume was significantly increased in high dose males and/or females during treatment periods and was still increased in high dose males after 16 weeks recovery.
- Specific gravity was reduced in high dose rats during treatment periods and returned to normal values thereafter.
- pH was also reduced in high dose and / or mid dose rats and returned to normal values thereafter
- Increased calcium levels and decreased sodium, potassium and phosphorus levels were seen in high and mid dose males and/or females during the treatment periods and returned to control range during recovery.
- No significant changes were seen in the low dose group and after the 21-week recovery period.

Table 5.8.2- 6: Urine analysis among various groups

Study week	Controls		8000 ppm		30 000 ppm		50 000 ppm	
	M	F	M	F	M	F	M	
Volume (mL)								
0	9.8±3.56	10.0±2.99						
8	19.0±5.66	17.0±6.3	19.0±1.6	25.4±12.6	19.8±6.6	15.9±4.[illegible]	42.8±9.4♣	28.7±[illegible]46
13	19.1±5.13	14.9±4.07	15.9±5.3	16.7±8.3	26.0±7.9	18.2±8.9	43.6±5.3♣	24.[illegible]6.5
8+16	25.5±12.6	14.9±8.2	27.0±7.9	11.1±1.9	19.8±5.3	17.[illegible]7.2	22.0±4.9	15.[illegible]20±5.6
13+21	20.8±2.9	19.3±8.0	16.0±5.9	20.0±7.6	25.0±4.2	[illegible].1±5.3	28.4±6.8	18.5±[illegible]
pH								
4	7.2±0.27	7.3±0.29	6.8±0.27	7.3±0.[illegible]7	5.1±0.22♣	5.3±0.27♣	5.0±0.0♣	5.2±0.45♣
8	7.4±0.22	7.7±0.22	6.9±0.22*	7.0±0.[illegible]5	5.8±0.45	5.3±0.27♣	5.0±0.00♣	5.0±0.00♣
13	7.2±0.27	7.7±0.22	6.7±0.27*	6.8±0.29*	6.0±0.35♣	5.[illegible]±0.55♣	5.0±0.00♣	5.0±0.00♣
8+8	6.8±0.27	6.2±0.27	6.9±0.22	6.[illegible]±0.96	6.9±0.22	7.3±0.91	6.5±0.00	6.4±0.65
13+21	7.1±0.22	6.9±1.24	6.8±0.27	6.7±0.[illegible]	7.[illegible]±0.87	6.6±0.65	6.8±0.27	6.[illegible]±.65
Calcium (mg/dL)								
0	13.3±5.43	10.0±5.15						
8	10.6±12.9	14.6±7.1	10.7±[illegible].6	11.9±3.[illegible]	181.[illegible]±17.6	91.7±[illegible]1*	64.5±7.9♣	73.7±[illegible]8.9♣
13	5.0±1.9	15.5±8.0	6.[illegible]±3.0	20.2±[illegible].3	15.[illegible]±6.2♣	45.3±[illegible].5*	37.6±[illegible]♣	70.[illegible]±12.1♣
8+16	4.3±2.8	15.5±11.2	[illegible]±2.6	6.6±2.3	[illegible].8±1.[illegible]	[illegible].6±3.1	3.4±[illegible].5	11.3±7.0
13+21	5.3±0.6	15.9±11.8	9.0±3.[illegible]	2[illegible]±5.9	5.[illegible]±1.9	[illegible]2.3±11.[illegible]	[illegible]±1.5	14.9±6.6
Sodium (meq/L)								
0	146.8±49.62	123.6±16.92						
8	139.0±57.4	105.4±[illegible].2	161.6±17.7	67.4±21.9	133.0±[illegible].0	124±30.4	40.0±[illegible].5♣	45.6±8.3♣
13	47.8±20.2	112.2±12.8	183.2±4[illegible]	168.0±44.6	65.0±[illegible].3	117.6±5[illegible].0	44.4±6.1	70.8±20.7
8+8	25.0±9.9	29.0±9.7	28.2±[illegible].0	31.4±14.5	38.0±10.6	32.8±[illegible].8	29.8±13.0	30.6±15.3
13+21	42.6±9.2	28.4±9.[illegible]	30.4±9.9	30.8±[illegible].6	[illegible].6±12.2	37.[illegible]±15.9	35.4±10.0	33.4±10.9
Potassium (meq/L)								
0	338.0±89.[illegible]	291.8±38.97						
8	291.8±[illegible].5	23[illegible].8±72.5	271.[illegible]±37.2	131.2±3[illegible]♣	201.[illegible]±37.3*	205.8±51.7	65.2±9.9♣	69.2±20.4♣
13	141.8±[illegible].1	241.6±[illegible].6	182.8±87.4	192.6±[illegible]4.2	115.8±5[illegible].6	19[illegible]±95.5	71.8±8.5	95.4±27.8♣
8+16	82.[illegible]±29.3	70.0±29.7	[illegible].8±22.[illegible]	76.4±14.0	100.6±16.7	67.4±27.2	83.5±13.5	90.2±41.6
13+21	107.2±19.[illegible]	8[illegible]±30.6	116.2±28.1	[illegible]28±14.[illegible]	94.[illegible]±13.8	83.0±24.0	88.8±20.0	37.4±14.6
Phosphorus (mg/dL)								
0	56.4±14.37	[illegible]0.4±[illegible]2						
8	108.6±23.0	80.2±[illegible].5	89.8±14.6	51.[illegible]±28.4	18.8±[illegible].5	23.3±13.2	1.3±0.35♣	1.3±0.35♣
13	56.9±[illegible].8	96.[illegible]±36.6	67.2±[illegible].5	61.9±16.[illegible]	25.[illegible]±20.6	28.2±13.9	1.1±0.28♣	1.5±0.58♣
8+16	74.3±[illegible].6	86.2±35.[illegible]	71.[illegible]±19.5	[illegible]89.8±13.1	[illegible]6.0±13.7	61.4±8.4	69.6±5.3	99.4±49.7
13+21	89.8±13.9	[illegible]00.8±[illegible].9	12[illegible].4±26.[illegible]*	77.[illegible]±16.9	[illegible]80.8±14.7	86.9±26.5	72.4±11.4	95.2±21.4

