Document Title

Summary of the toxicological and metabolism studies for Ethephon

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

2017-07-24

Author(s)

[redacted]

Bayer AG
Crop Science Division

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Version history

Date	Data points containing amendments or additions[1] and brief description	Document identifier and version number
2016-01-11	Initial document submitted for Annex I renewal Ethephon	M-544713-02-1
2017-06-01	Study summaries (M-588162-01-1) to M-187724-01-1, M-187699-01-1, M-187735-01-1 included (CA 5.5). Change of legal entity from Bayer CropScience AG to Bayer AG - Crop Science Division	M-544713-03-1

[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

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CA 5 TOXICOLOGICAL AND METABOLISM STUDIES ON THE ACTIVE SUBSTANCE

INTRODUCTION

Ethephon is a plant growth regulator and was included into Annex I of Directive 91/414 in 2006 (Directive 2006/85/EC, dated 23rd of October 2006, Entry into Force 1st of August 2007).

This dossier contains only summaries of studies, which were not available at the time of the first Annex I inclusion of ethephon and were, therefore, not evaluated during the first EU review of this compound. All other studies, which were already submitted by Bayer AG (formerly Bayer CropScience AG) for the first Annex I inclusion, are contained in the Monograph and in the Baseline Dossier (P-012067-01). These studies are summarised written in grey typeface in the dossier prepared for the renewal of approval.

The here presented and submitted studies used different synonyms and codes for the active substance ethephon, its metabolites and reference compounds. In order to present a common basis for the evaluation the following list summarises all names used.

Formula Report name used in summaries	**Codes used** *IUPAC index name* / Other names / codes
Ethephon	AE F016382 Ethephon technical concentrate Ethephon Base 250
Ethephon-2-hepa	HEPA, 2-HEPA (2-hydroxyethyl)phosphonic acid

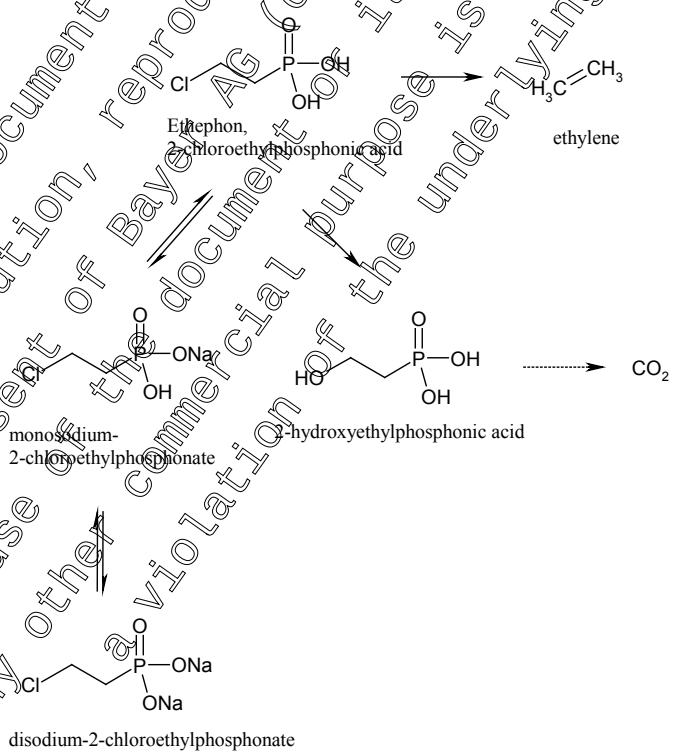
In addition, a list of metabolites which contains the structures, the synonyms and code numbers attributed to the compound is presented in Document N3 of this dossier. The matrices in which the metabolites were identified are also included in this list.

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

No new toxicokinetic study was carried out after ethephon Annex I inclusion. The existing relevant information was presented and evaluated during the EU process for Annex I listing.

The toxicokinetic behavior of ethephon was investigated in male and female rats after application of a single oral low (50 mg/kg), a single oral high dose (1000 mg/kg), a single intravenous dose (50 mg/kg) and after multiple applications of 14 daily doses (50 mg/kg) of non-radiolabelled ethephon followed by a single dose with radiolabelled ethephon.
Ethephon is rapidly and extensively absorbed via the gastrointestinal tract (78-84%, based on excretion data from urine, expired air/volatiles, cage wash, tissues and residual carcass) within 120 hours. The excretion is rapid, mainly via urine (50-60% within 120 hours) and expired air (≤20% within 120 hours). Ethylene and carbon dioxide were characterised as volatiles from the study. Less than 6.5% is excreted in faeces. Ethephon is widely distributed within the animal, however the amount retained in tissues and residual carcass is low (<0.5%) and the highest concentrations were found in liver, blood, kidneys, bone, spleen, lungs and heart. There was no potential for accumulation.
Ethephon is extensively metabolized. The fraction containing the disodium salt of ethephon was the major component in urine and faeces, representing on average 84-87% and 47-59% of the total radioactivity in the urine and faeces samples, respectively. Fractions other than the ones containing the disodium salt of ethephon individually accounted for ≤ 6.0% of applied radioactivity. The monosodium salt of ethephon was characterised. In a following study ethephon was applied in a single oral dose to male rats. After extraction of liver and kidney the presence of ethephon and the metabolite 2-hydroxyethyl phosphonic acid (HEPA) was confirmed.

The proposed metabolic pathway of ethephon in the rat can be depicted as follows:



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

No new toxicokinetic study was carried out after ethephon Annex I inclusion. The existing relevant information was presented and evaluated during the EU process for Annex I listing.

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

No new toxicokinetic study was carried out after ethephon Annex I inclusion. The existing relevant information was presented and evaluated during the EU process for Annex I listing.

CA 5.2 Acute toxicity

Ethephon in high concentration (>87%) is a waxy solid and difficult to handle, a liquid technical product (Ethephon Base 250) is therefore manufactured and marketed.

Ethephon Base 250, the liquid technical concentrate (TK), contains a nominal 71.3% of pure ethephon by weight, a nominal 21.37% of water. Most of the submitted acute toxicity studies were conducted with ethephon Base 250. Therefore, the values of the results of the acute toxicity studies have been corrected for the purity of ethephon.

The acute toxicity studies were already evaluated during the Annex I inclusion and no new studies have been conducted (see Table 5.2-1)

Table 5.2-1: Summary of acute toxicity

Type of test	Species	Results	References
Acute oral toxicity	Rat (♂&♀)	LD_{50} = 2664 mg/kg bw ♂ LD_{50} = 1563 mg/kg bw ♀	[redacted]; 1989a M-187938-01-1
Acute dermal toxicity	Rabbit (♂&♀)	LD_{50} = 1210 mg/kg bw ♂ LD_{50} = 983 mg/kg bw ♀	[redacted]; 1989b M-187936-01-1
Acute inhalation toxicity	Rat (♂&♀)	LC_{50}= 3.26 mg/L ♂& ♀	[redacted]; 1989 M-187658-01-1
Skin irritation	Rabbit (♂&♀)	Corrosive	[redacted]; [redacted]; [redacted]; 1983 M-187656-01-1
Eye irritation		Not required pH 1.6	
Skin sensitization Bühler	Guinea-pig (♂)	Not a sensitizer	[redacted], 1989 M-187667-01-1
Skin sensitization MaxK	Guinea-pig (♂)	Not a sensitizer	[redacted]; 2000a M-202329-01-1 & M-233609-01-1
Skin sensitization LLNA	Mouse	Not a sensitizer	[redacted]., 2005 M-247651-02-1
Phototoxicity in vitro		Not required : molar extinction coefficient = 10 L x mol^{-1} x cm^{-1}	

Ethephon oral LD_{50} following single administration by gavage of ethephon Base 250 to Hilltop-Wistar rats was 2664 mg/kg bw in males and 1563 mg/kg bw in females (M-187938-01-1) Clinical signs included sluggishness, piloerection, emaciation and prostration followed by mortality within 1 day. At necropsy, the visceral surfaces of livers were mottled tan and brown, the glandular portions of stomachs were black.

Ethephon dermal LD_{50} following single application of ethephon Base 250 to the shaved area of trunk skin surface of male and female New Zealand white rabbits was 1210 mg/kg bw in males and 983 mg/kg bw in females (M-187936-01-1) . Clinical signs included pinpoint pupils, salivation, unsteady gait, and prostration, followed by mortality within 3 days. At necropsy, findings included red lungs, red trachea, mottled livers, and intestines filled with paste-like fecal matter.

The results of the acute inhalation toxicity study indicate a 4-hr inhalation LC_{50} value of 3.26 mg/L for both male and female rats (M-187658-01-1). Hypothermia, tremors were noted in animals before death which occurred within the first day in animals receiving the top dose equivalent to 4.32 mg/mL. At macroscopic post-mortem examination unkempt fur, discoloration of lungs, liver, salivary glands and thymic region, brain haemorrhages and/or gaseous stomach and intestines were noted among the animals found dead during the study. No test substance related findings were noted in the other animals.

A skin irritation/corrosion study was performed by applying 0.5 ml ethephon Base 250 to the shaved skin of the trunk of six rabbits (3 males, 3 females) for 4 hours, and readings were made at 5, 24 and 48 hours after the initiation of the exposure. In addition, six rabbits (3 males, 3 females) were treated with 0.5 ml ethephon base 250 for 1 hour, and readings were made at 5, 24 and 48 hours after initiation of the exposure. Moderate to severe edema and necrosis were observed after 4 hours of test substance application. No necrosis following 1 hour exposure (M-187656-01-1).

No eye irritation study with ethephon was performed because of its low pH value of 1.6

The corrosive properties of ethephon base 250 made it difficult to conduct and to assess the skin sensitizing potential. Three skin sensitization studies were performed and assessed in the monograph and monograph addendum.

A modified Buehler test was performed with ethephon Base A-250, (M-187667-01-1). Five Hartley Guinea pigs per sex in the treatment group were tested instead of a total of 20/group. Doses of 25% w/v in distilled water were used for induction (3 topical inductions, for 6h under occlusion) and 10% w/v for the challenge. There was no evidence of contact sensitization observed following challenge with Base A-250. No or minimal dermal reactions were observed in both the induced and challenge control animals. Mean dermal scores following challenge were comparable between the Base A-250 treatment and the negative control. Clear dermal responses were seen with the positive control material.

In the Guinea pig maximization study, 10 controls (5/sex), 20 test animals (10/sex) were treated with ethephon (purity 74.1%) at 0.5% w/w via intradermal induction, or 50% w/w topical induction. A concentration of 25% was used for the topical challenge of 24 h under occlusion (M-202329-01-1). Results of the induction phase were not included in the study report, but during the discussion for Annex I inclusion, BCS submitted a statement prepared by the study director confirming that the concentration of 50% was irritant in the preliminary study (M-233609-01-1). In the treated group, at the 24-hour reading, a discrete erythema was noted in 5/20 animals. At 48-hour reading skin reactions faded and discrete erythema (grade 1) persisted in 1/20 animal only. As the cutaneous reactions observed in the animals of the treated group were non-persistent, they were attributed to the known irritant properties of the test substance but not to delayed contact hypersensitivity.

Ethephon Base 250 was tested in the Local Lymph Node Assay in female mice, (M-247651-02-1). To overcome the irritant properties an aqueous solution adjusted to pH 4.5 (±0.5) with aqueous sodium hydroxide (10M) was used. There were no lymphoproliferative responses (SI<3).

Overall the results of the studies indicated that ethephon Base 250 has no skin sensitizing potential.

Due to the new data requirements a photoxicity study has to be performed if the molar extinction coefficient is higher than 10 L x mol^{-1} x cm^{-1}. This is the not the case for ethephon so no photoxicity study has been conducted.

Comparison with criteria

According to the results of the acute oral, dermal and inhalation toxicity studies, ethephon has to be classified under current harmonized EU classification, according to the CLP regulation EC 1272/2008, Acute Toxicity Cat 4, H302 (limits 300 – 2000 mg/kg bw, oral), category 3, H311 (limits 200 – 1000 mg/kg bw, dermal) and Cat 4, H332 (limits aerosol 1-5 mg/L).

According to DSD criteria ethephon has to be classified as Xn, R20/R21/R22 because the oral LD_{50} is within of limits 200 – 2000 mg/kg bw, the dermal LD_{50} is within the of limits 400 – 2000 mg/kg bw, and the LC_{50} is within the limits of 1-5 mg/L.

Based on the low pH of ethephon (1.6) and the results of the skin irritation study, it can be concluded that ethephon is corrosive. As necrosis was observed after 4-hour exposure period and not after 1–hour, ethephon has to be classified as Skin corrosion 1C, H314 according to the criteria of CLP and with R34 according to the criteria of CLP.
Since ethephon is classified as corrosive and labeled as corrosive to the respiratory tract, additional classification STOT SE 3; H335 and SCL for STOT SE 3 H335 is not required as the classification as corrosive applies to mixtures at 5% and above.

No eye irritation study has been carried out due to the low pH of ethephon. No additional classification for eye need to be added as the classification for skin corrosion has to be assigned.

No reliable findings were obtained in the Buehler test, LLNA assay and/or GPMT study, due to the corrosive properties of ethephon. Therefore, as the compound has to be classified for skin corrosion, no further classification for skin sensitization is required.

Results of the UV-visible characteristics of ethephon in aqueous solution evaluated according to OECD TG 101 showed that ethephon molar extinction coefficient value is below 10 L x mol^{-1} x cm^{-1} and therefore no phototoxicity study is triggered and that there is no concern for phototoxic potential.

Conclusions on classification and labelling

In December 2012, the Committee for Risk Assessment (RAC) has adopted the following opinion for harmonized classification and labelling (CLH), of ethephon.

1. Classification and labelling in accordance with the CLP Regulation (Regulation (EC) 1272/2008)
   - Acute Tox 3; H311
   - Acute Tox 4; H332
   - Acute Tox 4; H302
   - Skin Corr 1C; H314

2. Classification and labelling in accordance with the criteria of Directive 67/548/EEC
   - Xn; R20/21/22
   - C; R34

CA 5.2.1 Oral

No new acute oral toxicity study was carried out after ethephon Annex I inclusion. The existing relevant information was presented and evaluated during the EU process for Annex I listing.

CA 5.2.2 Dermal

No new acute dermal toxicity study was carried out after ethephon Annex I inclusion. The existing relevant information was presented and evaluated during the EU process for Annex I listing.

CA 5.2.3 Inhalation

No new acute inhalation toxicity study was carried out after ethephon Annex I inclusion. The existing relevant information was presented and evaluated during the EU process for Annex I listing.

CA 5.2.4 Skin irritation

No new skin irritation study was carried out after ethephon Annex I inclusion. The existing relevant information was presented and evaluated during the EU process for Annex I listing.

CA 5.2.5 Eye irritation

No new eye irritation study was carried out after ethephon Annex I inclusion. The existing relevant information was presented and evaluated during the EU process for Annex I listing.

CA 5.2.6 Skin sensitization

No new skin sensitization study was carried out after ethephon Annex I inclusion. The existing relevant information was presented and evaluated during the EU process for Annex I listing.

CA 5.2.7 Phototoxicity

In vitro photoxicity study is required when an active substance absorbs electromagnetic radiation in the range of 290-700 nm and the molar extinction coefficient of the UV/VIS absorption maxima (calculated according to OECD TG 101) is above 10 L x mol^{-1}x cm^{-1}.

UV-visible characteristics of ethephon in aqueous solution have been determined (M-530716-01-1) according to the current requirements showed a maximum absorption at 200 nm at 25 L x mol^{-1}x cm^{-1} and of 0 between 290-700 nm.

Therefore, no phototoxicity study is required as Ethephon does not absorb in the relevant UV/VIS spectra range.

Table 5.2.7-1 Ethephon UV/VIS spectra (study submitted under point MCA 2.4)

Solvent	Wavelength [nm]	Molar extinction coefficient [L x mol^{-1}x cm^{-1}]	Reference
Distilled water	200	25	[redacted]; 2015; M-530716-01-1
	291	0	

CA 5.3 Short-term toxicity

The short term effects of ethephon were studied in two oral 28-day studies in the rat, two oral 28-day studies in the mouse, and one 1-year study in the dog (see table 5.3.1).
All these studies have been submitted and evaluated during the EU process for Annex I listing.

In addition two special studies investigating the effect of ethephon on the cholinesterase inhibition in dogs are available: the 28-day study was evaluated during the last phase of EU process for Annex I listing, whereas the 90-day study is a new study. Both studies are summarised under point CA 5.8.2, but as cholinesterase inhibition is a critical toxicological endpoint for ethephon, the results of these studies are also presented in this section.

In all the dietary studies the concentration of ethephon was measured and/or the diets were corrected for the purity of ethephon Base 250 (which has a content of ethephon of 71.3%). Therefore the calculated achieved intake represent the actual concentration of ethephon administered to the animals and no additional correction for purity is needed.

Table 5.3.1 Summary of short-term dietary toxicity studies – rodents

Study	NOAEL (mg/kg bw/day)	LOAEL (mg/kg bw/day)	Effects at LOAEL
28 day in Sprague-Dawley CD rats (10/sex/dose) 0, 625, 1250, 2500, 5000, 10000 ppm 0, 52, 106, 214, 431, 831 mg/kg bw/day in ♂and 0, 59, 119, 251, 487, 980 mg/kg bw/day in ♀ 0, 10000, 25000, 50000 ppm 0, 962, 2299 , 4742 mg/kg bw/day in ♂ and 0, 996, 2488, 4905 mg/kg bw/day in ♀ [redacted]; 1986. M-187685-01-1 & M-187683-01-1	52 (♂) - 59 (♀)	106 (♂) - 119 (♀)	LOAEL was based on inhibition of plasma and RBC cholinesterase activity Brain cholinesterase activity was not affected
28 day in CD-1 mice (10/sex/dose) 30, 100, 300, 1000 , 3000 ppm, 0, 5.3, 18, 51, 181, 546 mg/kg bw/day in ♂ and 0, 6.5, 22, 69, 209, 635 mg/kg bw/day in ♀ 0, 3000, 10000, 25000, 50000 ppm 0, 525, 1815, 4780, 10212 mg/kg bw/day in ♂ and 0, 632, 2231, 5852 or 14945 mg/kg bw/day in ♀ [redacted]; 1986 M-187702-01-1 & M-187703-01-1	18 (♂) - 69 (♀)	181 (♂) – 209 (♀)	LOAEL was based on inhibition of erythrocyte ChE activity Brain cholinesterase activity was not affected

Table 5.3.2 Summary of short-term and subchronic toxicity studies - Dogs

Study	NOAEL (mg/kg bw/day)	LOAEL (mg/kg bw/day)	Effects at LOAEL
14 day in Beagle-dogs (1/sex/dose) 0, 1, 3, 10, 100, 300, 1000, 3000, 10000 ppm and 2/sex/dose at 30 ppm 0, 0.16, 0.57, 2.1, 5.1, 17.2, 48, 164, 410 and 901 mg/kg bw/day in ♂ and 0, 0.12, 0.49, 2.1, 4.82, 24.6, 46, 202, 348, 1306 mg/kg bw/day in ♀ and [redacted]; 1989 M-187726-01-1 Preliminary study included in the 52-week study	17.2 (♂) - 24.6 (♀)	48 (♂) - 46 (♀)	≥ 300 ppm ↓ Erythrocyte ChE activity (≥ 20%) ≥ 1000 ppm ↓Brain ChE activity (≥ 20%) ↓ bodyweight 3000 ppm ↓ bodyweight and food consumption
28 day in Beagle-dogs (1/sex/dose) 0, 1, 3, 10, 30 ppm 0, 0.16, 0.57, 2.09, 5.09, mg/kg bw/day in ♂ and 0, 0.12, 0.49, 2.11, 4.82 mg/kg bw/day in ♀ [redacted]; 1989 M-187726-01-1 Preliminary study included in the 52-week study	5.09 (♂) / 4.82 (♀)	/	No effects up to highest dose
28 day in Beagle-dogs (3/females/dose) 0, 250 and 750 ppm, 0, 6 and 14 mg/kg bw/day. [redacted]; 2006 M-268126-01-1	6	14	LOAEL was based on inhibition of erythrocyte ChE activity Brain cholinesterase activity was not affected
90-day in Beagle dogs (4/sex/dose) 0, 70, 140, and 525 ppm 0, 2, 4, 15 mg/kg bw/day in ♀ and 2, 4, 18 mg/kg bw/day in ♂ [redacted]; 2006 M-276963-01-2	2 (♀) – 4(♂)	4 (♀) – 15 (♂)	LOAEL was based on inhibition of erythrocyte ChE activity Brain cholinesterase activity was not considered to be significantly affected
52-week in Beagle dogs (4/sex/dose) 0, 100, 300, 1000, 2000 ppm, 0, 2.79, 8.11, 27.4, 54.2 mg/kg bw/day in ♂ and 0, 2.55, 8.38, 29.7, 50.0 mg/kg bw/day in ♀ [redacted]; 1989 M-187726-01-1	27.4 (♂) – 29.7 (♀)	54.2 (♂) – 50.0 (♀)	↓terminal body weight , mean absolute and relative spleen weights in ♂ and mean spleen weights relative to body in ♀

Studies highlighted → not evaluated in the Monograph and/or monograph addendum

Table 5.3.3 Summary of short-term dermal toxicity study

Study	NOAEL (mg/kg bw/day)	LOAEL (mg/kg bw/day)	Effects at LOAEL
Dermal Toxicity 3-Week (6hours/day for 21 days) dermal toxicity in (NZW)SPF rabbit. (10/sex/ dose) at 0, 18, 54, 108 mg/ /kg bw/day [redacted]; 1989 M-188011-01-1	Skin: 54 Systemic: 108	Skin: 108	Treatment related effects associated with macroscopic and microscopic changes (acanthosis and chronic active inflammation) in the skin.

In the two 28-day rat studies (M-187685-01-1 and M-187683-01-1), ethephon was administered in the diets to group of Sprague-Dawley CD rats (10/sex/dose). The dietary concentration were between 0, 625, 1250, 2500, 5000, 10000, 25000 and 50000 ppm (equivalents to 0, 52, 106, 214, 431, 831-962, 2299, 4742 mg/kg bw/day in males and 0, 59, 119, 251, 487, 980-996, 2488 and 4905 mg/kg bw/day in females). Additional 5 animals/sex were dosed at 0, 1250, and 2500 ppm in order to determine cholinesterase (ChE) activities after 14 days.

Ethephon caused treatment-related inhibition of ChE activity in plasma (23-74%) at all doses and of erythrocytes (69-91%) in males and females at doses above 1250 ppm. A slight statistically significant inhibition of brain ChE activity (15% and 13% in males and females, respectively) was noted in rats given 50000 ppm, equivalent to 4673 mg/kg bw/day in males or 4905 mg/kg bw/day in females. No cholinergic effects were noted. Decreased spleen weights were noted at doses higher than 10000 ppm. Increased kidney weight was noted in females at from 25000 ppm. Decreased heart and lung weight, and increased brain, liver and kidney weights were noted at 50000 ppm or 4673 mg/kg bw/day in males and at 4905 mg/kg bw/day in females.

Table 5.3.4 Cholinesterase activity determinations in the short-term studies in the rats

Dietary concentrations (ppm)	% Inhibition of Cholinesterase (ChE) activity						Study reference
	Plasma ChE		RBC ChE		Brain ChE		
	2-wk	4-wk	2-wk	4-wk	2-wk	4-wk	
Males							
625	nd[a]	13	nd[a]	9	nd[a]	-[a]	[redacted]; 1986 M-187685-01-1
1250	21	27	16	22	7	-[a]	
2500	18	16	36	41	9	-[a]	
5000	nd[a]	30	nd[a]	58	nd[a]	13	
10000	nd[a]	35	nd[a]	73	nd[a]	1	
10 000	23	27	69	72	11	8	[redacted]; 1986 M-187683-01-1
25 000	31	34	84	82	4	-[a]	
50 000	42	45	91	91	15	14	
Females							
625	nd[a]	29	nd[a]	19	nd[a]	1	[redacted] 1986 M-187685-01-1
1250	44	50	14	35	-[a]	4	
2500	45	50	32	50	-[a]	1	
5000	nd[a]	49	nd[a]	67	nd[a]	4	
10000	nd[a]	63	nd[a]	78	nd[a]	4	
10 000	66	46	72	73	4	7	[redacted].; 1986 M-187683-01-1
25 000	66	58	82	80	2	3	
50 000	74	61	91	89	13	3	

nd[a] = Not determined
-[a]= Indicates that ChE activity was equivalent to or slightly greater than control values

Based on the biologically significant inhibition (higher than 20%) of ChE activity in erythrocyte, the overall NOAEL for the two studies is set at 625 ppm (52 and 59 mg/kg bw/day in males and females, respectively)

In the two 28-day mice studies (M-187702-01-1 and M-187703-01-1), ethephon was administered via the diets to CD-1 mice (10/sex/dose). Dietary dose levels ranged between 0, 30, 100, 300, 1000 and 3000, 10000, 25000 and 50000 ppm (equivalent to 0, 5.3, 18, 51, 181, 525-546, 1815, 4780 and 10212 mg/kg bw/day in males and 0, 6.5, 22, 69, 209, 632-635, 2231, 5852 and 14945mg/kg bw/day in females). Body weight was significantly reduced from 25000 ppm.

Ethephon caused treatment-related inhibition of ChE activity in plasma (22-79%) from 300 ppm and erythrocytes (29-90%) in males and females from 1000 ppm. The 23% inhibition of erythrocyte ChE inhibition observed at 300 ppm (22 mg/kg bw/day) in the females after two weeks of treatment was not considered to be biologically relevant as the inhibition following 4 weeks drop down to 14%. Slight statistically significant inhibition of ChE activity in brain was noted after 4 weeks in males in a consistent way from 10000 to 50000 ppm (12%, 14%, and 19%, respectively). No cholinergic effects or clinical signs were noted at any dose levels. Increased brain, kidney and lung weights were noted in males at 50000 ppm. Decreased spleen weights were noted in males and females at 50000 ppm. Decreased heart weights were noted among males at 50000.

Based on the biologically significant inhibition (higher than 20%) of ChE activity in erythrocyte the overall NOAEL for the two studies is set at 300 ppm (51 and 69 mg/kg bw/day in males and females, respectively).

Table 5.3.5 Cholinesterase activity determinations in the short-term studies in the mice

Ethephon Dietary concentrations (ppm)	% Inhibition of Cholinesterase (ChE) activity						Reference
	Plasma ChE		RBC ChE		Brain ChE		
	2-wk	4-wk	2-wk	4-wk	2-wk	4-wk	
Males							
30	nd[a]	3	nd[a]	3	nd[a]	14	[redacted]; [redacted]; 1986 M-187702-01-1
100	nd[a]	4	nd[a]	6	nd[a]	9	
300	18	22	9	18	-[a]	11	
1000	43	44	29	34	3	14	
3000	nd[a]	63	nd[a]	62	nd[a]	13	
3000	55	55	54	57	6	9	[redacted]; [redacted]; 1986 M-187703-01-1
10000	54	60	68	67	4	12	
25000	77	71	88	82	4	14	
50000	79	77	90	87	13	19	
Females							
30	ND[a]	2	ND[a]	-[b]	ND[a]	-[a]	[redacted]; [redacted]; 1986 M-187702-01-1
100	Nd[a]	4	Nd[a]	5	Nd[a]	3	
300	29	23	23	14	-[a]	-[a]	
1000	49	46	38	41	-[a]	-[a]	
3000	Nd[a]	55	Nd[a]	56	Nd[a]	-[a]	
3000	55	57	62	62	1	6	[redacted]; [redacted]; 1986 M-187703-01-1
10000	66	65	80	76	-[a]	-[a]	
25000	80	73	88	82	8	13	
50000	82	78	92	89	8	1	

Nd[a] = Not determined
-[a]= Indicates that ChE activity was equivalent to or slightly greater than control values

The effects of ethephon on ChE activity were investigated in a series of preliminary studies to select the doses for the one-year dog study. These data were already evaluated during the EU process for

Annex I listing as they were included in the report of the 52-week dog study. These preliminary studies were considered to be supplemental for understanding the effects of ethephon on erythrocytes and brain ChE inhibition, but not conclusive as only one animal/sex/ dose level was included in each of the study. There were a total of 3 dietary studies with ethephon doses of 0, 1, 3, 10, 30, 100, 300, 1000, 3000 and 10000 ppm, equivalent to about 0, 0.16, 0.57, 2.1, 5.1, 17.2, 48, 164, 410 and 901 mg/kg bw/day in males and 0, 0.12, 0.49, 2.1, 4.82, 24.6, 46, 202, 348 and 1306 mg/kg bw/day in females, respectively. Results indicated that the females were more susceptible than males, with a biologically relevant (> 20%) inhibition of erythrocyte from 300 ppm (equivalent to about 46 mg/kg bw/day) and of brain ChE activity from 1000 ppm (equivalent to 202 mg/kg bw/day).

