ETHEPHON

First draft prepared by
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Explanation

Ethephon is the International Organization for Standardization (ISO)–approved common name for 2-chloroethylphosphonic acid (International Union of Pure and Applied Chemistry), which has the Chemical Abstracts Service number 16672-87-0. Ethephon is a plant growth regulator that acts by release of ethylene, directly influencing several physiological processes, such as ripening and maturation, and stimulating the production of endogenous ethylene. Ethephon is used on a variety of crops, including fruits, vegetables, cereals and oilseed crops.

Ethephon was previously evaluated for toxicology by the Joint FAO/WHO Meeting on Pesticide Residues (JMPR) in 1977, 1978, 1993, 1995, 1997 and 2002. In 1993, the Meeting established an acceptable daily intake (ADI) of 0–0.05 mg/kg body weight (bw) on the basis of a no-observed-adverse-effect level (NOAEL) of 0.5 mg/kg bw per day in studies in humans given repeated ethephon doses and application of a 10-fold safety factor. In 2002, the Meeting established an acute reference dose (ARfD) of 0.05 mg/kg bw on the basis of human data.

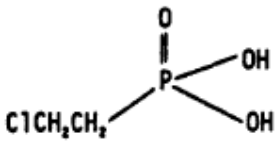
Ethephon was re-evaluated by the present Meeting as part of the periodic review programme of the Codex Committee on Pesticide Residues. Both new toxicity studies with ethephon in dogs and with the ethephon metabolite 2-hydroxyethyl phosphonic acid (or 2-hydroxyethephon; HEPA) in rats and previously submitted studies were considered by the present Meeting.

Some of the critical studies do not comply with good laboratory practice (GLP), as the data were generated before the implementation of GLP regulations. Overall, however, the Meeting considered that the database was adequate for the risk assessment.

Available studies with ethephon in humans were performed in accordance with the ethical standards at the time and were compliant with the Declaration of Helsinki.

The chemical structure of ethephon is shown in Fig. 1.

Fig. 1. Structure of ethephon



Evaluation for acceptable intake

1. Biochemical aspects

1.1 Absorption, distribution and excretion

Rats

The absorption, distribution and excretion of [U-^{14}C-ethyl]ethephon (radiochemical purity > 96%; batch no. CFQ.5611) dissolved in physiological saline adjusted to pH 3–4 using lactic acid were studied in groups of five male and five female Crl:CD(SD)BR rats. The experimental procedures are presented in Table 1.

At termination, blood and bone marrow, fat, heart, lungs, skeletal muscle, testes/ovaries, uterus, bone (femur), brain, kidney, liver, spleen and residual carcass were collected from the animals of groups A–D. Expired radioactivity was captured by a mercuric perchlorate and 2-ethoxyethanol:ethanolamine-containing trapping system. Bile was not collected. Clinical signs were observed daily, and body weights were measured at the start and termination of the study. The identification of ethephon and its metabolites in tissues and excreta is described in section 1.2 (Savage, 1990).

Recovery was 84–91%. At 120 hours after a single intravenous dose of 50 mg/kg bw, 67–71%, 0.9–1.7% and 8.9–10.8% of the radioactivity were recovered from urine, faeces and expired air, respectively. At 120 hours after a single oral dose of 50 and 1000 mg/kg bw or after an oral dose of 50 mg/kg bw following 14 oral daily pretreatments with unlabelled ethephon at 50 mg/kg bw, 47–60%, 4.0–6.5% and 18–22% of the radioactivity were recovered from urine, faeces and expired air, respectively. In all studies, radioactivity in expired air was mainly excreted as ethylene (96–98%) and a small fraction (2–4%) as carbon dioxide. Within 24 hours, excretion in urine and through expiration was 70–79% after a dose of 50 mg/kg bw and 62–68% after a dose of 1000 mg/kg bw, indicating that excretion occurred predominantly within the first 24 hours after dose administration. There were no remarkable differences in absorption and excretion between sexes and oral dosing regimens. Tissues and carcass contained only 0.05–0.06% (studies A and B), 0.4–0.5% (study C) or 0.01% (study D) of the administered radioactivity. Highest concentrations were found in bone, liver, blood and kidney. Radioactivity concentrations in brain and bone marrow were low. After single oral doses of 50 and 1000 mg/kg bw, peak blood concentrations were reached after 1.0–1.3 and 1.9–2.5 hours, respectively. Peak blood concentrations at 1000 mg/kg bw (63–66 mg equivalents/kg) were less than

Table 1. Study designs for absorption, distribution and excretion investigations in rats

Study	No. of animals of each sex	Treatment	Sampling times (hours after last dose)	Termination (hours after last dose)
A	5	Single intravenous dose at 50 mg/kg bw [U-^{14}C-ethyl]ethephon	0–4, 4–8, 8–12, 12–16, 16–24, 24–48, 48–72, 72–96 and 96–120: urine, faeces and cage debris 0–4, 4–8, 8–12, 12–16, 16–24, 24–36, 36–48, 48–72, 72–96 and 96–120: volatiles and CO_2 in expired air 120: cage wash 120: blood, selected organs and tissues	120
B	5	Single oral dose at 50 mg/kg bw [U-^{14}C-ethyl]ethephon	See study A	120
C	5	14 daily oral pretreatments of unlabelled ethephon (50 mg/kg bw per day, purity 96.1%) followed by one oral dose of 50 mg/kg bw [U-^{14}C-ethyl]ethephon	See study A	120
D	5	Single oral dose at 1 000 mg/kg bw [U-^{14}C-ethyl]ethephon	See study A	120
E	5	Single oral dose at 50 mg/kg bw [U-^{14}C-ethyl]ethephon	0.5, 1, 2, 3, 4, 6, 12, 24, 48, 72, 96, 120, 144 and 168: blood	168
F	5	Single oral dose at 1 000 mg/kg bw [U-^{14}C-ethyl]ethephon	0.5, 1, 2, 3, 4, 6, 12, 24, 48, 72, 96, 120, 144 and 168: blood	168

Source: Savage (1990)

proportional to dose, compared with peak concentrations at 50 mg/kg bw (10 mg equivalents/kg) (Savage, 1990).

Dogs

The absorption, distribution and excretion of [^{14}C]ethephon (radiochemical purity and batch number unknown) dissolved in a methanolic solution were studied in three male Beagle dogs dosed orally by gavage at a single dose of 180 mg/kg bw. Ethylene and carbon dioxide were collected from the expired air by a carbon dioxide and ethylene trapping system. Blood samples were collected at 0.5, 1, 2, 3, 4, 6, 8, 12, 24 and 48 hours after dosing. Urine and faeces were collected at intervals of 0–24, 24–48 and 48–72 hours. Blood samples were also used for the determination of erythrocyte and plasma cholinesterase levels. The animals were killed 72 hours after dosing, and radioactivity levels in brain, heart, lungs, liver, kidneys, spleen, small intestine, large intestine, stomach, testes, spinal cord, muscle and fat were determined.

Two of the three dogs vomited 15–30 minutes after dosing; thus, the actual dose received is unknown. About 35% of the dose appeared in the urine of two dogs within 24 hours after dosing. The radioactivity in urine dropped within 72 hours to levels below 1%. In one dog receiving the full dose,

4%, 0.1% and 0.03% of the dose were found in faeces at 24, 48 and 72 hours after dosing, respectively. In this dog, 30% of the dose was recovered as ethylene in the expired air, and only traces of carbon dioxide were recovered. At sacrifice, total selected organs retained a maximum of 0.25% of the administered dose. Peak plasma and red blood cell concentrations were observed 2 hours after dosing. Only traces were observed after 22 hours. Highest radioactivity concentrations were observed in liver, kidneys and spleen. Plasma cholinesterase levels were reduced at 2 hours, with recovery starting within a few hours. Erythrocyte cholinesterase levels responded more slowly, with few signs of recovery over the 72-hour period. In view of the uncertainties regarding the doses received, this study is considered to be of limited value (Stephen & Walker, 1971).

1.2 Biotransformation

Rats

The metabolism of [U-^{14}C-ethyl]ethephon (radiochemical purity > 96%; batch no. CFQ.5611) was studied in groups of five male and five female Crl:CD(SD)BR rats dosed intravenously with a single dose of 50 mg/kg bw or orally by gavage at a single dose of 50 or 1000 mg/kg bw or a single oral dose of 50 mg/kg bw after 14 daily pretreatments with unlabelled ethephon at the same dose. The study design and toxicokinetics are described in section 1.1 (Savage, 1990). Identification of metabolites in urine and faeces was performed using thin-layer chromatography (TLC) followed by liquid chromatography/mass spectrometry and nuclear magnetic resonance.

In urine and faeces, 10 regions of radioactivity were discernible from the autoradiograms. These could be grouped into three sets of closely migrating regions that were not fully resolved and a separate region. The fraction containing the disodium salt of ethephon constituted the major component in urine and faeces (about 64% and 3% of the administered radioactivity, respectively, in study A and about 38–47% and 2–8% of the administered radioactivity, respectively, in studies B, C and D). Up to 6% of the total radioactivity (urine and faeces combined) represented the monosodium salt of ethephon. The toxicokinetics data (Savage, 1990; see section 1.1) showed that about 10% (study A) and 18–22% (studies B, C and D) of the dose were excreted as ethylene in expired air, with small amounts exhaled as carbon dioxide (Hardy et al., 1990; Savage, 1990).

The metabolism of [U-^{14}C]ethephon (radiochemical purity 99.7%; batch no. CFQ12839) in dissolved saline was studied in groups of five male Sprague-Dawley CD rats dosed orally by gavage at a single dose of 50 mg/kg bw. At 1 hour after dosing, the animals were killed, and liver and kidney were collected. Identification of metabolites was performed using TLC and high-performance liquid chromatography.

Ethephon was the major radioactive component in the kidneys (87% of radioactivity recovered in kidney) and the liver (58% of radioactivity recovered in liver). The metabolite HEPA was also found in the kidneys (13% of radioactivity recovered in kidney) and liver (37% of radioactivity recovered in liver) (Odin-Feurtet, 2002).

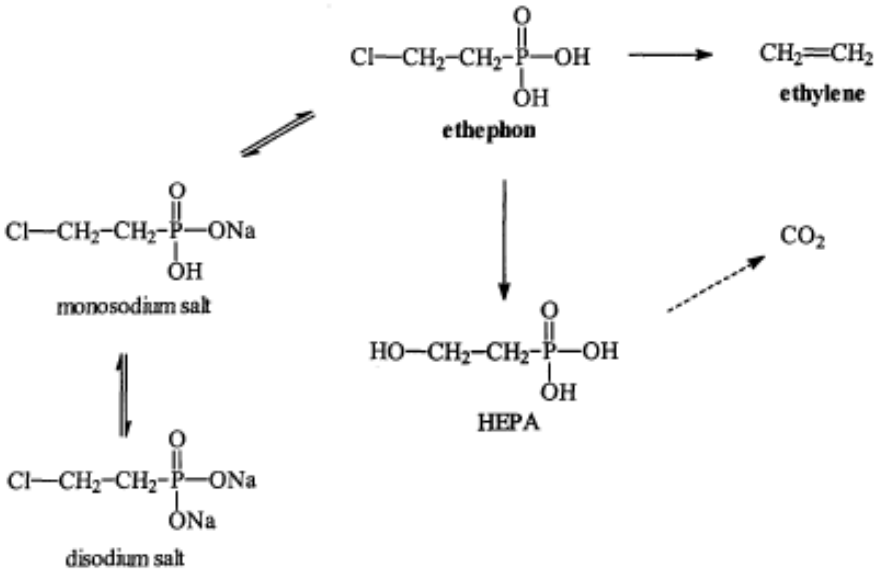
The proposed metabolic pathway of ethephon in rats is shown in Fig. 2.

Dogs

The metabolism of [^{14}C-ethyl]ethephon (radiochemical purity and batch number unknown) was studied in three male Beagle dogs dosed by gavage with a single dose of 180 mg/kg bw. The study design and toxicokinetics are described in section 1.1 (Stephen & Walker, 1971). Identification of metabolites in urine was performed using TLC and gas chromatography.

In the urine, unchanged ethephon was identified (Stephen & Stanovick, 1971).

Fig. 2. Proposed metabolic pathway of ethephon in rats



2. Toxicological studies

2.1 Acute toxicity

(a) Lethal doses

The results of studies of acute toxicity with ethephon are summarized in Table 2.

An acute oral toxicity study in rabbits (Weatherholtz, 1980) was not included, as the purity of the test material was not reported and only two females per dose were used.

(b) Dermal irritation

In an acute dermal irritation study, the intact skin of three male and three female New Zealand White rabbits was exposed for 1 or 4 hours under occlusion to 0.5 mL ethephon (purity 70%; batch no. RTC 2839AA; a slightly viscous liquid). Dermal irritation was scored at 24 and 72 hours after patch removal.

Contact with 0.5 mL of ethephon for 4 hours resulted in slight to well-defined erythema on 6/6 rabbits. Moderate to severe oedema was noted in 4/6 rabbits. Also evident on these four animals was necrosis, varying from one or two spots to a large area covering most of the dosed surface. Therefore, ethephon is considered to be corrosive. It is noted that the study was terminated after the 48-hour observation period. After 1 hour of exposure, less severe irritation was recorded. Five out of six rabbits had slight to well-defined erythema, but none developed oedema or necrosis (Myers, 1983).

(c) Ocular irritation

No studies were conducted, because the pH of ethephon is less than 2. Therefore, testing for eye irritation is not required.

Table 2. Results of studies of acute toxicity with ethephon

Species	Strain	Sex	Route	Vehicle	Purity (%)	LD_{50}/LC_{50}	Reference
Mouse	Carbia	M	Oral	Not reported	100	1 920 mg/kg bw[a]	Holsing (1969)[b]
Rat	Hilltop-Wistar	M + F	Oral	Water	70.75	2 639 mg/kg bw[a] (M) 1 564 mg/kg bw[a] (F)	Myers (1989)[c]
Rabbit	New Zealand White	M + F	Dermal	Water	70.75	1 210 mg/kg bw[a] (M) 983 mg/kg bw[a] (F)	Myers (1983)[d]
Rat	Sprague-Dawley	M + F	Inhalation	–	72.2	3.26 mg/L[a] (M/F)	Nachreiner & Klonne (1989)[e]

bw: body weight; F: female; LC_{50}: median lethal concentration; LD_{50}: median lethal dose; M: male

[a] Doses/concentrations are corrected for purity of the test material.

[b] At 2150 mg/kg bw, 2/5 mice died. At doses of 3160–10 000 mg/kg bw, all mice died. Depression, laboured respiration and prostrate appearance were noted at 2150, 3160 and 4640 mg/kg bw. At 6810 and 10 000 mg/kg bw, depression was noted 1 hour prior to mortality.

[c] The study design resembles Organisation for Economic Co-operation and Development (OECD) Test Guideline 401. The male rats were given a dose of 1738, 3475 or 6950 mg/kg bw, and the female rats were given a dose of 1738, 2460 or 3475 mg/kg bw. Signs of toxicity were observed at doses of 3475 mg/kg bw and higher in males and at doses of 2460 mg/kg bw and higher in females and included sluggishness, piloerection, emaciation (in one rat) and prostration. No mortality and no macroscopic changes at necropsy were observed at 1738 mg/kg bw. At higher doses, death occurred at 1 hour to 1 day. Survivors recovered at 1–3 days. At necropsy, the visceral surfaces of livers were mottled tan and brown, the glandular portions of stomachs were black and the lungs of two animals were red. Batch no. 47-20.

[d] The study design resembles OECD Test Guideline 402. All rabbits (five males and five females) at 2000 mg/kg bw and one male and two female rabbits at 1000 mg/kg bw died. Pinpoint pupils, salivation, unsteady gait and prostration were observed, followed by death at 1–3 days. Batch number was not reported.

[e] The study design resembles OECD Test Guideline 403. Rats were exposed (whole body) to actual ethephon concentrations of 6.12, 3.34 or 2.11 mg/L. At the highest concentration, all rats died. All rats displayed ocular and respiratory irritation and bright red extremities. Additional signs observed in the 6.12 and 3.34 mg/L groups included hypothermia (6.12 mg/L group only), tremors (6.12 mg/L group males only), a slow surface righting reflex and an absent tail pinch reflex. Depressed body weight gains were observed for some animals in the 3.34 mg/L group and for one female rat in the 2.11 mg/L group during the first week of the post-exposure period, but not during the second week after exposure. Treatment-related macroscopic lesions were found only in animals that died and included discoloration of the lungs, liver, salivary glands and thymic region, brain haemorrhage, and gaseous stomach and intestines. Mass mass median aerodynamic diameter ranged from 3.3 to 6.0 µm. The sample bore the reference number 4022193 and was assigned BRRC Sample No. 52-226.

(d) Dermal sensitization

In a dermal sensitization study using the Buehler test, performed in accordance with Organisation for Economic Co-operation and Development (OECD) Test Guideline 406, ethephon (purity 72.2%; batch no. 4022193) was tested in five male and five female Dunkin-Hartley guinea-pigs. The vehicle control group consisted of five males and five females. The dose levels were based on the results of a range-finding study in which two animals of each sex were treated topically with 1%, 5%, 10% or 25% weight per volume (w/v) of the test substance in distilled water. Slight patchy erythema was found after 24 hours at all test substance concentrations. In the induction phase, the animals received three topical inductions with 25% ethephon in distilled water once per week, followed by a topical challenge with 10% ethephon in distilled water after 2 weeks (6-hour exposure under occlusion). Dinitrochlorobenzene (DNCB) was used as a positive control.

Topical induction with ethephon at 25% w/v caused no dermal responses in any of the three inductions, with the exception of two animals that showed slight patchy erythema, one after the second induction and one after the third induction. Following challenge with 10% w/v, slight patchy erythema was noted after 24 hours in all animals of the treatment group and in 7/10 animals of the negative control group. After 48 hours, four animals of the treatment group and one animal of the negative control group showed slight patchy erythema. Sensitization of this strain was positively tested with DNCB. According to the reaction in the challenge phase, ethephon is not a skin sensitizer

(Rush, 1989). However, in view of the minimal reaction during the induction phase and the small number of animals in the test group, this study is considered to be of limited value.

In a dermal sensitization study using the Magnusson and Kligman maximization test, performed in accordance with OECD Test Guideline 406, ethephon (purity 72.4%; batch no. DA 588) was tested in 20 female Dunkin-Hartley guinea-pigs. The vehicle control group consisted of 10 males and 10 females. In the induction phase, the animals received intradermal injections of 0.75% ethephon followed by epidermal treatment with 50% ethephon on day 9 (48-hour exposure under occlusion). The challenge on day 22 was performed with epidermal application of 35% ethephon. DNCB was used as a positive control.

Equivocal macroscopic reactions were observed in 15/20 ethephon-treated animals. Histopathological examination of these lesions showed images of orthoergic irritation in nine animals. No reactions of cutaneous sensitization were observed in the 20 ethephon-treated guinea-pigs, but the lesions of orthoergic irritation noted in nine of them may have hidden possible reactions of cutaneous sensitization (Clement, 1989).