* $p<0.05$; ♣: $p<0.01$; ♠: $p<0.001$

G. FAECAL ANALYSIS

Analysis of **faeces** showed decreased calcium levels and increased phosphorus and aluminium levels in high-dose rats which returned to control range during recovery; such changes were occasionally seen in the mid-dose rats (see Table 5.8.2- 7).

Table 5.8.2- 7: Faecal analysis among various groups

Study week	Controls		8000 ppm		30 000 ppm		50 000 ppm	
	M	F	M	F	M	F	M	
Calcium (mg/g)								
0	35.97±5.61	37.23±6.8						
8	40.1±10.23	42.92±3.80	38.02±4.78	3.59±3.5	34.83±4.89	33.28±4.2♣	22.46±4.04*	24.96±3.58♣
13	29.33±12.78	37.52±2.48	31.13±6.1	33.27±7.23	29.51±7.34	29.76±8.19	24.64±3.65	26.3±4.04*
8+16	41.97±4.57	45.88±4.59	40.8±5.48	39.29±6.65	44.06±5.64	40.[illegible]93	36.92±[illegible]83	38.22±6.84
13+21	53.59±9.57	50.63±3.87	37.97±7.56	56.18±14.0	49.61±18.0	61.79±7.74	44.6[illegible]±6.27	64.99±[illegible]1
Phosphorus (mg/g)								
0	14.99±1.04	12.21±3.05						
8	15.74±6.25	20.02±2.08	17.89±1.30	22.21±[illegible]76	21.17±8.[illegible]	23.66±12.19	22.74±9.83	31.[illegible]4±1.7♣
13	10.71±10.48	19.75±1.64	20.38±8.37	14.6[illegible]87	24.21±12.9	20.[illegible]8±11.[illegible]	22.9[illegible]±11.7	21.66±9.6
8+16	21.15±2.81	20.07±3.30	18.87±2.55	21.[illegible]5±4.72	21.6[illegible]±3.46	23.63±1.82	21.44±8.[illegible]	24.9[illegible]±4.54
13+21	26.11±2.57	24.44±1.66	21.47±5.7	27.43±[illegible]5	27.[illegible]2±6.3[illegible]	30.2[illegible]±[illegible].69	2[illegible].92±1.[illegible]	29.[illegible]±4.38
Aluminium (mg/g)								
0	0.49±0.08	0.25±0.11						
8	0.409±0.22	0.42±0.14	3.41±[illegible].44	3.[illegible]3±.42	9.[illegible]5.17	12.25±[illegible]86	12.76±6.74♣	18.5[illegible]2.73♠
13	0.27±0.30	0.56±0.11	1.7[illegible]2.07	2.82±[illegible]3	6.[illegible]99±4.92	9.3[illegible]±7.28	4.22±[illegible]64	1[illegible]±7.76
8+16	0.55±0.04	0.59±0.09	0.[illegible]±0.0[illegible]	0.50±0.11	0.45±0.[illegible]	0.[illegible]6±0.03	0.32±0.21	0.54±0.05
13+21	0.58±0.07	0.63±0.29	0.58±0.[illegible]9	0.[illegible]±0.20	0.66±0.13	0.[illegible]76±0.[illegible]	0.[illegible]±0.49	0.67±0.13