In the dogs the effects of ethephon on cholinesterase inhibition were further investigated following dietary administration of ethephon for 28-day in the females (M-268126-01-1) and 90-day in both sexes (M-276963-01-2). A detailed summary of each study is presented under section CA 5.8.2.

In the 28-day study female Beagle dogs (3/dose levels) received dietary administration of ethephon at 0, 250 and 750 ppm (equivalent to 0, 6 and 14 mg/kg bw/day). Plasma and erythrocyte cholinesterase activity was determined during weeks 1, 2, 3, and 4, and brain cholinesterase activity was determined at study termination. Due to inter-animals variability of the values and the low number of animals, plasma and erythrocyte cholinesterase activities were evaluated as the change in activity between the average of the pretreatment values and the various days of treatment for each dog.

Plasma ChE activity was significantly depressed for all dose groups at all-time points, and erythrocyte ChE activity was depressed above 20% in the high-dose group on study days 14, 21, and 28. In the low-dose group (250 ppm), erythrocyte acetylcholinesterase activity was not inhibited. There was no effect on brain ChE activity.

The dose level of 6 mg/kg bw/day was the study NOAEL for inhibition of erythrocyte ChE activity in the in the female dogs following short-term exposure

Ethephon was administered in the diet to Beagle dogs (4/sex/dose) at dose levels of 0, 70, 140, and 525 ppm (equivalent to 2, 4 and 15 mg/kg bw/day in males and 2, 4, and 18 mg/kg bw/day in females) for 91 days (M-276963-01-2). Clinical observations were conducted daily. For determination of the dose in mg/kg/day, food consumption was measured daily and body weights were taken weekly. Plasma and erythrocyte cholinesterase activity was determined during weeks 1, 2, 4, 8, 10 and 12, and brain acetylcholinesterase activity was determined at study termination.

There were no mortality and/or treatment-related clinical signs.
Due to inter-animals variability of the values, plasma and erythrocyte cholinesterase activities were evaluated as the change in activity between the average of the pretreatment values and the various days of treatment for each dog. Ethephon significantly inhibited plasma cholinesterase activity at all doses tested in both sexes and erythrocyte cholinesterase activity at doses equivalent to 4 mg/kg bw/day in females and to 15 mg/kg bw/day in males.
Brain ChE esterase was inhibited up to 14% in the females at the top dose.

In conclusion, the NOAEL for this study was 70 ppm (2 mg/kg/day), based on the statistically significant inhibition of erythrocyte ChE activity in females.

A 1-year oral study was performed in the Beagle dogs in accordance with OECD 409 (M-187726-01-1). Dose levels of 0, 100, 300, 1000 and 2000 ppm, equivalent to 0, 2.79, 8.11, 27.4 and 54.2 mg/kg bw/day in males and 0, 2.55, 8.38, 29.7 and 50.0 mg/kg bw/day in females were used. No measurements of ChE activity in plasma, erythrocytes or brain were performed. Body weight, absolute and relative spleen weight and absolute thyroid weight were decreased at 49.96 mg/kg bw/day.

Relative thyroid weight was similar to control. There were no treatment-related histopathological findings in any organ.

The NOAEL in this study was 1000 ppm equivalent to 27.4 and 29.7 mg/kg bw/day in males and females, respectively.

The dermal toxicity of ethephon Base 250 was determined in a 21-day repeat dose study. Base 250 was applied for at least 6 hours/day to the intact skin of Hra:(NZW)SPF rabbits (10 sex/dose) at doses of 0, 25, 75, or 150 mg Base 250/kg bw/day (M-188011-01-1). Doses in this study are reported as Base 250, without correction for purity. Actual doses on pure active ingredient basis were 18, 54, and 108 mg as/kg bw/day.

No systemic toxicological effects occurred in rabbits following daily dermal application of 150 Base 250/kg bw/day equivalent to a NOAEL of 108 mg/kg bw/day over a period of 3 weeks.

The NOAEL for dermal irritation is nominal 75 mg Base 250/kg bw/day, equivalent to ethephon concentration of 54 mg as/kg bw/day, based on macroscopic and microscopic findings for treated skin.

CA 5.3.1 Oral 28-day study

No new 28-day toxicity study was carried out after ethephon Annex I inclusion. The existing relevant information was presented and evaluated during the EU process for Annex I listing. The special 28-day study in dogs focusing on ChE inhibition in dog is further summarized under point CA 5.8.2.

CA 5.3.2 Oral 90-day study

No new 90-day toxicity study was carried out after ethephon Annex I inclusion. The existing relevant information was presented and evaluated during the EU process for Annex I listing. The special 90-day study in dogs focusing on ChE inhibition in dog is further summarized under point CA 5.8.2.

CA 5.3.3 Other routes

No new toxicity study via non-oral route was carried out after ethephon Annex I inclusion. The existing relevant information was presented and evaluated during the EU process for Annex I listing. The special 90-day study in dogs focusing on ChE inhibition in dog is further summarized under point CA 5.8.2.

Specific target organ toxicity – (STOT-SE and STOT-RE) Comparison with criteria

The main effect of repeated exposure to ethephon is inhibition of cholinesterase. Plasma cholinesterase inhibition is not considered to be toxicologically relevant, and therefore not relevant for classification. In 2012, the ECHA RAC evaluated all the existing studies and considered that, based on JMPR '98 criteria for cholinesterase inhibition, the limit values should be based on inhibition of brain ChE instead of erythrocyte cholinesterase. Brain cholinesterase was inhibited at doses ≥ 164 mg/kg bw/day a 14 day study in dogs. Cholinergic effects were also seen in rats in the acute neurotoxicity study at dose levels below the cholinergic effects in repeated dose studies in the rat (see CA 5.7.1) . This indicates that these types of effects are acute effects and not repeated dose effects in the rat. It is assumed that this also applies to the dog. Therefore, no classification for specific target organ toxicity repeated exposure is proposed based. Inhibition of brain cholinesterase was also observed at doses ≥ 4673 mg/kg bw/day in a 28 day study in rats and doses ≥ 1815 mg/kg bw/day in a 28 day study in mice. In a 78 week study in mice, brain ChE activity was reduced (18%) in the females after 52 week of dietary exposure to 10000 ppm (equivalent to 1782 mg/kg bw/day) but not at final sacrifice after 78

weeks (see point CA.5.5.1). As the effect levels in the longer studies are above the guidance values for classification, classification was not considered necessary based on this endpoint.

Conclusions on classification and labelling
No classification for specific target organ toxicity following repeated exposure (STOT-RE) is required.

CA 5.4 Genotoxicity testing

The genotoxic potential of ethephon was investigated in a comprehensive range of in vitro assays designed to test different genotoxicity endpoints: gene mutation, chromosomal aberrations and unscheduled DNA synthesis. In addition an in vivo UDS study in the rat has been conducted. No new in vitro genotoxicity studies were carried out after ethephon Annex I inclusion. However, an *in vivo* mouse micronucleus assay was carried out upon request of the Japanese authorities. This study is summarised in detail.

Due to the new data requirements a photomutagenicity study has to be performed if the molar extinction coefficient is higher than 1000 L x mol^{-1} x cm^{-1}. This is not the case for ethephon which does not absorb light in the UV/visible range of 290-800 nm. Therefore no photomutagenicity study is triggered and/or required.

Table 5.4-1 Summary of genotoxicity studies

End point	Test system	Concentration	Results	Reference
Gene mutation (Reverse mutation)	*S. typhimurium* (TA 1535, TA 1537, TA 1538, TA 98, TA 100)	0.1, 0.5, 1.0, 2.5, 5.0, 10.0, 25.0, 50.0 µl/ plate without and with S9 mix	Positive in TA 1535 ± S9 at cytotoxic concentrations	[redacted]; 1987 M-187742-01-1
Gene mutation	Chinese Hamster Ovary cells HGPRT-locus	0.5, 1.0, 2.0, 2.5, 3.0, 3.5, 4.0, 5.0 mg/ml without S-9 mix 0, 1.0, 2.0, 2.2, 2.4, 2.6, 2.8, 3.0 mg/ml with S-9 mix	Negative	[redacted]; 1988 M-187751-01-1
Chromosomal aberration	Chinese Hamster Ovary cells,	752, 1000, 1510, 2010 µg/ml without S-9 mix 752, 1000, 1510, 2010 µg/ml with S-9 mix.	Negative	[redacted]; 1988 M-187762-01-1
UDS *in vitro*	Rat primary hepatocytes	25, 50, 100, 250, 100, 500, 1000, 2000 µg/ml	Negative	[redacted] 1988 M-187753-01-1
In vivo				
Study	**Species**	**Concentration mg/kg bw**	**Results**	**References**
UDS *in vivo* Oral route	Han Wistar rats (♂)	0, 800, 2000	Negative	[redacted], 2002 M-209739-01-1
Micronucleus Intraperitoneal	NMRI mice (♂)	0, 150, 300, 600	Negative	[redacted]; 2005 M-247916-01-1

CA 5.4.1 In vitro studies

No new in vitro genotoxicity study was carried out after ethephon Annex I inclusion. The existing relevant information was presented and evaluated during the EU process for Annex I listing.

CA 5.4.2 In vivo studies in somatic cells

Report:	KCA 5.4.2/02; [redacted]; 2005; M-247916-01-1
Title:	Micronucleus-test on the male mouse Ethephon
Report No.:	C047233
Document No.:	M-247916-01-1
Guideline(s):	EU (=EEC): 2000/32/EC, Method B12; OECD: 474; USEPA(=EPA): OPPTS 870.5395
Guideline deviation(s):	--
GLP/GEP:	**yes**

Executive Summary

A micronucleus test was carried out to investigate Ethephon in male NMRI mice for a possible clastogenic effect on the chromosomes of bone-marrow erythroblasts. The study complied with OECD TG 474 (1997).
The known clastogen and cytostatic agent; cyclophosphamide, served as positive control.

Male mice treated with ethephon received two intraperitoneal administrations of 150, 300 and 600 mg/kg, respectively, separated by 24 hours. Males of the positive control received a single intraperitoneal treatment with 20 mg/kg cyclophosphamide. The femoral marrow of all groups was prepared 24 hours after the last administration.

Males treated twice with ethephon at doses up to 600 mg/kg showed symptoms of toxicity after administration, starting at 150 mg/kg. These symptoms demonstrate systemic exposure to ethephon. However, all animals survived until the end of the test.

There was no altered ratio between polychromatic and normochromatic erythrocytes.

After two intraperitoneal treatments up to and including 600 mg/kg there was no indication of a clastogenic effect.

Cyclophosphamide, the positive control, had a clear clastogenic effect, as is shown by the biologically relevant increase in polychromatic erythrocytes with micronuclei. The ratio of polychromatic to normochromatic erythrocytes was not altered.

I. MATERIALS AND METHODS

MATERIALS:

1. Test Material:	Ethephon
Description:	Clear colourless liquid
Lot/Batch #:	040201
Purity:	71.4.% (analytical result dated July 1'th, 2004)
CAS #:	16672-87-0

Stability of test compound:	The batch used was analytically examined prior to study initiation and was approved for use for the test period. A stability test in the vehicle did not reveal significant degradation of the active ingredient.
Solvent used:	0.5 % aqueous Cremophor; the solution was set to pH 6 to 8by using 1N aqueous NaOH.

2. Control Materials:

Negative:	Deionized water
Solvent:	Physiological saline solution
Positive:	Cyclophosphamide (CP) in solvent.

3. Test animals:

Species:	Mouse (male and females for the pilot study)
Strain:	Hsd/Win: NMRI BR (Harlan Winkelmann GmbH, Borchen).
Age:	6-12 weeks old at start of administration
Weight at dosing:	Males: 36-41 g
Number of animals used:	Pilot study: 3 animals/sex/group, 1 group Main study: 5 animals/sex/group, 6 groups
Animal husbandry:	Housed individually in type II cages with Bedding of soft wood granules, type BK8/15
Diet:	Fixed formula feed 3883 (10 mm cubes), produced according to specification by [redacted] *ad libitum*.
Water:	Tap water provided in polycarbonate bottles, 300 mL volume ([redacted], 1, 38, 1961) and available *ad libitum*.

4. Environmental conditions

Temperature:	21± 1.5°C
Humidity:	40-70%
Air change:	10 air changes per hour
Photoperiod:	12 hour light / dark cycle

5. Dose Levels

(a) Pilot study:

1000 mg/kg bw administered by two intraperitoneal injections separated by 24 hours

(b) Main study:

0, 150, 300, 600 (2 groups) mg/kg bw administered by intraperitoneal injections in a volume of 10 mL/kg for all groups. The administration took place once a day for 2 consecutive days (at a 24-hour interval) in the negative control and test groups. In the positive control group, cyclophosphamide was administered only once.

A. Test Performance

The study was conducted from December 9th 2004 to January 11th 2005 at the [redacted].

1. Test procedure

The selection of Ethephon doses was based on a pilot test. This pilot test was performed in the laboratory which conducted the main study using animals of the same source, strain and age. Groups

consisting each of three males and three females received two intraperitoneal injections of 1000 mg ethephon /kg bw separated by 24 hours.

In the main study, animals were divided into groups by a randomization plan which distributed the animal numbers to the respective treatment group. Each group comprised five male mice.

Ethephon and the negative control were administered twice, separated by 24 hours, whereas cyclophosphamide was administered only once. Animals of the replacement groups were treated twice with 600 mg/kg bw.

Animals were sacrificed 24 hours after the last treatment.

Table 5.4.2- 1: Study design

Group	Dose (mg/kg bw/)	Application route	Number of applications
Negative control	0	i.p.	2
Ethephon	150	i.p.	2
	300	i.p.	2
	600	i.p.	2
	600	i.p.	2
Positive control (CP)	20	i.p.	2

CP = Cyclophosphamide

2. Slide preparation

Schmid's method was used to produce the smears.
At least one intact femur was prepared from each sacrificed animal (not pre-treated with a spindle inhibitor). A suitable instrument was used to sever the pelvic bones and lower leg. The femur was separated from muscular tissue. The lower-leg stump, including the knee and all attached soft parts, was separated in the distal epiphyseal cartilage by a gentle pull at the distal end. The proximal end of the femur was opened at its extreme end with a suitable instrument, e.g. fine scissors, making visible a small opening in the bone-marrow channel. A suitable tube was filled with sufficient foetal calf serum. A small amount of serum was drawn from the tube into a suitable syringe with a thin cannula. The cannula was pushed into the open end of the marrow cavity. The femur was then completely immersed in the calf serum and pressed against the wall of the tube, to prevent its slipping off. The contents were then flushed several times and the bone marrow was passed into the serum as a fine suspension. Finally, the flushing might be repeated from the other end, after it had been opened. The tube containing the serum and bone marrow was centrifuged in a suitable centrifuge at approximately 1000 rpm for five minutes. The supernatant was removed with a suitable pipette (e.g. Pasteur pipette), leaving only a small remainder. The sediment was mixed to produce a homogeneous suspension. One drop of the viscous suspension was placed on a well-cleaned slide and spread with a suitable object, to allow proper evaluation of the smear. The labelled slides were dried overnight. If fresh smears needed to be stained, they needed to be dried with heat for a short period.

Staining of Smears
The smears were stained manually 3 minutes in pure May-Grünwald solution, 1 minute in May-Grünwald solution [May-Grünwald/deionized water (1+2)], 28 minutes in Giemsa solution [Giemsa

solution/ deionized water (1+40)], rinsed in deionized water and allowed to dry for at least overnight. All solutions used during this preparation were freshly prepared each time. The May-Grünwald- and Giemsa solution was filtered before usage.

Covering of smears

Following this treatment, the smears were transferred to a holder. A cuvette was filled with xylene, into which the holder was immersed for approximately ten minutes. The slides were removed singly (e.g. with tweezers) to be covered. A small amount of covering agent was taken from a bottle with a suitable object (e.g. glass rod) and applied to the coated side of the slide. A cover glass was then placed in position without trapping bubbles. The slides were not evaluated until the covering agent had dried.

3. Evaluation

Coded slides were evaluated using a light microscope at a magnification of about 1000. Micronuclei appear as stained chromatin particles in the anucleated erythrocytes. They can be distinguished from artifacts by varying the focus. Normally, 2000 polychromatic erythrocytes were counted per animal. The incidence of cells with micronuclei was established by scanning the slides in a meandering pattern. The ratio of polychromatic to normochromatic erythrocytes is analysed for two reasons:

1. Individual animals with pathological bone-marrow depressions may be identified and excluded from the evaluation.
2. An alteration of this ratio may show that the test compound actually reaches the target.

Therefore, the number of normochromatic erythrocytes per 2000 polychromatic ones was noted. If the ratio for a single animal amounts to distinctly more than 6000 normochromatic erythrocytes per 2000 polychromatic ones, or if such a ratio seems likely without other animals in the group showing similar effects, then the case may be regarded as pathological and unrelated to treatment, and the animal may be omitted from the evaluation. A relevant, treatment-related alteration of the ratio polychromatic to normochromatic erythrocytes can only be concluded if it is clearly lower for a majority of the animals in the treated group than in the respective negative control. In addition to the number of normochromatic erythrocytes per 2000 polychromatic ones, the number of normochromatic erythrocytes showing micronuclei was also established. This information is useful in two ways. Firstly, it permits the detection of individuals already subject to damage before the start of the test. Secondly, combined with the number of micronucleated polychromatic erythrocytes, it permits a representation of the time-effect curve for positive substances. An increase in the number of micronucleated normochromatic erythrocytes, without a preceding increase in micronucleated polychromatic erythrocytes, is irrelevant to the assessment of a clastogenic effect, since normochromatic erythrocytes originate from polychromatic ones. Before an effect can be observed in normochromatic erythrocytes, there must be a much greater increase in micronucleated polychromatic erythrocytes, due to the "dilution effect" of the "old" cells, i.e. normochromatic erythrocytes already present at the start of the test, and this effect would have been observed previously.

In order to check the initial results obtained with male animals, an independent second evaluation was performed. Stained parallel slides from all male animals of the study, which were not used in the first evaluation, were coded and evaluated for micronuclei by scoring additionally 2000 polychromatic erythrocytes per animal. Furthermore, the frequency of micronuclei in normochromatic erythrocytes as well as the ratio of polychromatic to normochromatic erythrocytes was determined.

4. Assessment criteria

A test was considered positive if there was a relevant and significant increase in the number of polychromatic erythrocytes showing micronuclei in comparison to the respective negative control.

A test was considered negative if there was no relevant or significant increase in the rate of micronucleated polychromatic erythrocytes. A test was also considered negative if there was a significant increase in that rate which, according to the laboratory's experience was within the range of historical negative controls.

In addition, a test was considered equivocal if there was an increase of micronucleated polychromatic erythrocytes above the range of attached historical negative controls, provided the increase was not significant and the result of the negative control was not closely related to the data of the respective treatment group. A test was also considered equivocal, if its result was implausible. In both cases normally a second test will be performed.
An assay was considered acceptable if the figures of negative and positive controls were within the expected range, in accordance with the laboratory's experience and/or the available literature data.

5. Biometry

The ethephon group(s) with the highest mean (provided this exceeded the negative control mean) and the respective positive control were checked by Wilcoxon's non-parametric rank sum test with respect to the number of polychromatic erythrocytes having micronuclei and the number of normochromatic erythrocytes.

A variation was considered statistically significant if its error probability was below 5% and the treatment group figure was higher than that of the negative control.

The rate of normochromatic erythrocytes containing micronuclei was examined if the micronuclear rate for polychromatic erythrocytes was already relevantly increased. In this case, the group with the highest mean was compared with the respective negative control using the one-sided chi2-test. A variation was considered statistically significant if the error probability was below 5% and the treatment group figure was higher than that of the negative control.
In addition, standard deviations (Is ranges) were calculated for all the means.

II. RESULTS AND DISCUSSION

A. PILOT STUDY (RANGE FINDING TEST)

This pilot test was performed in the laboratory which conducted the main study using animals of the same source, strain and age. In males and females the following symptoms were recorded, following administration of 1000 mg/kg: apathy, roughened fur, loss of weight, spasm and difficulty in breathing. Symptoms were recorded for up to at least 24 hours after the second application. One animal of each sex dies.

Based on this findings, 600 mg ethephon/kg bw was chosen as MTD to be conducted in the males only, as no substantial differences in toxicity were observed between sexes.

B. MAIN STUDY (MICRONUCLEUS ASSAY)

1. Toxicity

After two intraperitoneal administrations of 150, 300 and 600 mg/kg ethephon, treated males showed the following compound-related symptoms for up to 15 minutes after the second administration: apathy, roughened fur and spasm. These symptoms demonstrate systemic exposure of males to ethephon.

Otherwise, their external appearance and physical activity remained unaffected. There was no substance-induced mortality. No symptoms were recorded for the control groups. No animals died in these groups

2. Microscopic evaluation

The ratio of polychromatic to normochromatic erythrocytes was not altered by the treatment with ethephon, being 2000:1488 (1s=488) in the negative control, 1952 (1s=830) in the 150 mg/kg group, 2000:1990 (1s=665) in the 300 mg/kg group and 2000:1884 (1s=132) in the 600 mg/kg group. No relevant variations were thus noted for males.
No biologically important or statistically significant variations existed for males between the negative control and the groups treated with intraperitoneal injection of ethephon, with respect to the incidence of micronucleated polychromatic erythrocytes. The incidence of these micronucleated cells was 3.2/2000 (1s=1.1) in the negative control, and 2.8/2000 (1s=1.6), 1.6/2000 (1s=1.3) and 2.0/2000 (1s=1.9) in the Ethephon groups.

Similarly, there was biologically significant variation between the negative control and Ethephon groups in the number of micronucleated normochromatic erythrocytes, since normochromatic erythrocytes originated from polychromatic ones. As expected, relevant variations were not observed.
The positive control, cyclophosphamide, caused a clear increase in the number of polychromatic erythrocytes with micronuclei. The incidence of micronucleated cells was 17.0/2000 (1s=4.6), which represents biologically relevant increases in comparison to the negative control.
There could not have been a biologically relevant effect on the number of micronucleated normochromatic erythrocytes in the positive control since, in conjunction with the cell-cycle duration, normochromatic erythrocytes originated from polychromatic ones.

No further effect of cyclophosphamide was found concerning the ratio of polychromatic to normochromatic erythrocytes, since this ratio did not vary to a biologically relevant degree [2000: 1895 (1s=1014), as against 2000:1488 in the negative control].
This clearly demonstrates that an alteration of the ratio of polychromatic to normochromatic erythrocytes is not necessary for the induction of micronuclei.

3. Assessment

Normally, cells with micronuclei (Howell-Jolly bodies) occur in polychromatic erythrocytes with an incidence of up to approximately 6.0/2000. The increase in micronucleated polychromatic erythrocytes, due, for example, to chromosome breaks or spindle disorders, is the criterion for clastogenic effects in this test model.

The results with Ethephon gave no indications of clastogenic effects for male mice after two intraperitoneal treatments with doses of up to and including 600 mg/kg.
The known mutagen and clastogen, cyclophosphamide, had a clear clastogenic effect at an intraperitoneal dose of 20 mg/kg. The number of micronucleated polychromatic erythrocytes increased to a biologically relevant degree.
The number of micronucleated normochromatic erythrocytes did not increase relevantly in any of the groups.

It is of further interest to establish the number of normochromatic cells, to learn whether the ratio of polychromatic to normochromatic erythrocytes was altered by treatment. This ratio did not vary to a biologically relevant degree in the ethephon groups in comparison to the negative control.

Cyclophosphamide did not change this ratio.

III. CONCLUSIONS

In conclusion, following intraperitoneal administration up to 600 mg/kg bw, there was no indication of a clastogenic effect of ethephon in the micronucleus test on the male mouse, i.e. in a somatic test system in vivo.

Table 5.4.2- 2: Summary of the micronucleus test results in male mice

Group	Number of evaluated PCEs	Number of NCE per 2000 PCE (mean ± SD)	MNNCE per 2000 NCE (mean ± SD)	MNPCE per 2000 PCE (mean ± SD)
Males				
Negative control	10000	1888 ± 488	3.4 ± 1.4	3.2 ± 1.1
Ethephon 2×150 mg/kg	10000	1952 ± 830	1.0 ± 1.1	2.8 ± 1.6
Ethephon 2×300mg/kg	10000	1990 ± 665	1.0 ± 1.4	1.6 ± 1.3
Ethephon 2×600 mg/kg	10000	1884 ± 132	0.9 ± 0.9	2.0 ± 1.9
CP 20 mg/kg	10000	1895 ± 1014	2.6 ± 2.0	13.0 ± 4.6 *

CP: Cyclophosphamide monohydrate (positive control)
*: $p < 0.01$, non-parametric Wilcoxon ranking test

NCE= normochromatic erythrocytes MNNCE= micronucleated NCE
PCE= polychromatic erythrocytes MNPCE= micronucleated PCE

CA 5.4.3 In vivo studies in germ cells

As ethephon is devoid of genotoxic potential in somatic cells, no genotoxic studies in the germ cells were carried out.

Comparison with the criteria
Although ethephon base 250 induced point mutations in S. typhimurium in the absence and presence of metabolic activation in tester strain TA 1535 at cytotoxic doses, in 4 other strains, was negative. Ethephon Base 250 was also negative in a gene mutation test with CHO Chinese hamster ovary cells, an UDS test with rat hepatocytes and a chromosome aberration test with CHO Chinese hamster ovary cells. Furthermore, ethephon Base 250 was negative in an *in vivo* UDS test in rats. In addition it was negative also in an *in vivo* micronucleus study in the mouse.
Therefore, ethephon Base 250 is considered to be non-genotoxic

Conclusions on classification and labelling
No classification is necessary for genotoxicity.

CA 5.5 Long-term toxicity and carcinogenicity

No new long-term toxicity and carcinogenity study was carried out after ethephon Annex I inclusion. The existing relevant information was submitted and evaluated during the EU process for Annex I listing.

In all the dietary studies the concentration of ethephon was measured and/or the diets were corrected for the purity of ethephon base 250 (which has a content of ethephon of 71.3%). Therefore the calculated achieved intake represent the actual concentration of ethephon administered to the animals and no additional correction for purity is needed.

The long term toxicity and carcinogenic potential of ethephon were assessed in one long term study in rats and one in mice.

Table 5.5-1 Summary of long-term toxicity and carcinogenicity studies

Study	NOAEL (mg/kg bw/day)	LOAEL (mg/kg bw/day)	Effects
Combined chronic toxicity oncogenicity study Sprague-Dawley rats (100/ sex/dose) 0, 300, 3000 , 10000, 30000 ppm, 0, 13, 131, 446, 1416 mg/kg bw/day in ♂ 0, 16, 161, 543,1794 mg/kg bw/day in ♀ [redacted]; 1989 M-187711-01-1	13 (♂) – 16(♀)	131(♂) - 161 (♀)	No carcinogenic effects found. At 3000 ppm: ↓body weight and food consumption in both sexes. ↑thyroglossal duct cysts, kidney glomerulo-sclerosis and nephritis and biliary hyperplasia cholangiofibrosis. At 10000 ppm ↓body weight in ♂ At 3000 ppm ↓RBC ChE activity
78 week dietary oncogenicity study in CD-1 mice (50/sex/ dose) 0, 100, 1000, 10000 ppm, 0, 14, 139, 1477 mg/kg bw/day in ♂ 0, 17, 173 or 1782 mg/kg bw/day in ♀ [redacted]; 1988 M-187730-01-1	14 (♂) – 173(♀)	139(♂) - 1782(♀)	No carcinogenic effects found. At 10000 ppm ↓body weights and weight gains in ♀ At 1000 ppm ↓RBC ChE activity

In a 24-months combined chronic toxicity/carcinogenicity study in rats, doses of 0, 300, 3000, 10000 and 30000 ppm were administered (equal to 0, 13, 131, 446 and 1416 mg ethephon/kg bw/day in males and 0, 16, 161, 543 and 1794 mg/kg bw/day in females. Final sacrifices were in week 97 for males and in week 104 for females (M-187711-01-1).

The main effect of ethephon administration was a dose-dependent inhibition of plasma and erythrocyte ChE activities, noted at all dose levels.