In a dermal sensitization study using the Magnusson and Kligman maximization test, performed in accordance with OECD Test Guideline 406, ethephon (purity 74.1%; batch no. 4120181) was tested in 10 male and 10 female Hartley Crl: (HA) BR guinea-pigs. The vehicle control group consisted of five males and five females. In the induction phase, the animals received intradermal injections of 0.5% ethephon followed by epidermal treatment with 50% ethephon on day 8 (48-hour exposure under occlusion). The challenge on day 22 was performed with epidermal application of 25% ethephon. In a contemporaneous study, the sensitivity of the experimental technique was demonstrated using mercaptobenzothiazole.

No clinical signs and no deaths related to treatment were noted during the study. After the challenge application, no cutaneous reactions were observed in the animals of the control group. In the treated group, at the 24-hour reading, a discrete erythema was noted in 5/20 animals. At the 48-hour reading, skin reactions faded, and discrete erythema (grade 1) persisted in 1/20 animals only. Dryness of the skin, which could have masked scoring of the cutaneous reactions in one animal, was observed at the 24- and 48-hour readings in 5/20 and 7/20 animals of the treated group, respectively. As the cutaneous reactions observed in the animals of the treated group were non-persistent and of low incidence and severity, they were attributed to the known irritating properties of the test substance, but not to delayed-contact hypersensitivity (Griffon, 2000).

2.2 Short-term studies of toxicity

(a) Oral administration

Mice

In a 28-day dietary study, ethephon (purity 71.3%; batch no. A51563) was administered to groups of 10 male and 10 female CD-1 mice at 0, 30, 100, 300, 1000 or 3000 parts per million (ppm) (equal to 0, 5.3, 18, 51, 181 and 546 mg/kg bw per day for males and 0, 6.5, 22, 69, 210 and 635 mg/kg bw per day for females, respectively). An additional five males and five females were included in the 0, 300 and 1000 ppm groups for cholinesterase activity determinations after 2 weeks of treatment. Mice were observed daily for mortality and clinical signs. Body weight and feed consumption were measured weekly. At day 14, blood was sampled from five rats of each sex (fasted overnight), after which the animals were killed and the brains were removed. Cholinesterase activity was determined in erythrocytes, plasma and brain. The same was done for the remaining animals on day 28. In addition, levels of aspartate aminotransferase (ASAT), sorbitol dehydrogenase, alkaline

Table 3. Cholinesterase activity in the erythrocytes, brain and plasma of mice treated with ethephon for 28 days

	% reduction compared with controls									
	30 ppm		100 ppm		300 ppm		1 000 ppm		3 000 ppm	
	M	F	M	F	M	F	M	F	M	F
Week 2										
Plasma ChE	–	–	–	–	18*	29*	43*	49*	–	–
Erythrocyte AChE	–	–	–	–	9	23*	29*	38*	–	–
Brain AChE	–	–	–	–	+	+	3	+	–	–
Week 4										
Plasma ChE	3	2	4	4	22*	23*	44*	46*	63*	55*
Erythrocyte AChE	3	+	6	5	18*	14*	34*	41*	62*	56*
Brain AChE	14	+	9	3	11	+	14	+	13	+

AChE: acetylcholinesterase; ChE: cholinesterase; F: females; M: males; ppm: parts per million; +: ChE activity was equivalent to or slightly greater than control values; –: not determined; *: $P < 0.05$ (Student's *t*-test)
Source: Van Miller & Troup (1986a)

phosphatase (ALP) and alanine aminotransferase (ALAT) were determined in blood. No haematology, ophthalmoscopy or histopathology were performed. At termination, the heart, lungs, liver, kidneys and spleen of each animal were weighed.

No effects of treatment on mortality, clinical signs, feed consumption, body weight, organ weights or clinical chemistry were observed.

The effects of ethephon on cholinesterase activity are presented in Table 3.

The NOAEL was 100 ppm (equal to 22 mg/kg bw per day), based on 23% reduction of erythrocyte acetylcholinesterase (AChE) activity observed in females at 300 ppm (equal to 69 mg/kg bw per day) (Van Miller & Troup, 1986a).

In a 28-day dietary study, ethephon (purity 71.3%; batch no. A51563) was administered to groups of 15 male and 15 female CD-1 mice at 0, 3000, 10 000, 25 000 or 50 000 ppm (equal to 0, 530, 1800, 4500 and 10 000 mg/kg bw per day for males and 0, 630, 2200, 5900 and 15 000 mg/kg bw per day for females, respectively). Mice were observed daily for mortality and clinical signs. Body weight and feed consumption were measured weekly. At day 14, blood was sampled from five rats of each sex (fasted overnight), after which the animals were killed and the brains were removed for analysis of brain cholinesterase activity. Cholinesterase activity was determined in erythrocytes, plasma and brain. No other clinical chemistry measurements, haematology, ophthalmoscopy or histopathology was performed. At termination, the heart, lungs, liver, kidneys and spleen of each animal were weighed.

No effects of treatment on mortality or clinical signs were observed. Feed consumption was significantly reduced (19–23%) for males and females during the first week of treatment at 50 000 ppm. Weight loss (15–16%) was observed for males and females at 50 000 ppm in week 1, followed by weight gains in subsequent treatment weeks. Compared with control mice, terminal body weight was lower in males (14%, statistically significant) and females (8%, not statistically significant). After 1 week of treatment, body weight at 25 000 ppm was slightly (6–9%), but statistically significantly, reduced compared with controls.

The effects of ethephon on cholinesterase activity are presented in Table 4.

Table 4. Cholinesterase activity in the erythrocytes, brain and plasma of mice treated with ethephon for 28 days

	% reduction compared with controls							
	3 000 ppm		10 000 ppm		25 000 ppm		50 000 ppm	
	M	F	M	F	M	F	M	F
Week 2								
Plasma ChE	55*	55*	54*	66*	77*	80*	79*	82*
Erythrocyte AChE	54*	62*	68*	80*	88*	88*	90*	92*
Brain AChE	6	1	4	+	4	8	13	8
Week 4								
Plasma ChE	55*	57*	60*	65*	71*	73*	77*	78*
Erythrocyte AChE	57*	62*	67*	76*	82*	82*	87*	89*
Brain AChE	9	6	12*	+	14*	13	19*	1

AChE: acetylcholinesterase; ChE: cholinesterase; F: females; M: males; ppm: parts per million; +: AChE activity was equivalent to or slightly greater than control values; *: $P < 0.05$ (Student's *t*-test)
Source: Van Miller & Troup (1986b)

A NOAEL could not be identified. The lowest-observed-adverse-effect level (LOAEL) was 3000 ppm (equal to 530 mg/kg bw per day), based on reductions in erythrocyte AChE activity (Van Miller & Troup, 1986b).

Rats

In a 2-week range-finding toxicity study (preliminary for the subchronic toxicity study), ethephon (purity 72.4%; batch no. A4051511) was administered to groups of six male and six female Sprague-Dawley Crl:CD(SD)BR rats by gavage at 0, 100, 300, 600 or 1000 mg/kg bw per day. Animals were checked daily for clinical signs of toxicity. A detailed physical examination was performed weekly. Body weights were measured twice per week, and feed consumption was measured weekly. Before the treatment was started and prior to dosing on days 2, 8 and 15, the rats were subjected to a functional observational battery, and blood was sampled. At termination on day 15, all animals were necropsied.

At 600 mg/kg bw per day, two males and four females were found dead. At 1000 mg/kg bw per day, all males and five females were found dead or were killed in a moribund condition. In most of the rats at 600 and 1000 mg/kg bw per day, fur staining, skin pallor, abnormal breathing, respiratory sounds, dehydration, cold to touch, decreased activity, weak appearance and abdominal distension were observed. In the lower-dose groups (i.e. 100 and 300 mg/kg bw per day), no treatment-related clinical signs were observed. Body weight loss and reduced feed consumption were observed at 600 and 1000 mg/kg bw per day.

In the functional observational battery, abnormal breathing, myosis, muzzle staining, diarrhoea and impaired gait were observed in a few rats at 300 mg/kg bw per day and above. Erythrocyte AChE levels were not affected by treatment. Dose-dependent decreases in plasma cholinesterase levels were observed in all treatment groups (Beyrouty, 1997a).

In a 28-day dietary range-finding study, ethephon (purity 71.3%; batch no. A51563) was administered to groups of 10 male and 10 female Sprague-Dawley CD rats at 0, 625, 1250, 2500,

Table 5. Cholinesterase activity in the erythrocytes, brain and plasma of rats treated with ethephon for 28 days

	% reduction compared with controls									
	625 ppm		1 250 ppm		2 500 ppm		5 000 ppm		10 000 ppm	
	M	F	M	F	M	F	M	F	M	F
Week 2										
Plasma ChE	ND	ND	21*	44	18*	45	ND	ND	ND	ND
Erythrocyte AChE	ND	ND	16	14*	36*	32*	ND	ND	ND	ND
Brain AChE	ND	ND	7	+	9	+	ND	ND	ND	ND
Week 4										
Plasma ChE	13*	29*	27*	50*	16*	50*	30*	49*	35*	63*
Erythrocyte AChE	9*	19*	22*	35*	41*	50*	58*	67*	73*	78*
Brain AChE	+	1	+	4	+	1	13	4	1	4

AChE: acetylcholinesterase; ChE: cholinesterase; F: females; M: males; ND: not determined; ppm: parts per million; +: AChE activity was equivalent to or slightly greater than control values; *: $P < 0.05$ (Student's *t*-test)
Source: Van Miller & Troup (1986c)

5000 or 10 000 ppm (equal to 0, 52, 106, 214, 431 and 831 mg/kg bw per day for males and 0, 59, 120, 251, 487 and 980 mg/kg bw per day for females, respectively). An additional five males and five females were included in the 0, 1250 and 2500 ppm groups for cholinesterase activity determinations after 2 weeks of treatment. The rats were observed daily for mortality and clinical signs. Body weight and feed consumption were measured weekly. At day 14, blood was sampled from five rats of each sex (fasted overnight), after which the animals were killed and the brains were removed. Cholinesterase activity was determined in erythrocytes, plasma and brain. The same was done for the remaining animals on day 28. In addition, haematology was performed, and levels of ASAT, ALAT, sorbitol dehydrogenase and ALP were determined in blood. At termination, the heart, lungs, liver, kidneys and spleen of each animal were weighed. No ophthalmoscopy or histopathology was performed.

No effects of treatment on mortality, clinical signs, feed consumption, haematology or clinical chemistry were observed. Slight reductions (up to 9%) in net body weight gains in males at 2500 and 10 000 ppm were considered to be not toxicologically relevant. Organ weights were not affected by treatment. The effects of ethephon on cholinesterase activity are presented in Table 5.

The NOAEL was 625 ppm (equal to 52 mg/kg bw per day), based on 22% reduction of erythrocyte AChE observed in males at 1250 ppm (equal to 106 mg/kg bw per day) (Van Miller & Troup, 1986c).

In a 28-day dietary range-finding toxicity study, ethephon (purity 71.3%; batch no. A51563) was administered to groups of 15 male and 15 female Sprague-Dawley CD rats at 0, 10 000, 25 000 or 50 000 ppm (equal to 0, 962, 2300 and 4673 mg/kg bw per day for males and 0, 996, 2488 and 4900 mg/kg bw per day for females, respectively). Groups of 14 male and 14 female rats received basal diet. Animals were checked daily for clinical signs of toxicity. Body weights and feed consumption were measured weekly. At day 14, blood was sampled from five rats of each sex, after which the animals were killed and the brains were removed. Cholinesterase activity was determined in erythrocytes, plasma and brain. The same was done for the remaining animals on day 28. No other clinical chemistry measurements, haematology, ophthalmoscopy or histopathology was performed. At termination, the heart, lungs, liver, kidneys and spleen of each animal were weighed.

Table 6. Cholinesterase activity in the erythrocytes, brain and plasma of rats treated with ethephon for 28 days

	% reduction compared with controls					
	10 000 ppm		25 000 ppm		50 000 ppm	
	M	F	M	F	M	F
Week 2						
Plasma ChE	23*	66	31*	66*	42*	74*
Erythrocyte AChE	69*	72*	84*	82*	91*	91*
Brain AChE	11	4	4	2	15*	13*
Week 4						
Plasma ChE	27*	46*	34*	58*	45*	61*
Erythrocyte AChE	72*	73*	82*	80*	91*	89*
Brain AChE	8	7	+	3	14	3

AChE: acetylcholinesterase; ChE: cholinesterase; F: females; M: males; ppm: parts per million; +: AChE activity was greater than control values; *: $P < 0.05$ (Student's *t*-test)
Source: Van Miller & Troup (1986d)

No mortality was observed. Loose faeces were observed in males and females at 50 000 ppm from day 10 onward. Feed consumption was statistically significantly reduced for males at 25 000 and 50 000 ppm in weeks 1 (10–24%) and 2 (7–8%), for females at 10 000 ppm in weeks 3 and 4 (10–11%), for females at 25 000 ppm in weeks 1, 3 and 4 (8–11%) and for females at 50 000 ppm throughout treatment (9–24%). Statistically significantly lower body weights compared with controls were recorded for males and females at 50 000 ppm throughout treatment, leading to total weight gain deficits relative to controls of approximately 27% and 34% for males and females, respectively. Statistically significant total weight gain deficits were approximately 15% and 18% for males and females, respectively, at 25 000 ppm and approximately 18% for females at 10 000 ppm. No effect on body weight development was noted for males at 10 000 ppm. Observed changes in organ weights at doses of 10 000 ppm or higher were attributed to differences in terminal body weights. The effects of ethephon on cholinesterase activity are presented in Table 6.

The LOAEL was 10 000 ppm (equal to 962 mg/kg bw per day), based on reduction of erythrocyte AChE activity in both sexes (Van Miller & Troup, 1986d).

Dogs

In a 1-year study, five male and five female Beagle dogs per dose group received ethephon (purity 71.1%; lot no. A62833) at a dietary concentration of 0, 100, 300, 1000 or 2000 ppm (equal to 0, 2.8, 8.1, 27 and 54 mg/kg bw per day for males and 0, 2.6, 8.4, 30 and 50 mg/kg bw per day for females, respectively) for 52 weeks. All animals were checked daily for mortality, moribundity and clinical signs. Feed consumption and body weights were measured weekly. Daily water consumption was measured at weeks 12, 25 and 51 (males) or 52 (females). Haematology, clinical chemistry and urine analysis were performed pretreatment (week −2) and at weeks 13, 26 and 52. Ophthalmology was tested pretreatment and at week 52. All dogs were necropsied, and weights of adrenals, brain, testes with epididymides/ovaries, heart, kidneys, liver with gallbladder drained, spleen, thyroid with parathyroid, and pituitary were recorded. Macroscopic and histopathological examinations were performed in all groups at termination. Reduction of erythrocyte, plasma and brain cholinesterase activities was not measured in this study.

There were no treatment-related effects on mortalities. The incidences of soft and/or mucoid stools were higher in both sexes in all treatment groups than in the controls, but the increases were not dose dependent, indicating that soft and/or mucoid stools are not treatment related. Frothy emesis, ataxia, tremors, head tilt and temporal high body temperature were observed from week 41 in one female at 1000 ppm without major abnormality in a neurological evaluation and X-ray examination. These symptoms, except for tremors, recovered essentially by termination after medication with sodium pentobarbital, suggesting that they were not caused by inhibition of cholinesterase activity. In the treated groups, mean weekly body weight gains in both sexes and terminal body weights in females did not show statistically significant changes compared with the controls. Statistically significantly lower terminal body weight in males in the 2000 ppm group (11.4 kg in the controls versus 9.3 kg in the 2000 ppm group) and slight decreases in mean body weight gain for 0–52 weeks at 2000 ppm in both sexes (males, 2.1 kg in the controls versus 1.7 kg in the 2000 ppm group; females, 1.3 kg in the controls versus 0.84 kg in the 2000 ppm group) were considered to be treatment related. There were no significant treatment-related effects on feed and water consumption, haematology, clinical chemistry, urine analysis or ophthalmological parameters. At 2000 ppm, spleen weight was decreased in both sexes. The statistically significantly lower absolute and relative (to body weight) spleen weights in males in the 2000 ppm group were considered to be treatment related (29.6 g and 0.26 g in the controls versus 16.5 g and 0.18 g in the 2000 ppm group for absolute and relative weights, respectively). In females of the 2000 ppm group, relative spleen weight was lower than the control value, with statistical significance (0.28 g in the controls versus 0.21 g in the 2000 ppm group). However, this finding was due to one female losing body weight and was considered not to be treatment related. As for other organ weights, statistically significant decreases in absolute heart and thyroid/parathyroid weights in males in the 2000 ppm group were caused by a statistically significant decrease in terminal body weight and were not a direct effect of treatment. In females at 2000 ppm, a statistically significant increase in kidney weight relative to brain weight was caused by two animals showing low relative brain weight and high relative kidney weight accompanied by slight mineralization and tubular regeneration, respectively, which are not related to the treatment. There were no treatment-related macroscopic or histopathological findings in any organs, including the spleen, in all treated groups.

The NOAEL was 1000 ppm (equal to 27 mg/kg bw per day), based on a lower body weight gain at 52 weeks in both sexes and low absolute and relative spleen weights in males at 2000 ppm (equal to 54 mg/kg bw per day) (Hamada, 1989).

In a 2-year study, six male and six female Beagle dogs per dose group received ethephon (purity 75.6%; batch no. AL 1030-42; for Source A) at a dietary concentration of 0, 30, 300 or 3000/2000/1000/1500 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) for 104 weeks. In the high-dose group, the dose was 3000 ppm in the first 3 weeks and was then changed to 2000 ppm (weeks 4–5), then to 1000 ppm (weeks 6–24) and finally to 1500 ppm (from week 25) owing to the persistent decrease in body weight gain in the first 3 weeks. In this study, another source of ethephon (purity 73.6%; batch no. AL-3096; for Source B) at a dietary concentration of 300 ppm (equal to 5.9 and 6.3 mg/kg bw per day for males and females, respectively) was administered to six dogs of each 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 4 weeks and every 4 weeks after week 5. Haematology and clinical chemistry, including plasma and erythrocyte cholinesterase activities, were performed in 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 dietary level for 10 days before 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 cholinesterase activity of ethephon was measured at termination. This study was not conducted in compliance with GLP.

There were no treatment-related effects on mortality. In the high-dose group, a high incidence of soft stools was recorded in the first 4 weeks (at 3000 or 2000 ppm) and observed persistently in

males or sporadically in females during the study. A high incidence of intermittent emesis was also observed in both sexes in the high-dose group. There were no statistically significant and treatment-related effects on body weight from week 25 or feed consumption in all treated groups. For haematology, clinical chemistry and absolute and relative organ weights, no significant treatment-related changes were observed in either sex in any treated group.

The effects of ethephon treatment on cholinesterase activity are presented in Table 7. From week 6, erythrocyte AChE activity was statistically significantly reduced by more than 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 in either sex at any dose.