* p<0.05; ♣: p<0.01 ;♠: 0.01<p<0.001

H. PATHOLOGY

Relative and/or absolute kidney weights were increased in high dose rats and also in mid dose males; such changes were also seen in high dose rats sacrificed at end of the various recovery periods. Changes in absolute and/or relative thyroid weights were seen in high dose rats during treatment periods are were considered as fortuitous.

Major gross pathology findings were related to the presence of calculi in the urinary system: uroliths occurred in the kidney pelvis and in the urinary bladder of mainly high dose rats and mid dose males (greater number and size in the urinary bladder, particularly after 2 weeks of treatment which declined progressively thereafter).

Major histopathological findings were related to the urolith phenomenon (see Table 5.8.2- 8):

In the **urinary bladder**, papillary hyperplasia occurred mostly in high-dose rats and mid-dose males and was already seen after 2 weeks of treatment. At end of the various recovery periods, hyperplasia was significantly reduced.

In the **kidney**, a high incidence of hydronephrosis was found in high dose rats and mid dose males and was considered to have caused pyelitis, pyelonephritis, papillary necrosis, dilatation of collecting tubules and transitional cell hyperplasia in the pelvic zone. In addition, a benign transitional cell papilloma was found in one high dose male which died on Week 22 (8 weeks of treatment + 16 weeks recovery group).

In the **ureters**, dilatation (occasionally accompanied by ureteritis or transitional cell hyperplasia) was seen in high dose rats and mid dose male and declined only slightly during the recovery periods.

Table 5.8.2- 8: Histopathological data

Organ (no. of rats examined)	Male Dose (ppm)				Female Dose (ppm)			
	0	8000	30 000	50 000	0	8000	30 000	50 000
URINARY BLADDER (n=10)								
Uroliths								
Week 2	0	0	5	3	0	0	0	0
Week 13	0	0	6	6 a	0	0	0	0
+ 21 weeks of recovery	1	0	2 b	3 c	0	0	0	1
Papillary hyperplasia								
Week 2	0	0	7	8	0	0	0	4
Week 13	0	0	5	6 a	0	0	0	1
+ 21 weeks of recovery	0	0	0 b	1 c	0	0	0	1
KIDNEY (n=10)								
Uroliths								
Week 2	0	0	1	4	0	0	0	7
Week 13	0	0	5	6 a	0	0	2	9
+ 21 weeks of recovery	0	0	0 b	2 c	0	2	1	6
Chronic interstitial nephritis								
Week 2	1	1	4	8	1	0	0	5
Week 13	0	0	2	6 a	0	0	1	10
+ 21 weeks of recovery	3	3	5 b	9 c	0	0	0	10
Hydronephrosis								
Week 2	0	1	3	5	0	2	0	3
Week 13	2	0	3	6 a	0	0	1	9
+ 21 weeks of recovery	2	0	4 b	9 c	1	0	1	9
Transitional cell hyperplasia								
Week 2	0	0	0	1	0	0	0	3
Week 13	0	0	0	6 a	0	0	0	8
+ 21 weeks of recovery	1	0	1 b	9 c	1	0	0	4
Tubular dilatation								
Week 2	0	0	0	0	0	0	0	0
Week 13	0	0	0	2 a	0	0	0	0
+ 21 weeks of recovery	0	0	1 b	3 c	0	0	0	1
URETERS (n=10)								
Dilatation								
Week 2	0	0	2	9	0	0	0	2
Week 13	0	0	2	6 a	1	0	1	8
+ 21 w of recovery	1	1	4 b	6 c	1	1	2	7

(a): 9 animals instead of 10 were examined; (b): 6 animals instead of 10 were examined; (c) 8 animals instead of 10 were examined.