Table 5.5-2 Summary of ChE activity in the rat long-term and carcinogenicity study

Group Males	13-week[a]		26-week[a]		51-week[a]		52 week[b]			56 week[c]			78 week[a]		97 week[d]		
	PC	RBC	PC	RBC	PC	RBC	PC	RBC	BC	PC	RBC	BC	PC	RBC	PC	RBC	BC
300	18	9	17	10	29	6	12	2	-	NA	NA	NA	26	4	44	8	2
3000	27	45	36	42	47	47	35	47	6	NA	NA	NA	35	47	32	39	4
10000	38	65	42	72	48	78	46	70	-	3	15	-	41	72	67	81	-
30000	45	83	51	86	56	84	62	86	7	24	22	8	48	87	56	86	-
Group Females	**13-week[a]**		**26-week[a]**		**51-week[a]**		**52 week[b]**			**56 week[c]**			**78 week[a]**		**104 week[d]**		
	PC	**RBC**	**PC**	**RBC**	**PC**	**RBC**	**PC**	**RBC**	**BC**	**PC**	**RBC**	**BC**	**PC**	**RBC**	**PC**	**RBC**	**BC**
300	23	13	24	11	15	19	22	11	-	NA	NA	NA	27	8	22	9	-
3000	59	55	58	58	53	63	48	50	-	NA	NA	NA	46	59	37	43	-
10000	61	72	65	79	64	78	62	75	-	9	16	-	58	77	47	73	2
30000	72	82	69	86	61	85	71	88	-	13	24	-	56	83	66	86	2

[a]Core/Chronic toxicity animals
[b]satellite/12 Month interim sacrifice animals
[c]recovery animals
[d]Core/Chronic toxicity animals except where Core/Oncogenicity animals were used to ensure 10 samples per group
- indicates equivalent or greater ChE activity than the controls
NA = Not Applicable (no animals in these groups included in the recovery period)

At the lowest dose of 13-16 mg/kg bw/day, the inhibition was considered not biologically significant (<20%). Complete recovery of plasma and erythrocyte CHE activity to control values was not observed in animals at 446 mg/kg bw/day and higher, and maintained under control conditions for 4 weeks following 52-week exposure. The observed inhibition of brain ChE activity (< 9%) was not considered biologically significant.

In the liver, the incidence of biliary hyperplasia was significantly higher in males at 30000 ppm at terminal sacrifice. The main cause of death in both sexes was pituitary adenoma. The incidence of this lesion as cause of death was equally distributed among groups and therefore is unrelated to treatment with ethephon.
There were no increases in neoplasms associated with ethephon treatment.
There was no evidence of carcinogenicity.

Table 5.5-3 Summary of tumor incidence in the rat long-term and carcinogenicity study

Observation	Males -Dose levels (ppm)					Females -Dose levels (ppm)				
	0	**300**	**3000**	**10000**	**30000**	**0**	**300**	**3000**	**10000**	**30000**
Number of animals in dose groups	100	90	90	100	100	100	90	90	100	100
Pituitary adenoma	51	44	54	40	45	69	62	67	58	68
Pituitary carcinoma	2	1	0	2	0	1	5	1	4	0
Hepatocellular adenoma	0	3	5*	2	5	0	2	1	1	0
Hepatocellular carcinoma	1	3	2	0	1	0	0	0	0	0

* Significantly different from control; p = 0.05

Based on inhibition of erythrocyte ChE activity at 300 ppm, the NOAEL for long-term toxicity and carcinogenity in the rat was 300 ppm, equivalent to 13 and 16 mg/kg bw/d in males and females, respectively,

In the 18 months carcinogenicity study in mice (M-187730-01-1), ethephon was given at dietary doses of 0, 100, 1000 and 10000 ppm (equal to mean intake of 14, 139 and 1477 mg/kg bw/day for males and 17, 173 and 1782 mg ethephon/kg bw/day for females) for 78 weeks.

Ethephon inhibited plasma and erythrocyte ChE activity with dose-related inhibition. At the lowest dose of 14-17 mg/kg bw/d, the inhibition was considered not biologically significant (<20%). Brain ChE activity was inhibited by 18% in females at the highest dose level after 52 weeks, but was comparable to the controls after 78 weeks at terminal sacrifice.

Table 5.5-4 Summary of ChE activity in the mice long-term and carcinogenicity study

Group		52 week			78 week		
		Plasma	RBC	Brain	Plasma	RBC	Brain
Males	100	--	--	--	2	--	--
	1000	35	36	--	41	35	--
	10000	65	70	--	71	72	--
Females	100	18	17	8	24	14	--
	1000	41	36	4	61	21	--
	10000	76	74	18	74	21	--

Activity expressed as % compared to the control group
- indicates equivalent or greater ChE activity than the controls

Two tumour types in males (hepatocellular adenoma and lung adenoma) and two types in females (lymphosarcoma and lung adenoma) were observed in frequencies above 5%, but only the increased incidence of lung adenomas in males at the intermediate dose of 1477 mg/kg bw/day reached the level of statistical significance.

Table 5.5-8 Incidence of lung tumours in the mice long-term and carcinogenicity study

Males	**0**	**100 ppm**	**1000 ppm**	**10000 ppm**	**Range HCD[a] (1985-1991)**	
Lung adenoma - N (%)	2/69 (2.9)	5/70 (7.1)	14/70* (20)	6/70 (8.6)	0 (0)	15/69 (21.74)
Lung carcinoma - N (%)	0/69 (0)	1/70 (1.45)	1/70 (1.45)	0/70 (0)	0 (0)	16.69 (23.19)
Females	**0**	**100 ppm**	**1000 ppm**	**10000 ppm**	**Range HCD[a] (1985-1991)**	
Lung adenoma - N (%)	7/70 (10)	4/69 (5.8)	7/69 (10.1)	7/70 (10)	0 (0)	11/70 (15.71)
Lung carcinoma - N (%)	0/70 (0)	1/69 (1.45)	1/69 (1.45)	0/70 (0)	0 (0)	7/70 (10)

* = p< 0.05 Fisher's test.
[a] = published compilations of Historical Control Data CD-1 mice ([redacted], document M-515478-01-1)

The incidence of lung adenomas observed in this study was not considered to be related to treatment, since lung adenomas commonly occur in this strain of mouse, the incidence observed in the study was within the historical control data and within the historical control data and the incidence was not dose-dependent.

Based on inhibition of erythrocyte ChE activity at 1000 ppm, the long term NOAEL was 100 ppm, equivalent to 14 and 17 mg/kg bw/day in males and females, respectively.

Report:	KCA 5.5/03; [redacted]; 2012; M-515478-01-1
Title:	Position paper - Ethephon (AE F016382) - Mouse oncogenicity study: spontaneous occurrence of lung tumours in CD-1 mice
Report No.:	M-515478-01-1
Document No.:	M-515478-01-1
Guideline(s):	not specified
Guideline deviation(s):	not specified
GLP/GEP:	**no**

This document reports the data published by [redacted] on the spontaneous incidence of lung tumours observed in the in the CD-1 mice in a series of 78-week oncogenicity studies. These data were submitted to the RAC/ECHA Committed during the discussion on Ethephon harmonised classification and labelling in EU (2010).

Comparison with criteria

There was no evidence of treatment-related carcinogenicity in the combined long-term and carcinogenicity studies in rat and mice following dietary administrations up to and above the limit dose of 1000 mg/kg bw/day.

Conclusions on classification and labelling

No classification for carcinogenicity is warranted.

Report:	KCA 5.5/04; [redacted]; [redacted]; 1977; M-187724-01-1
Title:	A two-year Study in Dogs Ethrel Final Report
Report No.:	R013319
Document No.:	M-187724-01-1
Guideline(s):	--
Guideline deviation(s):	--
GLP/GEP:	**no**
Restrictions:	Data are considered of questionable relevance for reference value derivation. The study has several limitations and was not performed according to GLP. The study was conducted using various sources of Ethephon, which were not well characterised by analytical data. This fact is of special relevance as different macroscopical as well as histopathological effects were observed at the same doses of Source A (thickened wall in stomach, smooth muscle hypertrophy in stomach/small intestine) and Source B (no effects). Body weight variation of single animals was high at the beginning of the study (7.8 – 13.5 kg for males; 7.3 – 10.4 kg for females) and also body weights at termination showed high variations. Males of the control group showed body weights from 12.7 to 18.2 kg at the end of termination. It is not clear if intermittent emesis that was observed throughout animals of all groups contributed to these high variations at the end of the study. No historical control data were available for further evaluation.

Report:	KCA 5.5/07; [redacted]; [redacted]; 2017; M-588162-01-1
Title:	Summaries of toxicity studies requested by RMS (the Netherlands) in the draft RAR - Volume 3 - Annex B (AS) - Ethephon - B.6 Toxicology and metabolism
Report No.:	M-588162-01-1
Document No.:	M-588162-01-1
Guideline(s):	none
Guideline deviation(s):	--
GLP/GEP:	**no**

Executive Summary

In a 2-year study, 6 male and female Beagle dogs per dose group received ethephon (purity 75.6%, batch no. AL 1030-42 for Source A) at dietary concentrations of 0, 30, 300 and 1500 ppm, equal to 0, 0.86, 7.6 and 42.2 mg/kg bw per day for males and 0, 0.86, 8.4 and 47.8 mg/kg bw per day for females for 104 weeks. In the highest dose group, the dose was 3000 ppm in the first three weeks and changed to 1500 ppm (from Week 25) through 2000 ppm (Weeks 4 to 5) and 1000 ppm (Weeks 6-24) due to the persistent decrease of body weight gain in the first three weeks. In this study, another source of ethephon (Source B, purity 73.6%, batch no. AL-3096) at dietary concentration of 300 ppm, equal to 8.0 and 8.6 mg/kg bw per day for males and females, respectively, was administered to 6 dogs/sex in the same manner.

All animals were checked daily for mortality, moribundity, and clinical signs. Feed consumption and body weights were measured weekly in the first four weeks and every four weeks after Week 5. Haematology and clinical chemistry including plasma and erythrocyte ChE activity were performed Weeks 0, 13, 26, 52, 78 and 104. Fasting blood glucose was also determined at Weeks 31 (in the controls and 1500 ppm group) and 39 (for all dogs). After a 104-week treatment, all animals were maintained at the appropriate diet level for 10 days before the sacrifice. All dogs were necropsied, and weights of thyroids, heart, liver, spleen, kidneys, adrenals and testes with epididymides were recorded. Histopathological examination was performed in all groups. The brain ChE activity of ethephon was measured at the termination. This study was not conducted in compliance with the GLP standards.

There were no treatment-related effects on mortalities. In the highest dose group, the high incidence of soft stools was recorded in the first four weeks (at 3000 or 2000 ppm) and observed persistently in males or sporadically in females during the study. High incidence of intermittent emesis was also observed in the highest group of both sexes. There were no statistically significant and treatment-related effects on body weight from Week 25 and food consumption in all the treated groups. In the haematology, clinical chemistry and absolute and relative organ weights, any treatment-related significant changes except of cholinesterase activity were not observed in all treated groups in both sexes. From week 6, erythrocyte AChE activity (AChE) was statistically significantly inhibited at >20% (42-56% in males and 47-56% in females at 300 ppm and 68-79% in males and 59-79% in females at 1500 ppm) in both sexes at the mid and high dose level, compared with the corresponding controls and the values at Week 0. Brain AChE activity was not affected at all the doses treated in both sexes. Morphologically, smooth muscle hypertrophy was observed in dogs treated with ethephon of source A, but not in any animal treated with ethephon of source B. Thereby, smooth muscle hypertrophy was observed mainly in females in the duodenum, the stomach or in jejunum and ileum, respectively. In males, smooth muscle hypertrophy of the duodenum was observed in one animal at the high dose group. However, the lesion was not observed in other small intestines in the same animal or other treated groups in males. In the stomach and small intestine, other findings such as chronic gastritis and congestion in the duodenum were noted at the mid and high dose group in males and/or females. However the affected animals were different from the ones showing smooth muscle hypertrophy. As it is reported that smooth muscle hypertrophy in the intestine is caused by obstruction, diverticulum, inflammation, infection or spontaneousness in animals (Murakami et al., 2010; Liu et al., 2014; Bettini et al., 2003), it is considered that the cause of the gastrointestinal effects,

only observed with ethephon source A, might be related to the different dietary preparation and/or source of ethephon tested and to the corrosive properties of ethephon (pH close to 2) on the gastric mucosa. This is also confirmed by the presence of findings like chronic gastritis, diffuse infiltration of inflammatory cells in the stomach and congestion at intestinal levels in the mid and top dose animals. There were no other treatment-related findings in other examined organs.

In a 2-year study in dogs administered ethephon at a dietary concentration of 0, 30, 300 or 1500 ppm (equal to 0, 0.86, 7.6 and 42.2 mg/kg bw per day for males and 0, 0.86, 8.4 and 47.8 mg/kg bw per day for females, respectively), the NOAEL was 30 ppm (equal to 0.86 mg/kg bw per day), based on reduction of erythrocyte AChE activity at 300 ppm (equal to 7.6 mg/kg bw per day).

The study is considered valid with restrictions, as two different sources of ethephon with not well characterized composition have been used. In addition, the study is not performed according to GLP.

I. Material and Methods

A. Materials

1. Test material

Test substance:	Ethephon
Common name:	2-Chloroethylphosphonic acid
Description:	Dark-brown liquids
Purity:	75.6% (Source A), 73.6% (Source B) (no analytical certificate available)
Batch no.:	Source A (Base 211, AL 1030-42) used for Groups 2, 3, and 4 and Source B, (Base 211, AL-30963096) used for Group 5
Stability:	Not reported (test substance was received on May 29, 1975)

2. Vehicle and/or positive control

Vehicle:	Basal diet, no positive control

3. Test animals

Species:	Dog
Strain:	Beagle, purebred
Age:	Young adults
Weight at start:	Males: 7.8 – 13.5 g Females: 7.3 – 10.4 g
Source:	[redacted]
Acclimation period:	Not reported
Diet:	Ground Wayne® Dog Food, *ad libitum*
Water:	Tap water, *ad libitum*
Housing:	Individually in elevated metal cages
Temperature:	not specified
Humidity:	not specified
Air changes:	not specified
Photoperiod:	not specified

B. Study design

1. In life dates: 24th June 1975 to 21th June 1977

2. Animal assignment and treatment

Before treatment commenced, all animals were weighed and assigned to treatment groups. Sixty (60) dogs, 30 males and 30 females were assigned to dosage groups. The treated animals were given the appropriate treated diets while control animals received basal diet *ad libitum* for 104 weeks.

Table 1: Study design and dose received

Group	**Dose (ppm)**	**Ethephon (mg/kg bw)**		**No of dogs (M/F)**
		Males	Females	Toxicity
1. Control	0	0	0	6/6
2. Low dose	30	0.86	0.86	6/6
3. Mid dose (Source A)	300	7.6	8.4	6/6
4. High dose	3000 (Week 0-3) 2000 (Week 4-5) 1000 (Week 6-24) 1500 (Week 25-104)	42.2	47.8	6/6
5. Mid dose (Source B)	300	8.6	8.0	6/6

3. Dose selection rational

No data about rationale for dose level selection were given in the report.

4. Diet preparation and analysis

Diets were prepared weekly by adding the required quantity of the test article to a small amount of Ground Wayne® Dog Food and by mixing using a mortar and pestle for Groups 2, 3, and 5, and in a Hobart blender for Group 4. The premix was then added to the remainder of the Ground Wayne® Dog Food to attain the required level and mixed in a twin-shell Patterson-Kelly blender fitted with an intensifier bar. Fresh diets were prepared each week.

No data about the check of the accuracy of formulations by analysis of samples taken from the diets prepared were presented in the report.

5. Statistics

Numerical data obtained during the conduct of the study were subjected to calculation of group mean values and standard deviations. Statistical analyses of the body weights, clinical laboratory data, and terminal body weights, organ weights and organ/body weight ratios were performed by Bartlett's test for homogeneity of variances ([redacted], 4:137, 1937) and the one-way classification analysis of variances ([redacted], 10:258-268, 1967). When differences were noted in the analysis of variances, Scheffe's method for judging all contrasts was utilized ([redacted], A Method for Judging All Contrasts in the Analysis of Variance, Biometrika, Vol. 40, Parts 1 and 2 June, 1953).

C. Methods

1. Observations

All animals were examined daily for mortality, moribundity, and clinical signs.

2. Body weight and feed intake

Individual body weights and feed consumption was measured weekly for the first four weeks of treatment and every four weeks thereafter. For all animals sacrificed after 104 weeks of treatment, fasted body weights were measured prior to terminal sacrifice.

3. Ophthalmoscopic examination

Treatment related effects on the eyes were not recorded.

4. Haematology and clinical chemistry

Laboratory investigations (haematology, clinical chemistry) were performed on all animals prior to commencement of treatment (Week 0) and at Week 13, 26, 52, 78, and 104.
Thereby, for haematology, haematocrit, haemoglobin levels, erythrocyte and total and differential leukocyte counts were recorded. Regarding clinical chemistry, serum glutamic pyruvic transaminase, alkaline phosphatase, blood urea nitrogen, plasma and erythrocyte cholinesterase, and fasting glucose were determined. Furthermore, plasma and erythrocyte cholinesterase values were measured on all dogs at Week 6 and additional fasting glucose values were recorded at Week 31 (in the controls and 1500 ppm group) and Week 39 (all dogs). Brain cholinesterase values were measured on all dogs at termination.
Food and water was removed overnight from animals selected for haematology and clinical chemistry. Blood samples for the haematology and clinical chemistry determinations were collected by jugular puncture.

5. Sacrifice and pathology

On study completion after the Week 104 of treatment all surviving animals were fasted overnight and sacrificed by exsanguination under Surital® anaesthesia and necropsied.

The necropsy consisting of an external examination including identification of all clinically recorded masses as well as a detailed internal examination (including retention of tissues) was performed on each animal found dead, sacrificed during the conduct of the study or sacrificed after 104 weeks of treatment. Selected organs such as thyroids, heart, liver, spleen, kidneys, adrenals, and testes with epididymides were weighted and organ weights relative to body weight were calculated.

On completion of the gross pathology examination of each animal, various organs and all clinically observed masses were taken and, in order to elucidate abnormal findings, preserved in 10% neutral buffered formalin: brain, pituitary, eyes, thoracic spinal cord, mandibular salivary gland, thyroids, heart, lung, liver, gallbladder, spleen, kidneys, adrenals, stomach, pancreas, small intestine (three sections), large intestine, mesenteric lymph node, urinary bladder, ovaries, uteri, prostate, sciatic nerve with adjacent muscle, femoral bone marrow, costochondral junction, and any found lesions. The testes with epididymides were preserved in Bouin's fixative. The following tissues were prepared for histopathological examination by embedding in paraffin wax, sectioning and staining with haematoxylin and eosin: thyroids, adrenals, liver, kidneys, stomach, and small intestine (three sections), large intestine, pancreas, sciatic nerve with adjacent muscle, femoral bone marrow, and costochondral junction from the control dogs and from the mid- and high-dose dogs receiving the test substance. Sections of the stomach, small intestine (three sections) and large intestine, taken from the low-dose dogs were treated in the same manner.

II. Results and discussion

1. Analytical results

Analytical data, such as accuracy of formulations checked by analysis of the concentrations of the test substance in the different treatment groups as well as storage stability and homogeneity of the preparations and storage conditions were not given in the report.

2. Clinical results

There were no treatment-related effects on mortality.

No treatment-related clinical signs were observed except for a high incidence of soft faeces observed in females and males in the first four weeks (at 3000 or 2000 ppm) and thereafter, after Week 16 persistently in males or sporadically in females. Furthermore, intermittent emesis was observed in the animals of all groups; however, a high incidence was found in the highest group of both sexes during Week one to four.

3. Body weight and feed intake

There were no statistically significant and treatment-related effects on the mean body weights of any of the treated groups from Week 25 and food consumption when compared to the mean control body weights. Minimal body weight losses and decreased food consumptions were noted in the high dose group dogs during the first three weeks of the study. Thereafter, a recovery in body weight and food consumption was noted.

Table 2: Body weight and mean food consumption

Dose [ppm]	wk	Males					Females				
		0	30	300	1500*	300	0	30	300	1500*	300
Body weight [kg]	0	10.60	10.83	10.55	11.47	10.98	8.83	8.53	8.73	8.90	8.83
	12	11.82	11.90	11.70	11.58	12.56	9.70	9.55	9.50	9.60	9.45
	24	12.27	11.58	11.58	11.48	12.93	9.92	9.17	9.72	10.02	9.32
	52	12.82	12.60	12.53	13.60	13.65	10.90	9.92	10.88	10.42	10.03
	76	13.93	12.92	13.07	14.07	14.42	11.43	10.80	11.73	10.78	10.37
	104	14.75	13.13	13.82	14.52	14.68	12.03	11.38	12.22	11.45	10.90
Body weight gain [g] *(% change vs control)*	0-12	+1.22	+1.07	+0.15	+0.60	+1.09	+0.87	+1.02	+0.77	+0.70	+0.62
	0-24	+1.67	+0.75	+1.03	+0.50	+1.46	+1.09	+0.64	+0.99	+1.12	+0.49
	0-52	+2.22	+1.77	+1.98	+2.60	+2.18	+2.07	+1.39	+2.15	+1.52	+1.20
	0-76	+3.33	+2.09	+2.52	+3.09	+2.95	+2.60	+2.27	+3.00	+1.88	+1.54
	0-104	+4.15	+2.30	+3.27	+3.54	+3.21	+3.20	+2.85	+3.49	+2.55	+2.07
Mean feed consumption (g/animal)	12	2.52 ±0.28	2.40 ±0.33	2.22 ±0.15	2.55 ±0.37	2.30 ±0.40	2.13 ±0.76	1.87 ±0.21	2.20 ±0.25	2.37 ±0.23	1.68 ±0.35
	24	2.43 ±0.10	2.57 ±0.48	2.03 ±0.31	2.33 ±0.43	2.53 ±0.45	2.10 ±0.22	1.98 ±0.41	1.98 ±0.32	2.27 ±0.23	2.03 ±0.27
	52	2.58 ±0.35	2.73 ±0.43	2.13 ±0.42	2.50 ±0.38	2.55 ±0.34	2.08 ±0.59	1.80 ±0.29	1.98 ±0.42	2.27 ±0.71	2.00 ±0.43

Dose [ppm]	wk	Males					Females				
		0	30	300	1500*	300	0	30	300	1500*	300
	76	2.58 ±0.45	2.52 ±0.04	2.12 ±0.47	2.48 ±0.36	2.50 ±0.46	2.03 ±0.46	2.12 ±0.26	2.00 ±0.25	1.95 ±0.45	1.90 ±0.25
	104	2.42 ±0.12	2.23 ±0.32	1.88 ±0.37	2.10 ±0.33	2.10 ±0.40	1.67 ±0.61	1.60 ±0.23	1.75 ±0.18	2.05 ±0.54	1.85 ±0.47

* 3000 ppm (Weeks 0-3), 2000 ppm (Weeks 4-5), 1000 ppm (Weeks 6-24), 1500 ppm (Weeks 25-104)

4. Ophthalmoscopic results

Treatment related effects on the eyes were not recorded in this study. Ophthalmology was tested in a 1-year study, in five male and five female Beagle dogs per dose group which received ethephon at a dietary concentration of up to 2000 ppm pretreatment for 52 weeks. In this study no significant treatment-related effects on ophthalmological parameters were observed.

5. Haematology and clinical chemistry

In the haematology and clinical chemistry, any treatment-related significant changes were not observed in all treated groups in both sexes. From week 6, statistically significant inhibition of mean plasma cholinesterase (AChE) occurred in all treatment groups. Erythrocyte AChE activity was statistically significantly inhibited at >20% (42-56% in males and 47-56% in females at 300 ppm and 68-79% in males and 59-79% in females at 1500 ppm) in both sexes at 300 ppm and higher, compared with the corresponding controls and the values at Week 0. Brain AChE activity was not affected at all the doses treated in both sexes (ranges from 20.60 to 52.29% above the mean male control value and for females from 3.68 to 38.95% above the mean female control value).

Table 3: Plasma Cholinesterase in dogs exposed to ethephon for 2 years (in the diet).

Dose [ppm]	wk	Males					Females				
		0	30	300	1500[1]	300	0	30	300	1500*	300
Plasma Cholinesterase Δ pH/min	0	1.878±0.168	1.918±0.085	1.901±0.161	1.974±0.117	1.901±0.116	1.932±0.122	1.965±0.166	1.906±0.160	1.931±0.168	1.992±0.070
			+2.13	+1.22	+5.11	+1.22		+1.71	-1.35	-0.05	+3.11
	6	1.786±0.261	1.353±0.273 *	0.925±0.201 *	0.821±0.120 *	0.854±0.110 *	1.912±0.119	1.262±0.178 *	0.921±0.098 *	0.740±0.121 *	0.904±0.100 *
			-24.24	-48.21	-54.03	-52.18		-34.00	-51.83	-61.30	-52.72
	13	1.810±0.085	1.418±0.168 *	0.981±0.100 *	0.929±0.190 *	0.984±0.091 *	1.524±0.150	1.159±0.210 *	0.741±0.074 *	0.622±0.054 *	0.687±0.092 *
			-21.66	-45.80	-44.81	-45.64		-23.95	-51.38	-59.19	-54.92
	26	1.865±0.118	1.372±1.161 *	0.905±0.077 *	0.867±0.102 *	0.908±0.100 *	1.823±0.153	1.135±0.228 *	0.782±0.120 *	0.683±0.093 *	0.715±0.087 *
			-26.43	-51.47	-53.51	-51.31		-37.74	-57.10	-62.53	-60.78
	52	1.553±0.148	1.217±0.149 *	0.811±0.136 *	0.723±0.135 *	0.754±0.085 *	1.797±0.199	1.241±0.154 *	0.853±0.117 *	0.627±0.098 *	0.773±0.096 *
			-21.64	-47.78	-53.44	-51.45		-30.94	-52.53	-65.11	-56.98
	78	1.842±0.161	1.277±0.208 *	0.700±0.124 *	0.613±0.144 *	0.763±0.096 *	2.052±0.180	1.465±0.211 *	0.872±0.095 *	0.793±0.069 *	0.940±0.139 *
			-30.67	-62.00	-66.72	-58.58		-28.61	-57.50	-61.35	-54.19
	104	1.704±0.165	1.184±0.157 *	0.736±0.156 *	0.628±0.089 *	0.737±0.079 *	1.787±0.377	1.253±0.159 *	0.745±0.087 *	0.603±0.106 *	0.745±0.102 *
			-30.52	-55.63	-63.15	-56.75		-29.88	-58.31	-66.26	-58.31

1 3000 ppm Weeks 0-3, 2000 ppm Weeks 4-5, 1000 ppm Weeks 6-24, 1500 ppm Weeks 25-104

* Statistically significant difference from control p<0.05 (Scheffe's method)

Table 4: Erythrocyte cholinesterase in dogs exposed to ethephon for 2 years (in the diet).

Dose [ppm]	wk	Males					Females				
		0	30	300 (A)	1500[1]	300 (B)	0	30	300 (A)	1500	300 (B)
RBC Cholinesterase Δ pH/min	0	1.325±0.160	1.299±0.174	1.181±0.221	1.192±0.150	1.182±0.198	1.176±0.236	1.224±0.188	0.964±0.156	1.126±0.205	1.196±0.175
			-1.96	-10.87	-10.04	-10.79		+4.08	-18.03	-4.25	+1.70
	6	1.296±0.211	1.176±0.151	0.685±0.139*	0.266±0.055*	0.690±0.112*	1.158±0.182	1.055±0.176	0.527±0.078	0.243±0.019*	0.638±0.129*
			-9.26	-47.15	-79.48	-53.70		-8.89	-54.49	-79.02	-44.91
	13	1.117±0.131	1.028±0.126	0.585±0.127*	0.330±0.051*	0.497±0.077*	0.962±0.181	0.980±0.136	0.451±0.068*	0.284±0.035*	0.456±0.049*
			-7.97	-47.63	-70.46	-55.51		+1.87	-53.12	-70.48	-52.59
	26	1.325±0.189	1.256±0.131	0.766±0.127*	0.425±0.035*	0.687±0.078*	1.130±0.152	1.045±0.168	0.565±0.040*	0.332±0.032*	0.689±0.107*
			-5.21	-42.19	-67.92	-48.15		-7.52	-50.00	-70.62	-39.03
	52	1.138±0.102	1.021±0.099	0.612±0.096*	0.334±0.039*	0.597±0.051*	0.969±0.204	0.970±0.171	0.503±0.095*	0.402±0.126*	0.469±0.054*
			-10.28	-46.22	-70.65	-55.49		+0.10	-48.09	-58.51	-51.60
	78	1.349±0.180	1.217±0.111	0.595±0.215*	0.287±0.056*	0.621±0.199*	1.196±0.260	1.316±0.289	0.636±0.106*	0.494±0.127*	0.760±0.094*
			-9.79	-55.89	-78.72	-52.97		+10.03	-46.82	-58.70	-36.45
	104	1.118±0.189	1.000±0.237	0.601±0.134*	0.308±0.031*	0.589±0.076*	1.108±0.275	0.969±0.189	0.486±0.063*	0.293±0.031*	0.568±0.089*
			-10.55	-46.24	-72.50	-47.32		-12.54	-56.14	-73.56	-48.74

1 3000 ppm Weeks 0-3, 2000 ppm Weeks 4-5, 1000 ppm Weeks 6-24, 1500 ppm Weeks 25-104

* Statistically significant difference from control p<0.05 (Scheffe's method)

Other statistically significant intergroup differences observed in clinical chemistry parameters were considered not to be treatment-related because the changes did not show a dose-response relationship. No urinalysis was performed.