Morphologically, smooth muscle hypertrophy in the duodenum was observed in two and three females at 300 ppm and 1500 ppm, respectively. In addition to the duodenal lesion, each female at 300 ppm had smooth muscle hypertrophy in the stomach or in both jejunum and ileum, respectively. One of three females bearing a duodenal lesion at 1500 ppm also had smooth muscle hypertrophy in the stomach and jejunum. In males, smooth muscle hypertrophy of the duodenum was observed in one animal at 1500 ppm. However, the lesion was not observed in other parts of the small intestine in the same animal or other treated groups of males. In the stomach and small intestine, other findings, such as chronic gastritis and congestion in the duodenum, were noted at 300 and 1500 ppm in males and/or females. However, the affected animals were different from the ones showing smooth muscle hypertrophy, indicating that the smooth muscle hypertrophy was not related to the observed gastrointestinal lesions. It is reported that smooth muscle hypertrophy in the intestine is caused by obstruction, diverticulum, inflammation or infection or may occur spontaneously in animals (Bettini et al., 2003; Murakami et al., 2010; Liu et al., 2014). Although the cause of smooth muscle hypertrophy in the stomach and small intestine could not be determined in this 2-year study, the increased incidence of the smooth muscle hypertrophy is considered to be treatment related. However, the biological significance of this effect is unclear, and it is not considered to be toxicologically adverse. There were no other treatment-related findings in other examined organs.

The NOAEL was 30 ppm (equal to 0.86 mg/kg bw per day), based on greater than 20% reduction of erythrocyte AChE activity in both sexes at 300 ppm (equal to 7.6 mg/kg bw per day) (Reno & Voelker, 1977).

(b) Dermal application

Rabbits

A 21-day toxicity test was carried out with dermal application of ethephon (purity 39.5%; batch number unknown) in rabbits. Groups of 5–10 male and female adult New Zealand White rabbits received ethephon at a dose of 0, 119 or 237 mg/kg bw per day (doses corrected for purity), 5 days/week, for 3 weeks, on the abdominal skin clipped free of hair. After 6–8 hours of exposure, the abdomen was washed with water. The general appearance, behaviour, body weight, clinical chemistry, signs of dermal irritation, gross pathology and histopathology were studied.

No systemic toxicity of ethephon was demonstrated at the two dose levels, apart from a severe dermal irritation characterized by subepidermal fibrosis, acanthosis, hyperkeratosis and ulceration of the epidermis (Holsing, 1969).

In a 21-day dermal toxicity test, ethephon (purity 72.2%; batch no. 4022193) was applied to the skin of groups of 10 male and 10 female Hra:(NZW)SPF rabbits at an ethephon dose of 0, 18, 53 or 107 mg/kg bw per day (corrected for purity), 5 days/week, for 3 weeks. After at least 6 hours of exposure, the abdomen was washed with water. Dermal irritation was scored immediately before each

Table 7. Cholinesterase activity in the erythrocytes, brain and plasma of dogs treated with ethephon for 2 years

	% reduction compared with controls					
Dietary concentration (ppm)	**Week 6**	**Week 13**	**Week 26**	**Week 52**	**Week 78**	**Week 104**
Erythrocyte AChE						
Males						
30 ppm (Source A)	−9	−8	−5	−10	−10	−11
300 ppm (Source A)	−47*	−48*	−42*	−46*	−56*	−46*
1 500 ppm (Source A)	−79*	−70*	−68*	−71*	−79*	−73*
300 ppm (Source B)	−54*	−56*	−48*	−55*	−53*	−47*
Females						
30 ppm (Source A)	−9	2	−8	0	10	−13
300 ppm (Source A)	−54*	−53*	−50*	−48*	−47*	−56*
1 500 ppm (Source A)	−79*	−70*	−71*	−59*	−59*	−74*
300 ppm (Source B)	−45*	−53*	−39*	−52*	−36*	−49*
Brain AChE						
Males						
30 ppm (Source A)	ND	ND	ND	ND	ND	53
300 ppm (Source A)	ND	ND	ND	ND	ND	42
1 500 ppm (Source A)	ND	ND	ND	ND	ND	20
300 ppm (Source B)	ND	ND	ND	ND	ND	40
Females						
30 ppm (Source A)	ND	ND	ND	ND	ND	39
300 ppm (Source A)	ND	ND	ND	ND	ND	9
1 500 ppm (Source A)	ND	ND	ND	ND	ND	17
300 ppm (Source B)	ND	ND	ND	ND	ND	4
Plasma ChE						
Males						
30 ppm (Source A)	−24*	−22*	−26*	−22*	−31*	−31*
300 ppm (Source A)	−48*	−46*	−51*	−48*	−62*	−56*
1 500 ppm (Source A)	−54*	−45*	−54*	−53*	−67*	−63*
300 ppm (Source B)	−52*	−46*	−51*	−51*	−59*	−57*
Females						
30 ppm (Source A)	−34*	−24*	−38*	−31*	−29*	−30*
300 ppm (Source A)	−52*	−51*	−57*	−53*	−58*	−58*
1 500 ppm (Source A)	−61*	−59*	−63*	−65*	−61*	−66*
300 ppm (Source B)	−53*	−55*	−61*	−57*	−54*	−58*

AChE: acetylcholinesterase; ChE: cholinesterase; ND: not determined; ppm: parts per million; *: $P < 0.05$ (Scheffe's method)
Source: Reno & Voelker (1977)

application (except day 0) and on the day of necropsy. The animals were checked daily for mortality and clinical signs. Body weights and feed consumption were recorded weekly. Haematology and clinical chemistry parameters were evaluated on the day prior to termination. All animals were examined macroscopically, and selected organs were weighed. Selected tissues from all animals in the 0 and 107 mg/kg bw per day groups were examined microscopically.

There were no treatment-related effects on behaviour, body weight, feed consumption, haematology, clinical chemistry or organ weights. In the low- and mid-dose groups, slight to moderate erythema and desquamation, slight oedema and slight fissuring in some of the animals were observed. In the high-dose group, there was slight to moderate erythema, slight to moderate oedema, slight to moderate desquamation and slight to moderate fissuring. Microscopic examination revealed acanthosis and chronic active inflammation of the skin in high-dose animals.

The NOAEL for systemic toxicity was 107 mg/kg bw per day, the highest dose tested (Henwood, 1989a).

2.3 Long-term studies of toxicity and carcinogenicity

Mice

In a 78-week dietary carcinogenicity study performed according to OECD Test Guideline 451, 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, respectively). 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 five mice of each sex per 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 examined macroscopically. 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. It is noted that because of a mistake in the allocation of animals of different sexes together, five females (one from the control group and four from the top-dose group) became pregnant. These animals were not included in analyses of group mortality, group mean body weight, group mean absolute and relative organ weights, or group mean feed consumption data (cages bearing these animals were eliminated). They were, however, included in necropsy and histopathological evaluations.

Statistically significant decreases in survival were noted in mid- and high-dose males beginning in weeks 64 and 72, respectively. The increased mortality was most likely related to the higher incidence of genitourinary infections, dermatitis and haematopoietic tumours in the mid- and high-dose animals during this period. As none of the above pathological entities was 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 to be 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 to be 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.

The effects of ethephon on cholinesterase activity are presented in Table 8.

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.

Table 8. Cholinesterase activity in the erythrocytes, brain and plasma of mice treated with ethephon for 78 weeks

	% reduction compared with controls					
	30 ppm		300 ppm		1 000 ppm	
	M	F	M	F	M	F
Week 26						
Plasma ChE	+	2	9	13*	40*	33*
Erythrocyte AChE	+	0	+	18	13	45*
Brain AChE	+	+	+	9	+	8
Week 52						
Plasma ChE	2	+	23*	19*	50*	39*
Erythrocyte AChE	1	17	25	36*	32	47*
Brain AChE	+	+	6	3	+	+
Week 78						
Plasma ChE	+	+	34*	28*	61*	64*
Erythrocyte AChE	+	31	11	56*	21	51*
Brain AChE	+	+	+	4	+	9

AChE: acetylcholinesterase; ChE: cholinesterase; F: females; M: males; ppm: parts per million; +: ChE activity was greater than control values; *: $P < 0.05$ (least significant difference test)
Source: Voss & Becci (1985)

The NOAEL was 30 ppm (equivalent to 4.5 mg/kg bw per day), based on a statistically significant reduction of erythrocyte AChE activity by more than 20% observed in females at 300 ppm (equivalent to 45 mg/kg bw per day) at weeks 52 and 78. No treatment-related tumours were observed in CD-1 mice under the conditions of the study (Voss & Becci, 1985).

In a 78-week dietary carcinogenicity study performed according to OECD Test Guideline 451, ethephon (purity 70.6–72.0%; batch nos A51563, HTS5841AA, A6041;18, 803A13-LJH, A62534, A70073) was administered to groups of 70 male and 70 female CD-1 mice at 0, 100, 1000 or 10 000 ppm (equal to 0, 14, 139 and 1477 mg/kg bw per day for males and 0, 17, 173 and 1782 mg/kg bw per day for females, respectively). Although there was initially a fifth group of males and females treated with 50 000 ppm, high incidences of mortality and morbidity were observed within 2 days of treatment. Therefore, this group was terminated, and the study proceeded with the four remaining groups. 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 13 weeks and every other week thereafter. Water consumption was measured over a 24-hour period at weeks 12, 25, 50, 51 (12-month interim sacrifice animals) and 55 (recovery animals). Ten animals of each sex per dose were used for haematology and urine analysis, and another 10 of each sex per dose were used for clinical chemistry and cholinesterase determinations pretest and in weeks 52 and 77/78. Twenty animals of each sex per dose were killed after 12 months for interim examination. The remaining rats were killed in week 78. Ophthalmoscopy was performed before treatment and before termination. All animals were examined macroscopically. The brain, liver, kidneys, spleen, heart, thyroid gland, adrenal glands and gonads were weighed. The brain of animals selected for brain cholinesterase activity measurement was divided in half. One half of the brain was retained for histological examination, and the other half was used for AChE activity measurement. Histological examinations were performed on a wide range of organs and tissues from all control and

Table 9. Cholinesterase activity in the erythrocytes, brain and plasma of mice treated with ethephon for 2 years

	% reduction compared with controls					
	100 ppm		1 000 ppm		10 000 ppm	
	M	F	M	F	M	F
Week 52						
Plasma ChE	+	18*	35*	41*	65*	76*
Erythrocyte AChE	+	17	36*	36*	70*	74*
Brain AChE	+	8	+	4	+	18
Week 78						
Plasma ChE	2	24	41*	61*	71*	74*
Erythrocyte AChE	+	14	35*	21	72*	60
Brain AChE	+	+	+	+	+	+

AChE: acetylcholinesterase; ChE: cholinesterase; F: females; M: males; ppm: parts per million; +: ChE activity was greater than control values *: $P < 0.05$ (Student's *t*-test)
Source: Van Miller (1988)

10 000 ppm mice and from all mice from the 100 and 1000 ppm groups that died or were killed in a moribund condition.

There were no effects of treatment on mortality, clinical signs, haematology, clinical chemistry or ophthalomoscopy. Slightly increased feed consumption occasionally seen in mice at 1000 and 10 000 ppm was attributed to increased spillage of test diets. Females at 10 000 ppm showed slightly (up to 6%), but statistically significantly, lower body weights in weeks 61, 63, 67 and 75, and body weight gains were significantly lower (up to 14%) in weeks 39, 47, 55, 57, 59, 61, 63, 67, 69, 71 and 75. The urinary pH was significantly lower in males at 1000 and 10 000 ppm after 77 weeks, which was attributed to the acidity of the test substance.

The effects of ethephon on cholinesterase activity are presented in Table 9.

A statistically significant increase in lung adenomas was observed in males at 1000 ppm. However, as no dose–response relationship was observed, and as this is a common finding in this strain of mice, this increase was not considered to be related to treatment.

The NOAEL was 100 ppm (equal to 14 mg/kg bw per day), based on a statistically significant reduction of erythrocyte AChE activity by more than 20% in males and females at 1000 ppm (equal to 139 mg/kg bw per day). No treatment-related tumours were observed in CD-1 mice under the conditions of the study (Van Miller, 1988).

Rats

In a 2-year dietary toxicity study, ethephon (purity 75.6%; batch no. AL-1030-42) 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 were measured. Five animals of each sex per dose were used for haematology, clinical chemistry and cholinesterase determinations in weeks 13, 26, 52, 78 and 104. Brain cholinesterase determinations were performed on five animals of each sex per 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 or clinical signs. Slightly lower net body weight gains (7–8%) were recorded for males at 300 ppm and for both sexes at 3000 ppm. Feed consumption was not affected by treatment. Brain AChE activity was not affected by ethephon. AChE activity in erythrocytes was significantly reduced (> 20%) at 3000 ppm in both sexes. Cholinesterase activity in plasma was significantly reduced (> 20%) at 300 and 3000 ppm in both sexes. No other toxicologically relevant changes in clinical chemistry parameters or organ weights were observed. Macroscopic and histopathological examination did not reveal any findings that could be attributed to the test material. There were no treatment-related increases in the incidence of neoplastic lesions.

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 (Reno, Serota & Voelker, 1978).

In a 2-year dietary toxicity study, ethephon (purity 70.6–72.1%; batch nos A51563, HTS5841AA, A6041;18, 803A13-LJH, A62534, A70073, A70471) was administered to groups of 90–100 male and 90–100 female Sprague-Dawley CD rats at 0, 300, 3000, 10 000 or 30 000 ppm (equal to 0, 13, 131, 446 and 1416 mg/kg bw per day for males and 0, 16, 161, 543 and 1794 mg/kg bw per day for females, respectively). The rats 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 13 weeks and every other week thereafter. Water consumption was measured over a 24-hour period at weeks 12, 25, 50, 51 (12-month interim sacrifice animals) and 55 (recovery animals). Ten animals of each sex per dose were used for haematology and urine analysis, and another 10 of each sex per dose were used for clinical chemistry and cholinesterase determinations in weeks 12/13, 25/26, 50/51, 77/78, 97 (males) and 103/104 (females). In weeks 13, 26, 51 and 78, only plasma and erythrocyte cholinesterase activities were determined. Ten animals of each sex per dose were killed after 12 months for interim examination. At 12 months, 10 animals of each sex in the control, 10 000 ppm and 30 000 ppm groups were placed on control diet for 1 month and served as recovery groups. The recovery animals were used for haematology, urine analysis, clinical chemistry and cholinesterase determinations during weeks 51/52 and 55/56. The remaining rats (50 and 30 animals of each sex for oncogenicity and chronic toxicity investigations, respectively) were killed in week 97 (males) or in week 104 (females). Ophthalmoscopy was performed before treatment and before termination. All animals were examined macroscopically. The brain, liver, kidneys, spleen, heart, thyroid gland, adrenal glands and gonads were weighed. The brains of animals selected for brain cholinesterase activity measurement were divided in half. One half of each brain was retained for histological examination, and the other half was used for AChE activity measurement. Histological examinations were performed on a wide range of organs and tissues from all rats.

There were no effects of treatment on mortality or ophthalmoscopy. In the high-dose rats, an increased incidence of loose faeces was observed from day 13 onward. An increased incidence of red and thickened ears was observed in females at 3000 ppm or higher. Histological examination showed increased incidences of aurocular chondropathy, the cause of which could not be established. This effect was considered to be not biologically significant. Statistically significantly lower body weights were recorded for males and females at 30 000 ppm throughout treatment, but particularly in the first year of treatment. A 28–31% reduction in body weight gain was observed in male and female rats at 30 000 ppm after the first week of treatment. The weight gain deficits at the end of the first year of the study (relative to untreated controls) at 30 000 ppm were 17% and 27% for males and females,

respectively. In the recovery period following 52 weeks of exposure, body weights remained significantly lower for males at 30 000 ppm. Males at 10 000 ppm had a weight gain deficit of 6% at the end of the first year of the study. Feed consumption was statistically significantly reduced (up to 11%) in males and females at 30 000 ppm in the majority of measurements in the first year of treatment, and the reduction was observed as early as in the first week of treatment. In the 4-week recovery period following 52 weeks of exposure, males and females at 30 000 ppm returned to normal feed consumption levels. The efficiency of feed utilization (body weight gain/feed consumed) in the first 13 weeks of treatment was significantly lower for males and females at 30 000 ppm.

Serum glucose level was significantly lower for males and females at 30 000 ppm after 26 weeks and for males at 30 000 ppm after 52 weeks. These differences were considered to be related to the reduced feed consumption and body weights observed for these animals. Serum phosphorus level was statistically significantly reduced (10%) for males at 30 000 ppm after 13 and 26 weeks. In males at 30 000 ppm, erythrocyte counts were up to 6% higher after 13 and 26 weeks, haematocrit was 7% higher after 13 weeks, and mean corpuscular haemoglobin concentration was 2–4% lower after 13, 26, 51 and 78 weeks. Prothrombin time was up to 5% lower for females at 30 000 ppm after 51 and 104 weeks. In view of the small magnitude of these haematological changes, they were not considered to be toxicologically relevant. Urinary pH was significantly lower throughout the study for males and females at 10 000 and 30 000 ppm, with a dose-related trend. A significantly lower urinary pH was also recorded for females at 300 ppm after 103 weeks and for females at 3000 ppm after 77 and 103 weeks. Under control conditions for 4 weeks following the 52-week exposure, the urinary pH returned to normal levels for males and females at 10 000 and 30 000 ppm. This effect is likely to be related to the acidity of the test substance. The specific gravity of urine was significantly higher for females at 10 000 and 30 000 ppm after 50 (high dose only) and 77 weeks. In the investigations during week 77, females at 10 000 and 30 000 ppm showed a strong decrease in urinary volume. As this effect was incidental, it was considered not to be treatment related.

The effects of ethephon on cholinesterase activity are presented in Table 10.

In the interim group at 52 weeks, relative kidney weights were significantly higher for males (27%) and females (22%) at 30 000 ppm. After 2 years of treatment, relative kidney weights were significantly higher for males at 3000 (31%) and 10 000 ppm (35%) and for males (25%) and females (31%) at 30 000 ppm. All other deviations in absolute or relative organ weights at 10 000 or 30 000 ppm at the interim and terminal kills reflected lower terminal body weights. In the kidneys of high-dose females at termination, the incidence of glomerulosclerosis (34/46) was significantly increased compared with controls (16/42). In the liver, the incidence of biliary hyperplasia was significantly higher in males at 30 000 ppm (35/48 versus 21/44 in controls). In females at 3000 ppm and higher, increased incidences of aurocular chondropathy were observed.

There were no treatment-related increases in the incidence of neoplastic lesions.

The NOAEL was 300 ppm (equal to 13 mg/kg bw per day), based on a statistically significant reduction by more than 20% in erythrocyte AChE activity observed in males and females at 3000 ppm (equal to 131 mg/kg bw per day). No treatment-related tumours were observed in Sprague-Dawley CD rats under the conditions of the study (Van Miller, 1989).