III. CONCLUSION

RMS conclusion: Dietary administration of 50 000 ppm and possibly 30 000 ppm in rats for up to 13 weeks induced some mortality, marked diuresis, reduction in food consumption and in bw gain, increased water consumption, increased BUN, phosphorus and calcium levels, decreased serum total protein; increased urine volume and decreased urine pH, specific gravity and electrolytes except calcium which was increased; all these changes, except bw, occurred shortly after initiation of treatment and reverse more or less completely during the recovery periods.
Urolithiasis, which occurred in high-dose rats and in mid-dose males, was then considered to cause irritation of the urinary tract and subsequent papillary hyperplasia which was partly reversible after cessation of treatment.
The NOAEL was 8000 ppm equivalent to 500 and 600 mg/kg bw/day in males and females, respectively.

CA 5.8.3 Endocrine disrupting properties

Designated studies on endocrine disrupting (ED) properties of fosetyl-Al have not been conducted by the applicant. The existing body of data is sufficient to exclude relevant ED-like potential of fosetyl-Al. This is based on the absence of effects on the weight and histopathological appearance of hormone-sensitive tissues like reproductive organs, thyroids and pituitary. In addition, the available fertility studies showed no effects on male or female fertility, which may be considered sensitive targets of ED-like activity.
There are **currently no scientific criteria for classification as ED**. However, the **interim criteria for ED** classification laid down in Regulation 1107/2009 **are not fulfilled**, since fosetyl-Al is neither classified as Carc 2 and/or Repr 2 nor does it fulfil the criteria for such a classification.

CA 5.9 Medical data

CA 5.9.1 Medical surveillance on manufacturing plant personnel and monitoring studies

For information on medical surveillance on manufacturing plant personnel and monitoring studies (M-527602-01-1) please refer to the CONFIDENTIAL part (Document JCA) because information on manufacturers is confidential.

CA 5.9.2 Data collected on humans

No human poisoning cases have been published.

CA 5.9.3 Direct observations

No human poisoning cases have been published.

CA 5.9.4 Epidemiological studies

No epidemiological studies have been published.

CA 5.9.5 Diagnosis of poisoning (determination of active substance, metabolites), specific signs of poisoning, clinical tests

Fosetyl-aluminium is an ethylphosphonate of low toxicity that does not inhibit cholinesterase!

Signs and Symptoms of Poisoning:
There are no reports on human cases.
In animal experiments symptoms have only been reported from chronic high dose studies.
Diuresis and a calcium decrease in urine cannot be ruled out for high dose ingestions.

CA 5.9.6 Proposed treatment: first aid measures, antidotes, medical treatment

First Aid:

- Remove patient from exposure/terminate exposure.
- Thorough skin decontamination with copious amounts water and soap, if available with polyethylene glycol 300 followed by water.
  Note: Most formulations with this active ingredient can be decontaminated with water (and soap) so for formulations polyethylene glycol 300 is not required.
- Flushing of the eyes with lukewarm water for 15 minutes.
- Induction of vomiting does not seem to be required in regard of the low toxicity. It should only be considered if a large amount has been swallowed, if the ingestion was less than one hour ago, and if the patient is fully conscious.
  Induced vomiting can remove maximum 50% of the ingested substance.
  Note: Induction of vomiting is forbidden, if a formulation containing organic solvents has been ingested!

Treatment:

- Gastric lavage does not seem to be required in regard of the low toxicity of the compound.
- The application of activated charcoal and sodium sulphate (or other carthartic) might be considered in significant ingestions.
- As there is no antidote, treatment has to be symptomatic and supportive.
  Atropine shall not be given.

CA 5.9.7 Expected effects of poisoning

No persisting effects are to be expected.