6. Organ weights

The absolute organ weights and organ weight relative to bodyweight of all treated animals were within acceptable laboratory limits and comparable to those of the control animals. There were no statistically significant and treatment-related effects on absolute organ weight or relative to bodyweight in all the treated groups.

7. Histopathology

At necropsy, no compound-related gross pathology findings were observed in all dogs treated with the test compound of source B. However, compound-related gross pathology findings including thickened stomach and intestinal walls in all treatment groups were found after treatment with the test compound of source A. No gross pathological changes were observed in the remaining organs of any of the test animals which could be considered as treatment related.
Additionally, histopathology revealed findings in the gastrointestinal tract, only in dogs receiving source A, not with source B. Smooth muscle hypertrophy in the duodenum was observed in 2/6 females (17184, 17189) at 300 ppm and 3/6 females (17170, 17176 and 17185) at 1500 ppm. In addition, females at the mid dose group (300 ppm) had smooth muscle hypertrophy in the stomach (17184) or in both jejunum and ileum (17189), respectively. One of three females (17176) bearing a duodenum lesion at 1500 ppm also had the smooth muscle hypertrophy in the stomach and jejunum. In males, smooth muscle hypertrophy of the duodenum was only observed in 1/6 animals at 1500 ppm. However, the lesion was not observed in other small intestines in the same animal or other treated groups in males. In the stomach and small intestine, other findings such as chronic gastritis and congestion in the duodenum were noted in treated males and/or females at 300 ppm (chronic gastritis ♂: 17140, 17115 (source B); congestion, 17153 (source B); ♀: 17179) and 1500 ppm (chronic gastritis ♀: 17179, 17190; ♂: 17122), but is also observed in control males and females (♀: 17156; ♂: 17137). However, the affected animals were different from the ones showing smooth muscle hypertrophy, indicating that the findings in the stomach and small intestine were not related. The observed clinical signs, such as emesis and increased incidence of soft stools, and the histopathological findings on the gastric and intestinal smooth muscle are generally considered as signs and symptoms of cholinesterase inhibitor toxicity. Indeed erythrocyte cholinesterase activity was inhibited in the animals presenting findings indicative of smooth muscle overstimulation. However, findings such as smooth muscle hypertrophy of the gastrointestinal should also have been observed in animals given ethephon source B at the same dose level of 300 ppm, a dose level that showed inhibition of erythrocytes cholinesterase activity above 20% compared to the control groups in both sexes. As it is reported that smooth muscle hypertrophy in the intestine is caused by obstruction, diverticulum, inflammation, infection or spontaneousness in animals (Murakami et al., 2010; Liu et al., 2014; Bettini et al., 2003), it is considered that the cause of the gastrointestinal effects, only observed with ethephon source A, might be related to the different dietary preparation and/or source of ethephon tested and to the corrosive properties of ethephon (pH close to 2) on the gastric mucosa. This is also confirmed by the presence of findings like chronic gastritis, diffuse infiltration of inflammatory cells in the stomach and congestion at intestinal levels in the mid and top dose animals. Nevertheless, the composition of the two sources tested in the two-year dog study could not be characterized.
There were no other treatment-related findings in other examined organs.
No data about non-neoplastic or neoplastic lesions have been reported.

III. Conclusion

In a 2-year study in dogs administered ethephon at a dietary concentration of 0, 30, 300 or 1500 ppm (equal to 0, 0.86, 7.6 and 42.2 mg/kg bw per day for males and 0, 0.86, 8.4 and 47.8 mg/kg bw per day

for females, respectively), the NOAEL was 30 ppm (equal to 0.86 mg/kg bw per day), based on reduction of erythrocyte AChE activity at 300 ppm (equal to 7.6 mg/kg bw per day).
The study is considered valid with restrictions, as two different sources of ethephon with not well characterized composition have been used. In addition, the study is not performed according to GLP.

References cited within study report:

[redacted] et al.: Hypertrophy of intestinal smooth muscle in cats. Res Vet Sci. 75 (2003), 3–53.

[redacted] et al.: Voltage dependent potassium channel remodeling in murine intestinal smooth muscle hypertrophy induced by partial obstruction. PLoS One. 9 (2014), e86109. doi:10.1371/journal.pone.0086109.

[redacted] et al.: Goblet cell hyperplasia and muscular layer thickening in the small intestine of a cynomolgus monkey. J Toxicol Pathol. 23 (2010), 85–9.

Report:	KCA 5.5/05; [redacted]; 1978; M-187699-01-1
Title:	104-week chronic Toxicity Study in Rats Ethrel Final Report
Report No.:	R013314
Document No.:	M-187699-01-1
Guideline(s):	--
Guideline deviation(s):	--
GLP/GEP:	**no**
Restrictions:	Data are considered of questionable relevance. The study has several limitations and was not performed according to GLP. The study was conducted using various sources of Ethephon, which were not well characterised by analytical data.

Report:	KCA 5.5/07; [redacted]; 2017; M-588162-01-1
Title:	Summaries of toxicity studies requested by RMS (the Netherlands) in the draft RAR - Volume 3 - Annex B (AS) - Ethephon - B.6 Toxicology and metabolism
Report No.:	M-588162-01-1
Document No.:	M-588162-01-1
Guideline(s):	none
Guideline deviation(s):	--
GLP/GEP:	**no**

Executive Summary

In a 2-year dietary toxicity study, ethephon (Source A: purity 75.6%; batch no. AL-1030-42; Source B (only at 300 ppm): purity 73.6%, batch no. AL-3096) was administered to groups of 55 male and 55 female Sprague-Dawley CD rats at 0, 30, 300 or 3000 ppm (equal to 0, 1.2, 13 and 129 mg/kg bw per day for males and 0, 1.6, 16 and 171 mg/kg bw per day for females, respectively). The rats were checked daily for mortality and clinical signs. Every fourth week a detailed clinical examination was performed and body weights and feed consumption was measured. Five animals/sex/dose were used for haematology, clinical chemistry and cholinesterase determinations in week 13, 26, 52, 78 and 104. Brain cholinesterase determinations were performed on five animals/sex/group killed at week 52 and on all surviving animals at 104 weeks. All animals were necropsied and the liver, kidneys, spleen, heart, thyroid gland, adrenal glands, and testes with epididymides were weighed. Histological

examinations were performed on a wide range of organs and tissues of 20 males and 20 females in the control and high dose groups. In addition all gross tissue masses and suspected tumours from all dose groups were examined.
There were no effects of treatment on mortality and clinical signs. Slightly lower net body weight gains (-7 to -8%) were recorded for males at 300 ppm and in both sexes at 3000 ppm. Feed consumption was not affected by treatment. Cholinesterase activity was consistently inhibited in erythrocytes: 1.1 to 17.0% (source A) and 12.8 to 18.6% (source B) at 300 ppm and 20.0 to 31.4% at 3000 ppm in males and 2.9 to 14.4% (source A) and 4.8 to 21.7% (source B) at 300 ppm and 15.7 to 33.6% at 3000 ppm in females. Brain cholinesterase activity was not affected by ethephon (0-10%). Cholinesterase activity in plasma was inhibited at all doses (4.5 to 6.6% at 30 ppm, 4.1 to 18.9% (source A) and 6.5 to 27.7% (source B) at 300 ppm and 17.5 to 34.4% at 3000 ppm in males and 2.1% at 30 ppm, 7.8 to 31.9% (source A) and 18.5 to 27.1% (source B) at 300 ppm and 15.7 to 61.5% at 3000 ppm in females). No other toxicologically relevant changes in clinical chemistry parameters and organ weights were observed. Macroscopic and histopathological examination did not reveal any findings which could be attributed to the test material. There were no treatment-related increases in the incidence of neoplastic lesions.
The study is considered not valid, as two different sources of ethephon with not well characterized composition have been used. In addition, the study is not performed according to GLP.

1. Material and Methods

A. Materials

1. Test material

Test substance:	Ethephon
Common name:	2-Chloroethylphosphonic acid
Description:	Dark brown liquids
Purity:	75.6% (Source A), 73.6% (Source B)
Batch no.:	Source A (Base 211, AL 1030-42) used for Groups 2, 3, and 4 and Source B, labelled Ethephon Base 211, AL-3096) used for Group 5
Stability:	Not reported

2. Vehicle and/or positive control

Vehicle:	Basal diet, no positive control

3. Test animals

Species:	Rat
Strain:	Sprague-Dawley
Age:	five to six weeks
Weight at start:	Males: 120 – 188 g Females: 103 – 148 g
Source:	[redacted]
Acclimation period:	Not reported
Diet:	Ground Purina® Laboratory Chow®, *ad libitum*
Water:	Tap water, *ad libitum*
Housing:	Individually in elevated metal cages
Temperature:	not specified
Humidity:	not specified
Air changes:	not specified
Photoperiod:	not specified

B. Study design

1. In life dates: 18th December 1975 to 15th December 1977

2. Animal assignment and treatment

Before treatment commenced, all animals were randomized according to body weight and assigned to treatment groups. The treated animals were given the appropriate treated diets while control animals received basal diet *ad libitum* for 104 weeks.

Table1: Study design and dose received

Group	Dose (ppm)	Ethephon (mg/kg bw)		No of rats (M/F)
		Males	Females	Toxicity
1. Control	0	0	0	55/55
2. Low dose	30	1.2	1.6	55/55
3. Mid dose (Source A)	300	13	16	55/55
4. High dose	3000	129	171	55/55
5. Mid dose (Source B)	300	13	16	30/30

3. Dose selection rational

No data about rationale for dose level selection were given in the report.

4. Diet preparation and analysis

Diets were prepared weekly by adding the required quantity of the test article to a small amount of Ground Purina® Laboratory Chow® and by mixing using a mortar, and pestle for Groups 2, 3, and 5, and in a twin-shell blender fitted with intensifier bar for Group 4. The premix was then added to the remainder of the Ground Purina® Laboratory Chow® to attain the required level and mixed in a twin-shell Patterson-Kelly blender fitted with an intensifier bar. Fresh diets were prepared each week.

No data about the check of the accuracy of formulations by analysis of samples taken from the diets prepared were presented in the report.

5. Statistics

Numerical data obtained during the conduct of the study were subjected to calculation of group mean values and standard deviations. Statistical analyses of the terminal body weights, food consumption, clinical laboratory data, organ weights and organ/body weight ratios were performed by Bartlett's test for homogeneity of variances ([redacted]., 4:137, 1937) and the one-way analysis of variances ([redacted] 10:258-268, 1967). When differences were noted in the analysis of variances, Scheffe's method for judging all contrasts was utilized ([redacted] A Method for Judging All Contrasts in the Analysis of Variance, Biometrika, Vol. 40, Parts 1 and 2 June, 1953). Statistical analyses of survival were performed using the life table technique ([redacted] Life table technique in the analysis of response time data from laboratory experiments on animals, 1959). All evaluations were conducted using the 5% probability level as the criterion for significance.

C. Methods

1. Observations

All animals were examined daily for mortality, moribundity, and clinical signs.

2. Body weight and feed intake

Individual body weights and feed consumption was measured every four weeks. For all animals sacrificed after 104 weeks of treatment, terminal body weights were measured prior to terminal sacrifice.

3. Ophthalmoscopic examination

Ophthalmoscopic examinations were not conducted.

4. Haematology and clinical chemistry

Haematological and clinical chemistry investigations were performed on blood samples from five animals per sex and per group at Week 13, 26, 52, 78, and 104. Thereby, haematocrit, haemoglobin, erythrocyte and total and differential leukocyte counts were recorded. Blood samples were obtained by segmental tail amputation.
For clinical chemistry serum glutamic pyruvic transaminase, serum alkaline phosphatase, blood urea nitrogen, plasma and erythrocyte cholinesterase, and fasting glucose were determined.

Blood samples taken at Week 104 were obtained from the abdominal aorta, except the samples for cholinesterase determinations which were obtained by segmental tail amputation.

Brain cholinesterase values were measured on five animals per sex per group sacrificed at Week 52 and on all surviving animals sacrificed at termination.

Analysis of faeces and urine were not performed.

5. Sacrifice and pathology

On study completion after the Week 104 of treatment all surviving animals were sacrificed by exsanguination under sodium pentobarbital anaesthesia. Necropsies were performed on all animals sacrificed by design at Week 104, and on all rats which died or were sacrificed in extremis during the course of study.

Selected organs such as thyroids, heart, liver, spleen, kidneys, adrenals, and testes with epididymis were weight and organ weights relative to body weight were calculated. Thyroids and adrenals were weighed after fixation in 10% neutral buffered formalin and testes with epididymis was weighed after fixation in Bouin's solution.

On completion of the gross pathology examination of each animal, various organs and tissues were taken and, in order to elucidate abnormal findings, preserved in 10% neutral buffered formalin: brain, trachea, pituitary, eyes, thoracic spinal cord, salivary gland, thyroids, heart, lung, liver, spleen, kidney, adrenals, stomach, duodenum, jejunum, ileum, caecum, pancreas, esophagus, large intestine, mesenteric lymph node, urinary bladder, ovary, uterus, prostate, nerve with adjacent muscle, femoral bone marrow, rib junction, and any found lesions. The testes with epididymides were preserved in Bouin's fixative.

For histopathological examination all tissues of 20 males and females of the control and high dose group were embedding in Paraplast®, sectioned and stained with haematoxylin and eosin.

In addition, all gross tissue masses or expected tumours from animals of the low- and mid-dose groups (Source A) and mid-dose group (Source B) were examined.

II. Results and discussion

1. Analytical results

Analytical data, such as accuracy of formulations checked by analysis of the concentrations of the test substance in the different treatment groups as well as storage stability and homogeneity of the preparations and storage conditions were not given in the report.

2. Clinical results

There were no treatment-related effects on mortality and clinical signs.

3. Body weight and feed intake

There were no statistically significant and treatment-related effects on the body weights of any of the treated groups and on food consumption. Slightly lower net body weight gains (-7 to 8%) were recorded for males in the mid dose group and in both sexes at the highest treatment level.

4. Ophthalmoscopic results

Treatment related effects on the eyes were not recorded in this study.

5. Haematology and clinical chemistry

Statistically significant inhibition of cholinesterase (AChE) was observed in different treatment groups. Cholinesterase activity was consistently inhibited in erythrocytes; 1.1 to 17.0% (source A) and 12.8 to 18.6% (source B) at 300 ppm and 20.0 to 31.4% at 3000 ppm in males and 2.9 to 14.4% (source A) and 4.8 to 21.7% (source B) at 300 ppm and 15.7 to 33.6% at 3000 ppm in females. Brain cholinesterase activity was not affected by ethephon (0-10%). Cholinesterase activity in plasma was inhibited at all doses (4.5 to 6.6% at 30 ppm, 4.1 to 18.9% (source A) and 6.5 to 27.7% (source B) at 300 ppm and ~~45-67%~~ 17.5 to 34.4% at 3000 ppm in males and 2.1% at 30 ppm, 7.8 to 31.9% (source A) and 18.5 to 27.1% (source B) at 300 ppm and 15.7 to 61.5% at 3000 ppm in females).

Other statistically significant intergroup differences observed in haematology or clinical chemistry parameters were considered not to be treatment-related because the changes were either within the normal physiological range for rats of this age and strain or did not show a dose-response relationship.

No urinalysis was performed.

Table 2: Plasma Cholinesterase in rats exposed to ethephon for 2 years (in the diet).

Dose [ppm]	wk	Males					Females				
		0	30	300 (Source A)	1500[1]	300 (Source B)	0	30	300 (Source A)	1500[1]	300 (Source B)
Plasma Cholinesterase Δ pH/min	13	0.500±0.129	0.467±0.052	0.430±0.087	0.328±0.015*	0.378±0.030	1.683±0.286	1.768±0.134	1.246±0.154	0.648±0.101*	1.372±0.302
			-6.6	-14.0	-34.4	-24.4		+5.1	-26.0	-61.5	-18.5
	26	0.750±0.138	0.716±0.112	0.608±0.083	0.504±0.036*	0.542±0.048*	2.233±0.171	2.254±0.143	1.746±0.159*	0.924±0.149*	1.730±0.277*
			-4.5	-18.9	-32.8	-27.7		+0.9	-21.8	-58.6	-22.5
	52	0.570±0.090	0.677±0.172	0.516±0.083	0.470±0.074	0.570±0.085	2.220±0.195	2.260±0.100	1.746±0.333	1.276±0.129*	1.662±0.434
			+18.8	-9.5	-17.5	0.0		+1.8	-21.4	-42.5	-25.1
	78	0.588±0.193	0.554±0.161	0.564±0.092	0.430±0.046	0.550±0.306	1.360±0.281	1.332±0.219	0.966±0.224	0.804±0.130*	0.992±0.202
			-5.8	-4.1	-26.9	-6.5		-2.1	-31.9	-40.9	-27.1
	104[#]	0.6±0.0	1.1±0.5	0.5±0.1	0.5±0.1	0.5±0.0	1.2±0.5	1.6±0.2	1.1±0.1	1.0±0.1	0.9±0.3
			75.8	-14.8	-19.9	-15.4		+28.9	-7.8	-15.7	-22.7

2 3000 ppm Weeks 0-3, 2000 ppm Weeks 4-5, 1000 ppm Weeks 6-24, 1500 ppm Weeks 25-104

* Statistically significant difference from control $p<0.05$ (Scheffe's method)

\# Results (absolute values) were only readable in the study report up to 1 decimal place and are copied in the table. Relative values were fully readable.

Table 3: Erythrocyte cholinesterase in rats exposed to ethephon for 2 years (in the diet).

Dose [ppm]	wk	Males					Females				
		0	30	300 (Source A)	1500[1]	300 (Source B)	0	30	300 (Source A)	1500[1]	300 (Source B)
RBC Cholinesterase Δ pH/min	13	0.870±0.019	0.938±0.081	0.800±0.074	0.600±0.037*	0.712±0.073*	0.844±0.046	0.788±0.053	0.756±0.054	0.560±0.054*	0.742±0.026
			+7.8	-8.0	-31.0	-18.2		-6.6	-10.4	-33.6	-12.1
	26	1.076±0.065	1.050±0.087	0.910±0.042	0.738±0.045*	0.876±0.139*	0.856±0.036	0.894±0.057	0.830±0.023	0.654±0.050*	0.816±0.091
			-2.4	-15.4	-31.4	-18.6		+4.6	-3.0	-23.7	-4.8
	52	1.092±0.242	0.854±0.087	0.906±0.111	0.874±0.323	0.812±0.071	0.844±0.101	0.706±0.079	0.722±0.082	0.610±0.140*	0.660±0.116
			-21.8	-17.0	-20.0	-12.8		-16.3	-14.4	-27.6	-21.7
	78	0.548±0.026	0.536±0.033	0.554±0.065	0.418±0.043*	0.478±0.044*	0.474±0.034	0.496±0.024	0.486±0.042	0.362±0.023*	0.434±0.035
			-2.2	+1.1	-23.7	-13.5		+4.6	+2.5	-23.6	-8.4
	104	0.754±0.049	0.656±0.148	0.670±0.105	0.566±0.109	0.656±0.040	0.622±0.078	0.682±0.021	0.604±0.073	0.524±0.065	0.590±0.037
			-13.0	-11.1	-24.9	-13.0		+9.6	-2.9	-15.7	-5.1

2 3000 ppm Weeks 0-3, 2000 ppm Weeks 4-5, 1000 ppm Weeks 6-24, 1500 ppm Weeks 25-104

* Statistically significant difference from control p<0.05 (Scheffe's method)

Table 4: Brain cholinesterase in rats exposed to ethephon for 2 years (in the diet).

Dose [ppm]	wk	Males					Females				
		0	30	300 (Source A)	1500[1]	300 (Source B)	0	30	300 (Source A)	1500[1]	300 (Source B)
Brain Cholinesterase Δ pH/min	52	2.038±0.156	1.930±0.143	1.862±0.120	1.898±0.063	1.956±0.173	1.924±0.163	1.878±0.068	1.728±0.143	1.764±0.168	1.740±0.160
			-5.2	-8.6	-6.9	-4.0		-2.4	-10.2	-8.3	-9.6
	104	1.875±0.242	1.964±0.223	1.879±0.223	1.884±0.224	1.780±0.494	1.964±0.203	1.944±0.204	2.033±0.305	1.997±0.294	1.841±0.189
			+4.7	+0.2	+0.5	-5.1		-1.0	+3.5	+1.7	-6.3

1 3000 ppm Weeks 0-3, 2000 ppm Weeks 4-5, 1000 ppm Weeks 6-24, 1500 ppm Weeks 25-104
* Statistically significant difference from control p<0.05 (Scheffe's method)

6. Organ weights

The absolute organ weights and organ weight relative to bodyweight of all treated animals were within acceptable laboratory limits and comparable to those of the control animals. There were no statistically significant and treatment-related effects on absolute organ weight or relative to bodyweight in all treatment groups.

7. Histopathology

At necropsy, macroscopic and histopathological examination did not reveal any treatment-related findings in all animals treated with the test compound. There were no treatment-related increases in the incidence of neoplastic lesions.

III. Conclusion

The NOAEL was 300 ppm (equal to 13 mg/kg bw per day), based on reduction of erythrocyte AChE activity in both sexes at 3000 ppm (equal to 129 mg/kg bw per day). No treatment-related tumours were observed in Sprague-Dawley CD rats under the conditions of the study.

The study is considered valid with restrictions, as two different sources of ethephon with not well characterized composition have been used. In addition, the study is not performed according to GLP.

References cited within study report:

[redacted], 4:137, 1937

[redacted] Ames, 10:258-268, 1967

[redacted], Biometrika, Vol. 40, Parts 1 and 2 June 1953

[redacted] Life table technique in the analysis of response time data from laboratory experiments on animals, 1959

Report:	KCA 5.5/06; [redacted]; [redacted]; 1985; M-187735-01-1
Title:	78-week Oncogenic Evaluation in Swiss Albino Mice (Amended Report)
Report No.:	R013322
Document No.:	M-187735-01-1
Guideline(s):	--
Guideline deviation(s):	--
GLP/GEP:	**no**
Restrictions:	Study of doubtful quality: males and females were mixed in the same cage by mistake. No tumorigenic effects. Results of non-tumorigenic potential confirmed the more recent mouse carcinogenicity study evaluated during the Annex I inclusion and submitted to JMPR in 2014.

Report:	KCA 5.5/07; [redacted]; [redacted]; 2017; M-588162-01-1
Title:	Summaries of toxicity studies requested by RMS (the Netherlands) in the draft RAR - Volume 3 - Annex B (AS) - Ethephon - B.6 Toxicology and metabolism
Report No.:	M-588162-01-1
Document No.:	M-588162-01-1
Guideline(s):	none
Guideline deviation(s):	--
GLP/GEP:	**no**

Executive Summary

In a 78-week dietary carcinogenicity non-GLP study, ethephon (purity 75%; batch no X00782) was administered to groups of 85 male and 85 female CD-1 mice at 0, 30, 300 or 1000 ppm (equivalent to 0, 4.5, 45 and 150 mg/kg bw per day). The mice were checked daily for mortality and clinical signs. A detailed clinical examination was performed weekly. Group feed consumption and individual body weights were recorded weekly for the first 26 weeks and every month thereafter. Haematology and cholinesterase determinations in plasma, erythrocytes and brain were performed on 5 mice per/sex/dose in weeks 26, 52 and 78. No macroscopic or histopathological examinations were done on these animals. The remaining rats were killed in week 78. All animals were macroscopically examined. The brain, liver, kidneys, spleen, heart, thyroid gland with parathyroid, adrenal glands, pituitary, testes, epididymides, seminal vesicles, prostate, ovaries and uterus were weighed. Histological examinations were performed on a wide range of organs and tissues.

Statistically significant decreases in survival were noted in mid and high dose males beginning on weeks 64 and 72 respectively. The increased mortality was most likely related to the higher incidence of genitourinary infections, dermatitis and hematopoietic tumours in the mid and high dose animals during this time period. Since none of the above pathological entities were considered to be related to administration of the test compound, the increased mortality in male animals at the 300 and 1000 ppm levels was not considered compound-related. Clinical signs, body weight gain and feed consumption were not affected by treatment. A statistically significant increase in total leukocytes in high dose females at week 26 was not considered a treatment-related effect because it was an isolated finding and the value was within the accepted normal range for this parameter. No other differences in haematological parameters were found.

Plasma cholinesterase (AChE) activity was decreased significantly in high dose males (40 to 61%), high dose females (33 to 64%), and mid dose females (13 to 28%) at Week 26, 52, and 78 and mid dose males (23 to 34%) at Week 52 and 78. Statistically significant reductions in erythrocyte AChE activity occurred in females in the high dose group (45 to 51%) at Week 26, 52, and 78 and mid dose group (36 to 56%) at Week 52 and 78. In males decreased erythrocyte AChE activity was observed in the mid (25 and 11%) and high (32 and 21%) dose group at Week 52 and 78, however, statistical significance was not demonstrated. A statistically significant increase in brain AChE activity occurred in high dose females, at week 52. This was an isolated finding of no toxicological significance. No other differences in brain AChE activities occurred during the study.

No toxicologically relevant differences in organ weight, macroscopic findings or histopathological findings were observed. There was no indication of a neoplastic effect of the test compound on any organ in either sex.

The study is considered of doubtful quality based on the mistake in the allocation of animals of different sexes together, which led to the exclusion of females (one control animal and 4 animals of the top dose group) because they became pregnant.

I. Material and Methods

A. Materials

1. Test material

Test substance:	Ethephon
Common name:	2-Chloroethylphosphonic acid
Description:	Not reported
Purity:	75% (w:w)
Batch no.:	Ethephon, Base #211 from Lot 1X00782
Stability:	Not reported

2. Vehicle and/or positive control

Vehicle:	Basal diet, no positive control

3. Test animals

Species:	Mouse
Strain:	Swiss Albino
Age:	Three to four weeks
Weight at start:	Males (mean): 16.0 g Females (mean):16.4 g
Source:	[redacted]
Acclimation period:	Approximately 1 week
Diet:	Basal diet, *ad libitum*
Water:	Tap water, *ad libitum*
Housing:	Five per cage in wire mesh bottom cages
Temperature:	21±2°C
Humidity:	not specified
Air changes:	not specified
Photoperiod:	12 hour light / 12 hour dark

B. Study design

1. In life dates: 23rd June 1978 to 15th January 1980

2. Animal assignment and treatment

Before treatment commenced, all animals were randomized and assigned to treatment groups. The treated animals were given the appropriate treated diets while control animals received basal diet *ad libitum* for 78 weeks. Eighteen animals which were found dead during the first four weeks of the study were replaced. In addition, several animals were assigned to the wrong group due to errors in sex determination and thereafter returned to their proper group for the duration of the study. These animals were not included in analyses of group mortality, group mean body weight, group mean absolute and relative organ weight, or group mean food consumption data (cages bearing these animals were eliminated). They were, however, included in necropsy and histopathological evaluations. Five females became pregnant during the study, and were therefore not included in analyses of toxicological data.

Table 1: Study design and dose received

Group	Dose (ppm)	Ethephon (mg/kg bw)		No of mice (M/F)
		Males	Females	Toxicity
1. Control	0	0	0	85/85
2. Low dose	30	4.5	4.5	85/85
3. Mid dose	300	45	45	85/85

4. High dose	1000	150	150	85/85

3. Dose selection rational

No data about rationale for dose level selection were given in the report.

4. Diet preparation and analysis

Diets were prepared by dissolving an appropriate amount of the test article in water and subsequently incorporated into the feed utilizing a Hobart mixer. Fresh diets were prepared each week. Correction was made for purity of the test article (75% ethephon by weight).

No data about the check of the accuracy of formulations by analysis of samples taken from the diets prepared were presented in the report.

5. Statistics

Numerical data obtained during the conduct of the study were subjected to calculation of group mean values and standard deviations. Statistical analyses of the terminal body weights, food consumption, haematology, clinical laboratory data (cholinesterase data), absolute and relative organ weight ratios were performed by the one-way analysis of variances ([redacted] Statistical Methods, Iowa State University Press, Ames, 10:258-268, 1967). Differences among groups were identified using the Least Significant Difference test and significance was judged at the level of $p \leq 0.05$. Group mortality and pathology incidence data were analyzed using a chi-square test with Yates correction for 2 x 2 contingency tables ([redacted] Probability, Statistics and Data Analysis, Iowa State University Press, Ames, Iowa, 284-286, 1971). Significance was judged at the level of $p \leq 0.05$.