2.4 Genotoxicity

Ethephon was tested for genotoxicity in a range of assays. A number of these studies, although indicating no genotoxic effect of ethephon, were considered as supportive evidence only, as the purity of the test material and often batch number were not reported. An unscheduled DNA synthesis test in vitro (Cifone, 1988) that showed negative results was considered supportive only, as no doses with appropriate cytotoxicity were tested. Ethephon induced a positive response in a gene mutation test (Ames test). In the absence of metabolic activation, ethephon induced up to 4-fold

Table 10. Cholinesterase activity in the erythrocytes, brain and plasma of rats treated with ethephon for 2 years

	% reduction compared with controls							
	300 ppm		3 000 ppm		10 000 ppm		30 000 ppm	
	M	F	M	F	M	F	M	F
Week 13								
Plasma ChE	18*	23*	27*	59*	38*	61*	45*	72*
Erythrocyte AChE	9*	13*	45*	55*	65*	72*	83*	82*
Week 26								
Plasma ChE	17	24	36*	58*	42*	65*	51*	69*
Erythrocyte AChE	10*	11*	42*	58*	72*	79*	86*	86*
Week 51								
Plasma ChE	29*	15	47*	53*	48*	64*	56*	61*
Erythrocyte AChE	6	19*	47*	63*	78*	78*	84*	85*
Week 52								
Plasma ChE	12	22	35*	48*	46*	62*	62*	71*
Erythrocyte AChE	2	11*	47*	50*	70*	75*	86*	88*
Brain AChE	+	+	6	+	+	+	7	+
Week 56 recovery								
Plasma ChE	ND	ND	ND	ND	3	9	24	13
Erythrocyte AChE	ND	ND	ND	ND	15*	16*	22*	24*
Brain AChE	ND	ND	ND	ND	+	+	8	+
Week 78								
Plasma ChE	26*	28	35*	47*	41*	59*	48*	57*
Erythrocyte AChE	4	8*	47*	59*	72*	77*	87*	83*
Weeks 97/104								
Plasma ChE	44	22	32	37*	67*	47*	56*	86*
Erythrocyte AChE	8	9	39*	43*	81*	73*	86*	86*
Brain ChE	2	+	4	+	+	2	+	2

AChE: acetylcholinesterase; ChE: cholinesterase; ND: not determined; F: females; M: males; ppm: parts per million; +: ChE activity was greater than control values; *: $P < 0.05$ (Student's *t*-test)
Source: Van Miller (1989)

increases in the number of revertant colonies of the *Salmonella typhimurium* tester strain TA1535. In the presence of metabolic activation, the test substances induced up to 9-fold increases in the number of revertant colonies. Ethephon did not induce point mutations in *S. typhimurium* in the absence or presence of metabolic activation in tester strains TA98, TA100, TA1537 or TA1538. In all other available studies, ethephon gave negative results (Table 11).

2.5 Reproductive and developmental toxicity

(a) Multigeneration studies

In a two-generation dietary reproductive toxicity study performed according to OECD Test Guideline 416, Sprague-Dawley Crl:CD(SD)BR rats (28 of each sex per group for the F_0 and F_1 generations) were fed ethephon (purity 71.14–72.14%; batch nos A6041, 18,803A, 13-LJH, A62534, A70073, A70471) at a dietary concentration of 0, 300, 3000 or 30 000 ppm (equal to 0, 22, 220 and 2260 mg/kg bw per day for F_0 males and 0, 25, 260 and 2570 mg/kg bw per day for F_0 females; and 0, 20, 200 and 2220 mg/kg bw per day for F_{1b} males and 0, 24, 245 and 2520 mg/kg bw per day for F_{1b} females, respectively). F_0 adults were treated over a 10-week premating period and throughout the 3-

Table 11. Overview of genotoxicity tests with ethephon[a]

Test	Test object	Concentration	Purity (%)	Results	Reference
In vitro					
Gene mutations	*Salmonella typhimurium* strains TA98, TA100, TA1535, TA1537, TA1538	0.1–50 µg/plate (±S9)	72.3	Positive	Jagannath (1987)[b]
Gene mutations	Chinese hamster ovary cells, HPRT test	500–2 500 µg/mL (±S9)	Not reported	Negative *Study considered supportive*	Godek, Naismith & Matthews (1983)[c]
Gene mutations	Chinese hamster ovary cells, HPRT test	166–5 000 µg/mL (±S9)	Not reported	Negative *Study considered supportive*	Godek, Naismith & Matthews (1984)[d]
Gene mutations	Chinese hamster ovary cells, HPRT test	500–2 500 µg/mL (−S9); 500–2 600 µg/mL (+S9)	72.3	Negative	Young (1988)[e]
Chromosomal aberrations	Chinese hamster ovary cells	753–2 010 µg/mL (−S9); 502–2 010 µg/mL (+S9)	71.3	Negative	Murli (1988)[f]
Unscheduled DNA synthesis	Male Fischer 344 rat hepatocytes	10–1 000 µg/well	Not reported	Negative *Study considered supportive*	Barfknecht, Naismith & Matthews (1984)[g]
Unscheduled DNA synthesis	Male Fischer 344 rat hepatocytes	Experiment 1: 25–1 000 µg/mL Experiment 2: 500–2 000 µg/mL	71.3	Negative *Study considered supportive*	Cifone (1988)[h]
In vivo					
Micronucleus test	Male and female CD-1 mice, bone marrow	Experiment 1: intraperitoneal dose of 200 mg/kg bw, harvesting after 30 h Experiment 2: intraperitoneal dose of 200 mg/kg bw, harvesting after 480 h Experiment 3: two intraperitoneal doses of 200 mg/kg bw, separated by 24 h, harvesting 48 h after first dose Experiment 4: two intraperitoneal doses of 200 mg/kg bw, separated by 24 h, harvesting 72 h after first dose	Not reported	Negative *Study considered supportive*	Sorg, Naismith & Matthews (1981)[i]

Table 11 (continued)

Test	Test object	Concentration	Purity (%)	Results	Reference
Dominant lethal mutation	Sprague-Dawley COBS CD (SD) rats	Oral dose of 250, 500 or 1 000 mg/kg bw per day for 5 days	Not reported	Negative *Study considered supportive*	Naismith & Matthews (1979)[j]
Unscheduled DNA synthesis	Male Han Wistar rat hepatocytes	Experiment 1: oral dose of 800 or 2 000 mg/kg bw, hepatocyte harvesting after 12–14 h Experiment 2: oral dose of 800 or 2 000 mg/kg bw, hepatocyte harvesting after 2–4 h	71.14	Negative	Howe (2002)[k]

bw: body weight; DNA: deoxyribonucleic acid; HPRT: hypoxanthine–guanine phosphoribosyltransferase; S9: 9000 × *g* supernatant fraction from rat liver homogenate

[a] Positive and negative (solvent) controls were included in all studies.

[b] Test substance: Ethephon; active ingredient 72.3%. Batch number not reported. In the absence of metabolic activation, the test substance induced up to 4-fold increases in the number of revertant colonies of the tester strain TA1535. In the presence of metabolic activation, the test substances induced up to 9-fold increases in the number of revertant colonies. Ethephon did not induce point mutations in *S. typhimurium* in the absence or presence of metabolic activation in tester strains TA98, TA100, TA1537 or TA1538. The *Escherichia coli* strain WP2uvrA was not included in the present study.

[c] Test substance: Ethephon. Batch number not reported. Study design resembles OECD Test Guideline 476. Vehicle was culture medium with 5% fetal calf serum. Post-treatment survival at 2500 µg/mL was 25–30% in the absence of S9 and 14–15% in the presence of S9. Ethephon did not induce gene mutations in mammalian cells.

[d] Test substance: Ethephon. Batch number not reported. Study design resembles OECD Test Guideline 476. Vehicle was culture medium with 5% fetal calf serum. Cytotoxicity was observed at 5 mg/mL in the absence and presence of metabolic activation. Ethephon did not induce gene mutations in mammalian cells.

[e] Test substance: Ethephon, purity 72.3%. Batch number not reported. The study was performed in accordance with OECD Test Guideline 476. Cytotoxicity below 50% was observed only in the first experiment without metabolic activation and in the second experiment with metabolic activation.

[f] Test substance: Ethephon. Batch number not reported. Study design resembles OECD Test Guideline 473. Solvent was McCoys 5a culture medium. Doses used in this assay were based on results from cytotoxicity assays. Cytotoxicity was observed at 1990 µg/mL (–S9). Ethephon did not induce chromosomal aberrations in Chinese hamster ovary cells.

[g] Test substance: Ethephon, batch no. A-41213. Study design resembles OECD Test Guideline 482. Vehicle was deionized water. Cytotoxicity was observed at 3333 and 10 000 µg/well.

[h] Test substance: Ethephon. Study design resembles OECD Test Guideline 482. Vehicle was WME culture medium. No dose levels with appropriate toxicity were tested. At the highest dose of 1000 µg/mL in the first experiment, survival was 89.5%. At the highest dose of 2000 µg/mL in the second experiment, survival was 83.2%. Only five dose levels in the first experiment and two dose levels in the second experiment were used for counting grains.

[i] Purity and batch number not reported. Doses were based on a range-finding test. Following a single dose of 200 mg/kg bw, the mice exhibited writhing, decreased activity, abnormal gait, ptosis and a decreased body tone. No increases in micronuclei were observed in ethephon-treated mice. Polychromatic erythrocyte/normochromatic erythrocyte ratio was not reported. A toxicokinetic study indicates that following an oral or intravenous dose of radiolabelled ethephon to rats, radioactivity concentrations in bone marrow are low (Savage, 1990).

[j] Purity not reported. Batch no. 56375. Three groups of 10 male rats were administered ethephon orally at a dose of 250, 500 or 1000 mg/kg bw per day for 5 consecutive days. Twenty-four hours after the fifth dose, each male was co-housed with two virgin females for 7 days. The matings were repeated weekly with two virgin females for a total of 8 weeks. The females were killed 14 days from mid-week of co-housing, and the numbers of corpora lutea and live and dead implantations were counted and recorded. Ethephon did not produce dominant lethal effects in the male rats at the doses administered, as measured by preimplantation and postimplantation losses. Postimplantation fetal deaths were significantly increased after treatment with the positive control, triethylenemelamine.

[k] Test substance: Ethephon, batch no. C1045. Test performed according to OECD Test Guideline 486. Groups of four male rats were treated once with the solvent (purified water), ethephon (at 800 or 2000 mg/kg bw) or the required positive control, by gavage, at a dosing volume of 10 mL/kg bw. The positive controls used were 2-acetamidofluorene (75 mg/kg bw) suspended in corn oil (12- to 14-hour experiment) and dimethylnitrosamine (10 mg/kg bw) dissolved in purified water (2- to 4-hour experiment).

week mating period, gestation and 21-day lactation of two litters (F_{1a} and F_{1b}). The second mating was made specifically with males and females that failed at the first mating (so-called alternative pairing). F_0 parents were killed after weaning of the F_{1b} pups.

Twenty-eight rats of each sex (predominantly F_{1b} pups) selected to produce the next generation followed the same protocol. Each litter (F_{1a}, F_{1b}, F_{2a}, F_{2b}) was randomly culled to eight pups on postnatal day (PND) 4. Parental (F_0) rats were exposed from 10 weeks before mating until termination, and F_1 rats were exposed from postnatal week 3 until termination. Clinical examination was performed daily. Feed consumption was recorded weekly during the premating period. Body weights of parental rats were recorded weekly; in addition, females were weighed on gestation days (GDs) 0, 4, 7, 14 and 20 and PNDs 0, 4, 7, 14 and 21. At termination, necropsy was performed on all F_0 and F_{1b} parental rats, and weights of brain, ovaries and testes were recorded. Histological examination was performed on gross lesions of all F_0 and F_{1b} parental rats. In addition, in F_0 and F_{1b} control and high-dose adults, histopathology was extended to the reproductive tract (ovaries, uterus, vagina/prostate with seminal vesicles, testes with epididymides) and pituitary. All litters were examined for number of pups, sex of pups, number of stillbirths, number of live births and gross external anomalies. Survival indices were calculated at 0, 4, 7 and 14 days after birth and at weaning. All litters were kept until the youngest litter was 28 days old. Pups were weighed on PNDs 0, 4, 7, 14, 21 (at weaning) and 28. Ten pups of each sex per dose of the F_{1a}, F_{1b}, F_{2a} and F_{2b} generations were necropsied. The remaining offspring were examined for gross external abnormalities. Cholinesterase activity measurements were not performed.

No treatment-related mortality was observed in the F_0 or F_1 parental animals. At 30 000 ppm, increased incidences of unkempt appearance and urinary stains were observed in F_0 male rats, and loose faeces were observed in F_0 and F_1 parental animals of both sexes. Loose faeces were also observed in F_{1b} males at 3000 ppm.

At the end of the premating period, F_0 males and females at 30 000 ppm exhibited a lower body weight (up to 10% in males and 12% in females) and body weight gain (15% in males and 25% in females) compared with controls. The largest reduction in body weight gain (32%) was observed in the first week of treatment. A slight reduction in feed consumption (generally less than 10%) was observed at 30 000 ppm. At the F_0 breeding to produce F_{1a} litters, gestational parameters were unaffected by treatment. Although high-dose F_0 females had a lower body weight than controls throughout gestation and lactation of the F_{1a} and F_{1b} generations, the body weight gain throughout these periods was not lower than that of controls. At birth, the F_{1a} and F_{1b} pups at 30 000 ppm had an 8% lower body weight compared with control pups. During lactation, the weight gain in high-dose pups was lower, so that at weaning, the body weights of these pups were 26–30% lower than those of controls. A similar reduction in body weight was observed at PND 28. In the F_{1b} generation, an increased number of stillborn pups was observed at 30 000 ppm. The number of deaths of F_{1b} pups was increased at 30 000 ppm on PNDs 0–4 (19 versus 2 in the control group) and on PNDs 4–7 at 3000 ppm (8 versus 0 in the control group). Necropsy of F_0 adults or F_{1a} and F_{1b} pups revealed no treatment-related findings. At histopathological examination of high-dose F_0 adults, no treatment-related lesions were observed. Whereas terminal body weights were reduced in F_0 males and females at 30 000 ppm, absolute organ weights were unaffected by treatment. There were also no differences in organ weights relative to brain weight.

During the 10-week premating exposure, the F_{1a} and F_{1b} parental animals at 30 000 ppm had a 15–17% lower body weight gain compared with controls. At 3000 ppm, body weight gain was slightly decreased (4–7%). Feed consumption was decreased at 30 000 ppm.

At the F_{1b} breeding to produce F_{2a} litters, gestational parameters were unaffected by treatment. In the F_{2a} pups, perinatal deaths and lactational survival were unaffected by treatment. The number of stillborn F_{2b} pups (n = 15) and deaths from PNDs 1 to 4 (n = 9) were increased at 30 000 ppm compared with controls (n = 2 and 1, respectively). At 30 000 ppm, maternal body weight was about

15% lower than that of control dams throughout gestation and lactation of the F_{2a} and F_{2b} generations. However, body weight gain during these periods was similar. At birth, the F_{2a} and F_{2b} pups at 30 000 ppm had a 10–11% lower body weight compared with control pups. During lactation, the weight gain in high-dose pups was lower, so that at weaning, the body weights of these pups were 26–30% lower than those of controls. A similar reduction in body weight was observed at PND 28. A slight reduction in body weight gain during lactation (up to 10%) was observed in F_{2b} pups at 3000 ppm.

There were no treatment-related lesions observed in the necropsy of F_{2a} and F_{2b} pups or F_{1b} adults. There were also no treatment-related lesions observed in the histopathological examination of selected organs from high-dose and control F_{1b} adults. Terminal body weights were reduced in F_{1b} males (18%) and females (16%) at 30 000 ppm.

The NOAEL for parental toxicity was 300 ppm (equal to 20 mg/kg bw per day), based on an increased incidence of loose faeces in F_{1b} males at 3000 ppm (equal to 200 mg/kg bw per day).

The NOAEL for offspring toxicity was 300 ppm (equal to 22 mg/kg bw per day), based on an increased mortality in F_{1b} pups from PNDs 4 to 7 and a reduction in body weight gain during lactation in F_{2b} pups at 3000 ppm (equal to 220 mg/kg bw per day).

The NOAEL for reproductive toxicity was 30 000 ppm (equal to 2220 mg/kg bw per day), the highest dose tested (Neeper-Bradley & Tyl, 1990).

(b) Developmental toxicity

Rats

In a developmental toxicity study, groups of 25 pregnant Charles River COBS CD rats were treated orally, by gavage, with ethephon (purity and batch number not reported) in 0.5% aqueous Methocel at a dose level of 0, 200, 600 or 1800 mg/kg bw per day from days 6 through 15 of gestation (day 0 = day on which sperm were detected in the vaginal smear). Clinical signs and mortality were recorded daily from GDs 6 to 20. Body weight was measured on GDs 0, 6, 9, 12, 16 and 20. All females were killed on day 20 of gestation and subjected to gross examination. The uterus was examined, and the numbers of live and dead fetuses, corpora lutea, implantations, and early and late resorptions were counted. Body weight and sex of the fetuses were recorded. About two thirds of the fetuses from each litter were selected for skeletal examinations, and one third for visceral examinations. Cholinesterase activity measurements were not performed.

At 1800 mg/kg bw per day, 14/25 dams died during the treatment period, and only nine litters with viable fetuses were available for evaluation. Gastroenteritis, respiratory disease and necrotic hepatitis were noted as the immediate causes of death in several of these animals. Mortality was first observed on GD 10 in four dams. Dry red matter around the mouth and/or nose and laboured breathing were noted in the majority of animals that died at this dose. Several rats in this treatment group had excessive salivation and matting and/or staining of the anogenital region towards the end of the treatment period. In the high-dose dams, a body weight loss (4 g) was observed from GD 6 to GD 9, compared with a body weight gain of 7 g in control dams. In the high-dose dams surviving to termination, net body weight gain minus uterine weight (13 g) was lower than in control dams (39 g). In these high-dose dams, necropsy revealed hydronephrosis, distension of the stomach and intestines with gas, enlarged spleen with white coloration on the outer surface, yellowish-brown discoloration of the kidneys and a dark red depressed area on the quadrate lobe of the liver. Histopathological examination of the high-dose dams revealed focal lymphoid hyperplasia of the spleen and focal parenchymal fibrosis of the liver. No effects of treatment on mortality, clinical signs, body weight, macroscopy or histopathology were observed at 200 and 600 mg/kg bw per day. The number of implantations, number of early and late resorptions, number of live fetuses, sex ratio and uterus weight were not affected by treatment. A slightly lower fetal weight (–6%) at 1800 mg/kg bw per day may be due to maternal toxicity or an increased number of viable fetuses at the high dose (14.4 versus 11.9 in controls). Visceral and skeletal examination revealed no treatment-related findings.

The NOAEL for maternal toxicity was 600 mg/kg bw per day, based on increased mortality, clinical signs (salivation), reduced body weight gain, and various macroscopic findings and histological changes (focal lymphoid hyperplasia of the spleen and focal parenchymal fibrosis of the liver) at 1800 mg/kg bw per day.

The NOAEL for embryo and fetal toxicity was 1800 mg/kg bw per day, the highest dose tested (Rodwell, 1980).