C. Methods

1. Observations

All animals were examined daily for mortality, moribundity and clinical signs. A detailed clinical examination was performed weekly.

2. Body weight and feed intake

Individual body weights were recorded prior to initiation of the study (week 0), weekly for the first 26 weeks and every month thereafter. For all animals sacrificed after 78 weeks of treatment, terminal body weights were measured prior to terminal sacrifice.

Group feed consumption was recorded weekly for the first 26 weeks and every month thereafter.

3. Haematology and clinical chemistry

Haematological and clinical chemistry investigations were performed on blood samples from five animals per sex and per dose group at Week 26, 52 and 78. Thereby, total and differential leukocyte counts were recorded. The method for blood sampling was not reported.
For clinical chemistry plasma, and erythrocyte cholinesterase activities were determined.
Subsequent to blood sampling, animals were sacrificed by chloroform vapour, the brains removed, and analysed for brain cholinesterase activity. No macroscopic or histopathological examinations were done on animals used for clinical studies.

4. Sacrifice and pathology

On study completion after the Week 78 of treatment all surviving animals were sacrificed by carbon dioxide. Necropsies were performed on all animals sacrificed by design at Week 78, and on all rats which died during the course of study. Selected organs such as brain, liver, kidneys, spleen, heart,

thyroid gland with parathyroid, adrenal glands, pituitary gland, testes, epididymides, seminal vesicles, prostate, ovaries and uterus were weighed and organ weights relative to body weight were calculated. On completion of the gross pathology examination of each animal, various organs and tissues were taken and, in order to elucidate abnormal findings, preserved in 10% neutral buffered formalin: adrenal glands, altered tissues and masses, brain, epididymides, eyes, heart, large intestine, small intestine, kidneys, liver, lungs, lymph nodes, mammary glands, ovaries, pancreas, pituitary, prostate, salivary glands, seminal vesicle, skeletal muscle with sciatic nerve, skin, spinal cord, spleen, sternum (with marrow), stomach, testes, thymus, thyroid (with parathyroids), urinary bladder and uterus. For histopathological examination all tissues of 70±2 males and 70±2 females of the control and all dose groups were embedding in paraffin, sectioned and stained with haematoxylin and eosin.

II. Results and discussion

1. Analytical results

Analytical data, such as accuracy of formulations checked by analysis of the concentrations of the test substance in the different treatment groups as well as storage stability and homogeneity of the preparations and storage conditions were not reported.

2. Mortality and clinical results

Statistically significant decreases in survival were noted in mid and high dose males beginning on weeks 64 and 72 respectively. The increased mortality was most likely related to the higher incidence of genitourinary infections, dermatitis and hematopoietic tumours in the mid and high dose animals during this time period. Since none of the above pathological entities were considered to be related to administration of the test compound, the increased mortality in male animals at the 300 and 1000 ppm levels was not considered compound-related. Clinical signs were not affected by treatment. A statistically significant increase in total leukocytes in high dose females at week 26 was not considered a treatment-related effect because it was an isolated finding and the value was within the accepted normal range for this parameter. No other differences in haematological parameters were found.

3. Body weight and feed intake

There were no statistically significant and treatment-related effects on the body weights and on food consumption of any of the treated groups.

Intermittent statistically significant differences in group mean body weights (both increases and decreases) occurred between control and all test groups of both sexes. These differences were transient and no dose dependent effect was evident at any time.

Intermittent statistically significant differences on food consumption between groups did occur during the study. These differences, however, did not constitute any discernible test-article related effect.

4. Haematology and clinical chemistry

A statistically significant increase in total leukocytes in high dose females at week 26 was not considered a treatment-related effect because it was an isolated finding and the value was within the accepted normal range for this parameter. No other differences in haematological parameters were found.

Plasma cholinesterase (AChE) activity was decreased significantly in high dose males (40 to 61%), high dose females (33 to 64%), and mid dose females (13 to 28%) at Week 26, 52, and 78 and mid dose males (23 to 34%) at Week 52 and 78. Statistically significant reductions in erythrocyte AChE activity occurred in females in the high dose group (45 to 51%) at Week 26, 52, and 78 and mid dose group (36 to 56%) at Week 52 and 78. In males decreased erythrocyte AChE activity was observed in the mid (25 and 11%) and high (32 and 21%) dose group at Week 52 and 78, however, statistical significance was not demonstrated. A statistically significant increase in brain AChE activity occurred in high dose females, at week 52. This was an isolated finding of no toxicological significance. No

other differences in brain AChE activities occurred during the study.
The effects of ethephon on AChE activity are presented in the table below.
Other statistically significant intergroup differences observed in clinical chemistry parameters were considered not to be treatment-related because the changes were either within the normal physiological range for mice of this age and strain or did not show a dose-response relationship.

Table 2: Plasma Cholinesterase in mice exposed to ethephon for 78 weeks (in the diet).

Dose [ppm]	wk	Males				Females			
		0	30	300	1000	0	30	300	1000
Plasma Cholinesterase Units /% inhibition	26	2230±196	2444± 95	2032±190	1330±121*	2869± 28	2822± 41	2507±108*	1800± 63*
			-	-9	-40*		-2	-13*	-33*
	52	1952±153	1920±126	1505±157*	967± 70*	2664± 85	2709±87	2137± 83*	1624±260*
			-2	-23*	-50*		-	-19*	-39*
	78	1921±109	2043±103	1268±124*	747±36*	2395±31	2423±36	1720±214*	861±99*
			-	-34*	-61*			-28*	-64*

* Statistically significant difference from control $p \leq 0.05$

Table 3: Erythrocyte cholinesterase in mice exposed to ethephon for 78 weeks (in the diet).

Dose [ppm]	wk	Males				Females			
		0	30	300	1000	0	30	300	1000
Erythrocyte Cholinesterase Units /% inhibition	26	351±25	448±30*	356±21	307±7	583±49	581±41	477±33	318±13*
			-	-	-13		0	-18	-45*
	52	631±87	627±76	474±38	429±61	1002±68	830±72	637±46*	531±68*
			-1	-25	-32		-17	-36*	-47*
	78	310±32	436±103	276±21	246±14	673±167	464±25	297±54*	330±70*
			-	-11	-21		-31	-56*	-51*

* Statistically significant difference from control $p \leq 0.05$

Table 4: Brain cholinesterase in mice exposed to ethephon for 78 weeks (in the diet).

Dose [ppm]	wk	Males				Females			
		0	30	300	1000	0	30	300	1000
Brain Cholinesterase Units	26	1563±123	1787± 73	1604±158	1579±124	1591±124	1658±63	1440±139	1465±166
			-	-	-		-	-9	-8
	52	2039± 21	2173± 49	1922± 84	2178± 47	2061± 22	2068±22	1992± 29	2169± 35*
			-	-6	-		-	-3	-
	78	676±95	811±162	1005±146	745±147	669±168	726±117	645±106	609±93
			-	-	-		-	-4	-9

* Statistically significant difference from control $p \leq 0.05$

6. Organ weights

The absolute organ weights and organ weight relative to bodyweight of all treated animals were within acceptable laboratory limits and comparable to those of the control animals. No toxicologically relevant differences in organ weight were observed. Statistically significant decreases in relative epididymal and relative pituitary weights occurred in high dose males and low dose females, respectively. Increased group mean body weights within these groups partially accounted for these findings. Therefore, these differences were not considered to be treatment-related.

7. Histopathology

No toxicologically relevant differences in macroscopic or histopathological findings were observed. There was no indication of a neoplastic effect of the test compound on any organ in either sex. Statistically significant intergroup differences observed were considered not to be treatment-related because the changes were either within the normal range for mice of this age and strain or did not show a dose-response relationship.

III. Conclusion

The NOAEL was 30 ppm (equivalent to 4.5 mg/kg bw per day) based on a statistically significant inhibition of erythrocyte AChE activity of more than 20%, observed in females at 300 ppm (equal to 45 mg/kg bw per day) at weeks 52 and 78. Ethephon was not carcinogenic in CD-1 mice under the conditions of the study.

The study is considered of doubtful quality based on the mistake in the allocation of animals of different sexes together, which led to the exclusion of females (one control animal and 4 animals of the top dose group) because they became pregnant.

References cited within study report:

[redacted] Probability, Statistics and Data Analysis, [redacted] 1971, 284-286.

[redacted] Statistical Methods, [redacted], 1967, 258-298.

CA 5.6 Reproductive toxicity

No new reproductive and/or developmental toxicity study was carried out after ethephon Annex I inclusion. The existing relevant information was presented and evaluated during the EU process for Annex I listing. However, to provide a full picture of the effects at maternal toxic doses and of the rationale for the selection of the dose levels in the studies evaluated during the Annex I inclusion, the dose-range studies in rats and rabbit and first developmental toxicity study in the rat are submitted and summarised in detailed.

Table 5.6-1 Summary of reproductive and developmental toxicity studies

Study	NOAEL/NOEL (mg/kg bw/day)	LOAEL (mg/kg bw/day)	Effects
Reproduction toxicity			
CD (Sprague-Dawley) rats (F0: 28/sex/dose group) 0, 300, 3000, 30000 ppm 0, 23, 231, 2444 mg/kg bw/day in both sexes [redacted] 1990 M-187771-01-1	Offspring and adults 23	Offspring and adults 231	≥3 000 ppm parental toxicity ↓ Body weight, weight gain depression and food consumption Postnatal toxicity ↓ pup body weights
Developmental toxicity			
Crl:CD Sprague-Dawley rats (5/ dose) 0, 450, 900, 1350 or 1800 mg/kg bw/day GD 6-15 [redacted]; 1988 M-457655-01-1	Maternal 450 Developmental 1350	Maternal 900 Developmental 1800	≥ 900 mg/kg bw/day Maternal : ↓Body weight and mortality Developmental ↓few pups evaluated due to maternal deaths at 1800 mg/kg bw/day
Crl:CD Sprague-Dawley rats (25/ dose). 0, 300, 600, 1200 mg/kg bw/day GD 6-15 [redacted]; 1988 M-188150-01-1	Maternal 300	Maternal 600	≥ 900 mg/kg bw/day maternal death and clinical signs Developmental toxicity not evaluated in this study
Crl:CD Sprague-Dawley rats (25/ dose). 0, 125, 250, 500 mg/kg bw/day GD 6-15 [redacted]; 1989 M-187750-01-1	500 (maternal & developmental)	No test material-related clinical observation in the main study up to the top dose at 500 mg/kg bw.	
Range-finding teratology study Hra (NZW)SPF rabbit (8/dose group)0, 50, 100, or 200 mg/kg/day GD 7-19 [redacted]; 1988 M-457641-01-1			Excessive number of deaths at all dose levels. The study was repeated
Range-finding Hra (NZW)SPF rabbit (8/dose group) 0, 25, 50, 100, or 200 mg/kg/day GD 6-19 [redacted]; 1988 M-188152-01-1	100 (maternal) 200 (developmental)	200	≥ 200 Maternal: ↓ Body weight, weight gain No developmental effects
Hra (NZW)SPF rabbit (22/dose group) receiving by gavage at 0, 62.5, 125 or 250 mg/kg/day GD 6-19 [redacted]; 1990 M-187739-01-1	125 (maternal & developmental)	250	At 250 mg/kg/ Maternal mortality and clinical signs Developmental ↓ number of live fetuses ↑ early resorptions and post-implantation loss

Studies highlighted in yellow: studies not evaluated during the EU process for Annex I listing

In both the dietary and oral gavage studies, the concentrations of ethephon were measured and corrected for the purity of ethephon base 250 (which has a content of ethephon of 71.3%). Therefore the calculated achieved intake represent the actual concentration of ethephon administered to the animals and no additional correction for purity is needed.

In the two-generation reproduction study (M-187771-01-1), Sprague-Dawley rats received ethephon via the diet at 0, 300, 3000 and 30000 ppm (equal to 0, 23, 231 and 2444 mg/kg bw/day (mean values for F0, F1A and F1B).

There were no reproductive effects following any of the mating periods (F1A, F1B, F2A or F2B) or in the combined F0 breeding or F1B breeding performance. In addition there was no effect on the mating index (number of copulation's/ number of oestrus cycles required) or on the length of gestation. The NOAEL for reproductive toxicity was 30000 ppm (equivalent to 2444 mg/kg bw/day)

Toxicity to immature/adult rats (reduced body weight gain, decreased food consumption, increased incidence of loose feces) was clearly indicated at 30 000 ppm. There was also evidence of substance-related effects on these animals (notably on body weight gain) at 3000 ppm.

Clear adverse effects on fetuses/pups were seen at 30 000 ppm and consisted in reduced mean litter weight at birth, during lactation and immediately post weaning (increased stillbirths and mortality in early lactation was mainly demonstrated by total pup data rather than by the more rigorous mean litter data). A slight reduction in body weight was also observed at 3000 ppm.

At 30000 ppm there was an increase of still births and deaths during early lactation period and reduced weight. Reduced weight was also observed at 3000 ppm. Increased perinatal pup deaths at 3 000 ppm for the F1B litters (day 4 post-cull day 7) and at 300 ppm for the F2B litters (day 0-day 4 pre cull) both showed statistically significant differences from controls but were not considered to be substance-related by the study investigators. It is notable that there was no dose response and that most of the deaths at 300 ppm were from just 2 litters, also there was no evidence of an effect on lactation index or 4-day survival index at 3000 or 300 ppm.

Table 5.6-2 Summary of litter survival

Dose level ppm	0		300		3 000		30 000	
1st Generation	**F1A**	**F1B**	**F1A**	**F1B**	**F1A**	**F1B**	**F1A**	**F1B**
Total born (alive or dead)	305	331	346	292	353	311	333	302
Total stillborn	5	6	3	9	3	4	6	18*
No dead days 0-4 pre cull	7	2	4	7	4	8	17	19**
No dead days 4 post cull to 7	0	0	0	1	0	8**	0	0
No dead days 4 post cull to 21	0	0	0	1	0	8	0	1
Live birth index	98.4	98.4	99.3	96.4	99.2	98.9	97.7	95.3
4-day survival index	97.8	98.9	99.0	98.2	98.8	97.7	95.3	91.4
Lactation index	100	100	100	99.4	100	95.7	100	97.7
2nd Generation	**F2A**	**F2B**	**F2A**	**F2B**	**F2A**	**F2B**	**F2A**	**F2B**
Total born (alive or dead)	294	263	330	300	296	309	351	321
Total stillborn	8	2	4	4	3	1	6	15**
No dead days 0-4 pre cull	7	1	6	11*	4	3	9	9*
No dead days 4 post cull to 7	0	0	0	1	0	0	0	0
No dead days 4 post cull to 21	2	2	0	1	2	0	0	1
Live birth index	97.3	99.4	98.7	98.6	97.2	99.7	98.5	95.6
4-day survival index	97.6	99.7	96.9	96.4	98.8	99.2	97.6	97.3
Lactation index	97.3	98.7	100	99.4	99.0	100	100	99.5

Statistically different from control *=p<0.05, **=p<0.01 (2-tailed)

No ChE activity was measured in this study. However, ChE activity was measured at equivalent dose levels in the rat short-term study and in the long-term carcinogenicity study and showed no significant inhibition of erythrocyte ChE activity up to doses equivalent to 59 mg/kg bw/day and brain ChE activities up to doses above 1000 mg/kg bw/day.

The NOAEL for toxicity to adults is 300 ppm (23 mg/kg bw/day) based on clear substance-related adverse effects at 30000 ppm and effects on body weight at 3000 ppm.

The NOAEL for developmental toxicity is 300 ppm (23 mg/kg bw/day) based on mortality at 30000 ppm and effects on body weight from 3000 ppm.

Ethephon irritant and corrosive properties are more evident when the compound is administered as a unique bolus by gavage than when it is mixed in the diet. For this reason, several preliminary studies have been conducted to select the doses to be administered in the developmental toxicity studies in rats and rabbit. These studies are submitted for evaluation under section 6.2 in order to explain the final selection of the dose levels tested in the main studies.

In the main developmental toxicity study in Crl:CD (SD)BR female rats (M-187750-01-1), ethephon was administered by gavage at 0, 125, 250 or 500 mg /kg bw/day on gestational days 6- 15. No treatment-related effects on dams or on foetuses were observed up to highest dose. The highest dose of 500 mg/kg bw/day was selected based on the results of two preliminary studies in which the dose equivalent and higher than 600 mg/kg bw/day were associated with adverse effects and mortality in the dams (M-188150-01-1 and M-457655-01-1). No ChE was measured in this study.
However, ChE activity was measured following administration by gavage for 2 and 13 weeks in the neurotoxicity studies (M-188213-01-1 and M-188217-01-1). Results showed no inhibition of erythrocyte ChE up to doses 300 mg/kg bw/day and no effects on brain ChE up to doses 200-400 following 90-day administration by gavage.
The NOAEL for maternal and developmental toxicity is 500 mg/kg bw/day.

In the teratogenicity study in rabbits (M-187739-01-1), ethephon was administered at dose levels equivalent to 0, 62.5, 125 or 250 mg/kg bw/day during gestational days 7-19. The doses were selected based on the results of two preliminary studies (M-457641-01-1 and M-188152-01-1).

Severe effects were observed at the top dose of 250 mg/kg bw/day (mortality and increased incidence of stomach lesions in dams).
At the top dose, post-implantation loss and the percent of early resorptions were considerably higher and the percent of live fetuses was lower than the control values. There were no substance-related differences in mean fetal body weights even though there were fewer live fetuses/litter at the top dose.

Table 5.6-3 Summary of Cesarean section data

Dose levels (mg/kg bw/day)	**0**	**62.5**	**125**	**250**
Females mated	22	22	22	22
Females pregnant	21	21	20	18
Females aborted	0	0	0	0
Females died[a]	0	2	1	16
Females with viable fetuses	21	17	19	2
Females with no viable fetuses	0	2	0	0
Resorptions (total)	17	8	20	4
Live fetuses (total)	143	119	112	9
Post-implantation loss (mean %)	11.6	5.3	15.8	43.1
Early resorptions (mean %)	8.0	14.8	10.9	43.1
Live fetuses (mean %)	88.4	84.7	84.2	56.9
Mean male fetal body weight, g	43.9	44.4	44.7	44.4
Mean female fetal body weight, g	41.9	42.8	45.0	41.4

[a] = pregnant females only (includes females sacrificed moribund and unscheduled sacrifices)

Although too few fetuses were available for examination of malformations at the top dose, there were not any clear indications of teratogenic effects at this dose level when compared to the control data. Moreover, at the next lower dose (which was only 50% less than the top dose) there was clearly no substance-related teratogenic response.

The NOAEL for maternal and developmental toxicity was set at 125 mg/kg bw/day.

CA 5.6.1 Generational studies

No new reproductive and developmental toxicity study was carried out after ethephon Annex I inclusion. The existing relevant information was presented and evaluated during the EU process for Annex I listing.

CA 5.6.2 Developmental toxicity studies

No new developmental toxicity study was carried out after ethephon Annex I inclusion. The existing relevant information was presented and evaluated during the EU process for Annex I listing. However, to provide a full picture of the effects at maternal toxic doses and on the rationale for the selection of the dose levels in the studies evaluated during the Annex I inclusion, the dose-range studies in rats and rabbit and first developmental toxicity study in the rat are submitted and summarised in detailed here below.

Report:	KCA 5.6.2/03; [redacted]; 1988; M-457655-01-1
Title:	Range-finding teratology study with ethephon technical - base 250 in rats
Report No.:	HLA 6224 -11 4
Document No.:	M-457655-01-1
Guideline(s):	83 -3
Guideline deviation(s):	not specified
GLP/GEP:	**yes**

Executive summary

Mated female rats were treated with ethephon technical Base 250 by oral gavage at 0, 450, 900, 1350, or 1800 mg/kg on gestation days 6 through 15. Dams were observed daily for indications of toxic effects. Body weight and physical data were recorded on days 0, 6, 9, 12, 16, and 20 of gestation. Cesarean sections were done on day 20 of gestation and included a gross internal examination of the dam. All viable fetuses were examined externally for gross abnormalities, weighed, sacrificed, and then discarded.

Results showed 100% survival rates for the 0, 450 and 1350 mg/kg bw/day groups and 80% for the 900 mg/kg bw/day group. In the top dose group of 1800 mg/kg bw/day survival was only 20%.

Pregnancy rates were 100% for all groups. Wheezing, gasping, and languidness were observed for animals at 1800 mg/kg and all animals but one were dead by gestation day 9.

There were no significant differences in body weights, body weight gains, or litter fetal weights, although there was a mean weight loss in the 900 and 1350 mg/kg bw/day groups during the interval 6 to 9 gestation days. There were discolored livers in three of the five animals at 1800 mg/kg, and two of these animals had necrotic stomach mucosa

There were no significant differences in the number of corpora lutea or implantations, implantation efficiency, the number or percentage of live or resorbed fetuses, or mean fetal weights in all the treated groups including one surviving animal in the 1800 mg/kg bw/day group .

There were no test material-related fetal external abnormalities.

The NOAEL for maternal toxicity was 450 mg/kg bw/day and for embryotoxicity 1350 mg/kg bw/day.

Based on the results of this study, doses of 0, 300, 600 and 1200 were selected for the main study.

I. MATERIALS AND METHODS

A. MATERIALS

Test Material:	Ethephon
Description:	Pale yellow liquid
Lot/Batch #:	A70471
Purity:	71.7 %
CAS #:	16672-87-0
Stability of test compound:	Stable at room temperature for at least 8 days.
Vehicle and or positive control:	Distilled water
Test Animals	
Species:	Rat (females)
Strain:	Crl:CD®(SD)BR rats
Age:	55 days old
Weight:	Females: 189-258 g
Source:	[redacted]. Males from the same strain and source (from an in - house colony) were used for breeding females.
Acclimation period:	13 days
Diet:	Certified Rodent Chow® #5002 ([redacted]) *ad libitum*.
Water:	Tap water *ad libitum*.
Housing:	Individual housing of pregnant females in suspended stainless steel cages.
Environmental conditions	
Temperature:	72°F ±3° (~22 – 24 °C)
Humidity:	30 - 70 %
Air change:	Not provided
Photoperiod:	12-h dark/ 12 h light (7 am- 7 pm)

B. STUDY DESIGN

1. In life dates:

The study was carried out from 15th March 1988 to 6th April 1988 at [redacted].

2. Mating

Females were mated by housing each female with one male with a double-sized computer generated random numbers table. Vaginal smears were taken daily, and the presence of a copulatory plug or sperm in the vaginal smear was considered evidence of mating. The day on which such evidence was found was Day 0 of gestation, and the female was then removed and housed individually.

3. Animal assignment and treatment

The females were assigned to control and treated groups using a computer-generated randomization. The dose groups are indicated in table 5.6.2-1.

Table 5.6.2-1 Study design

Group	Test Substance	Dose levels (mg/kg/day)	Volume (mL/kg)	Number of animals
1	Vehicle	0	10	5
2	Ethephon	450	10	5
3		900	10	5
4		1350	10	5
5		1800	10	5

4. Dosing solution and analysis

Suspensions of each concentration were prepared daily in distilled water.

Homogeneity of test material content was established from mixtures prepared for the first day of dosing. One sample from each dose level was taken from the top, bottom, and middle of the containers and assayed.

Stability analysis was done concurrently with the homogeneity analysis. One sample was taken from each dose level; retained at animal room temperature, humidity, and lighting for 7 days; and then assayed.

Homogeneity and concentration analysis results: dose preparations were determined to be homogeneous and were stable at room temperature for at least 8 days. The results of the test material analysis were within an acceptable range (101-106%).

5. Dosage administration

The test suspensions were administered by oral gavage at a volume of 10 mL/kg of body weight once daily on Days 6 through 15 of gestation. Animals were dosed at approximately the same time each day. The dose administered to each female was based on individual body weights on Day 6.

6. Statistics

Standard one -way analysis of variance (ANOVA) was used to analyze the following data for each pregnant female: body weights and body weight gains (corrected and uncorrected); the number of corpora lutea and implantations; implantation efficiency; and the number and percent of live and resorbed fetuses. Fetal body weights were analyzed by covariate analysis using the number of live fetuses in the litter as the covariate.

Levene's test was done before ANOVA to test for variance homogeneity. In the case of heterogeneity of variance at $p < 0.05$, the following transformations were done to stabilize the variance:

- Log X = Data analyzed following log10 transformation
  X^2 - data analysed following square transformation
- $X^{1/2}$ = Data analyzed following square root transformation
- 1/X = Data analyzed following reciprocal transformation
- Arcsine $X^{1/2}$ = Data analyzed following angular transformation
- Rank X = Data analyzed following rank transformation

The ANOVA was then done on the homogeneous or ranked data. If the ANOVA was significant, Dunnett's t –test was used for pairwise comparisons between groups. When no transformation established variance homogeneity at $p \leq 0.001$, the data were also examined by nonparametric techniques. These statistics included the Kruskal-Wallis H-test ANOVA and, if this test was significant, the Nemenyi-Kruskal-Wallis test for multiple comparisons or the Wilcoxon-Mann-Whitney two-sample rank tes. All group comparisons were evaluated at the 5.0% two-tailed probability level.

C. METHODS – MATERNAL OBSERVATIONS AND EVALUATIONS

1. Observations

All animals were observed twice daily (a.m. and p.m.) for moribundity and mortality and once daily for obvious indications of a toxic effect.

2. Body weight and food consumption

Individual body weights were recorded on Days 0, 6, 9, 12, and 16 and at sacrifice on Day 20. Physical examinations were done at each weighing interval.

3. Cesarean sections

All dams were examined macroscopically. On Day 20 of gestation, the surviving animals in each group were weighed and then euthanized with carbon dioxide. A midline laparotomy was done, the uterus was ligated at the cervix, and the entire reproductive tract was removed. The ovaries were evaluated for gross abnormalities, and the number of corpora lutea was recorded. After the dam was examined internally, the uterus was opened along the entire length, conceptuses were removed, and placental membranes were incised. The number of live and dead fetuses, early and late resorptions, and any other abnormalities were recorded. All viable fetuses were examined for external abnormalities, weighed, euthanized, and discarded.

II. RESULTS AND DISCUSSION

A. MATERNAL OBSERVATIONS

1. Mortality

Survival rates in this study were 100% for the 0, 450 and 1350 mg/kg bw/day groups; survival in the 900 mg/kg bw/day group was 80% and only 20% for the 1800 mg/kg bw/day group.

2. Clinical observations

Wheezing, dysphea, thinness, and few or no feces were observed at 900, 1350, and 1800 mg/kg. In addition, languidness, and gasping were observed at 900 and 1800 mg/kg. One incidence of soft feces was noted at 1800 mg/kg bw/day. No observations were noted at 450 mg/kg.

3. Body Weights

Body weights were slightly lower for all treated groups beginning at Day 9 through Day 20; however, there were no statistically significant differences.
There were no significant differences in body weight gains between control and treated groups.

4. Gross pathology

There were discolored livers in three of the five animals at 1800 mg/kg bw/day. In addition, two animals had necrotic stomach mucosa along with reddened, distended intestines. One animal at 900 mg/kg had a discolored liver, black foci, and gas-filled stomach and intestines. All of these animals died during administration.

B. CESAREAN SECTION DATA

There were no significant differences in fetal litter weights between control and treated groups.

There were no statistically significant differences in the mean number of corpora lutea or implantations, implantation efficiency, the number or percentage of live or resorbed fetuses or fetal weight. There were no dead fetuses.

III. CONCLUSIONS

Based on the results of this study, the NOAEL for maternal toxicity was 450 mg/kg bw/day and the NOAEL for embryo/foetotoxicity was 1350. There was no evidence of teratogenicity at the dose levels tested.
Based on the results of this study, doses of 0, 300, 600 and 1200 mg/kg bw/day were selected for the main developmental toxicity study (M-188150-01-1)

Report:	KCA 5.6.2/04; [redacted]; 1988; M-188150-01-1
Title:	Teratology Study with Ethephon Technical- Base 250 in Rats
Report No.:	R013543
Document No.:	M-188150-01-1
Guideline(s):	USEPA (=EPA): 83-3
Guideline deviation(s):	--
GLP/GEP:	**yes**

Executive summary

The purpose of this study was to assess the embryo/fetal toxicity and teratogenic potential of ethephon technical Base 250 when administered by oral gavage to pregnant rats during the period of fetal organogenesis.

[redacted]:C(SD)BR mated female rats (25 animals/group) received 0, 300, 600, or 1200 mg ethephon technical Base 250/kg bw on gestation days 6 through 15 of gestation.

Animals were observed daily for signs of toxicity, and body weights were recorded on days 0, 6, 9, 12, 16, and 20. On Day 20 of gestation, all surviving animals were sacrificed and discarded due to the fact that a maximum tolerated dose (MTD) was exceeded as demonstrated by high mortality in the mid and high dose groups. The study was continued through gestation day 20 to obtain data to be used in the selection of doses for a new study. Statistical analysis was limited to means and standard deviations for bodyweight and bodyweight gains.

Survival rates were 100% for controls, 96% at 300 mg/kg bw/day, 88% at 600 mg/kg bw/day and 72% at 1200 mg/kg bw/day. All deaths (moribund sacrifice and died on test) at 600 and 1200 mg/kg bw /day occurred between Day 7 and 11.

Test material-related ante-mortem observations noted at 1200 mg/kg bw/day included thinness, low body temperature, rhinorrhea, gasping, dyspnea, wheezing, urine stains, few or no feces, and piloerection.

Body weights were lower at day 9 for animals receiving 1200 mg/kg bw/day when compared with those of controls. Body weight gains were lower between days 6 and 9 of gestation for all treated animals. Body weight gains for intervals during the remainder of the study were similar to or greater than those of controls.

Observations for animals at 1200 mg/kg bw/day, which died or were sacrificed moribund, included small thymus, necrotic areas in the stomach mucosa, distended (i.e., gas-filled) stomach, and greenish-black stomach contents.

Overall, excessive maternal toxicity was induced by the mid- and high-dose levels (i.e., 600 and 1200 mg/kg bw/day). Therefore data in this study formed the basis for the selection of doses of 125, 250, and 500 mg/kg for the definitive main study (M-187750-01-1).

All original paper data, all magnetically coded records, and a copy of the termination report were retained in the archives of [redacted] in accordance with 40 CFR 160.

I. MATERIALS AND METHODS

The information on materials and methods is obtained from the study protocol, as the study report (consisting of 30 pages) presented only the results and the study protocol, because the study was terminated and a new study was started.

A. MATERIALS

Test Material:	Ethephon
Description:	Pale yellow liquid
Lot/Batch #:	A70471
Purity:	71.7 %
CAS #:	16672-87-0
Stability of test compound:	Stable at room temperature for at least 8 days.
Vehicle and or positive control:	Distilled water
Test Animals	
Species:	Rat (females)
Strain:	Crl:CD®(SD)BR rats
Age:	8 to 10 weeks old
Weight:	Females: 200 - 268g
Source:	[redacted]. Males from the same strain and source (from an in - house colony) were used for breeding females.
Acclimation period:	at least 1 week
Diet:	Certified Rodent Chow® #5002 ([redacted]) *ad libitum.*
Water:	Tap water *ad libitum.*
Housing:	Individual housing of pregnant females in suspended stainless steel cages.
Environmental conditions	
Temperature:	72°F ±3° (~22 – 24 °C)
Humidity:	30 - 70 %
Air change:	Not provided
Photoperiod:	12 h dark/ 12 h light (7 am- 7 pm)

B. STUDY DESIGN

1. In life dates:

The study was carried out from 25th April 1988 to 27th May 1988 at [redacted].

2. Mating

Females were mated by housing each female with one male in a double-sized
Computer generated random numbers table. Vaginal smears were taken daily, and the presence of a copulatory plug or sperm in the vaginal smear was considered evidence of mating. The day on

which such evidence was found was Day 0 of gestation, and the female was then removed and housed individually.

3. Animal assignment and treatment

The females were assigned to control and treated groups using a computer-generated randomization.

Table 5.6.2-2 Study design

Group	Test Substance	Dose levels (mg/kg/day)	Volume (mL/kg)	Number of animals
1	Vehicle	0	10	25
2	Ethephon	300	10	25
3		600	10	25
4		1200	10	25

4. Dosing solution and analysis

Suspensions of each concentration were prepared daily in distilled water.
Samples of each mixed batch, including control vehicle, from the first day of preparation and from the last day of dosing were analysed for dosage confirmation.

5. Dosage administration

The test suspensions were administered by oral gavage at a volume of 10 mL/kg of body weight once daily on Days 6 through 15 of gestation. Animals were dosed at approximately the same time each day.

6. Statistics

Statistical analysis was limited to means and standard deviations for bodyweight and bodyweight gains

C. METHODS – MATERNAL OBSERVATIONS AND EVALUATIONS

1. Observations

All animals were observed twice daily (a.m. and p.m.) for moribundity and mortality and once daily for obvious indications of a toxic effect.

2. Body weight and food consumption

Individual body weights were recorded on days 0, 6, 9, 12, 16 and at sacrifice on day 20 of gestation. Physical examinations were done at each weighing interval.

3. Cesarean sections

Dams that were found dead or that were sacrificed in moribund conditions were examined for any macroscopically abnormal thoracic, abdominal, or pelvic viscera.
On day 20 of gestation, animals were sacrificed with carbon dioxide and discarded, due to high mortality at the mid and high dose.

II. RESULTS AND DISCUSSION

A. MATERNAL OBSERVATIONS

1. Mortality

Survival rates were 100% for controls, 96% at 300 mg/kg mg/kg bw/day, 88% at 600 mg/kg bw/day and 72% at 1200 mg/kg bw/day. All deaths (moribund sacrifice and found dead) at 600 and 1200 mg/kg occurred between Day 7 and 11.

Table 5.6.2-3 Summary of mortality

Dose levels (mg/kg bw/day	0	300	600	1200
Number of animal on test	25	25	25	25
Found dead	0	1	3	5
Sacrificed moribund	0	0	0	2
Schedules sacrifice	25	24	22	18

2. Clinical observations

Test material-related antemortem observations noted at 1200 mg/kg included thinness, low body temperature, rhinorrhea, gasping, dyspnea, wheezing, urine stains, few or no feces, and piloerection

3. Body Weights

Body weights were lower at day 9 for animals receiving 1200 mg/kg when compared with those of controls. Body weights for these animals were slightly lower than those of controls at study initiation (i.e., on day 0). Body weights continued to be lower than those of controls throughout the study, but not at the end of the study when the surviving animals were considered.
Body weight gains were lower between days 6 and 9 of gestation for all treated animals. Body weight gains for intervals during the remainder of the study were similar to or greater than those of controls.
There were no significant differences in body weight gains between control and treated groups.

4. Gross pathology

Observations performed on animals at 1200 mg/kg which died or were sacrificed moribund included small thymus, necrotic areas in the stomach mucosa, distended (i.e., gas-filled) stomach, and greenish-black stomach contents.

B. CESAREAN SECTION DATA

Data on foetuses were not recorded.

III. CONCLUSIONS

Based on the results of this study, treatment of rats with ethephon technical Base 250 during the period of organogenesis (i.e., Days 6 through 15) at doses of 600 or 1200 mg/kg bw/day resulted in 12% and 28% mortality, respectively.
Toxicity at 1200 mg/kg bw/day was also demonstrated by test material-related ante-mortem observations and necropsy findings. These results indicated that excessive maternal toxicity was induced by the mid- and high-dose levels (i.e., 600 and 1200 mg/kg bw/day). The data in this study therefore formed the basis for the selection of doses of 125, 250, and 500 mg/kg bw/day for the main developmental toxicity study with ethephon technical (M-187750-01-1).
All original paper data, all magnetically coded records, and a copy of the termination report were retained in the archives of [redacted] in accordance with 40 CFR 160.

Report:	KCA 5.6.2/05; [redacted]; 1988; M-457641-01-1
Title:	Range-finding teratology study with ethephon technical-base 250 in rabbits
Report No.:	[redacted]6224-120
Document No.:	M-457641-01-1
Guideline(s):	not specified
Guideline deviation(s):	not specified
GLP/GEP:	**yes**

Executive summary

The purpose of this study was to obtain data to set levels for a definitive teratology study based on evaluation of the maternal toxicity, the embryo/fetotoxicity, and teratogenic potential of ethephon technical-Base 250 when administered by oral gavage to pregnant rabbits during the fetal period of organogenesis (i.e., Days 7 through 19 of gestation).

Artificially inseminated Hra:(NZW)SPF rabbits (eight/group) were treated with ethephon technical Base 250 by oral gavage at 0, 25, 50, 100, or 200 mg/kg on Days 7 through 19 of gestation. Animals were observed daily for signs of toxicity. Body weight and physical examination data were recorded on Days 0, 7, 10, 13, 16, 20, 24, and 29 of gestation. Cesarean sections were done on Day 29 of gestation and included a gross internal examination of the doe. All viable fetuses were examined externally for gross abnormalities, weighed, sacrificed, and then discarded.

The percent of animals at scheduled necropsy were 100% in the control and at 100 mg/kg bw/day, 60% at 25 and 50 mg/kg bw/day, 62% at 100 mg/kg bw/day and 40% at 200 mg/kg bw/day.
There were no test material-related necropsy observations, although there was one animal at 25 mg/kg and two animals each at 50 and 200 mg/kg bw/day showing lung discoloration, indicating possible gavage errors.
There was an increased incidence of hunched body, soft stool, diarrhea, and thickened urine at 200 mg/kg. Single incidents of soft stool and diarrhea were also observed at 100 mg/kg bw/day.
There were no statistically significant differences in body weights or body weight gains.

Pregnancy rates were 63% at 0 and 100 mg/kg, 100% at 25 mg/kg, and 75% at 50 and 200 mg/kg.

There were no statistically significant differences in pre-implantation or post-implantation loss, the percent of live and or resorbed (early, late, and total) fetuses, or live fetal body weights

Because of the excessive number of technical-related deaths at 25, 50, and 200 mg/kg bw/day, this study was repeated (M-488152-01-1).

I. MATERIALS AND METHODS

A. MATERIALS

Test Material:	Ethephon
Description:	Pale yellow liquid
Lot/Batch #:	A70471
Purity:	71.7 %
CAS #:	16672-87-0
Stability of test compound:	Stable at room temperature for at least 8 days.
Vehicle and or positive control:	Distilled water

Test Animals

Species:	Rabbit (females)
Strain:	Hra:(NZW)SPF
Age:	5 months old
Weight:	3.0 - 4.0 kg
Source:	[redacted].
Acclimation period:	20 days
Diet:	Certified Rabbit Chow® #5322 ([redacted]) *ad libitum.*
Water:	Tap water *ad libitum.*
Housing:	individually housed in stainless steel, screen-bottom cages, with absorbent pan liners in the urine- and feces-collecting pans

Environmental conditions

Temperature:	70°F ±3° (~21 – 23 °C)
Humidity:	50 - 70 %
Air change:	Not provided
Photoperiod:	16 h light/ 8 h dark

B. STUDY DESIGN

1. In life dates:

The study was carried out from 7th June1988 to 6th July, 1988 (in-life dates) at [redacted].

2. Mating

Twenty females per day were impregnated by artificial insemination. Approximately 3 hours before insemination, each doe was injected with human chorionic gonadotropin solution (Lypho Med, Inc., Melrose Park, Illinois, 100 USP units/kg of body weight) via the marginal ear vein. Semen was collected from proven breeder males of the same strain and source as the females. Each semen collection was evaluated for motility, morphology, and concentration. Semen pooled from at least two collections was diluted with saline and deposited into the uterus. The day of insemination was designated day 0 of gestation.

3. Animal assignment and treatment

The females were assigned to control and treated groups using a computer-generated randomization. The dose groups are indicated in table 5.6.2-4.

Table 5.6.2-4 Study design

Group	Test Substance	Dose levels (mg/kg/day)	Volume (mL/kg)	Number of animals
1	Vehicle	0	2	8
2	Ethephon	25	2	8
3		50	2	8
4		100	2	8
5		200	2	8

Test material solutions were prepared weekly in distilled water and adjusted for purity. Test solutions were stored at room temperature between dosing days. The test solutions were administered by oral gavage at a volume of 2 ml/kg of body weight/day on Days 7 through 19 of gestation. Animals were

dosed approximately the same time each day. The dose administered to each female was based on individual body weights on Day 7

4. Dosing solution and analysis

Suspensions of each concentration were prepared weekly in distilled water.
Samples of all dose levels from each preparation were taken and analyzed for test material content, homogeneity and stability.
Stability, homogeneity and concentration analysis: Results of analyses showed that the dose preparations were homogeneous, ranging from 93.6% to 107% of theoretical.
The test material was shown to be stable in the vehicle for at least 7 days under the same conditions as used in the study, ranging from 88.9% to 100.0% of theoretical. Confirmation of dose preparations at Week 2 ranged from 98.7% to 104.0% of theoretical..

5. Dosage administration

The test suspensions were administered by oral gavage at a volume of 10 mL/kg of body weight once daily on Days 6 through 15 of gestation. Animals were dosed at approximately the same time each day. The dose administered to each female was based on individual body weights on Day 6.

6. Statistics

Standard one -way analysis of variance (ANOVA) was used to analyze the following data for each pregnant female: body weights and body weight gains (corrected and uncorrected); the number of corpora lutea and implantations; implantation efficiency; and the number and percent of live and resorbed fetuses. Fetal body weights were analyzed by covariate analysis using the number of live fetuses in the litter as the covariate.
Levene's test was done before ANOVA to test for variance homogeneity. In the case of heterogeneity of variance at p ~ 0.05, the following transformations were done to stabilize the variance:

- Log X = Data analyzed following log10 transformation
  X^2 - data analysed following square transformation
- $X^{1/2}$ = Data analyzed following square root transformation
- 1/X = Data analyzed following reciprocal transformation
- Arcsine $X^{1/2}$ = Data analyzed following angular transformation
- Rank X = Data analyzed following rank transformation

The ANOVA was then done on the homogeneous or ranked data. If the ANOVA was significant, Dunnett's t –test was used for pairwise comparisons between groups. When no transformation established variance homogeneity at $p \leq 0.001$, the data were also examined by nonparametric techniques. These statistics included the Kruskal-Wallis H-test ANOVA and, if this test was significant, the Nemenyi-Kruskal-Wallis test for multiple comparisons or the Wilcoxon-Mann-Whitney two-sample rank tes. All group comparisons were evaluated at the 5.0% two-tailed probability level.
Standard one-way ANOVA was used to analyze maternal body weight and body weight changes. ANOVAwas used to analyze the following data for each pregnant female: the number of corpora lutea and implantations, implantation efficiency, pre-implantation loss, number and percent of live (male and female) fetuses and early, late, and total resorptions.
Standard one-way analysis of covariance (ANCOVA) was used to analyze fetal body weights, with the number of live fetuses as the covariate. Group comparisons found to be statistically significant at the 5.0% and 1.0% two-tailed probability level were indicated with an "a" and "b," respectively.

The proportion of litters and fetuses with external abnormalities in the treated groups were compared with the control group by the Cochran-Armitage test of trend and departure and by a Fisher-Irwin exact test.

C. METHODS – MATERNAL OBSERVATIONS AND EVALUATIONS

1. Observations

All animals were observed twice daily (a.m. and p.m.) for moribundity and mortality and once daily for obvious indications of a toxic effect.

2. Body weight and food consumption

Individual body weights were recorded on days 0, 7, 10, 13, 16, 20, 24, and 29 of gestation. Physical examinations were done at each weighing interval.

3. Cesarean sections

On Day 29 of gestation, all does were weighed, euthanized, necropsied, and examined macroscopically. A midline laparotomy was done and the entire reproductive tract was removed. The ovaries were evaluated for gross abnormalities and the number of corpora lutea was recorded. After the doe was examined internally, the uterus was opened along its entire length, conceptuses were removed, and placental membranes were incised. The number of live and dead fetuses, early and late resorptions, and any abnormalities were recorded. All viable fetuses were examined for external abnormalities, weighed, sacrificed and discarded.

II. RESULTS AND DISCUSSION

A. MATERNAL OBSERVATIONS

1. Mortality

Survival rates were 100% for the controls and at 100 mg/kg, 60% at 25 and 50 mg/kg, and 20% at 200 mg/kg. Five of the deaths in this study (one at 25 mg/kg and two each at 50 and 200 mg/kg) were attributed to gavage error based on post-dose observations and necropsy findings (e.g., discolored lungs).

2. Clinical observations

There was an increased incidence of hunched body, soft stool, diarrhea, and thickened urine at 200 mg/kg. The observation "few feces" was present in all groups. Other observations, including prostration, thrashing, chattering teeth, salivation, and cyanosis, are probably a result of gavage error.

3. Body Weights

There were no statistically significant differences in body weights or body weight gains.

4. Gross pathology

There were no test material-related findings at necropsy observations

B. CESAREAN SECTION DATA

Pregnancy rates were 100% for the controls and at 50 and 100 mg/kg bw/day, 80% at 25 and 200 mg/kg bw/day.

There were no statistically significant differences in pre-implantation or post-implantation loss (i.e., implantation efficiency), the percent of live or resorbed (early, late, and total) fetuses, or live fetal body weight.

There were no abnormal fetal external observations.

III. CONCLUSIONS

The no-observable-effect level (NOEL) for maternal toxicity was 100 mg/kg bw/day and for embryo/fetotoxicity was 200 mg/kg bw/day.

Because of the excessive number of technical-related deaths at 25, 50, and 200 mg/kg bw/day, this study was repeated (M-188152-01-1).

Report:	KCA 5.6.2/06; [redacted]; 1989; M-188152-01-1
Title:	Range-Finging Teratology Study with Ethephon Technical-Base 250 in Rabbits
Report No.:	R013544
Document No.:	M-188152-01-1
Guideline(s):	--
Guideline deviation(s):	--
GLP/GEP:	**yes**

Executive summary

The purpose of this study was to obtain data to set levels for a definitive teratology study based on evaluation of the maternal toxicity, the embryo/fetotoxicity, and teratogenic potential of ethephon technical-Base 250 when administered by oral gavage to pregnant rabbits during the fetal period of organogenesis (i.e., Days 7 through 19 of gestation).

Artificially inseminated Hra:(NZW)SPF rabbits (eight/group) were treated with ethephon technical Base 250 by oral gavage at 0, 25, 50, 100, or 200 mg/kg on Days 7 through 19 of gestation. Animals were observed daily for signs of toxicity. Body weight and physical examination data were recorded on Days 0, 7, 10, 13, 16, 20, 24, and 29 of gestation. Cesarean sections were done on Day 29 of gestation and included a gross internal examination of the doe. All viable fetuses were examined externally for gross abnormalities, weighed, sacrificed, and then discarded.

The percent of animals at scheduled necropsy were 88% in the control, 100% at 25 and 50 mg/kg bw/day, 62% at 100 mg/kg bw/day, and 75% at 200 mg/kg bw/day.
Clinical signs consisted of increase in the incidence of diarrhea at 100 and 200 mg/kg.

There were no statistically significant differences in body weights, but a decrease in body weight gains was observed at 200 mg/kg bw/day. There were no test material-related necropsy observations.

Pregnancy rates were 63% at 0 and 100 mg/kg bw/day, 100% at 25 mg/kg bw/day, and 75% at 50 and 200 mg/kg bw/day.

There were no statistically significant differences in the number of corpora lutea or implantations, implantation efficiency, pre-implantation loss, the number or percent of live or resorbed (early, late, and total) fetuses or live fetal body weights.

There were no test material-related fetal external observations.

The no-observable-effect level (NOEL) for maternal toxicity was 100 mg/kg and for embryo/ fetotoxicity was greater than 200 mg/kg. Based on the results of this study doses of 0, 62.5, 125, and 250 mg/kg were recommended for the definitive teratology study (M-187739-01-1).

I. MATERIALS AND METHODS

A. MATERIALS

Test Material:	Ethephon
Description:	Pale yellow liquid
Lot/Batch #:	A70471
Purity:	71.7 %
CAS #:	16672-87-0
Stability of test compound:	Stable at room temperature for at least 8 days.
Vehicle and or positive control:	Distilled water
Test animals	
Species:	Rabbit (females)
Strain:	Hra:(NZW)SPF
Age:	5 months old
Weight:	3.0 kg
Source:	[redacted].
Acclimation period:	21 days
Diet:	Certified Rabbit Chow® #5322 ([redacted]) *ad libitum*
Water:	Tap water *ad libitum*.
Housing:	individually housed in stainless steel, screen-bottom cages, with absorbent pan liners in the urine- and feces-collecting pans.
Environmental conditions	
Temperature:	70°F ±3° (~21 - 23 °C)
Humidity:	30 - 70 %
Air change:	Not provided
Photoperiod:	12 h dark, 12 h light (7 am- 7 pm)

B. STUDY DESIGN

1. In life dates:

The study was carried out from 6th September 1988 to 7th October, 1988 (in-life dates) at [redacted].

2. Mating

Twenty females per day were impregnated by artificial insemination. Approximately 3 hours before insemination, each doe was injected with human chorionic gonadotropin solution (Lypho Med, Inc., Melrose Park, Illinois, 100 USP units/kg of body weight) via the marginal ear vein. Semen was collected from proven breeder males of the same strain and source as the females. Each semen collection was evaluated for motility, morphology, and concentration. Semen pooled from at least two collections was diluted with saline and deposited into the uterus. The day of insemination was designated day 0 of gestation.

3. Animal assignment and treatment

The females were assigned to control and treated groups using a computer-generated randomization.

Table 5.6.2-5 Study design

Group	Test Substance	Dose levels (mg/kg/day)	Volume (mL/kg)	Number of animals
1	Vehicle	0	2	8
2	Ethephon	25	2	8
3		50	2	8
4		100	2	8
5		200	2	8

Test material solutions were prepared weekly in distilled water and adjusted for purity. Test solutions were stored at room temperature between dosing days. The test solutions were administered by oral gavage at a volume of 2 ml/kg of body weight/day on Days 7 through 19 of gestation. Animals were dosed approximately the same time each day. The dose administered to each female was based on individual body weights on Day 7

4. Dosing solution and analysis

Suspensions of each concentration were prepared weekly in distilled water.
Samples of all dose levels from each preparation were taken and analyzed for test material content, homogeneity and stability.

Results of stability, homogeneity and concentration analysis: results of analyses showed that the dose preparations were homogeneous, ranging from 93.6% to 107% of theoretical.
The test material was shown to be stable in the vehicle for at least 7 days under the same conditions as used in the study, ranging from 88.9% to 100.0% of theoretical. Confirmation of dose preparations at Weeks 1 through 3 ranged from 88.0% to 114.0% of theoretical.

5. Dosage administration

The test suspensions were administered by oral gavage at a volume of 10 mL/kg of body weight once daily on Days 6 through 15 of gestation. Animals were dosed at approximately the same time each day. The dose administered to each female was based on individual body weights on Day 6.

6. Statistics

Standard one -way analysis of variance (ANOVA) was used to analyze the following data for each pregnant female: body weights and body weight gains (corrected and uncorrected); the number of corpora lutea and implantations; implantation efficiency; and the number and percent of live and resorbed fetuses. Fetal body weights were analyzed by covariate analysis using the number of live fetuses in the litter as the covariate.
Levene's test was done before ANOVA to test for variance homogeneity. In the case of heterogeneity of variance at $p \sim 0.05$, the following transformations were done to stabilize the variance:

- Log X = Data analyzed following log10 transformation
  X^2 - data analysed following square transformation
- $X^{1/2}$ = Data analyzed following square root transformation
- 1/X = Data analyzed following reciprocal transformation
- Arcsine $X^{1/2}$ = Data analyzed following angular transformation
- Rank X = Data analyzed following rank transformation

The ANOVA was then done on the homogeneous or ranked data. If the ANOVA was significant, Dunnett's t –test was used for pairwise comparisons between groups. When no transformation established variance homogeneity at $p \leq 0.001$, the data were also examined by nonparametric techniques. These statistics included the Kruskal-Wallis H-test ANOVA and, if this test was significant, the Nemenyi-Kruskal-Wallis test for multiple comparisons or the Wilcoxon-Mann-Whitney two-sample rank tes. All group comparisons were evaluated at the 5.0% two-tailed probability level.

Standard one-way ANOVA was used to analyze maternal body weight and body weight changes. ANOVAwas used to analyze the following data for each pregnant female: the number of corpora lutea and implantations, implantation efficiency, pre-implantation loss, number and percent of live (male and female) fetuses and early, late, and total resorptions.

Standard one-way analysis of covariance (ANCOVA) was used to analyze fetal body weights, with the number of live fetuses as the covariate. Group comparisons found to be statistically significant at the 5.0% and 1.0% two-tailed probability level were indicated with an "a" and "b," respectively.

The proportion of litters and fetuses with external abnormalities in the treated groups were compared with the control group by the Cochran-Armitage test of trend and departure and by a Fisher-Irwin exact test.

C. METHODS – MATERNAL OBSERVATIONS AND EVALUATIONS

1. Observations

All animals were observed twice daily (a.m. and p.m.) for moribundity and mortality and once daily for obvious indications of a toxic effect.

2. Body weight and food consumption

Individual body weights were recorded on days 0, 7, 10, 13, 16, 20, 24, and 29 of gestation. Physical examinations were done at each weighing interval.

3. Cesarean sections

On Day 29 of gestation, all does were weighed, euthanized, necropsied, and examined macroscopically. A midline laparotomy was done and the entire reproductive tract was removed. The ovaries were evaluated for gross abnormalities and the number of corpora lutea was recorded. After the doe was examined internally, the uterus was opened along its entire length, conceptuses were removed, and placental membranes were incised. The number of live and dead fetuses, early and late resorptions, and any abnormalities were recorded. All viable fetuses were examined for external abnormalities, weighed, sacrificed and discarded.

II. RESULTS AND DISCUSSION

A. MATERNAL OBSERVATIONS

1. Mortality

The percent of animals at scheduled necropsy were 88% in the controls, 100% at 25 and 50 mg/kg bw/day, 62% at 100 mg/kg, and 75% at 200 mg/kg. Two females at 100 mg/kg bw/day were found dead on days 15 and 16. In addition, one control female and one female at 100 mg/kg bw/day were sacrificed on day 20 upon evidence of aborting. Two females at 200 mg/kg bw/day were sacrificed on day 20, one in a moribund condition and one upon evidence of aborting.

2. Clinical observations

There was a test material-related increase in the incidence of diarrhea was observed at 100 and 200 mg/kg bw/day.

3. Body Weights

There were no statistically significant differences in body weights. A transient decrease was present only during the dosing period (days 7 - 19) and did not persist after the completion of dosing period.

4. Gross pathology

There were no test material-related findings at necropsy observations

B. CESAREAN SECTION DATA

Pregnancy rates were 63% at 0 and 100 mg/kg, 100% at 25 mg/kg, and 75% at 50 and 200 mg/kg. There were no test material-related necropsy observations and no statistically significant differences in the number of corpora lutea or implantations, implantation efficiency, pre-implantation loss, the number or percent of live or resorbed (early, late, and total) foetuses or live fetal body weight. There were no test material-related abnormal fetal external observations. One fetus at 25 mg/kg had gastroschisis, but this finding was not considered to be treatment-related.

III. CONCLUSIONS

The no-observable-effect level (NOEL) for maternal toxicity was 100 mg/kg and for embryo/ fetotoxicity was 200 mg/kg bw/day.

Based on the results of this study, doses of 0, 62.5, 125, and 250 mg/kg bw/day were selected for the definitive teratology study (M-187739-01-1).

Comparison with criteria

Based on the observed effects, classification for reproductive toxicity is not triggered according to the criteria of DSD and CLP since developmental effects regarding pup survival and post implantation loss were only observed in presence of severe maternal toxicity in the rat 2-generation study (at dose equivalent to 2444 mg/kg bw) and mortality in the rabbit developmental toxicity study (at dose equivalent to 250 mg/kg/day).

Conclusions on classification and labelling

No classification for reproductive toxicity is warranted

CA 5.7 Neurotoxicity studies

The effects of ethephon on the nervous system were studied following acute, short-term and subchronic exposure in the rat and the potential of inducing delayed polyneuropathy in the hen (see table 5.7-1). All these studies have been submitted and evaluated during the EU process for Annex I listing.

No new studies have been carried out after the Annex I inclusion.

Table 5.7-1 Summary of neurotoxicity studies

Study	NOAEL (mg/kg bw/day)	LOAEL (mg/kg bw/day)	Effects
Preliminary and full acute neurotoxicity studies Sprague-Dawley rat 0, 250, 500, 1000, 2000 mg/kg bw [redacted] 1996. M-188166-01-2 and M-188081-01-2	250	500	≥ 500 mg/kg bw/day ↑mortality and ↓motor activity at 1000 and 2000 mg/kg bw ≥ 500 mg/kg bw/day ↑pinpoint pupils
2-week study Sprague-Dawley rat 0, 100, 300, 600, 1000 mg/kg bw/day [redacted]; 1997 M-188213-01-1	300	600	Mortality and clinical signs from 600 mg/kg bw/day
13-week study Sprague-Dawley rat 0, 75, 150 or 300/400 mg/kg bw/day [redacted]; 1997 M-188217-01-1	75	150	300/400 mg/kg bw/day Deaths, and clinical signs ↓body weight gain 150 mg/kg bw/day ↓RBC ChE activity in females
Acute delayed neurotoxicity study with ethephon Base 250 in mature white leghorn chickens (30 birds/group) at 0, 1040, 3039, 3290 mg/kg bw [redacted], [redacted] 1983 M-187671-01-1	1040	3039	≥3039 mortality No evidence of delayed neurotoxicity
Acute Delayed neurotoxicity in leghorn hens (14 birds in control and 20 treated at 2000 mg/kg bw) [redacted], 2005 M-247629-01-1	2000	/	No effects.