In a developmental toxicity study, groups of 25 mated female Crl:CD(SD)BR rats were treated orally, by gavage, with ethephon (purity 71.7%; batch no. A70471) in distilled water at a dose of 0, 125, 250 or 500 mg/kg bw per day from days 6 through 15 of gestation (day 0 = day on which sperm were detected in the vaginal smear). Clinical signs and mortality were recorded daily. Body weight was measured on GDs 0, 6, 9, 12, 16 and 20. All females were killed on day 20 of gestation and subjected to gross examination. The uterus was examined, and the numbers of live and dead fetuses, corpora lutea, implantations, and early and late resorptions were counted. Body weight and sex of the fetuses were recorded. About half of the fetuses from each litter were selected for skeletal examinations, and the other half for cross-sectional visceral examinations. Cholinesterase activity measurements were not performed.

No treatment-related effects on mortality, clinical signs, abortions or body weight were observed. Necropsy of the dams revealed no effect of ethephon. The number of implantations, number of early and late resorptions, number of live fetuses, fetal weight, sex ratio and uterus weight were not affected by treatment. Visceral and skeletal examination revealed no treatment-related findings.

The NOAEL for maternal toxicity was 500 mg/kg bw per day, the highest dose tested.

The NOAEL for embryo and fetal toxicity was 500 mg/kg bw per day, the highest dose tested (Henwood, 1989b).

Rabbits

In a developmental toxicity study, groups of 17 pregnant female New Zealand White rabbits were treated orally, by gavage, with ethephon (purity unknown; batch no. aa) in deionized water at a dose of 0, 50, 100 or 250 mg/kg bw per day from days 6 through 19 of gestation. Clinical signs and mortality were recorded daily. Body weight was measured on GDs 0, 6, 11, 15, 19 and 29. Feed consumption was measured daily. All females were killed on day 29 of gestation. The uterus was examined, and the numbers of live and dead fetuses, corpora lutea, implantations and resorptions were counted. Body weight and length and sex of the fetuses were recorded. All fetuses were examined for skeletal and visceral anomalies. Cholinesterase activity measurements were not performed.

One control animal, two low-dose animals, four mid-dose animals and eight high-dose animals were found dead or killed in extremis during the study. An increased incidence of inactive animals was noted in the maternal 250 mg/kg bw per day group during the treatment and post-treatment phases. At the middle and high doses, body weight losses of 105 and 187 g, respectively, were observed from GD 6 to GD 11, whereas control and low-dose rabbits gained weight during this period. At the high dose, feed consumption was reduced by about 23% during treatment. Necropsy revealed no effect of treatment. The mean number of resorptions was higher at the middle (2.4) and high doses (1.8) compared with controls (0.8). It is not indicated whether these were early or late resorptions. At 100 and 250 mg/kg bw per day, the mean number of live fetuses (4.7 and 3.1, respectively) and fetal viability (63% and 47%, respectively) were lower than in controls (mean number of live fetuses 5.9, viability 79%), although the differences were not statistically significant. The mean fetal weights and lengths were comparable between the control and treated groups. Visceral and skeletal examination revealed no effect of treatment.

The NOAEL for maternal toxicity was 50 mg/kg bw per day, based on a body weight reduction from GD 6 to GD 11 and increased number of resorptions at 100 mg/kg bw per day.

The NOAEL for embryo and fetal toxicity was 50 mg/kg bw per day, based on the reduced number of live fetuses and viability of fetuses at 100 mg/kg bw per day (Weatherholtz, Wolfe & Durloo, 1981).

In a developmental toxicity study, groups of 22 artificially inseminated female Hra(NZW)SPF rabbits were treated orally, by gavage, with ethephon (purity 72.2%; batch no. 4022193) in deionized water at a dose of 0, 62.5, 125 or 250 mg/kg bw per day from days 7 through 19 of gestation. Clinical signs and mortality were recorded daily. Body weight was measured on GDs 0, 7, 10, 13, 16, 20, 24 and 29. All females were killed on day 29 of gestation. The uterus was examined, and the numbers of live and dead fetuses, corpora lutea, implantations, and early and late resorptions were counted. Body weight and sex of the fetuses were recorded. About half of the fetuses from each litter were selected for skeletal examinations, and the other half for cross-sectional visceral examinations. Cholinesterase activity measurements were not performed.

At 250 mg/kg bw per day, three does died and 14 does were killed in a moribund condition. Additionally, one dam at 250 mg/kg bw was euthanized because of an eye lesion (buphthalmos and corneal opacity). At the high dose, the majority of the does showed ataxia, reduced activity, prostration and/or yellow-stained anogenital area. The high-dose females lost weight (about 8% on GD 13), whereas the other groups showed a slight increase in body weight (1–2%) during this period. In the two remaining dams at 250 mg/kg bw, the incidences of early resorptions (2 versus 0.5 in controls) and postimplantation loss (43% versus 12% in controls) were high, and the number of live fetuses per litter was low. Visceral and skeletal examination revealed no treatment-related findings.

The NOAEL for maternal toxicity was 125 mg/kg bw per day, based on mortality, clinical signs of toxicity and body weight loss at 250 mg/kg bw per day.

The NOAEL for embryo and fetal toxicity was 125 mg/kg bw per day (Henwood, 1990). The Meeting considered that the number of fetuses in the high-dose group was insufficient to conclude on the effects of ethephon at 250 mg/kg bw per day on prenatal development.

2.6 Special studies

(a) Mechanistic studies on butyrylcholinesterase inhibition

The mechanistic basis for inhibition of butyrylcholinesterase (cholinesterase) was investigated in vitro. The sensitivity of plasma cholinesterase to ethephon (90-minute preincubation at 25 °C) was greatest for humans, dogs and mice (median inhibitory concentration [IC_{50}] 6–23 µmol/L), intermediate for chickens, rabbits, rats and guinea-pigs (IC_{50} 26–53 µmol/L) and lowest for pigs and horses (IC_{50} 92–172 µmol/L). The IC_{50} decreased linearly with time on a log–log scale to values of 0.15–0.3 µmol/L for human, dog and horse cholinesterase at 24 hours. The inhibition rate was generally related to ethephon concentration, consistent with a bimolecular reaction (e.g. phosphorylation). The extent of inhibition of the esteratic activity of cholinesterase by ethephon was directly proportional to the extent of inhibition of [^{3}H]diisopropyl phosphorofluoridate postlabelling, which is not reversible on removing the ethephon, either directly or after further incubation for 24 hours at 25 °C. These observations strongly suggest that ethephon, similar to diisopropyl phosphorofluoridate, phosphorylates human plasma cholinesterase at the Ser-198 of the esteratic site, leading to the formation of a phosphobutyrylcholinesterase (Haux, 2000).

The metabolism and reactivity as ethylene generators (on the basis of plant growth regulator activity), in vitro inhibition of plasma cholinesterase, and alkylating and phosphorylating properties of the phosphorus-containing components in technical ethephon were investigated. Urinary products of technical ethephon in rats were the parent compound, $HOP(O)(OH)_2$ and unmetabolized $(HO)_2P(O)CH_2CH_2P(O)(OH)_2$. Ethephon was more potent than the impurities present in the technical-grade material as plant growth regulators (tomato epinasty assay), in vitro inhibitors of plasma

cholinesterase and phosphorylating agents. The ethephon metabolite HEPA did not cause inhibition of plasma cholinesterase activity. The authors concluded that the biological activity of technical-grade ethephon appears to be associated with the reactions of its principal component, particularly ethylene liberation, and possibly phosphorylating activity (Segall et al., 1991).

Ethephon as the dianion phosphorylates cholinesterase at its active site. To define the structure–activity relationships and mechanism of cholinesterase inhibition by ethephon (2-chloroethylphosphonic acid), this compound and substituted phenyl moieties (3- and 4-nitrophenyl-, 3- and 4-dimethylaminophenyl- and 3- and 4-trimethylammoniumphenylethylphosphonic acid) were investigated. The study showed that the substituted phenyl moieties decompose under basic conditions about 100-fold faster than ethephon to yield the corresponding styrene derivatives. Electron-withdrawing substituents on the phenyl ring decrease the hydrolysis rate, whereas electron-donating substituents increase the rate. The 4-trimethylammonium analogue had the highest affinity (dissociation constant [K_i] = 180 mmol/L) and potency (IC_{50} = 19 mmol/L) in first binding reversibly at the substrate site (possibly with stabilization in a dianion–monoanion environment) and then progressively and irreversibly inhibiting the enzyme activity. The authors concluded that it is likely that dissociation of chloride is the first and rate-limiting step both in the hydrolysis and, by analogy, in the phosphorylation of cholinesterase by ethephon bound at the active site (Zhang & Casida, 2002).

(a) Acute and subchronic neurotoxicity

Rats

In a pilot study aimed at finding the time to peak effect after a single dose of ethephon, groups of 18 male and 18 female Sprague-Dawley Crl:CDR(SD)BR rats were given ethephon (purity 72.4%; batch no. 4051511) by gavage at 0, 250, 500, 1000 or 2000 mg/kg bw. The animals were observed daily for mortality and clinical signs. Body weight was measured on days 1 and 7. Three rats of each sex per dose were subjected to a functional observational battery 0.5, 1, 2, 4, 6, 8 and 24 hours or on day 7 after dosing. The remaining rats were bled pre-dosing (via the tail vein) for plasma and erythrocyte cholinesterase levels, and from these, three rats of each sex per group were killed 0.5, 1, 2, 4 or 8 hours post-dosing for plasma, erythrocyte and brain cholinesterase determinations.

No treatment-related mortality and no clinical signs were observed for animals treated with ethephon. Moderate to large body weight losses (at least 14 g) were observed for all males and one female in the 2000 mg/kg bw group and for one male in the 1000 mg/kg bw group at the first day after treatment. These same animals tended to gain more weight from days 1 to 7 compared with the controls.

Erythrocyte and brain AChE levels were not affected by treatment. Plasma cholinesterase levels were decreased for both males and females of all treated groups and in a dose-related manner from 0.5 hour post-dosing for the 1000 and 2000 mg/kg bw groups and from 1 hour post-dosing for the other treated groups. The maximum suppression for all groups was at 4–8 hours following treatment (Beyrouty, 1996a).

In an acute neurotoxicity study, performed according to OECD Test Guideline 424, groups of 12 male and 12 female Sprague-Dawley Crl:CDR(SD)BR rats were given ethephon (purity 72.4%; batch no. 4051511) by gavage at 0, 500, 1000 or 2000 mg/kg bw. The animals were observed daily for mortality and clinical signs. A detailed physical examination was performed weekly. Body weight and feed consumption were measured weekly. The rats were subjected to a functional observational battery and a motor activity test on day 0 at about 5–5.5 hours after dosing and on days 7 and 14. At study completion on day 15, all rats were necropsied. Neurohistological examination of the brain, ganglions, nerves, eyes and gastrocnemius muscle was performed on six rats of each sex per dose.

Two females dosed at 2000 mg/kg bw died within 2 days after dosing, and one female at 1000 mg/kg bw was found dead on day 5. At 2000 mg/kg bw, laboured breathing, piloerection, prominent backbone, thin body condition, swollen abdominal region, cold to touch, reduced activity and fur staining were observed. At 2000 mg/kg bw, body weight was 6–7% lower than in controls at day 7 after dosing. Feed consumption during the first week after treatment was 9% lower than in controls in mid-dose females and high-dose males and females. Increased incidences of myosis were observed at 2000 mg/kg bw (6/12 in males; 10/12 in females), 1000 mg/kg bw (3/12 males; 5/12 females) and 500 mg/kg bw (3/12 males; 5/12 females), compared with controls (1/12 males and females). The myosis persisted up to day 14 in a few treated animals. Hypothermia was observed in high-dose rats on day 0. A statistically significant increase in urination was noted for males at 2000 mg/kg bw on day 0, and reduced motor activity was observed in males at 1000 and 2000 mg/kg bw and females at 2000 mg/kg bw on day 0. Brain weight, length and width were not affected. Macroscopic postmortem examination and histopathological examination of nervous tissues did not reveal any abnormalities.

A NOAEL could not be identified. The LOAEL was 500 mg/kg bw, based on increased incidences of myosis at all doses (Beyrouty, 1996b).

In a 2-week range-finding toxicity study, ethephon (purity 72.4%; batch no. A4051511) was administered to groups of six male and six female Sprague-Dawley Crl:CD(SD)BR rats by gavage at 0, 100, 300, 600 or 1000 mg/kg bw per day (doses corrected for purity). Animals were checked daily for clinical signs of toxicity. A detailed physical examination was performed weekly. Body weights were measured twice per week, and feed consumption was measured weekly. Before the treatment was started and prior to dosing on days 2, 8 and 15, the rats were subjected to a functional observational battery, and blood was sampled. At termination on day 15, all animals were necropsied.

At 600 mg/kg bw per day, two males and four females were found dead. At 1000 mg/kg bw per day, all males and five females were found dead or were killed in a moribund condition. In most of the rats at 600 and 1000 mg/kg bw per day, fur staining, skin pallor, abnormal breathing, respiratory sounds, dehydration, cold to touch, decreased activity, weak appearance and abdominal distension were observed. In the lower-dose groups (i.e. 100 and 300 mg/kg bw per day), no treatment-related clinical signs were observed. Body weight loss and reduced feed consumption were observed at 600 and 1000 mg/kg bw per day.

In the functional observational battery, abnormal breathing, myosis, muzzle staining, diarrhoea and impaired gait were observed in a few rats at 300 mg/kg bw per day and above. Red blood cell cholinesterase levels were not affected by treatment. Dose-dependent decreases in plasma cholinesterase levels were observed in all treatment groups (Beyrouty, 1997a).

In a 13-week neurotoxicity study, ethephon (purity 72.4%; batch no. A4051511) was administered to groups of 22 male and 22 female Sprague-Dawley Crl:CD(SD)BR rats by gavage at 0, 75, 150 or 400 mg/kg bw per day. The high dose level was decreased to 300 mg/kg bw per day during week 10/11 of treatment. Animals were checked daily for clinical signs of toxicity. A detailed physical examination was performed weekly. Body weights and feed consumption were measured weekly. Before the treatment was started and prior to dosing in weeks 4, 8 and 13, 12 rats of each sex per dose were subjected to a functional observational battery and motor activity test. In the remaining 10 animals of each sex per dose, blood was sampled for cholinesterase measurements in weeks 4 and 8 and at termination in week 13. At termination, all animals were necropsied. The brains of 10 animals of each sex per dose, with the exception of nine females at the top dose, were sampled for AChE measurements. Neuropathological examination was performed on six rats of each sex per dose.

At 400 mg/kg bw per day, three males and three females were found dead on week 5 (one male and one female) and week 10 of treatment (two males and two females). Abnormal breathing was shown by 13 males and 11 females at 400/300 mg/kg bw per day. Six high-dose males were cold to touch, and five males and one female at this dose showed a weak/thin/dehydrated appearance. Slightly reduced body weight gains (up to 9%) were noted in rats at 400/300 mg/kg bw per day. There

Table 12. Cholinesterase activity in the erythrocytes, brain and plasma of rats treated with ethephon for 13 weeks

	% reduction compared with controls					
	75 mg/kg bw per day		150 mg/kg bw per day		400/300 mg/kg bw per day	
	M	F	M	F	M	F
Week 4						
Plasma ChE	18.6*	21.2*	19.0*	34.7*	32.3*	53.5*
Erythrocyte AChE	4.6	16.7*	9.6	21.7*	16.8*	23.6*
Week 8						
Plasma ChE	15.1*	34.6	17.1*	48.2*	31.5*	62.6*
Erythrocyte AChE	2.5	19.1*	7.6	24.7*	15.5*	30.2*
Week 13						
Plasma ChE	20.3*	43.3	20.6*	56.0*	25.2*	63.9*
Erythrocyte AChE	8.1*	10.0	12.3*	9.1	21.8*	18.6*
Brain AChE	+	4.5	+	5.5	+	8.5*

AChE: acetylcholinesterase; bw: body weight; ChE: cholinesterase; F: females; M: males; +: AChE activity was greater than control values; *: $P < 0.05$ (Dunnett's)
Source: Beyrouty (1997b)

were no behavioural changes observed in the functional observational battery and motor activity tests that were indicative of neurotoxicity.

The effects of ethephon on cholinesterase activity in this study are presented in Table 12.

Macroscopic and histopathological examination of nervous tissues did not reveal any abnormalities.

The NOAEL was 75 mg/kg bw per day, based on a statistically significant reduction of erythrocyte AChE activity in females at 150 mg/kg bw per day (Beyrouty, 1997b).

Dogs

To determine a NOAEL for reduction of cholinesterase activity, three female Beagle dogs per dose group received ethephon (purity 71.3%; batch no. 2250197) at a dietary concentration of 0, 250 or 750 ppm (equal to 0, 6 and 14 mg/kg bw per day, respectively) for 28 days (doses corrected for purity). All animals were checked daily for clinical signs. Detailed clinical examinations were performed weekly. Feed consumption was measured daily and body weights were measured weekly to calculate the intake of ethephon. Plasma cholinesterase and erythrocyte AChE activity were measured for all animals once prior to administration of the test substance and during days 7, 14, 21 and 28, and brain AChE activity was determined at study termination. Other examinations, including haematology, clinical chemistry and pathological analysis, were not performed.

There were no animals found dead or moribund and no compound-related clinical observations. The effects of ethephon on cholinesterase activity are presented in Table 13. It is noted that the cholinesterase measurements were not performed at the time of peak plasma cholinesterase inhibition after a gavage dose (4–8 hours post-dosing; see Beyrouti, 1996a).

The NOAEL for cholinesterase inhibition was 250 ppm (equal to 6 mg/kg bw per day), based on greater than 20% inhibition of erythrocyte AChE activity with statistical significance at 750 ppm (equal to 14 mg/kg bw per day) (Eigenberg, 2006a).

Table 13. Cholinesterase activity in the erythrocytes, brain and plasma of female dogs treated with ethephon for 28 days

	% reduction relative to controls[a]			
Dietary concentration (ppm)	**Day 7**	**Day 14**	**Day 21**	**Day 28**
Erythrocyte AChE				
250	+	+	+	5
750	19	39	50*	58*
Brain AChE				
250	ND	ND	ND	4
750	ND	ND	ND	+
Plasma ChE				
250	30	46*	48*	49*
750	56*	63*	62*	60*

AChE: acetylcholinesterase; ChE: cholinesterase; ND: not determined; ppm: parts per million; +: AChE activity was greater than control values; *: $P < 0.05$ (ANOVA + Student's *t*-tests, two-sided)
Source: Eigenberg (2006a)

In a 91-day toxicity study to evaluate the effects of ethephon on blood and brain cholinesterase inhibition, four male and four female Beagle dogs per dose group received ethephon (purity 71.9%; batch no. 040201) at a dietary concentration of 0, 70, 140 or 525 ppm (equal to 0, 2, 4 and 15 mg/kg bw per day for males and 0, 2, 4 and 18 mg/kg bw per day for females, respectively). All animals were checked daily for clinical signs. Detailed clinical examinations were performed weekly. Feed consumption was measured daily, and body weights were measured weekly. Plasma cholinesterase and erythrocyte AChE activity were measured on all animals twice prior to administration of the test substance and during days 3, 10, 25, 53, 70 and 87. At study termination, brain AChE activity was measured. Other examinations, including haematology, clinical chemistry and pathological analysis, were not performed.