A time of peak effects study (M-188166-01-2) was conducted to determine an appropriate time, within 8 hours of dosing, to assess the peak behavioral effects of ethephon Base 250 for use in a subsequent acute neurotoxicity study and to evaluate time-course effects on cholinesterase activity.
Sprague-Dawley Crl:CD(SD)BR rats (18/sex/dose) were administered Ethephon Base 250 (72.4% active) orally by gavage at dose levels of 0, 250, 500, 1000 or 2000 mg as/kg bw. Doses were corrected for purity.

There was no effect on ChE activity. No behavioral changes associated with treatment were observed using the functional observational battery. Based on the findings in this study, the following times were selected as appropriate for assessing peak behavioral effects in a subsequent acute neurotoxicity study: FOB, at approximately 5 to 5.5 hours post dosing, and motor activity, commencing at approximately 5.5 to 6 hours post-dosing.

In the acute neurotoxicity study (M-188081-01-2), Sprague-Dawley Crl:CD(SD)BR rats were given ethephon by gavage at a single dose of 0, 250, 500, 1000 or 2000 mg/kg bw. Cholinesterase activity was not determined. One or two animals at the two higher doses died, and abnormal clinical signs and some changes in a battery of functional tests were observed at these doses on the day of treatment, which persisted for a few days in one or two animals. Pinpoint pupils were seen at 500, 1000 and 2000

mg/kg bw, although not in all animals at the lower doses, and this effect persisted for several days in a few animals.
The NOAEL was 250 mg/kg bw.

Groups of at least 6 male and 6 female Sprague-Dawley rats were treated for 14 consecutive days (M-188213-01-1), by gavage, with ethephon Base 250, at dose levels of 0, 100, 300, 600 or 1000 mg/kg bw/day in order to assess its potential toxicity and to provide information on dose levels for use on a subsequent 13-week neurotoxicity study.
All males and five females in the 1000 mg/kg bw/day group and two males and four females in the 600 mg/kg bw/day group died or were sacrificed due to poor condition prior to study completion. In these two groups, body weights and food intake were significantly decreased. Gross pathological findings such as dilatation of the stomach and/or intestine, dark, raised and/or depressed areas in the stomach, dark areas on the thymus and small spleen thymus were seen for pre-terminal animals. Plasma ChE activity was inhibited in all treated groups, but there was no evidence of inhibition or erythrocyte ChE in animals up to 300 mg/kg bw/day. Brain ChE activity was not measured. Based on these findings, dose levels of 75,150 and 400 mg/kg bw/day were selected for use on the subsequent 13-week neurotoxicity study (M-188217-01-1).

In the 90-day study of neurotoxicity, (M-188217-01-1), Sprague-Dawley Crl:CD(SD)BR rats (6 sex/dose group) received ethephon by oral gavage at 75, 150 or 400 mg/kg bw/day. The highest dose was reduced to 300 mg/kg bw/ day at week 10–11 because of excessive mortality. These were the only deaths observed. Abnormal clinical signs were observed at the highest dose.
Erythrocyte ChE activity was significantly inhibited by > 20% at the higher dose and from 150 mg/kg bw/day in females. There was no toxicologically significant effect on brain ChE activity, as only an inhibition below 10% was observed at the highest dose. There were no behavioral changes using the FOB and motor activity tests considered indicative of neurotoxicity.

The NOAEL was 75 mg/kg bw/day on the basis of toxicologically relevant inhibition (> 20%) erythrocyte ChE activity at 150 mg/kg bw/day.

Ethephon showed no potential to induce delayed neuropathy based on the results two acute delayed neurotoxicity studies carried out with leghorn hens in 1983 (M-187671-01-1) and in 2005 (M-247629-01-1).

CA 5.7.1 Neurotoxicity studies in rodents

No new neurotoxicity study was carried out after ethephon Annex I inclusion. The existing relevant information was presented and evaluated during the EU process for Annex I listing.

CA 5.7.2 Delayed polyneuropathy studies

No new delayed polyneuropathy study was carried out after ethephon Annex I inclusion. The existing relevant information was presented and evaluated during the EU process for Annex I listing.

CA 5.8 Other toxicological studies

CA 5.8.1 Toxicity studies of metabolites

The metabolite 2-hydroxyethyl phosphonic acid (HEPA) was found to be a major metabolite of ethephon in plant metabolite and therefore a series of studies have been carried out and evaluated during EU process for Annex I listing.

No new toxicity studies have been carried out on metabolites after the Annex I inclusion.

Table 5.8.1-1 Summary of toxicity studies with 2-hydroxyethyl phosphonic acid (HEPA)

Type of study	Species/test system	Results
HEPA: Acute oral toxicity study in rat [redacted]; 2001 M-209737-01-1	Crl:WI(Glx/BRL/Han)BR) rats (5 ♂ 5 ♀)	LD_{50} > 2000 mg/kg bw
Ames test [redacted]; 2001; M-209742-01-1	S. typhimurium (TA 1535, TA 1537, TA 102, TA 98, TA 100)	Negative
In vitro chromosome aberration [redacted]; 2001; M-209529-01-1	Human lymphocytes	Negative
In vitro forward mutations [redacted]; 2001; M-211768-01-1	Mouse lymphoma L5178Y cells	Negative

Type of study	NOAEL (mg/kg bw/day)	NOAEL (mg/kg bw/day)	Effect
15-day toxicity in Sprague-Dawley rats by gavage 0, 125, 250, 500 mg/kg bw/day [redacted]; 2003 M-231000-01-1	500	/	No adverse effects up to the top dose
28-day toxicity in Sprague-Dawley rats by gavage 0, 125, 350, 1000/700 mg/kg bw/day [redacted]; 2003 M-233065-01-1	350	1000/700	Mortality Clinical signs

5.8.1-2 Other relevant information

Reference	Results
Composition of technical ethephon ((2-chloroethyl) phosphonic acid) and some analogues relative to their reactivity and biological activity [redacted]; [redacted]; [redacted]; [redacted]; 1991 M-211768-01-1	Unlike ethephon, HEPA does not inhibit plasma cholinesterase activity when tested *in vitro*
Position paper on the toxicological relevance of 2-hydroxyethyl-phosphonic acid (HEPA), a rat and plant metabolite of ethephon [redacted]; 2006 M-271001-01-1	A comparison of the result of the toxicity profile of ethephon and HEPA, indicate that HEPA is not a toxicologically relevant metabolite

The acute oral LD_{50} of HEPA in female and male rats is higher than 2000 mg/kg (M-209737-01-1).

The genotoxic potential of HEPA was tested in three *in vitro* studies: Ames test (M-209742-01-1), chromosomal aberration in human lymphocytes (M-209529-01-1) and gene mutation in mammalian cells (M-211768-01-1). Results of all the three studies showed no genotoxic potential.

The systemic toxic effects of HEPA were investigated in Sprague Dawley rats following oral administration for 15 days and for 28 days. In these studies HEPA was administered by gavage because it was too viscous to be mixed homogeneously in the rodent diet.

In the 15-day study (M-231000-01-1) dose levels of 0, 125, 250 or 500 mg/kg bw/day were administered by gastric intubation to 5 rats /sex/dose level. There were no mortalities or treatment-related clinical signs during the study. Administration of HEPA up to 500 mg/kg did not affect body weight or body weight gain in either sex during the course of the study. There were no treatment-related changes in haematology and clinical chemistry parameters. Mean absolute and relative liver weights were found statistically significantly higher in males and females at 500 mg/kg bw/day but not correlated with findings at macroscopic examination. The highest dose administered of 500 mg/kg bw/ day was considered to be the study NOAEL.

In the 28-day study (M-233065-01-1), ten Sprague Dawley rats/sex/dose group were given HEPA at dose levels of 0, 125, 350 and 1000/700 mg/kg/day by gastric intubation. The dose level of 1000 mg/kg bw/day was too high, provoking mortality and marked body weight loss. On day 5, the high dose level was reduced to 700 mg/kg bw /day.
Clinical signs were observed at the top dose of 1000/700 mg/kg bw/day, and consisted of pilo-erection, nasal discharge, few faeces, laboured/noisy respiration, reduced motor activity and wasted appearance.
However no signs were observed during the neurotoxicity assessment and at ophthalmological examination in any dose group. There were no changes in mean terminal body weights or in mean organ weights in treated animals when compared to controls. Gross pathology and histopathology examination of terminal sacrifice animal did not reveal any treatment-related changes.

Based on the mortality and clinical signs observed at the high dose level (1000/700 mg/kg day), the NOAEL of HEPA after 28 days of treatment in Sprague-Dawley rats was determined to be 350 mg/kg/day in both sexes.

There are no *in vivo* data on the effects of HEPA on either plasma or erythrocyte inhibition. However, the effects of HEPA on plasma cholinesterase activity have been investigated *in vitro* as impurity of ethephon and showed that HEPA does not inhibit plasma cholinesterase activity (M-211768-01-1).

A comparison of the result of the toxicity studies with Ethephon and HEPA, indicate that HEPA is less toxic than ethephon as it does not inhibit ChE activity and higher NOAELs were observed after administration by gavage (M-271001-01-1).

Therefore, HEPA is not a toxicological relevant metabolite and it does need to be included in the residue definition of crops treated with ethephon. No ADI or ARfD has been set by EFSA during ethephon and HEPA evaluation for Annex I inclusion.
http://www.efsa.europa.eu/sites/default/files/scientific_output/files/main_documents/1347.pdf

CA 5.8.2 Supplementary studies on the active substance

During and following the Annex I inclusion of ethephon, two special studies were carried out in dogs to further investigate the effect of ethephon on ChE activity in this species and a immunotoxicity study was carried out in the mouse to address a question from US EPA.

Detailed summary is provided for each of these studies also for the 28-day dog toxicity study that was considered in setting the ARfD during the EU evaluation but was not included in the dossier and/or DAR addendum.

Table 5.8.2-1 Summary of supplementary studies

Study	NOAEL (mg/kg bw/day)	LOAEL (mg/kg bw/day)	Effects
28 day in Beagle-dogs (3/females/dose) 0, 250 and 750 ppm, ~0, 6 and 14 mg/kg bw/day. [redacted]; 2006 M-268126-01-1	6	14	LOAEL was based on inhibition of erythrocyte ChE activity Brain cholinesterase activity was not affected
90-day in Beagle dogs (4/sex/dose) 0, 70, 140, and 525 ppm ~ 2, 4, 15 mg/kg bw/day in ♂ and 2, 4, 18 mg/kg bw/day in ♀ [redacted]; 2006 M-276963-01-2	2 (♀) – 4(♂)	4 (♀) – 15 (♂)	LOAEL was based on inhibition of erythrocyte ChE activity Brain cholinesterase activity was not considered to be significantly affected
28-day immunotoxicity in the C57BL/6J female mice (10/dose group) 0, 1000, 3000 and 7000 ppm 0, 187, 575, 1373 mg/kg bw/day [redacted]; 2012 M-429804-01-1	1373	/	No immunotoxic potential

In the 28-day study female Beagle dogs (3/dose levels) received dietary administration of ethephon at 0, 250 and 750 ppm (equivalent to 0, 6 and 14 mg/kg bw/day). Plasma and erythrocyte ChE activity was determined during weeks 1, 2, 3, and 4, and brain cholinesterase activity was determined at study termination.

Plasma cholinesterase activity was significantly depressed for all dose groups at all-time points, and erythrocyte ChE was depressed above 20% in the high-dose group on study days 14, 21, and 28. In the low-dose group (250 ppm), erythrocyte ChE activity was not inhibited. There was no effect on brain ChE activity.

The dose level of 6 mg/kg bw/day was the study NOAEL for inhibition of erythrocyte ChE activity in the in the female dogs following short-term exposure

Ethephon was administered in the diet to Beagle dogs (4/sex/dose) at dose levels of 0, 70, 140, and 525 ppm (equivalent to 2, 4 and 15 mg/kg bw/day in males and 2, 4, and 18 mg/kg bw/day in females) for 91 days (M-276963-01-2). Clinical observations were conducted daily. For determination of the

dose in mg/kg/day, food consumption was measured daily and body weights were taken weekly. Plasma and erythrocyte ChE activity was determined during weeks 1, 2, 4, 8, 10 and 12, and brain ChE activity was determined at study termination.

Ethephon significantly inhibited plasma ChE activity at all doses tested in both sexes and erythrocyte ChE activity at doses equivalent to 4 mg/kg bw/day in females and to 15 mg/kg bw/day in males. Brain ChE esterase was inhibited up to 14% in the females at the top dose.

In conclusion, the NOAEL for this study was 70 ppm (2 mg/kg/day), based on the statistically significant inhibition of erythrocyte ChE activity in females.

The potential immunotoxic properties of ethephon were tested in female C57BL/6J mice (10 animals/dose group) by dietary administration of ethephon for 28 days (M-429804-01-1). The tested concentrations were 1000, 3000 and 7000 ppm (equating to 187, 575, 1373 mg/kg bw/day). A similarly constituted group received untreated diet and acted as a control group. An additional group of 10 female mice were administered cyclophosphamide (immunosuppressive agent) daily by gavage for 28 days at concentration of 7.5 mg/kg bw /day and acted as positive control group.

Four days before necropsy, all animals were immunized with Sheep Red Blood Cell (SRBC) antigen by intravenous injection of 10^8 SRBC/animal via the tail vein. Animals were observed daily for mortality and clinical signs. Body weight and food consumption were recorded once weekly. A detailed physical examination was performed once during the acclimatization phase and at least weekly throughout the study. On study day 30, blood samples were collected from the retro-orbital venous plexus of each animal (just before necropsy) for specific anti-SRBC immunoglobulin M (IgM) analysis. All animals were necropsied, gross pathology observations were performed and selected organs (spleen and thymus) weighed.

Dietary administration of ethephon to female mouse at dose levels up to 7000 ppm did not cause any adverse effect. No impairment of the immunological IgM response was observed after immunization with SRBC at any dose levels. Therefore, ethephon is considered not to have immunotoxic potential.

Report:	KCA 5.8.2/02; [redacted] 2006; M-268126-01-1
Title:	A 28-day cholinesterase inhibition study via dietary administration in the beagle dog with ethephon base 250
Report No.:	201302
Document No.:	M-268126-01-1
Guideline(s):	not specified
Guideline deviation(s):	not specified
GLP/GEP:	**yes**

Executive summary

Ethephon Base 250 (purity 7.3%) was administered in the diet to Beagle dogs (3/females/dose) at dose levels of 0, 250, and 750 ppm (equivalent to 6 and 14 mg/kg bw/day) for 28 days. The designated nominal concentrations was corrected for the test material purity.

Clinical observations were conducted daily. For determination of the dose in mg/kg/day, food consumption was measured daily and body weights were taken weekly.

Plasma and erythrocyte ChE activity was determined during weeks one, two, three, and four, and brain cholinesterase activity was determined at study termination.

At 750 ppm, there were no death and no compound-related clinical observations. Plasma ChE activity was significantly inhibited, erythrocyte ChE was also biologically significantly inhibited but brain cholinesterase was not inhibited.

At 250 ppm only plasma ChE was significantly inhibited and there were no effects on erythrocyte and brain ChE activities.

As inhibition of plasma cholinesterase is not considered to be an adverse effect, the study NOAEL was 250 ppm (equivalent to 6) based on inhibition of erythrocyte ChE activity at 750 ppm.

I. MATERIALS AND METHODS

A. MATERIALS

Test Material:	Ethephon
Description:	Clear liquid
Lot/Batch #:	2250197
Purity:	71.3 %
CAS #:	16672-87-0
Stability of test compound:	Not measured in this study
Vehicle:	none
Test Animals	
Species:	Female Dog (nulliparous and nonpregnant)
Strain:	Beagle
Age:	11-12 months
Weight:	Males 8.6 – 9.7 kg, females 6.2 – 8.5 kg
Source:	[redacted]
Acclimation period:	At least 2 weeks
Diet:	Purina Mill Lab Canine Diet Meal 5007 was presented to the animals for 2 - 2.5 hours/day. Fresh feed was given daily.
Water:	Tap water (Kansas City, Missouri municipal water, dispensed by automatic watering system) was provided continuously for ad libitum consumption during the acclimation period and throughout the study.
Housing:	Animals were housed individually in stainless steel cages.
Environmental conditions	
Temperature:	64 to 84 °F equivalent to 17.8 – 28.9 ºC
Humidity:	30-70 %
Air change:	Target of 15.67 changes per hour
Photoperiod:	12 hrs dark/ 12 hrs light

B. STUDY DESIGN

1 In life dates:

The study was carried out at the [redacted] from May 21st to June 29th, 2004

2 Animal assignment and treatment

Animals were allocated to dose groups without using a formal randomization program.

Table 5.8.2-2 Study design

Group	Test substance	Dose levels (ppm)	Number of animals per group
Females			
1	Control	0	3
2	ethephon	250	3
3		750	3

The dose levels were selected from results obtained in toxicity studies previously performed in dogs, where the selected doses were well tolerated and in order to measure effect of ethephon on plasma, erythrocyte and brain ChE activities.

3 Dose preparation analysis

Ethephon was mixed in the feed at the designated nominal concentrations by correcting for the test material purity. All feed mixtures were prepared weekly and stored under freezer conditions until presented to the animals. The diet was prepared by dissolving the ethephon in acetone prior to being mixed in the diet. The control diet was prepared the same as the treated diet, excluding only the test substance.

4 Statistics

Statistical significance was determined at $p<0.05$ for all tests with the exception of Bartlett's test, in which a probability value of $p<0.001$ was used. All tests were two-tailed.

Due to inter-animals variability of the values and the low number of animals, plasma and erythrocyte cholinesterase activities were evaluated as the change in activity between the average of the pretreatment values and the various days of treatment for each dog.

Cholinesterase activity was analyzed by Bartlett's test for homogeneity. If the data was homogeneous, an ANOVA was performed followed by a Student's t-test on data points showing a significant effect by ANOVA. If the data was non-homogeneous, a Kruskal-Wallis ANOVA was performed followed by the Mann-Whitney U-test to identify statistical significance between groups.

C. METHODS

1. Clinical signs

All study animals were observed at least once daily for clinical signs of toxicity (except once daily on weekends). Detailed clinical observations for clinical signs of toxicity were performed on all animals at study initiation and on a weekly basis thereafter.

2. Body weight

Individual body weights were measured weekly throughout the study.

3. Food consumption

Food consumption was measured daily. The intake of test compound in mg/kg/day was calculated for the animals using the nominal concentration of ethephon in the feed and the following equation:

(Average food consumption per week/average body weight per week) x ppm in the feed/1000

4. Clinical chemistry

Plasma and erythrocyte ChE activity were measured on all animals once prior to administration of the test substance and during weeks one, two, three, and four. Brain ChE activity was determined

at study termination. The method used to measure plasma, erythrocyte, and brain ChE activities was a modification of the method described by [redacted]: "A new Rapid Colorimetric Determination of Acetylcholinesterase Activity", Biochem. Pharm, 7, p. 88-95, 1961.]. The modification involves using 6,6'-dithio-dinicotinic acid (DTNA) as the coupling reagent and measuring the change in absorbance at 340 nm.

5. Sacrifice and pathology

Animals were euthanized at the end of the study by intravenous injection of Fatal-Plus® ([redacted]).

No gross necropsy was performed. Only the brain was collected for determination of ChE activity.

II. RESULTS AND DISCUSSION

A. OBSERVATIONS

1. Clinical signs of toxicity

There were no clinical signs related to the compound administration.

2. Mortality

There were no mortalities during the study.

B. ChE activity evaluation

1. Plasma ChE

Plasma ChE activity was statistically significantly depressed for all dose groups at all time-points, with the following ranges of depression for the various dose groups: low: -30% to -49%; high: -56% to -63%.

Table 5.8.2-3 Ethephon effect on plasma cholinesterase activity

Dose level ppm (mg/kg bw/day)	Plasma cholinesterase activity (IU/g)			
	Day 7	Day 14	Day 21	Day 28
Control (0)	1.69 ± 0.34	1.62 ± 0.25	1.72 ± 0.33	1.58 ± 0.28
250 (6)	1.18 ± 0.24	0.88* ± 0.20	0.90* ± 0.23	0.80* ± 0.25
750 (14)	0.74* ± 0.13	0.60* ± 0.09	0.66* ± 0.05	0.63* ± 0.08

Anova + Student's t-tests (Two-Sided) *p<0.05

2. Erythrocyte ChE

Erythrocyte ChE activity was biologically depressed in the high-dose group on study days 14, 21, and 28. The depression in erythrocyte ChE observed on study day 7 was not considered to be biologically significant as this was below 20% inhibition compare with the controls.

For the low-dose group, the erythrocyte ChE activity was not inhibited to biologically significant extent over the 28-day exposure period.

Table 5.8.2-4 Ethephon effect on erythrocyte cholinesterase activity

Dose level ppm (mg/kg bw/day)	Erythrocyte cholinesterase activity (IU/g)			
	Day 7	Day 14	Day 21	Day 28
Control (0)	1.70 ± 0.62	1.82 ± 0.53	1.77 ± 0.48	1.70 ± 0.49
250 (6)	2.03 ± 0.04	1.98 ± 0.13	1.77 ± 0.05	1.61 ± 0.19
750 (14)	1.37 ± 0.21	1.11 ± 0.14	0.88* ± 0.11	0.71*± 0.02

Anova + Student's t-tests (Two-Sided) *p<0.05

3. Brain ChE

The percent depression of the treated groups versus the control group for brain ChE activity on study day 28 was -4% and +1% for the low- and high-dose groups, respectively. Thus, there was no effect of ethephon on brain acetylcholinesterase activity at both the doses tested in this study.

Table 5.8.2-5 Ethephon effect on brain cholinesterase activity

	Control	250 ppm	750 ppm
Activity (IU/g)	6.9 ± 0.6	6.6 ± 1.0	7.0 ± 0.4
% of control	/	-4%	+1%

III. CONCLUSIONS

In conclusion, a dose level of 250 ppm (equivalent 6 mg/kg bw/day) is considered to be the NOAEL for biologically relevant erythrocyte ChE activity in dogs following 28-day dietary administration of ethephon.

Report: KCA 5.8.2/02; [redacted]; 2006; M-276963-01-2
Title: A 90 -day cholinesterase inhibition study via dietary administration in the beagle dog with technical ethephon
Report No.: 201261
Document No.: M-276963-01-2
Guideline(s): U.S. EPA OCSPP 870.SUPP
Guideline deviation(s): not specified
GLP/GEP: yes

Executive summary

Ethephon was administered in the diet to Beagle dogs (4/sex/dose) at dose levels of 0, 70, 140, and 525 ppm (equivalent to 2, 4 and 15 mg/kg bw/day and 2, 4 and 18 mg/kg bw/day in males and females, respectively) for 91 days. For determination of the dose in mg/kg/day, food consumption was measured daily and body weights were taken weekly. Clinical observations were conducted daily. Plasma and erythrocyte ChE activities were determined during weeks one, two, four, eight, ten, and twelve, and brain ChE activity was determined at study termination.

There were no mortalities and no clinical signs at any dose levels.

Plasma ChE activity was significantly inhibited at all dose levels. Erythrocyte acetylcholinesterase was statistically significantly inhibited from doses of 140 ppm in females and at the top dose of 525 ppm in males.

Brain cholinesterase activity in females was inhibited at in a dose-related extent, with an effect above 10% at the top dose. There was no evidence of inhibition of brain cholinesterase in males.

The NOAEL was 70 ppm (equivalent to 2 mg/kg bw/day) in females and 140 ppm (equivalent to 4 mg/kg bw/day) in males.

Erythrocyte inhibition data were also analyzed using a Benchmark Dose (BMD) procedure which predicted the BMD for 20% inhibition of erythrocyte ChE to be mg/kg/day and 5 mg/kg/day for males and females, respectively.

MATERIALS AND METHODS

A. MATERIALS

Test Material:	Ethephon Technical Grade
Description:	Clear liquid
Lot/Batch #:	040201
Purity:	71.4 to 71.9% %
CAS #:	16672-87-0
Stability of test compound:	
Vehicle and or positive control:	None. Doses administered in diet
Test Animals	
Species:	Male and Female Dog (nulliparous and nonpregnant)
Strain:	Beagle
Age:	6 months
Weight:	Males 7.8 - 9.4 kg, females 5.3 - 7.2 kg
Source:	[redacted]
Acclimation period:	At least 10 days
Diet:	Purina Mill Certified Lab Canine Diet Meal 5007 was presented to the animals for 2 - 2.5 hours/day, beginning during the acclimation period. Fresh feed was given daily.
Water:	Tap water (Kansas City, Missouri municipal water) was provided continuously for *ad libitum* consumption during the acclimation period and throughout the study
Housing:	Individually housed in stainless steel cages (MRI) and stainless steel runs (Bayer Toxicology).
Environmental conditions	
Temperature:	64 to 84°F (18-29°C).
Humidity:	30-70 %
Air change:	Averaged 14.02 changes per hour
Photoperiod:	12 h dark/ 12 h light

B. STUDY DESIGN

1. In life dates:

The study was carried out at the [redacted] from September to December 2004

2. Animal assignment and treatment

The nominal concentrations were 0 (concurrent vehicle control) 70, 140, and 525 ppm (4/sex/ dose) of ethephon mixed with dog ration (target doses of 2, 4, and 15 mg/kg/day of ethephon). Selection of these dose levels was based on the results of the special study on the effects of ethephon on ChE activity in Beagle dogs following 28-day feeding.

Table 5.8.2-6 Animal assignment

Group	Test substance	Dose levels (ppm)	Number of animals per sex and group
1	Control	0	4
2	ethephon	70	4
3		140	4
4		525	4

3. Diet preparation and analysis

The appropriate amount of test substance was incorporated in the feed in the feed at the designated nominal concentrations by correcting for ethephon purity. All feed mixtures were prepared weekly and stored under freezer conditions until presented to the animals. The diet was prepared by dissolving the ethephon in ethanol prior to being mixed in the diet. The control diet was prepared the same as the treated diet, excluding the test substance.

The homogeneity of the test substance in the feed and the stability of the active ingredient in the test substance in feed stored at room temperature for 7 days and at freezer temperatures for 28 days were confirmed analytically.
The concentration of the active ingredient in the feed was verified for study weeks 1, 2, 3, 7, and 11.
Results were as follows:

- Homogeneity Analysis 85-91% nominal concentration
- Stability Analysis: 89-93% of nominal concentration for 7 day
- Concentration Analysis: 86-90% of nominal concentration

The homogeneity of the test substance in the feed and the stability of the active ingredient in the test substance in feed stored at room temperature for 7 days and at freezer temperatures for 28 days were confirmed analytically.

Results were within the in-house target range of 85 to 115% of nominal concentration and were therefore considered to be acceptable for use on the current study.

4. Statistics

Statistical significance was determined at $p<0.05$ for all tests with the exception of Bartlett's test, in which a probability value of $p<0.001$ was used. All tests were two-tailed.

Due to the high inter-animal variability, plasma and erythrocyte acetylcholinesterase activity was evaluated as the change in activity between the average of the pretreatment values and the various days of treatment. The data was analyzed by Bartlett's test for homogeneity. If the data was homogeneous, an ANOVA was performed followed by a Student's t-test on data points showing a significant effect by ANOVA. If the data was non-homogeneous, a Kruskal-Wallis ANOVA was performed followed by the Dunn test on data points showing a significant effect by the Kruskal-Wallis ANOVA. Statistical analyses were carried out using SAS programs.

Brain acetylcholinesterase activity was analyzed by Bartlett's test for homogeneity. If the data was homogeneous, an ANOVA was performed followed by a Student's t-test on data points showing a significant effect by ANOVA. If the data was non-homogeneous, a Kruskal-Wallis ANOVA was performed followed by the Mann-Whitney U-test to identify statistical significance between groups. This analysis was done using INSTEM DATATOX®.

For the Benchmark Dose (BMD) analysis the data using a computer program developed and maintained by the U.S. EPA (Benchmark Dose Software (BMDS, version 1.3.2, U.S. EPA, National Center for Environmental Assessment, http://www.epa.gov/ncea/bmds.htm).

The method used was for continuous data and a benchmark response factor of 20% or 0.2 relative deviation was employed. The data were considered to be non-homogeneous by the BMD software and the linear model was chosen.

C. METHODS

1. Observations

All study animals were observed once daily for clinical signs of toxicity. Detailed clinical observations for clinical signs of toxicity were performed on all animals at initiation of dosing and on a weekly basis thereafter.