There was no mortality or treatment-related clinical signs. There was no compound-related effect on body weight or feed consumption. The effects of ethephon on cholinesterase activity are presented in Table 14. Erythrocyte AChE activity was statistically significantly inhibited at 525 ppm from day 10 in males and at 140 ppm from day 53 and 525 ppm from day 25 in females, compared with the mean pretreatment values for each treated group. In the female 140 ppm and male and female 525 ppm groups, these AChE inhibitions were greater than 20% compared with the corresponding control groups at the time points when the cholinesterase inhibition was statistically significant compared with the pretreatment values. Brain AChE activity was not affected by the treatment in males.

The NOAEL for cholinesterase inhibition was 70 ppm (equal to 2 mg/kg bw per day), based on greater than 20% reduction of erythrocyte AChE activity with statistical significance in females at 140 ppm (equal to 4 mg/kg bw per day) (Eigenberg, 2006b)

(c) Delayed neurotoxicity

Groups of 10 white Vantress chickens received ethephon by intubation at 1000 mg/kg bw per day (purity 88%; batch number not reported) on days 1 through 5 or at 1000 mg/kg bw on day 1 and thereafter at 500 mg/kg bw per day on days 2 through 10. Two positive control groups received tri-*o*-cresyl phosphate at 60 mg/kg bw per day. Two negative control groups received olive oil at 300 mg/kg bw per day.

Table 14. Cholinesterase activity in erythrocytes, plasma and brain of dogs treated with ethephon for 91 days

	% reduction compared with controls[a]					
	70 ppm		140 ppm		525 ppm	
	Males	Females	Males	Females	Males	Females
Erythrocyte AChE						
Day 3	6	22	10	+	9	28
Day 10	6	29	16	7	22*	37
Day 25	13	34	35	16	58*	59*
Day 53	18	36	35	31*	73*	70*
Day 70	21	39	40	32*	75*	72*
Day 87	3	37	40	29*	72*	70*
Brain AChE						
Day 91	+	8#	+	10#	+	14#
Plasma ChE						
Day 3	9*	42	23*	45	50*	52*
Day 10	33*	62*	38*	65*	46*	65*
Day 25	35*	57*	43*	57*	62*	63*
Day 53	34*	60*	39*	56*	61*	61*
Day 70	28*	60*	41*	58*	61*	63*
Day 87	34*	58*	37*	55*	58*	60*

AChE: acetylcholinesterase; ChE: cholinesterase; ppm: parts per million; +: AChE activity was greater than control values; *: $P < 0.05$ (ANOVA + Dunnett's tests) versus the pretreatment value; #: $P < 0.05$ (ANOVA + Student's *t*-tests) versus the control

Source: Eigenberg (2006b)

No clinical signs of neurotoxicity and no gross pathology were observed in any of the necropsied chickens treated with ethephon. Microscopic examination showed no cytopathological changes in the spinal cord or sciatic nerve of the animals receiving ethephon. Administration of tri-*o*-cresyl phosphate caused clinical signs of neurotoxicity and some spinal axonal dystrophy in 10 chickens and sciatic neuropathy in one chicken.

There was no evidence of delayed neurotoxicity induced by ethephon (Weatherholtz & Shott, 1970).

Groups of 15–30 white leghorn chickens received a single oral dose of ethephon (purity 71%; batch number not reported) of 0, 3160 or 3850 mg/kg bw. A positive control group received tri-*o*-tolyl phosphate orally at 500 mg/kg bw. Twenty-one days following dosing, all surviving birds (n = 21) were treated orally with a single dose of ethephon at 2370 mg/kg bw.

Twenty-eight of 30 birds in the 3850 mg/kg bw group were found dead within 24 hours after the first dose. One additional mortality was recorded in this group on test day 8. Ten of 30 birds in the 3160 mg/kg bw group were found dead within 24 hours after the first dose. One bird from this group was found dead within 48 hours after the second dose. Signs of lethargy and anorexia were present

following dosing. Complete recovery of all surviving birds was seen during both 21-day test periods. There were no signs of locomotor disturbances or other clinical signs of delayed neurotoxicity among any of the ethephon-treated chickens during the 42-day test period. Ethephon-treated birds exhibited decreased feed intake and body weight loss during the test period. The positive control birds lost weight and exhibited behavioural signs of neurotoxicity by day 9 of the investigation. All positive control birds were killed in extremis on test day 17 or 18. Gross pathological examination of birds found dead within 24 hours after dosing revealed diffuse red discoloration with severe dilatation of the vessels in the intestinal tract and diffuse light grey discoloration with transparent gel circumscribing the crop area in all birds. Histopathology of neural tissues from the ethephon-treated birds revealed no changes. Treatment-related lesions were noted with respect to the positive control birds.

No evidence of delayed neurotoxicity was observed in this study at 3160 mg/kg bw. The 3850 mg/kg bw dose could not be evaluated, as only one bird survived the observation period at this high dose (Fletcher, 1983).

In a delayed neurotoxicity study performed according to OECD Test Guideline 418, a group of 20 Hyline hens received ethephon (purity 71.3%; batch no. 040201) at a single oral dose of 2000 mg/kg bw. Sixteen control hens received water. Observations for mortality, adverse clinical signs, assessment of delayed locomotor ataxia and body weight were performed at scheduled intervals during the study. Twenty-four and 48 hours after dosing, three birds from each treatment group at each time point were killed, and brain and spinal cord tissues were examined for AChE and neurotoxic esterase activities. Positive control data using tri-*o*-cresyl phosphate were generated and reported as a separate study.

No clinical signs of delayed locomotor ataxia were observed in any treated or control birds during the study. There was no sign of any enzymatic inhibition of either AChE or neurotoxic esterase in either the group dosed with ethephon or the control group at any sampling time. No treatment-related findings on body weight gain or macroscopic or histological examination were detected (Rodgers, 2005).

(d) Studies with metabolites

Acute and short-term toxicity and genotoxicity studies with HEPA, the major metabolite of ethephon, were available. HEPA is a major metabolite in kidneys of male rats and also the main plant metabolite.

Acute toxicity

The results of studies of acute toxicity with HEPA are summarized in Table 15.

Table 15. Results of acute toxicity studies with HEPA

Species	Strain	Sex	Route	Vehicle	Purity (%)	LD_{50} (mg/kg bw)	Reference[a]
Rat	Crl:WI(Glx/BRL/Han)BR)	M + F	Oral	Water	95.7	> 2 000 (M/F)	Denton (2001)

bw: body weight; F: female; LD_{50}: median lethal dose; M: male

[a] Statements of adherence to quality assurance and GLP were included in all studies.

[b] Batch no. B907. Study was performed according to OECD Test Guideline 423. A dose of 2000 mg/kg bw was administered by gavage to three male and three female rats. Vehicle was water. There were no deaths. Clinical signs were diarrhoea and lethargy. A slight body weight loss was observed in all rats 1 day after treatment and in one male and one female 1 week after treatment. Necropsy revealed no macroscopic changes.

Short-term studies of toxicity

In a 2-week range-finding toxicity study, HEPA (purity 95.9%; batch no. B960/LJ33246) was administered to groups of five male and five female Sprague-Dawley ICO: OFA. SD. (IOPS Caw) rats by gavage at 0, 125, 250 or 500 mg/kg bw per day. Animals were checked daily for clinical signs of toxicity. Body weights were measured before the start of treatment and on days 1, 7 and 15. Feed consumption was measured weekly. On day 16, blood was sampled for haematology and clinical chemistry. Urine analysis was performed on day 14. After termination on day 16, all animals were necropsied, and brain, liver, kidneys, spleen, ovaries/testes and thyroids were weighed.

At 500 mg/kg bw per day, liver weights were increased (15–16%). No other treatment-related effects were observed (Bigot, 2003a).

In a 28-day toxicity study, HEPA (purity 95.9%; batch no. B960/LJ33246) was administered to groups of 10 male and 10 female Sprague-Dawley ICO: OFA. SD. (IOPS Caw) rats by gavage at 0, 125, 350 or 1000/700 mg/kg bw per day. The highest dose level was reduced from 1000 to 700 mg/kg bw per day from day 5 onwards, as a result of mortality. Animals were checked daily for clinical signs of toxicity. Detailed physical examinations were performed at least weekly. Body weights were measured before the start of treatment, on days 1, 4, 8, 15, 22 and 28 and before necropsy. Feed consumption was measured weekly. Ophthalmoscopy was performed before treatment and on day 28. On day 24 or 25, blood was sampled for haematology and clinical chemistry. Urine analysis was performed on day 29, 30 or 31. After termination on day 29, 30 or 31, all animals were necropsied, and adrenal glands, brain, epididymis, heart, kidney, liver, ovary, pituitary gland, prostate gland, spleen, testis, thymus, thyroid gland (with parathyroid gland) and uterus (including cervix) were weighed. Histopathological examination of a wide range of organs and tissues was performed on all control and high-dose rats, as well as all animals that died before termination in the intermediate-dose group.

At 1000 mg/kg bw per day, one male and two females were found dead on day 4, and one female was killed for humane reasons on day 4. After lowering the dose to 700 mg/kg bw per day on day 5, one male was found dead on day 21. Seven animals showed body weight loss from days 1 to 4, and on day 8, after reduction of the dose from 1000 to 700 mg/kg bw per day, the mean body weight gain was reduced by 28–42% when compared with controls. Feed consumption was reduced (−12%) in females during the first week of treatment. At 1000/700 mg/kg bw per day, wasted appearance, laboured/noisy respiration, piloerection, few/soft/mucoid faeces, reduced motor activity, increased salivation, cold to touch, skin lesions, hair loss and scabs were observed. No changes were noted during the neurotoxicity assessment and at ophthalmological examination. Haematology and clinical chemistry showed no effect of treatment. Urine analysis showed lower pH and ketone levels in males with fewer crystals than usually observed. In animals found dead or killed for humane reasons, macroscopic findings were gaseous distension of stomach/intestines, pale/small spleen, red foci/mottled thymus, dark red lung, dark liver, small prostate gland and small seminal vesicles. Microscopic examination of these animals revealed epithelial necrosis and intraluminal inflammatory exudates in trachea.

The NOAEL was 350 mg/kg bw per day, based on mortality, clinical signs, reduced body weight gain and feed consumption (females only), changes in urinary parameters and various macroscopic findings and histological changes (epithelial necrosis and intraluminal inflammatory exudates in trachea) observed at 1000/700 mg/kg bw per day. The effects observed at the high dose are considered related to the gavage administration and the physicochemical properties of HEPA (Bigot, 2003b).

Table 16. Results of studies on the genotoxicity of HEPA

End-point	Test object	Concentration	Purity (%)	Results	Reference[a]
In vitro					
Gene mutation	*Salmonella typhimurium* strains TA98, TA100, TA1535, TA1537, TA1538	1.6–5 000 µg/plate (±S9)	95.7	Negative	Johnson (2002)[b]
Gene mutation	Mouse lymphoma L5178Y (*TK*) locus	40–1 260 µg/mL (−S9), 2.5–40 µg/mL (±S9), experiment 1 200–1260 µg/mL (±S9), experiment 2	95.7	Negative	Ballantyne (2002)[c]
Chromosomal aberrations	Human peripheral blood lymphocytes	516.5–1 261 µg/mL (−S9, 3 h); 404.3–1 261 µg/mL (−S9, 20 h); 807–1 261 µg/mL (+S9, 3 h); 911–1 261 µg/mL (+S9, repeat assay, 3 h)	95.7	Negative	Whitwell (2002)[d]

S9: 9000 × *g* supernatant fraction from rat liver homogenate; *TK*: thymidine kinase

[a] Positive and negative (solvent) controls were included in all studies. In all studies, statements of adherence to GLP and quality assurance were included.

[b] Batch no. B907. Performed according to OECD Test Guideline 471. Vehicle was purified water. Toxicity was observed at 1000 and 5000 µg/plate. HEPA did not induce point mutations in *S. typhimurium* under the conditions of the test.

[c] Batch no. B907. Performed in accordance with OECD Test Guideline 476. Vehicle was purified water. No cytotoxicity was observed up to the highest tested dose of 1260 µg/mL. HEPA did not induce gene mutations in mouse lymphoma cells under the conditions of the test.

[d] Batch no. B907. Performed in accordance with OECD Test Guideline 473. Vehicle was purified water. Cytotoxicity was observed at a dose level of 1072 µg/mL (−S9, 20-hour exposure). HEPA did not induce chromosomal aberrations in cultured human peripheral blood lymphocytes under the conditions of the test.

Genotoxicity

The results of genotoxicity studies with HEPA are summarized in Table 16.

3. Observations in humans

In a range-finding study with ethephon, two volunteers received ethephon (purity 88%; batch no. 514A) at increasing doses of 5.4–120 mg/day by oral capsule, in three divided doses, one immediately after each meal over a 7-week period. The dose reportedly ranged from 0.06 to 1.25 mg/kg bw per day. Blood was sampled twice per week for assessment of cholinesterase activity. Haematology, clinical chemistry and urine analysis were performed weekly.

No effect of ethephon on haematology and plasma or erythrocyte cholinesterase activity was observed. Transient, subjective feelings of urinary urgency were experienced by both volunteers. No persistent side-effects were observed during the course of the study. A slight elevation in ALAT activity was observed from day 26 to day 46. Laboratory studies performed 2 weeks following the last ethephon administration gave test results within normal biological limits (Reese, 1971).

Five male and five female volunteers received technical ethephon (formulation consisting of 10% ethephon, hydrated silica and cornstarch) at a dose of 124 mg ethephon per day for 28 days by oral capsule, approximately equal to 1.5 mg/kg bw per day for males and 2.2 mg/kg bw per day for females. Three males and three females were given a placebo. Each subject received two capsules postprandially for the first two dosing periods; the third dose (two capsules) was given at the end of the workday. All subjects were monitored constantly during the first 8 hours following ingestion of

the test material for adverse effects or symptoms related to compound administration and once daily thereafter. Cholinesterase activity in plasma and red blood cells was measured before the start of dosing, on days 1, 2, 7, 14 and 28 of the study and 2 weeks following the last dose. Haematology, clinical chemistry and urine analysis were performed weekly.

No changes in plasma or erythrocyte cholinesterase activity and no persistent side-effects were observed. However, transient subjective complaints, such as diarrhoea or urgency of bowel movements, were observed on 1–4 days in the first week of treatment in four volunteers receiving ethephon, but not in control subjects. Urgency or an increased frequency of urination was observed during the course of the study in one control and five treated volunteers. In addition, loose stools, stomach cramps and/or gas, flank pain, and loss or increase of appetite were occasionally reported by some volunteers treated with ethephon. No treatment-related changes in haematology, clinical biochemistry or urine analysis were noted (Reese, 1972).

Ten male and 10 female volunteers received technical ethephon (formulation consisting of 2.5% ethephon, hydrated silica and cornstarch) at a dose of 0.5 mg/kg bw per day by oral capsule, divided over three daily dosages, for 16 days. Six males and four females were given placebo. All subjects were monitored constantly during the first 8 hours following ingestion of the test material for adverse effects or symptoms related to compound administration and once daily thereafter. Cholinesterase activity in plasma and red blood cells was measured before the start of dosing, on days 4, 8, 12 and 16 of treatment, and on days 15 and 29 of a recovery period. Haematology and clinical chemistry were performed before the treatment started, on days 8 and 16 of treatment and on day 29 of the recovery period. Urine analysis was performed on days 1, 9 and 16 of the dosing period.

No treatment-related clinical signs or changes in erythrocyte AChE values, haematology, clinical chemistry or urine analysis were observed. Plasma cholinesterase activity was significantly inhibited (54–62% of pre-dosing levels) in a reversible manner.

The NOAEL was 0.5 mg/kg bw per day, based on lack of inhibition of erythrocyte AChE activity (Weir, 1977a).

In a volunteer study, ethephon (formulation consisting of 21.6% ethephon, hydrated silica and cornstarch) was administered orally for 22 days in capsules to three males and four females at 0.17 mg/kg bw per day and to four males and three females at 0.33 mg/kg bw per day, divided into three doses, for 22 days, followed by a 14-day recovery period. Three males and three females were given placebo. All subjects were monitored constantly during the first 8 hours following ingestion of the test material for adverse effects or symptoms related to compound administration and once daily thereafter. Haematology and clinical chemistry and assessment of cholinesterase activity in plasma and red blood cells were performed before the start of dosing, on days 8, 15 and 22 of treatment and on days 8 and 14 of the recovery period. Urine analysis was performed on days 1, 9 and 16 of the dosing period.

No treatment-related clinical signs or changes in erythrocyte AChE activity, haematology, clinical chemistry or urine analysis were observed. Plasma cholinesterase activity was significantly inhibited (59–74% of pre-dosing levels). The plasma cholinesterase inhibition was not reversible during the 14-day recovery period.

The NOAEL was 0.33 mg/kg bw per day, the highest dose tested (Weir, 1977b).

Comments

Biochemical aspects

In rats, absorption of ethephon was rapid, with a T_{max} of 1.0–1.3 hours after a single oral dose of 50 mg/kg bw and 1.9–2.5 hours after a single oral dose of 1000 mg/kg bw. Peak blood concentrations at 1000 mg/kg bw were less than proportional to dose, compared with those after 50 mg/kg bw. Six days after a single dose, tissues and carcass contained at most 0.06% of the administered radioactivity. Highest concentrations were found in bone, liver, blood and kidney. Radioactivity concentrations in brain were low. Radioactivity was excreted in urine (47–60%), expired air (18–22%, mainly ethylene) and faeces (4.0–6.5%), indicating that at least 65% of the administered dose was absorbed. Excretion was largely complete within the first 24 hours after dose administration (Savage, 1990). Ethephon was mainly recovered as its monosodium and disodium salts, ethylene and, to a lesser extent, HEPA (Hardy et al., 1990; Savage, 1990; Odin-Feurtet, 2002). There were no remarkable differences in absorption and excretion between sexes and between oral dosing regimens (Savage, 1990).

Ethephon inhibits butyrylcholinesterase (cholinesterase) activity in plasma and, to a lesser extent, AChE activity in erythrocytes. Ethephon has virtually no effect on brain AChE activity in vivo. In vitro studies showed that cholinesterase in plasma of dog, human and mouse was more sensitive to ethephon inhibition than cholinesterase in plasma of rabbit, rat, chicken and guinea-pig (Haux, 2000). Mechanistic investigations indicate that ethephon inhibits cholinesterase activity by phosphorylation at Ser-198 of the esteratic site, leading to the formation of a phosphobutyryl-cholinesterase (Haux, 2000).

Toxicological data

The acute toxicity of ethephon is low (rat oral LD_{50} = 1564 mg/kg bw [Myers, 1989]; rabbit dermal LD_{50} = 983 mg/kg bw [Myers, 1983]; rat inhalation LC_{50} = 3.26 mg/L [Nachreiner & Klonne, 1989]). Ethephon was severely irritating to the skin of rabbits (Myers, 1983). No eye irritation study was required, as technical ethephon has a pH of less than 2 and is therefore assumed to be corrosive to the eye. Ethephon was not a skin sensitizer in a Magnusson and Kligman test in guinea-pigs (Griffon, 2000).