2. Body weight

Individual body weights were measured (weekly).

3. Food consumption and compound intake

Food intake was measured daily. The intake of test compound in mg/kg/day was calculated for males and females using the analytical concentration of ethephon in the feed and the following equation:

$$\text{Achieved body weight (mg/kg bw/day)} = \left(\frac{\text{Mean weekly food consumption (g/day)} \times \text{Dose level (ppm)}}{\text{Mean body weight per week} \times 1000} \right)$$

4. Clinical pathology

Plasma and erythrocyte ChE activity were measured on all animals once prior to administration of the test substance and during weeks one, two, three, and four. Brain ChE activity was determined At study termination.
The method used to measure plasma, erythrocyte, and brain cholinesterase activities was a modification of the method described by [redacted]. A new Rapid Colorimetric Determination of Acetylcholinesterase Activity", Biochem. Pharm, 7, p 88-95, 1961.]. The modification involves using 6,6'-dithio-dinicotinic acid (DTNA) as the coupling reagent and measuring the change in absorbance at 340 nm.

5. Sacrifice and pathology

Animals were euthanized at the end of the study by intravenous injection of Fatal-Plus® ([redacted]).

No gross necropsy or histopathology was performed. Only the brain was collected for the determination of ChE activity.

II. RESULTS AND DISCUSSION

A. OBSERVATIONS

1. Clinical signs of toxicity

There were no treatment-related sign of toxicity

2. Mortality

There were no mortalities during the study.

B. BODY WEIGHT AND BODY WEIGHT GAIN

There was no effect on bodyweight or body weight gain between control and treated grops

C. FOOD CONSUMPTION AND COMPOUND INTAKE

The average intake of ethephon in mg/kg/day, calculated using the analytical concentration of ethephon in the feed, is shown the table below.

Table 5.8.2-7 Achieved intake

Dose group (ppm)	70	140	525
Males	2	4	15
Females	2	4	18

B. ChE activity

1. Plasma ChE

To evaluate the effect of ethephon on plasma ChE activity, the enzyme activity was mathematically adjusted for the control and treatment groups prior to statistical analysis of the data.

This adjustment was done by taking the average of the enzyme activity for each animal prior to treatment and subtracting this value from the acetylcholinesterase activity for each animal at each treatment time evaluated.

Plasma ChE activity was statistically significantly decreased in all treated groups

Table 5.8.2-8 Inhibition of Plasma ChE

MALES						
Dose level ppm (mg/kg bw/day)	Plasma cholinesterase activity (IU/g)					
	Day 3	Day 10	Day 25	Day 53	Day 70	Day 87
Control (0)	0.04	-0.15	-0.15	-0.12	-0.10	-0.09
70 (2)	-0.46*	-0.94*	-0.98*	-0.94*	-0.85*	-0.92*
140 (4)	-0.76*	-1.10*	-1.18*	-1.11*	-1.12*	-1.06*
525 (15)	-1.03*	-1.08*	-1.29*	-1.27*	-1.26*	-1.21*
FEMALES						
Dose level ppm (mg/kg bw/day)	Plasma cholinesterase activity (IU/g)					
	Day 3	Day 10	Day 25	Day 53	Day 70	Day 87
Control (0)	0.00	0.03	-0.25	-0.27	-0.23	-0.29
70 (2)	-0.37	-0.73*	-0.76*	-0.81*	-0.80*	-0.79*
140 (4)	-0.70	-1.09*	-1.04*	-1.05*	-1.05*	-1.04*
525 (18)	-1.12*	-1.37*	-1.43*	-1.39*	-1.41*	-1.39*

Anova; Dunnett's test *p≤0.05

2. Erythrocyte ChE

To evaluate the effect of ethephon on erythrocyte acetylcholinesterase activity, the enzyme activity was mathematically adjusted for the control and treatment groups prior to statistical analysis of the data.

This adjustment was done by taking the average of the enzyme activity for each animal prior to treatment and subtracting this value from the acetylcholinesterase activity for each animal at each treatment time evaluated..

Erythrocyte ChE activity was statistically significantly decreased from 140 ppm in the females and at the top dose of 525 ppm in males.

Table 5.8.2-9 Inhibition of Erythrocyte ChE

MALES						
Dose level	**Erythrocyte cholinesterase activity (IU/g)**					
ppm (mg/kg bw/day)	**Day 3**	**Day 10**	**Day 25**	**Day 53**	**Day 70**	**Day 87**
Control (0)	0.09	-0.15	-0.14	-0.37	-0.24	-0.25
70 (2)	0.09	-0.14	-0.25	-0.52	-0.47	-0.55
140 (4)	0.15	-0.17	-0.50	-0.65	-0.64	-0.65
525 (15)	-0.03	-0.47*	-1.09*	-1.42*	-1.42*	-1.38*
FEMALES						
Dose level	**Erythrocyte cholinesterase activity (IU/g)**					
ppm (mg/kg bw/day)	**Day 3**	**Day 10**	**Day 25**	**Day 53**	**Day 70**	**Day 87**
Control (0)	0.17	0.02	-0.07	-0.27	-0.17	-0.22
70 (2)	0.20	-0.08	-0.25	-0.43	-0.43	-0.43
140 (4)	0.29	-0.26	-0.56	-1.02*	-0.96*	-0.95*
525 (18)	-0.11	-0.42	-0.98*	-1.27*	-1.30*	-1.27*

Anova + Dunnett's test *p≤0.

3. Brain ChE activity

Brain ChE activity was not affected in males at all, whereas a dose –relationship was observed in the females and with a decrease above 10% at the top dose of 525 ppm.

Table 5.8.2-10 Inhibition of Brain ChE

Males	**Control**	**70 ppm**	**140 ppm**	**525 ppm**
Activity (IU/g)	6.9± 0.3	7.1 ±1.0	7.7±2.0	6.8±0.5
% of control	/	+3	+12	+1
Females	**Control**	**70 ppm**	**140 ppm**	**525 ppm**
Activity (IU/g)	7.2± 0.2	6.6*±0.4	6.5*±0.4	6.2*±6.2
% of control	/	-8%	-10%	-14

Anova + Student's t-tests (Two-Sided) *p≤0.05

1. Benchmark Analysis

A method used continuous data and a benchmark response factor of 20% or 0.2 relative deviation was employed. The data on ChE erythrocyte inhibition were considered to be non-homogeneous by the BMD software and the linear model was chosen.

Results gave a BMD of 4 mg/kg/day (BMD and BMDL of 4.44 and 3.99 mg/kg/day respectively) in males and of 5 mg/kg bw/day (BMD and BMDL 4.81 and 5.88, respectively).

III. CONCLUSIONS

In conclusion, the NOAEL for this study was 70 ppm (2 mg/kg/day), based on inhibition of erythrocyte acetylcholinesterase activity. The Benchmark Dose (mg/kg/day) for 20% inhibition of erythrocyte acetylcholinesterase activity is 4 mg/kg/day for males and 5 mg/kg/day for females.

Immunotoxicity study

Report:	KCA 5.8.2/04; [redacted]; 2012; M-429804-01-1
Title:	Ethephon - 28-Day immunotoxicity study in the female mouse by dietary administration
Report No.:	SA 10361
Document No.:	M-429804-01-1
Guideline(s):	U.S.E.P.A., OPPTS Series 870, Health Effects Testing Guidelines, No. 870.7800 (August 1998)
Guideline deviation(s):	not specified
GLP/GEP:	**yes**

Executive Summary

The objective of this study was to assess the potential immunotoxic properties of Ethephon in female C57BL/6J mice following daily administration by oral gavage for at least 28 days.

Ethephon (batch number 0022141: a light yellow liquid, 70.8% purity), was administered continuously via dietary administration to separate groups of female C57BL/6J mice (10/group) at concentrations of 1000, 3000 and 7000 ppm (equating approximately to 187, 575, 1373 mg/kg bw/day) for 28 days. A similarly constituted group received untreated diet and acted as a control group. An additional group of 10 female mice were administered cyclophosphamide (immunosuppressive agent) daily by gavage for 28 days at concentration of 7.5 mg/kg body weight/day and acted as positive control group.

Four days before necropsy, all animals were immunized with Sheep Red Blood Cell (SRBC) antigen by intravenous injection of 10^8 SRBC/animal via the tail vein. Animals were observed daily for mortality and clinical signs. Body weight and food consumption were recorded once weekly. A detailed physical examination was performed once during the acclimatization phase and at least weekly throughout the study. On Study Day 30, blood samples were collected from the retro-orbital venous plexus of each animal (just before necropsy) for specific anti-SRBC immunoglobulin M (IgM) analysis. All animals were necropsied, gross pathology observations were performed and selected organs (spleen and thymus) weighed.

Dietary administration of ethephon to female mouse at dose levels up to 7000 ppm did not cause any mortality or clinical signs and did not cause any effect on body weight parameters, food consumption, terminal body weight or macroscopic examination.

For the immunological response, the results obtained in control animals after immunization with SRBC and those obtained with the positive control confirmed the ability of the test system to detect immune-suppressive effects and confirmed the validity of the test design. Up to the highest ethephon dose of 7000 ppm, no relevant change was noted in anti-SRBC IgM concentrations compared to controls.

In conclusion, no impairment of the immunological IgM response was observed after immunization with SRBC of mice receiving ethephon in the diet at dose level up to 7000 ppm for at least 28 days (corresponding to 1373 mg/kg bw/day). Therefore, ethephon was considered not to have an immunotoxic potential.

I. MATERIALS AND METHODS

A. MATERIAL:

1. Test Material:	Ethephon
Description:	light yellow liquid
Lot/Batch:	0022141
Purity:	70.8%
CAS:	16672-87-0
Stability of test compound:	Stable in rodent diet for at least 27 days frozen followed by 10 days at room temperature
2. Vehicle and /or positive control:	Cyclophosphamide
Description:	white powder
Lot/Batch:	Purchased by [redacted]
Purity:	100.6%
CAS:	6055-19-2
Stability of test compound:	Stable at 1 and 3 g/mL for a time period which covers the period of storage and usage for the current study
3. Test animals:	
Species:	Female mice
Strain:	C57BL/6J mice
Age:	7 weeks
Weight at dosing:	14.9 to 18.7 g
Source:	[redacted]
Acclimation period:	12 days
Diet:	Certified rodent powdered and irradiated diet A04CP-10 from [redacted]
Water:	Tap water filtered and softened water from the municipal water supply ad libitum
Housing:	Mice were housed individually in suspended, stainless steel, wire-mesh cages
Environmental conditions:	
Temperature:	20°C - 24 °C
Humidity:	40-70% %
Air changes:	Approximately 10 changes per hour
Photoperiod:	Alternating 12-hour light and dark cycles (7 am - 7 pm)

B. STUDY DESIGN

1. In life dates
From October 12th, 2011 to November 25th, 2011 performed at the [redacted]

2. Animal assignment and treatment
The test item dose levels were based on the results of 28-day and oncogenicity study in the mouse. The cyclophosphamide dose level was based on a validation study carried out in the laboratory.

All groups treated by the test item received the appropriate dietary concentrations at a constant dose level. Control group and the group treated by the immunosuppressive agent cyclophosphamide received untreated diet.
Mice received the cyclophosphamide formulation by gavage at a dosage volume of 5 mL/kg body weight. The volume administered to each rat was adjusted on the most recently recorded body weight.

Table 5.8.2-11 Study design

Group	Test Substance	Dose level (ppm)	Number of animals Per group
1	Control	0	10
2	Ethephon	1000	10
3		3000	10
4		7000	10
5	Cyclophosphamide	7.5 (mg/kg bw/day)	10

3. Diet preparation and analysis of the test substances
Ethephon was incorporated into the diet to provide the required dietary concentrations. The test item was ground to a fine powder before being incorporated into the diet by dry mixing. There was one preparation for each concentration. When not in use, the diet formulations were stored at approximately -18° C.

The homogeneity of ethephon in diet was verified before the study for all concentrations to demonstrate adequate formulation procedures. Dietary levels of the test item were verified for each concentration.

Homogeneity and concentration results ranged from 80 to 92% of the nominal concentration and were within the in-house target range.

The stability of the ethephon dietary formulation was determined during the study at 1000 and 7000 ppm. The mean value obtained from the homogeneity check was taken as measured concentration. Diet samples from the highest and lowest concentrations were taken and frozen. They were analyzed after having been frozen for at least 27 days then thawed and kept at room temperature for 10 days. Ethephon was found to be stable at 1000 and 7000 ppm in rodent diet for at least 27 days frozen storage followed by 10 days at room temperature. After storage under those conditions, concentrations were 91% and 102% of the nominal concentration at 1000 and 7000 ppm, respectively. Therefore, formulations were considered to be acceptable for the study.

Results were obtained using the calculation software Empower 2 (Build 2154).

The dosing formulation of the positive control cyclophosphamide was prepared by suspending it in sterilized water to produce the required dosing concentration. They were prepared and stored in air-tight light resistant containers at approximately +4°C when not in use. There were two preparations during the study.

4. Statistics
Mean and standard deviation were calculated for each group.
Statistical evaluations on body and organ weight data were done using the Dunnett-test in connection with a variance analysis.

All variables that were not dichotomous were described by sex, dose group and time point using appropriate measures of central tendency (mean, median) and general variability (standard deviation, minimum, maximum).
For the statistical evaluation of samples drawn from continuously distributed random variables three types of statistical tests were used, the choice of the test being a function of prior knowledge obtained in former studies. Provided that the variables in question were approximately normally distributed with equal variances across treatments, the Dunnett test was used, if heteroscedasticity appeared more likely, a p value adjusted Welch test was applied. If the evidence based on experience with historical data indicated that the assumptions for a parametric analysis of variance cannot be maintained, distribution-free tests in lieu of ANOVA were carried out, i.e. the Kruskal-Wallis test followed by adjusted Mann- Whitney-Wilcoxon tests (U tests) where appropriate.

C. METHODS

1. Daily observations

All animals were checked for moribundity and mortality twice daily (once daily on weekends or public holidays). All Animals were observed for clinical signs at least once each day starting on study Day 1 and every day throughout the study. The nature, onset, severity, reversibility and duration of clinical signs were recorded. Detailed physical examinations were performed at least weekly during the treatment period. Cages and cage-trays were inspected daily for evidence of ill-health such as blood or loose feces.

2. Body weight

Each animal was weighed at least weekly during the acclimatization period, on the start of treatment (Study Day 1), then at weekly intervals throughout the treatment period and before necropsy.

3. Food consumption

The weight of food supplied and of that remaining at the end of the food consumption period was recorded weekly for all animals during the treatment period.
The weekly mean achieved dosage intake in mg/kg body weight/day for each week and for Weeks 1 to 4 was calculated (except for the group exposed to the immunosuppressive agent cyclophosphamide) using the following formula:

$$\text{Achieved dosage intake (mg/kg bw/day)} = \frac{\text{Dose level (ppm)} \times \text{Group mean food consumption (g/day) per week}}{\text{Group mean body weight (g) at end of week}}$$

4. Immunotoxicity

- ***Sheep Red Blood Cell (SRBC) sensitization***
  a) SRBC identification:
     Antigen: SRBC
     Supplier: BioMérieux
     Reference No.: 72 141
  b) Storage: SBRC were stored at approximately 5 ± 3°C

SRBC was selected as an appropriate antigen, since it has a large size ensuring proper immunization of animals and since it is recommended by the guideline.

On the day of injection, Sheep Red Blood Cells were washed in PBS (Phosphate Buffered Saline), counted using a cell counting instrument (Siemens Advia 120) and diluted in PBS in order to obtain a 10^9 cells/mL preparation. SRBC preparation was kept on ice until use.

On Study Day 26 after the start of treatment, all animals in all groups were immunized by intravenous injection in tail vein (0.1 mL/animal i.e. 10^8 cells/animal) with Sheep Red Blood Cell (SRBC) preparation. Prior to intravenous injection, animals were anesthetized with Isoflurane (Virbac, Carros, France).

5. Clinical pathology

Blood sampling

Blood samples were taken from all animals in all groups by puncture of the retro-orbital venous plexus 4 days after SRBC immunization (terminal sacrifice). Animals were not diet fasted. Animals were anesthetized by inhalation of Isoflurane (Virbac, Carros, France). Blood (approximately 0.5 ml) was placed into tubes with clot activator (for serum preparation). After centrifugation, serum aliquots were frozen (approximately -74°C) for future analysis.

6. SRBC-specific IgM assay

Enzyme-Linked Immunosorbent Assay (ELISA) was used to determine the level of SRBC-specific immunoglobulin M in response to antigen administration.
Mouse Anti-Sheep Red Blood Cell IgM ELISA kits from [redacted]) were used.
Results were obtained using the software KC4 (version 3.4 Revision 12).

7. Post-mortem examinations

Necrospy

On Study Day 30, all animals from all groups were sacrificed by exsanguination while under deep anesthesia (Isoflurane inhalation).
All animals were necropsied. The necropsy included the examination of all major organs, tissues and body cavities. Macroscopic abnormalities were recorded but not sampled.

Organ weights

At the end of the treatment spleen and thymus were weighed.

Histopathology

No histopathological examination was performed.

II. RESULTS

A. Mortality

There was no mortality in any group throughout the study.

B. Clinical Signs

There were no clinical signs observed in any groups throughout the study.

C. Body weight

Mean body weight and mean body weight gain parameters were unaffected at any dose level during the study.

D. Food and water consumption

There was no effect on food and water consumption.
Mean achieved intake is presented in table 5.8.2-12

Table 5.8.2-12 Mean achieved dietary intake

	Ethephon		
Dose levels (ppm)	**1000**	**3000**	**7000**
Intake (g/kg bw/day)	187	575	1373

E. Immunotoxicity assessment

<u>SRBC-specific IgM response</u>
A slight inter-individual variability was noted in all the groups.
The high mean anti-SRBC IgM concentration observed in the control group reflected the normal response after SRBC immunization and confirmed the sensitization of the animals.
No treatment-related change was noted in the animals treated with ethephon up to 7000 ppm.
In the group treated with cyclophosphamide, mean anti-SRBC IgM concentration was markedly lower (-72%, p≤0.01) than the controls.
This variation corresponds to the range usually observed with cyclophosphamide (7.5 mg/kg/day) within the laboratory conditions.

Table 5.8.2-13 SRBC-specific IgM (u/mL) levels

	Ethephon				Cyclophosphamide
Dose level (ppm)	**0**	**1000**	**3000**	**7000**	**7.5 mg/kg bw/day**
Mean	1654±725	1858±710	1902±608	1814±430	457** ±274
% control	/	+12	+15	+10	-72

**: p≤0.01

F. Post-mortem examinations

In animals treated with ethephon up to 7000 ppm, there were no treatment-related changes at the macroscopic examination and there were no effects on spleen and thymus weight.
Spleen weights were significantly decreased in animals treated with cyclophosphamide

Table 5.8.2-14 Terminal body and spleen and thymus weights

	Ethephon				Cyclophosphamide
Dose levels (ppm)	**0**	**1000**	**3000**	**7000**	**7.5 mg/kg bw/day**
Terminal bw (g)	20.5 ± 0.9	20.5 ± 1.1	20.2 ± 1.0	19.9 ± 1.0	19.4*±1.1
Spleen (absolute, mg)	0.096 ±0.013	0.097 ±0.025	0.092 ±0.018	0.089 ±0.023	0.076** ±0.014
Spleen (% bw)	0.4654±0.060	0.4690±0.106	0.4567±0.381	0.4447±0.106	0.3905** ±0.054
Thymus (absolute, mg)	0.045±0.009	0.047±0.009	0.051±0.008	0.046±0.001	0.041±0.007
Thymus (% bw)	0.2182±0.046	0.2297±0.036	0.2531±0.048	0.2275±0.042	0.2126±0.034

**: p≤0.01

III. CONCLUSION

In conclusion, no impairment of the immunological IgM response was observed after immunization with SRBC of mice receiving ethephon in the diet at dose level up to 7000 ppm for at least 28 days (corresponding to 1373 mg/kg bw/day). Therefore, ethephon is not considered to have an immunotoxic potential.

CA 5.8.3 Endocrine disrupting properties

Ethephon data base does not trigger classification for reproductive and developmental toxicity and for carcinogenicity. Therefore it does not meet the current interim criteria for endocrine disrupting properties. Moreover, the comprehensive data base does not show any direct toxic effect on an endocrine organ and/or any evidence of direct effect on estrogen and androgen receptors.

CA 5.9 Medical data

CA 5.9.1 Medical surveillance on manufacturing plant personnel and monitoring studies

For further information on medical surveillance on manufacturing plant personnel and monitoring studies please refer to the respective CONFIDENTIAL part (Document JCA).

CA 5.9.2 Data collected on humans

No detailed cases of overexposures or intoxications with ethephon have been reported in literature.

CA 5.9.3 Direct observations

No poisoning cases have come to the attention of Bayer CropScience.

A preliminary study in two volunteers and three human volunteer studies have been conducted at the same laboratory in the 1970s.

These studies have been evaluated during the EU process for Annex I listing and have been considered the key studies for setting the reference values in various countries.

Table 5.9.3-1 Human studies

Study	NOAEL/NOEL (mg/kg bw/day)	LOAEL (mg/kg bw/day)	Effects
Preliminary dose range study in two human volunteers. ~0.05 to 1.33 mg/kg bw/day [redacted]; 1971 M-187795-01-1			Sighting study.: no firm conclusions about the potential toxicity of ethephon
28-day oral study with human volunteers (8 ♂ and 2 ♀) 1.49 mg/kg bw/day in ♂ and 2.17 mg/kg bw/day in ♀ [redacted]; 1972 M-187790-01-1	Not determined		No significant inhibitory effect on human plasma or erythrocyte cholinesterase activity was observed. Subjective complaints of urinary urgency, sudden onset of diarrhea, effect on appetite and dyspepsia were recorded. Based on clinical symptoms a NOEL was not determined.
22-day oral study with human volunteers (10 ♂ and 10 ♀) 0, 0.17 or 0.33 mg/kg bw/day in both sexes [redacted];1977 M-187792-01-1	0.33	/	Plasma cholinesterase activity was inhibited and did not recover within the recovery period of 14 days. The NOAEL was 0.33 mg/kg bw/day in both males and females based upon the lack of inhibition of erythrocyte cholinesterase
16-day oral study with human volunteers (16 ♂ and 14 ♀) 0, 0.5 mg/kg bw/day in both sexes [redacted], 1977 M-187794-01-1	0.5	/	Plasma cholinesterase activity was inhibited and recovered within the recovery period of 29 days. The NOAEL of 0.5 mg/kg bw/day was established based upon the lack of inhibition of erythrocyte cholinesterase.

All these studies showed similarities in experimental design and included investigation of hematology, clinical chemistry (including measurement of cholinesterase activity in plasma and erythrocytes) and urinary parameters. Dosing was performed three times/day to simulate ingestion of ethephon as a residue in food. In the three main studies the dosing period lasted a working day (which suggests that the time between the first and last dose would have been at most about 8h).

However these studies present a series of limitations regarding their quality, due to the poor reporting of the volunteer data. In addition, there was also some lack of consistency between the cholinesterase findings from different studies conducted at the same laboratory. This could be related to the lack of information on the time of blood sampling which makes it difficult to judge if blood sampling coincided with peak cholinesterase inhibition or to know the number of doses received per day before blood was sampled.

In a sighting study (M-187795-01-1), two healthy male volunteers (90 and 102 kg) initially were given ethephon at 5.4 mg per day, increasing to 120 mg per day over the 44-day exposure period which was preceded by a 3-day pre-dose period and was followed by an 18-day post-exposure period. Overall doses were ranged from 0.06 to 1.33 mg ethephon/kg bw/day for one volunteer and 0.05 to 1.18 mg ethephon/kg bw/day for the other volunteer. Cholinesterase activity was measured by the Michel method approximately every 1 to 5 days during the exposure period and at the end of the post-exposure period. There was no suggestion of a toxicologically significant effect on plasma or red blood cell cholinesterase activity at any time during the study apart from on day 15 when both individuals showed a 23-24% reduction in plasma cholinesterase activity compared with the mean pre-dose value.

A total 16 adult human volunteers (8 male, 8 female) took part to the second study (M-187790-01-1). The test material was described as ethephon 10% w/w in silica and cornstarch. Of the total number, 6 volunteers (3 male and 3 female) were randomly assigned to the control group and 10 volunteers (5 male and 5 female) to the treatment group. Dosing was based on 1.24 mg/day. However, when the amount ingested was calculated on body weight of individual subjects, the dose averaged 1.83 mg/kg bw/day overall, with average dose in males of 1.49 mg/kg bw/day and average dose in females of 2.17 mg/kg bw/day. There was a 7-day pre-dose period followed by a 28 day dose period. Final evaluations were made approximately 2 weeks after the last dose was ingested.

The treatment was administered in a gelatin capsule in 3 divided daily doses, one after breakfast, one after lunch, and one at the end of the work day.

There were no persistent side-effects, but there were transient subjective complaints of urinary urgency, sudden onset of diarrhea, effect on appetite and dispepsia.
There were no treatment effects on hematology, clinical chemistry, or urinalysis. There were no effects on either plasma or red blood cell cholinesterase activity in any individual during the study. Values in individuals ranged from approximately 90 to 130% of pretreatment levels throughout the study for both control and treatment groups. However, based on the transient subjective complaints, and the lack of other dose levels, no NOEL was set in this study.

A total of 20 adult human volunteers (10 male, 10 female) participated to the third study (M-187792-01-1). Of the total number 6 volunteers (3 male, 3 female) were randomly assigned to the control group and 14 volunteers (7 male, 7 female) to two treatment groups. Treatment groups were 0.17 mg/kg bw/day (3 male, 4 female) and 0.33 mg/kg bw/day (4 male, 3 female). All volunteers received a placebo (pure cornstarch) over a 21 day pre-dosage period and over a recovery period of 14 days. During the dosage period (15 days), the control group continued to receive the placebo, while the two treatment groups received the carrier plus ethephon.

Test material or placebo was given in 3 daily divided doses, taken after breakfast, after lunch, and at the end of the workday. Doses were administered in gelatin capsules.

There were no effects of treatment on hematologic parameters, clinical biochemistry, or urinary output. There were no reports of clinical symptoms associated with either dose level.
Based on average values for the group, plasma cholinesterase activity was significantly reduced in the control group on day 14 of the recovery period only and in both treatment groups at all evaluations during the dosage and recovery periods. There were no statistical differences in red blood cell cholinesterase at any time in any group.
The NOAEL was equivalent to 0.33 mg/kg bw/day.

A total of 30 adult human volunteers (16 male, 14 female) took part to the fourth study (M-187794-01-1). Of the total number, 10 volunteers (6 male, 4 female) were randomly assigned to the control group and 20 volunteers (10 male, 10 female) to the treatment group. The treatment group received 0.5 mg/kg bw/day of ethephon. All volunteers received a placebo (pure cornstarch) over a 6-day pre-dosage period and over a recovery period of 29 days. During the dosage period (10 days), the control group continued to receive the placebo, while the two treatment groups received the carrier plus ethephon.
Test material or placebo was given in 3 daily divided doses, taken after breakfast, after lunch, and at the end of the workday. Doses were administered in gelatin capsules. There were no effects of treatment on hematological parameters, clinical biochemistry, or urinalysis. No clinical symptoms were reported. Both control and treatment groups showed statistically significant changes in both plasma and RBC cholinesterase activity throughout the dosing and recovery periods.

In these volunteer studies with ethephon, the overall NOAEL was 0.5 mg ethephon/kg bw/day based on the clinical symptoms recorded at 1.49 mg ethephon/kg bw and above.

CA 5.9.4 Epidemiological studies

With the exception of the human volunteers studies described under section CA 5.9.3 there are no additional epidemiological data.

CA 5.9.5 Diagnosis of poisoning (determination of active substance, metabolites), specific signs of poisoning, clinical tests

No cases of human poisoning have been reported.

Animal experiments revealed an inhibition of plasma and red blood cell cholinesterases, whereas the brain cholinesterase was not affected. Symptoms of poisoning were not observed.

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 of water and soap, if available with polyethylenglykol 300 followed by water.
  Note: Most formulations with this active ingredient can be decontaminated with water (and soap), so for formulations polyethyleneglykol 300 is not required.
- Flushing of the eyes with lukewarm water for 15 minutes
- Induction of vomiting is forbidden due to the caustic effect.

Treatment:

- Gastric lavage should be considered in cases of significant ingestions within the first (2) hour(s
- The application of activated charcoal and sodium sulphate (or other carthartic) may be considered in significant ingestions.
- As there is no antidote, treatment has to be symptomatic and supportive.
- After oral ingestion of undiluted ethephon, treatment must follow the regimens for acid ingestion.

CA 5.9.7 Expected effects of poisoning

No persisting effects of acute poisoning are to be expected.