In repeated-dose oral toxicity studies with ethephon in mice, rats and dogs, the main effect was reduction of erythrocyte AChE activity.

In a 28-day study in mice administered ethephon at a dietary concentration of 0, 30, 100, 300, 1000 or 3000 ppm (equal to 0, 5.3, 18, 51, 181 and 546 mg/kg bw per day for males and 0, 6.5, 22, 69, 210 and 635 mg/kg bw per day for females, respectively), the NOAEL was 100 ppm (equal to 22 mg/kg bw per day), based on reduction of erythrocyte AChE activity observed in females at 300 ppm (equal to 69 mg/kg bw per day) (Van Miller & Troup, 1986a). In a second 28-day study in mice administered ethephon at a dietary concentration of 0, 3000, 10 000, 25 000 or 50 000 ppm (equal to 0, 530, 1800, 4500 and 10 000 mg/kg bw per day for males and 0, 630, 2200, 5900 and 15 000 mg/kg bw per day for females, respectively), no NOAEL could be identified, as reductions in erythrocyte AChE activity were observed at all doses (Van Miller & Troup, 1986b).

In a 28-day range-finding study in rats administered ethephon at a dietary concentration of 0, 625, 1250, 2500, 5000 or 10 000 ppm (equal to 0, 52, 106, 214, 431 and 831 mg/kg bw per day for males and 0, 59, 120, 251, 487 and 980 mg/kg bw per day for females, respectively), the NOAEL was 625 ppm (equal to 52 mg/kg bw per day), based on reduction of erythrocyte AChE activity observed in males at 1250 ppm (equal to 106 mg/kg bw per day) (Van Miller & Troup, 1986c). In a second 28-day range-finding study in rats administered ethephon at a dietary concentration of 0, 10 000, 25 000 or 50 000 ppm (equal to 0, 962, 2300 and 4673 mg/kg bw per day for males and 0, 996, 2488 and 4900 mg/kg bw per day for females, respectively), no NOAEL could be identified, as reduction of AChE activity in erythrocytes was observed at all doses (Van Miller & Troup, 1986d).

In a 1-year study in dogs administered ethephon at a dietary concentration of 0, 100, 300, 1000 or 2000 ppm (equal to 0, 2.8, 8.1, 27 and 54 mg/kg bw per day for males and 0, 2.6, 8.4, 30 and 50 mg/kg bw per day for females, respectively), the NOAEL was 1000 ppm (equal to 27 mg/kg bw per day), based on a lower body weight gain at 52 weeks in both sexes and low absolute and relative spleen weights in males at 2000 ppm (equal to 54 mg/kg bw per day). The effect of ethephon treatment on cholinesterase activity was not assessed in this study (Hamada, 1989).

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) (Reno & Voelker, 1977).

In a 78-week carcinogenicity study in mice administered ethephon at a dietary concentration of 0, 30, 300 or 1000 ppm (equivalent to 0, 4.5, 45 and 150 mg/kg bw per day, respectively), the NOAEL was 30 ppm (equivalent to 4.5 mg/kg bw per day), based on reduction of erythrocyte AChE activity observed at weeks 52 and 78 in females at 300 ppm (equivalent to 45 mg/kg bw per day). No treatment-related tumours were observed in mice in this study (Voss & Becci, 1985).

In a second 78-week carcinogenicity study in which mice were administered ethephon at a dietary concentration of 0, 100, 1000 or 10 000 ppm (equal to 0, 14, 139 and 1477 mg/kg bw per day for males and 0, 17, 173 and 1782 mg/kg bw per day for females, respectively), the NOAEL was 100 ppm (equal to 14 mg/kg bw per day), based on reduction of erythrocyte AChE activity in both sexes at 1000 ppm (equal to 139 mg/kg bw per day). No treatment-related tumours were observed in mice in this study (Van Miller, 1988).

The overall NOAEL for the two 78-week studies in mice was 100 ppm (equal to 14 mg/kg bw per day). The overall LOAEL was 300 ppm (equivalent to 45 mg/kg bw per day).

In a 2-year toxicity and carcinogenicity study in rats administered ethephon at a dietary concentration of 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 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 rats in this study (Reno, Serota & Voelker, 1978).

In a second 2-year toxicity and carcinogenicity study in which rats were administered ethephon at a dietary concentration of 0, 300, 3000, 10 000 or 30 000 ppm (equal to 0, 13, 131, 446 and 1416 mg/kg bw per day for males and 0, 16, 161, 543 and 1794 mg/kg bw per day for females, respectively), the NOAEL was 300 ppm (equal to 13 mg/kg bw per day), based on reduction of erythrocyte AChE activity observed in both sexes at 3000 ppm (equal to 131 mg/kg bw per day). No treatment-related tumours were observed in rats in this study (Van Miller, 1989).

The overall NOAEL for the two 2-year studies in rats was 300 ppm (equal to 13 mg/kg bw per day). The overall LOAEL was 3000 ppm (equal to 129 mg/kg bw per day).

The Meeting concluded that ethephon is not carcinogenic in mice or rats.

Ethephon was tested for genotoxicity in an adequate range of assays, both in vitro and in vivo. There was no evidence of genotoxicity in vitro (Godek, Naismith & Matthews, 1983, 1984; Barfknecht, Naismith & Matthews, 1984; Cifone, 1988; Murli, 1988; Young, 1988), except for a positive response in *Salmonella typhimurium* strain TA1535 in both the absence and presence of metabolic activation (Jagannath, 1987). There was no evidence of genotoxicity in vivo (Naismith & Matthews, 1979; Sorg, Naismith & Matthews, 1981; Howe, 2002).

Based on the weight of evidence, the Meeting concluded that ethephon is unlikely to be genotoxic in vivo.

In view of the lack of genotoxicity in vivo and the absence of carcinogenicity in mice and rats, the Meeting concluded that ethephon is unlikely to pose a carcinogenic risk to humans.

In a two-generation reproductive toxicity study in rats administered ethephon at a dietary concentration of 0, 300, 3000 or 30 000 ppm (equal to 0, 22, 220 and 2260 mg/kg bw per day for F_0 males and 0, 25, 260 and 2570 mg/kg bw per day for F_0 females, respectively; and 0, 20, 200 and 2220 mg/kg bw per day for F_{1b} males and 0, 24, 245 and 2520 mg/kg bw per day for F_{1b} females, respectively), the NOAEL for parental toxicity was 300 ppm (equal to 20 mg/kg bw per day), based on an increased incidence of loose faeces in F_{1b} males at 3000 ppm (equal to 200 mg/kg bw per day). The NOAEL for offspring toxicity was 300 ppm (equal to 22 mg/kg bw per day), based on an increased mortality in F_{1b} pups from PND 4 to PND 7 and a reduction in body weight gain during lactation in F_{2b} pups at 3000 ppm (equal to 220 mg/kg bw per day). The NOAEL for reproductive toxicity was 30 000 ppm (equal to 2220 mg/kg bw per day), the highest dose tested. The effect of ethephon treatment on cholinesterase activity was not assessed in this study (Neeper-Bradley & Tyl, 1990).

In a developmental toxicity study in rats administered ethephon by gavage at a dose of 0, 200, 600 or 1800 mg/kg bw per day, the NOAEL for maternal toxicity was 600 mg/kg bw per day, based on increased mortality, clinical signs (salivation), reduced body weight gain, and various macroscopic findings and histological changes (focal lymphoid hyperplasia of the spleen and focal parenchymal fibrosis of the liver) at 1800 mg/kg bw per day. The NOAEL for embryo and fetal toxicity was 1800 mg/kg bw per day, the highest dose tested. The effect of ethephon treatment on cholinesterase activity was not assessed in this study (Rodwell, 1980).

In a second developmental toxicity study in rats administered ethephon by gavage at a dose of 0, 125, 250 or 500 mg/kg bw per day, the NOAEL for maternal toxicity and for embryo and fetal toxicity was 500 mg/kg bw per day, the highest dose tested (Henwood, 1989b). The effect of ethephon treatment on cholinesterase activity was not assessed in this study.

In a developmental toxicity study in rabbits administered ethephon by gavage at a dose of 0, 50, 100 or 250 mg/kg bw per day, the NOAEL for maternal toxicity was 50 mg/kg bw per day, based on a body weight reduction from GD 6 to GD 11 and an increased number of resorptions at 100 mg/kg bw per day. The NOAEL for embryo and fetal toxicity was 50 mg/kg bw per day, based on a reduced number of live fetuses and reduced viability of fetuses at 100 mg/kg bw per day. The effect of ethephon treatment on cholinesterase activity was not assessed in this study (Weatherholtz, Wolfe & Durloo, 1981).

In a second developmental toxicity study in rabbits administered ethephon by gavage at a dose of 0, 62.5, 125 or 250 mg/kg bw per day, the NOAEL for maternal toxicity was 125 mg/kg bw per day, based on mortality, clinical signs of toxicity and decreased body weight at 250 mg/kg bw per day. The NOAEL for embryo and fetal toxicity was 125 mg/kg bw per day. As three does died and 14 does were killed in a moribund condition at 250 mg/kg bw per day, the number of fetuses in the high-dose group was insufficient to conclude on the effects of ethephon on prenatal development at 250 mg/kg bw per day (Henwood, 1990). The effect of ethephon treatment on cholinesterase activity was not assessed in this study.

In a pilot neurotoxicity study in rats aimed at finding the time to peak effect after a single gavage dose of ethephon of 0, 250, 500, 1000 or 2000 mg/kg bw, the maximum suppression of plasma cholinesterase activity for all groups occurred at 4–8 hours following treatment. Erythrocyte and brain AChE levels were not affected by treatment in this study (Beyrouty, 1996a).

In an acute neurotoxicity study in rats administered ethephon by gavage at a dose of 0, 500, 1000 or 2000 mg/kg bw, no NOAEL could be identified, as increased incidences of myosis were observed at all dose levels. The effect of ethephon treatment on cholinesterase activity was not assessed in this study (Beyrouty, 1996b).

In a 13-week neurotoxicity study in rats administered ethephon by gavage at a dose of 0, 75, 150 or 400 mg/kg bw per day (the high dose was decreased to 300 mg/kg bw per day during week

10/11 of treatment), the NOAEL was 75 mg/kg bw per day, based on reduction of erythrocyte AChE activity in females at 150 mg/kg bw per day (Beyrouty, 1997b).

In a 28-day neurotoxicity study in dogs administered ethephon at a dietary concentration of 0, 250 or 750 ppm (equal to 0, 6 and 14 mg/kg bw per day, respectively), the NOAEL was 250 ppm (equal to 6 mg/kg bw per day), based on reduction of AChE activity in erythrocytes at 750 ppm (equal to 14 mg/kg bw per day) (Eigenberg, 2006a).

In a 91-day neurotoxicity study in dogs administered ethephon at a dietary concentration of 0, 70, 140 or 525 ppm (equal to 0, 2, 4 and 15 mg/kg bw per day for males and 0, 2, 4 and 18 mg/kg bw per day for females, respectively), the NOAEL was 70 ppm (equal to 2 mg/kg bw per day), based on reduction of AChE activity in erythrocytes at 140 ppm (equal to 4 mg/kg bw per day) (Eigenberg, 2006b).

The Meeting noted that in the neurotoxicity studies, no clinical signs of neurotoxicity were observed, even though erythrocyte AChE activity was reduced.

No evidence for delayed neurotoxicity was observed in three studies in chickens (Weatherholtz & Shott, 1970; Fletcher, 1983; Rodgers, 2005).

Toxicological data on metabolites and/or degradates

HEPA is a significant metabolite of ethephon in rats (Odin-Feurtet, 2002) and is also the main plant metabolite. Acute and short-term toxicity and genotoxicity studies with HEPA were available. The acute oral toxicity of HEPA was low (rat LD_{50} > 2000 mg/kg bw) (Denton, 2001). HEPA did not cause inhibition of plasma cholinesterase activity in vitro (Segall et al., 1991).

In a 28-day toxicity study in rats administered HEPA by gavage at a dose of 0, 125, 350 or 1000/700 mg/kg bw per day (the highest dose was reduced from 1000 to 700 mg/kg bw per day from day 5 onwards, as a result of mortality), the NOAEL was 350 mg/kg bw per day, based on mortality, clinical signs, reduced body weight gain and feed consumption (females only), changes in urinary parameters, and various macroscopic findings and histological changes (epithelial necrosis and intraluminal inflammatory exudates in trachea) observed at 1000/700 mg/kg bw per day. The effects observed at the high dose are considered related to the gavage administration and the physicochemical properties of HEPA (Bigot, 2003b).

HEPA was negative in a gene mutation test in bacteria and in a gene mutation test and a chromosomal aberration test in mammalian cells in vitro (Ballantyne, 2002; Johnson, 2002; Whitwell, 2002).

In gavage studies in rats, the toxicity of HEPA was similar to that of ethephon. The effects observed in these studies with high doses of HEPA or ethephon are likely the result of a local gastrointestinal effect due to the physicochemical properties of these compounds and are therefore not relevant to the risk assessment. As HEPA does not reduce cholinesterase activity and as the NOAEL for HEPA in a 28-day gavage study is at least 2 orders of magnitude higher than the NOAEL of 0.5 mg/kg bw in humans that forms the basis of the ADI and ARfD (see below), HEPA is not considered to be a toxicologically relevant metabolite.

Human data

In a 28-day study in human volunteers, five males and five females received ethephon at oral (capsule) doses of approximately 1.5 mg/kg bw per day for males and 2.2 mg/kg bw per day for females, divided over three daily dosages. Three males and three females received placebo. Transient, subjective complaints, such as diarrhoea or urgency of bowel movements, were observed on 1–4 days in the first week of treatment in four volunteers receiving ethephon, but not in control subjects.

Urgency or an increased frequency of urination was observed during the course of the study in one control and five treated volunteers. In addition, loose stools, stomach cramps and/or gas, flank pain, and loss or increase of appetite were occasionally reported by some volunteers treated with ethephon. No changes in plasma and erythrocyte cholinesterase activities and no persistent side-effects were observed. No treatment-related changes in haematology, clinical biochemistry or urine analysis parameters were noted (Reese, 1972).

In a 16-day study, volunteers received ethephon orally (by capsule) at a dose of 0 or 0.5 mg/kg bw per day (divided over three daily dosages). Ten males and 10 females received ethephon, and six males and four females received placebo. No treatment-related clinical signs or changes in erythrocyte AChE values or in haematology, clinical chemistry or urine analysis parameters were observed (Weir, 1977a).

In a 22-day volunteer study using ethephon at oral (capsule) doses of 0 (three males and three females), 0.17 (three males and four females) and 0.33 mg/kg bw per day (four males and three females), no treatment-related clinical signs or changes in erythrocyte AChE activities or haematology, clinical chemistry or urine analysis parameters were observed (Weir, 1977b).

The Meeting concluded that the existing database on ethephon was adequate to characterize the potential hazards to the general population, including fetuses, infants and children.

Toxicological evaluation

The Meeting reaffirmed the ADI of 0–0.05 mg/kg bw, established on the basis of the overall NOAEL of 0.5 mg/kg bw per day in studies in humans, based on transient, subjective complaints, such as diarrhoea and urgency of bowel movements, loose stools, stomach cramps and/or gas, urgency or an increased frequency of urination, flank pain, and loss or increase of appetite, with the application of a 10-fold safety factor.

The Meeting reaffirmed the ARfD for ethephon of 0.05 mg/kg bw, established on the basis of the overall NOAEL of 0.5 mg/kg bw per day in studies in humans, based on transient, subjective complaints, such as diarrhoea and urgency of bowel movements, loose stools, stomach cramps and/or gas, urgency or an increased frequency of urination, flank pain, and loss or increase of appetite observed during the first week of treatment, with the application of a 10-fold safety factor.

Levels relevant to risk assessment of ethephon

Species	Study	Effect	NOAEL	LOAEL
Mouse	Seventy-eight-week studies of toxicity and carcinogenicity[a,b]	Toxicity	100 ppm, equal to 14 mg/kg bw per day	300 ppm, equivalent to 45 mg/kg bw per day
		Carcinogenicity	10 000 ppm, equal to 1 477 mg/kg bw per day[c]	–
Rat	Two-year studies of toxicity and carcinogenicity[a,b]	Toxicity	300 ppm, equal to 13 mg/kg bw per day	3 000 ppm, equal to 129 mg/kg bw per day
		Carcinogenicity	30 000 ppm, equal to 1 416 mg/kg bw per day[c]	–
	Two-generation study of reproductive	Reproductive toxicity	30 000 ppm, equal to 2 220 mg/kg bw per day[c]	–

Species	Study	Effect	NOAEL	LOAEL
	toxicity[a]	Parental toxicity	300 ppm, equal to 20 mg/kg bw per day	3 000 ppm, equal to 200 mg/kg bw per day
		Offspring toxicity	300 ppm, equal to 22 mg/kg bw per day	3 000 ppm, equal to 220 mg/kg bw per day
	Developmental toxicity study[d]	Maternal toxicity	600 mg/kg bw per day	1 800 mg/kg bw per day
		Embryo and fetal toxicity	1 800 mg/kg bw per day[c]	–
Rabbit	Developmental toxicity study[d]	Maternal toxicity	50 mg/kg bw per day	100 mg/kg bw per day
		Embryo and fetal toxicity	50 mg/kg bw per day	100 mg/kg bw per day
Dog	Thirteen-week study of neurotoxicity[a]	Toxicity	70 ppm, equal to 2 mg/kg bw per day	140 ppm, equivalent to 4 mg/kg bw per day
	Two-year study of toxicity[a]	Toxicity	30 ppm, equal to 0.86 mg/kg bw per day	300 ppm, equal to 7.6 mg/kg bw per day
Human	Sixteen- and 28-day studies of toxicity[b,e]	Toxicity	0.5 mg/kg bw per day	1.5 mg/kg bw per day

[a] Dietary administration.
[b] Two or more studies combined.
[c] Highest dose tested.
[d] Gavage administration.
[e] Capsule administration.

Estimate of acceptable daily intake (ADI)

0–0.05 mg/kg bw

Estimate of acute reference dose (ARfD)

0.05 mg/kg bw

Information that would be useful for the continued evaluation of the compound

Results from epidemiological, occupational health and other such observational studies of human exposure

Critical end-points for setting guidance values for exposure to ethephon

Absorption, distribution, excretion and metabolism in mammals	
Rate and extent of oral absorption	Rapid; > 65% at 50 and 1 000 mg/kg bw (rat)
Dermal absorption	No data

Distribution	Widespread distribution, highest concentrations found in bone, liver, blood and kidney; low concentrations in brain (rat)
Potential for accumulation	Low
Rate and extent of excretion	Rapid; largely complete within the first 24 h after dose administration
Metabolism in animals	Converted to its monosodium and disodium salts, ethylene and, to a lesser extent, HEPA
Toxicologically significant compounds in animals and plants	Ethephon
Acute toxicity	
Rat, LD_{50}, oral	1 564 mg/kg bw
Rabbit, LD_{50}, dermal	983 mg/kg bw
Rat, LC_{50}, inhalation	3.26 mg/L
Rabbit, dermal irritation	Severely irritating
Rabbit, ocular irritation	Assumed to be corrosive, pH < 2
Guinea-pig, dermal sensitization	Not sensitizing (maximization test)
Short-term studies of toxicity	
Target/critical effect	Reduction of erythrocyte AChE activity
Lowest relevant oral NOAEL	0.86 mg/kg bw per day (dog)
Lowest relevant dermal NOAEL	237 mg/kg bw per day (highest dose tested); severe dermal irritation at 119 mg/kg bw per day (rabbit)
Lowest relevant inhalation NOAEC	No data
Long-term studies of toxicity and carcinogenicity	
Target/critical effect	Reduction of erythrocyte AChE activity
Lowest relevant NOAEL	13 mg/kg bw per day (rat)
Carcinogenicity	Not carcinogenic in rats or mice[a]
Genotoxicity	
	Unlikely to be genotoxic in vivo[a]
Reproductive toxicity	
Target/critical effect	No reproductive effect
Lowest relevant parental NOAEL	20 mg/kg bw per day (rat)
Lowest relevant offspring NOAEL	22 mg/kg bw per day (rat)
Lowest relevant reproductive NOAEL	2220 mg/kg bw per day (highest dose tested; rat)
Developmental toxicity	
Target/critical effect	Reduced viability and number of live fetuses
Lowest relevant maternal NOAEL	50 mg/kg bw per day (rabbit)
Lowest relevant embryo/fetal NOAEL	50 mg/kg bw per day (rabbit)
Neurotoxicity	
Acute neurotoxicity LOAEL	500 mg/kg bw (rat)
Subchronic neurotoxicity NOAEL	2 mg/kg bw per day (dog)

Developmental neurotoxicity NOAEL	No data
Delayed neurotoxicity	Negative
Other toxicological studies	
Studies with HEPA	Oral LD_{50} > 2 000 mg/kg bw (rat) 28-day study: NOAEL = 350 mg/kg bw per day (rat) Negative in a gene mutation test in bacteria and in a gene mutation test and a chromosomal aberration test in mammalian cells in vitro
Human data	
	NOAEL 0.5 mg/kg bw per day. Transient, subjective clinical signs were reported in a 28-day oral (capsule) study in human volunteers, using ethephon doses of approximately 1.5–2.2 mg/kg bw per day. No effects on plasma or erythrocyte cholinesterase activities.

[a] Unlikely to pose a carcinogenic risk to humans from the diet.

Summary

	Value	Study	Safety factor
ADI	0–0.05 mg/kg bw	Sixteen-day and 28-day studies in humans	10
ARfD	0.05 mg/kg bw	Sixteen-day and 28-day studies in humans	10

References

Ballantyne B (2002). HEPA: mutation at the thymidine kinase (*tk*) locus of mouse lymphoma L5178Y cells (MLA) using the Microtitre® fluctuation technique. Unpublished report no. M-209531-01-1 from Covance Laboratories Ltd, North Yorkshire, England, United Kingdom. Submitted to WHO by Bayer CropScience.

Barfknecht TR, Naismith RW, Matthews RJ (1984). Rat hepatocyte primary culture/DNA repair test. Unpublished report no. PH 311-UC-002-84 from Pharmakon Research International, Inc., Waverly, PA, USA. Submitted to WHO by Bayer CropScience.

Bettini G, Muracchini M, Della Salda L, Preziosi R, Morini M, Guglielmini C et al. (2003). Hypertrophy of intestinal smooth muscle in cats. Res Vet Sci. 75:3–53.

Beyrouty P (1996a). A time of peak effects study of a single orally administered dose of ethephon in rats. Unpublished report no. M-188166-01-2 from Bio-Research Laboratories Ltd, Senneville, Quebec, Canada. Submitted to WHO by Bayer CropScience.

Beyrouty P (1996b). An acute study of the potential effects of a single orally administered dose of ethephon, technical grade, on behaviour and neuromorphology in rats. Unpublished report no. M-188166-01-2 from Bio-Research Laboratories Ltd, Senneville, Quebec, Canada. Submitted to WHO by Bayer CropScience.

Beyrouty P (1997a). A 2-week range-finding toxicity study of orally administered ethephon technical grade base 250 in rats. Unpublished report no. M-188213-01-1 from Bio-Research Laboratories Ltd, Senneville, Quebec, Canada. Submitted to WHO by Bayer CropScience.

Beyrouty P (1997b). A 13-week study of the potential effects of orally administered ethephon, technical grade base 250 on behavior, neurochemistry and neuromorphology in rats. Unpublished report no. M-188217-01-1 from ClinTrials BioResearch Ltd, Senneville, Quebec, Canada. Submitted to WHO by Bayer CropScience.

Bigot D (2003a). Exploratory 15-day toxicity study in the rat by gavage, code: HEPA (2-hydroxyethylphosphonic acid). Unpublished report no. M-231000-01-1 from Bayer CropScience, Sophia Antipolis, France. Submitted to WHO by Bayer CropScience.

Bigot D (2003b). 28-day toxicity study in the rat by gavage, HEPA (2-hydroxy-ethylphosphonic acid). Unpublished report no. M-233065-01-2 from Bayer CropScience, Sophia Antipolis, France. Submitted to WHO by Bayer CropScience.

Cifone MA (1988). Mutagenicity test on ethephon in the rat primary hepatocyte unscheduled DNA synthesis assay. Unpublished report no. M-187753-01-1 from Hazleton Laboratories America Inc., Kensington, MD, USA. Submitted to WHO by Bayer CropScience.

Clement C (1989). Test to evaluate the sensitizing potential in the guinea pig. Guinea-pig maximization test. Unpublished report no. 903326 from Hazleton France, L'Arbresle, France. Submitted to WHO by Bayer CropScience.

Denton SM (2001). HEPA: acute oral toxicity study in the rat. Unpublished report no. M-209737-01-1 from Covance Laboratories Ltd, North Yorkshire, England, United Kingdom. Submitted to WHO by Bayer CropScience.

Eigenberg DA (2006a). A 28-day cholinesterase inhibition study via dietary administration in the Beagle dog with ethephon base 250. Unpublished report no. M-268126-01-1 from Bayer CropScience LP Toxicology, Stilwell, KS, USA. Submitted to WHO by Bayer AG.

Eigenberg DA (2006b). A 90-day cholinesterase inhibition study via dietary administration in the Beagle dog with technical ethephon. Unpublished report no. M-276963-01-1 from Bayer CropScience LP Toxicology, Stilwell, KS, USA. Submitted to WHO by Bayer AG.

Fletcher DW (1983). 42-day neurotoxicity study with ethephon base 250 in mature white leghorn chickens. Unpublished report no. M-187671-01-1 from Bio-Life Associates, Ltd, Neillsville, WI, USA. Submitted to WHO by Bayer CropScience.

Godek EG, Naismith RW, Matthews RJ (1983). CHO/HGPRT, mammalian cell forward gene mutation assay. Unpublished report no. PH-314-UC-003-83 from Pharmakon Research International, Inc., Waverly, PA, USA. Submitted to WHO by Bayer CropScience.

Godek EG, Naismith RW, Matthews RJ (1984). CHO/HGPRT, mammalian cell forward gene mutation assay. Unpublished report no. PH-314-UC-001-84 from Pharmakon Research International, Inc., Waverly, PA, USA. Submitted to WHO by Bayer CropScience.

Griffon B (2000). Skin sensitization test in guinea-pigs (maximization method of Magnusson, B. and Kligman, A.M.). Unpublished report no. M-202329-0101 from Centre International de Toxicologie (CIT), Evreux, France. Submitted to WHO by Bayer CropScience.

Hamada NN (1989). One-year oral toxicity study in Beagle dogs with ethephon. Unpublished report no. M-187726-01-1 from Hazleton Laboratories America, Inc., Reston, VA, USA. Submitted to WHO by Bayer CropScience.

Hardy IAJ, Chem C, Marshall IR, Outram JR (1990). Plant growth regulators: ethephon. Spectroscopic identification of metabolites from a ^{14}C-ethephon ADME study in the rat. Unpublished report no. D. Ag. 1523 from Rhone-Poulenc Agriculture Ltd, Essex, England, United Kingdom. Submitted to WHO by Bayer CropScience.

Haux JE (2000). Phosphobutylcholinesterase: phosphorylation of the esteratic site of butyrylcholinesterase by ethephon [(2-choroethyl) phosphonic acid] dianion. Chem Res Toxicol. 13:646–51.

Henwood SM (1989a). 3-week dermal toxicity study with ethephon technical in rabbits. Unpublished report no. M-188011-01-1 from Hazleton Laboratories America Inc., Madison, WI, USA. Submitted to WHO by Bayer CropScience.

Henwood SM (1989b). Teratology study with ethephon technical – base 250 in rats. Unpublished report no. M-187750-01-1 from Hazleton Laboratories America Inc., Madison, WI, USA. Submitted to WHO by Bayer CropScience.

Henwood SM (1990). Teratology study with ethephon technical – base 250 in rabbits. Unpublished report no. M-187739-01-1 from Hazleton Laboratories America Inc., Madison, WI, USA. Submitted to WHO by Bayer CropScience.

Holsing GC (1969). Acute oral – mice, five compounds. Unpublished and unnumbered report (Project No. 141-197) from Hazleton Laboratories, Inc., Reston, VA, USA. Submitted to WHO by Bayer CropScience.

Howe J (2002). Ethephon measurement of unscheduled DNA synthesis in rat liver using an in vivo/in vitro procedure. Unpublished report no. M-209739-01-1 from Covance Laboratories Ltd, North Yorkshire, England, United Kingdom. Submitted to WHO by Bayer CropScience.

Jagannath DR (1987). Mutagenicity test on ethephon base 250 in the Ames *Salmonella*/microsome reverse mutation assay. Unpublished report no. M-187742-01-1 from Hazleton Laboratories America Inc., Kensington, MD, USA. Submitted to WHO by Bayer CropScience.

Johnson M (2002). HEPA reverse mutation in five histidine-requiring strains of *Salmonella typhimurium*. Unpublished report no. M-209742-01-1 from Covance Laboratories Ltd, North Yorkshire, England, United Kingdom. Submitted to WHO by Bayer CropScience.

Liu D-H, Huang X, Guo X, Meng X-M, Wu Y-S, Lu H-L et al. (2014). Voltage dependent potassium channel remodeling in murine intestinal smooth muscle hypertrophy induced by partial obstruction. PLoS One. 9(2):e86109. doi:10.1371/journal.pone.0086109.

Murakami Y, Okazaki Y, Okayama S, Fujihara S, Noto T, Nakatsuji S et al. (2010). Goblet cell hyperplasia and muscular layer thickening in the small intestine of a cynomolgus monkey. J Toxicol Pathol. 23:85–9.

Murli H (1988). Mutagenicity test on ethephon base 250 in an in vitro cytogenetic assay measuring chromosomal aberration frequencies in Chinese hamster ovary (CHO) cells. Unpublished report no. M-187762-01-1 from Hazleton Laboratories America Inc., Kensington, MD, USA. Submitted to WHO by Bayer CropScience.

Myers RC (1983). Ethephon base 250, acute percutaneous toxicity study. Unpublished report no. 46-122 from Bushy Run Research Center, Pittsburgh, PA, USA. Submitted to WHO by Bayer CropScience.

Myers RC (1989). Ethephon base 250 – Acute peroral toxicity study. Unpublished report no. M-187938-01-1 from Bushy Run Research Center, Pittsburgh, PA, USA. Submitted to WHO by Bayer CropScience.

Nachreiner DJ, Klonne DR (1989). Ethephon base 250, acute aerosol inhalation toxicity test in rats. Unpublished report no. 52-580 from Bushy Run Research Center, Pittsburgh, PA, USA. Submitted to WHO by Bayer CropScience.

Naismith RW, Matthews RJ (1979). Dominant lethal study. Unpublished report no. 56375 from Pharmakon Laboratories, Harrisburg, PA, USA. Submitted to WHO by Bayer CropScience.

Neeper-Bradley TL, Tyl RW (1990). Two-generation reproduction study in CD albino rats exposed to ethephon by dietary inclusion. Unpublished report no. M-187771-01-1 from Bushy Run Research Center, Pittsburgh, PA, USA. Submitted to WHO by Bayer CropScience.

Odin-Feurtet M (2002). [^{14}C]-Ethephon: tissue metabolism study in the rat. Unpublished report no. M-210828-01-1 from Bayer CropScience, Sophia Antipolis, France. Submitted to WHO by Bayer AG.

Reese WH (1971). Preliminary dose range study in two human volunteers. Final report – Phase I. Unpublished report no. M-187795-01-1 from Bionetics Research Laboratories Inc., Bethesda, MD, USA. Submitted to WHO by Bayer CropScience.

Reese WH (1972). Final report – Phase II. Evaluation of ethrel in human volunteers. Unpublished report no. M-187790-01-1 from Bionetics Research Laboratories Inc., Bethesda, MD, USA. Submitted to WHO by Bayer CropScience.

Reno FE, Voelker RW (1977). A two-year study in dogs – Ethrel – Final report. Unpublished report no. M-187724-01-1 from Hazleton Laboratories America Inc., Reston, VA, USA. Submitted to WHO by Bayer CropScience.

Reno FE, Serota DG, Voelker RW (1978). 104-week chronic toxicity study in rats. Unpublished and unnumbered report (Project No. 141-263) from Hazleton Laboratories America Inc., Reston, VA, USA. Submitted to WHO by Bayer CropScience.

Rodgers MH (2005). Ethephon assessment to determine acute delayed neurotoxicity to the domestic hen. Unpublished report no. M-247629-01-1 from Huntingdon Life Sciences Ltd, Cambridgeshire, England, United Kingdom. Submitted to WHO by Bayer CropScience.

Rodwell DE (1980). Teratology study in rats. Unpublished report no. 369-042 from International Research and Development Corporation, Mattawan, MI, USA. Submitted to WHO by Bayer CropScience.

Rush RE (1989). Dermal sensitization study in guinea-pigs with base A-250. Unpublished and unnumbered report (Study No. SLS 3147.44) from Springborn Laboratories, Inc., Mammalian Toxicology Division, Spencerville, OH, USA. Submitted to WHO by Bayer CropScience.

Savage EA (1990). ^{14}C-Ethephon: absorption, distribution, metabolism, and excretion in the rat. Unpublished report no. M-206918-01-1 from Hazleton UK, North Yorkshire, England, United Kingdom. Submitted to WHO by Bayer CropScience.

Segall Y, Grendell RL, Toia RF, Casida JE (1991). Composition of technical ethephon [(2-chloroethyl)phosphonic acid] and some analogues relative to their reactivity and biological activity. J Agric Food Chem. 39:380–5.

Sorg RM, Naismith RW, Matthews RJ (1981). Genetic toxicology micronucleus test (MNT). Unpublished report no. PH 309A-US-001-81 from Pharmakon Laboratories, Waverly, PA, USA. Submitted to WHO by Bayer CropScience.

Stephen W, Stanovick RP (1971). Identification of ^{14}C-ethephon metabolites in the dog. Unpublished and unnumbered report from Hazleton Laboratories America Inc., Reston, VA, USA. Submitted to WHO by Bayer CropScience.

Stephen W, Walker D (1971). Metabolism of ^{14}C-ethephon in the dog. Unpublished and unnumbered report from Hazleton Laboratories America Inc., Reston, VA, USA. Submitted to WHO by Bayer CropScience.

Van Miller JP (1988). Lifetime dietary oncogenicity study with ethephon in albino mice. Unpublished report no. M-187730-01-1 from Bushy Run Research Center, Pittsburgh, PA, USA. Submitted to WHO by Bayer CropScience.

Van Miller JP (1989). Lifetime dietary combined chronic toxicity and oncogenicity study with ethephon in albino rats. Unpublished document no. M-187711-01-1 by Bushy Run Research Center, Pittsburgh, PA, USA. Submitted to WHO by Bayer CropScience.

Van Miller JP, Troup CM (1986a). Twenty-eight day dietary toxicity study with ethephon in mice. Unpublished report no. M-187702-01-1 from Bushy Run Research Center, Pittsburgh, PA, USA. Submitted to WHO by Bayer CropScience.

Van Miller JP, Troup CM (1986b). Twenty-eight day dietary toxicity study with ethephon in mice – Study II. Unpublished report no. M-187703-01-1 from Bushy Run Research Center, Pittsburgh, PA, USA. Submitted to WHO by Bayer CropScience.

Van Miller JP, Troup CM (1986c). Twenty eight day dietary toxicity study with ethephon in rats. Unpublished report no. M-187685-01-1 from Bushy Run Research Center, Pittsburgh, PA, USA. Submitted to WHO by Bayer CropScience.

Van Miller JP, Troup CM (1986d). Twenty-eight day dietary toxicity study with ethephon in rats – Study No. II. Unpublished report no. M-187683-01-1 from Bushy Run Research Center, Pittsburgh, PA, USA. Submitted to WHO by Bayer CropScience.

Voss KA, Becci PJ (1985). 78-week oncogenic evaluation in Swiss albino mice. Unpublished and unnumbered report (Study No. 5754) from Food and Drug Research Laboratories, Inc., Waverly, NY, USA. Submitted to WHO by Bayer CropScience.

Weatherholtz WM (1980). Acute oral toxicity study in rabbits. Unpublished and unnumbered report (Project No. 400-630) from Hazleton Laboratories America Inc., Reston, VA, USA. Submitted to WHO by Bayer CropScience.

Weatherholtz WM, Shott LD (1970). Neurotoxicity study – hens, ethrel, formulated, ethrel, technical. Unpublished and unnumbered report (Project No. 141-218) from Hazleton Laboratories America Inc., Reston, VA, USA. Submitted to WHO by Bayer CropScience.

Weatherholtz WM, Wolfe GW, Durloo RS (1981). Teratology study in rabbits, technical ethephon. Unpublished and unnumbered report (Project No. 400-635) from Hazleton Laboratories America Inc., Reston, VA, USA. Submitted to WHO by Bayer CropScience.

Weir RJ (1977a). Evaluation of ethephon in human volunteers – Final report. Unpublished report no. M-187792-01-1 from Litton Bionetics, Inc., Frederick, MD, USA. Submitted WHO by Bayer CropScience.

Weir RJ (1977b). Evaluation of ethephon in human volunteers – Final report. Unpublished report no. M-187794-01-1 from Litton Bionetics, Inc., Frederick, MD, USA. Submitted WHO by Bayer CropScience.

Whitwell J (2002) HEPA: induction of chromosome aberrations in cultured human peripheral blood lymphocytes. Unpublished report no. M-209529-01-1 from Covance Laboratories Ltd, North Yorkshire, England, United Kingdom. Submitted to WHO by Bayer CropScience.

Young RR (1988). Mutagenicity test on ethephon base 250 in the CHO/HGPRT forward mutation assay. Unpublished report no. M-187751-01-1 from Hazleton Laboratories America Inc., Kensington, MD, USA. Submitted to WHO by Bayer CropScience.

Zhang N, Casida JE (2002). Novel irreversible butyrylcholinesterase inhibitors: 2-chloro-1-(substituted-phenyl)ethylphosphonic acids. Bioorg Med Chem. 10:1281–90.