**CALIFORNIA ENVIRONMENTAL PROTECTION AGENCY**
**DEPARTMENT OF PESTICIDE REGULATION**
**HUMAN HEALTH ASSESSMENT BRANCH**

# SUMMARY OF TOXICOLOGY DATA

Ethephon
(2-Chloroethylphosphonic acid)

**Chemical Code #** 1626**, Document Processing Number (DPN)** 300
**SB 950 #** 261
July 29, 1986
Revised: 6/10/87, 2/9/90, 9/20/91, 11/24/97, and Jan. 13, 2016

## DATA GAP STATUS

| | |
|---|---|
| **Chronic toxicity, rat:** | No data gap, no adverse effect |
| **Chronic toxicity, dog:** | No data gap, no adverse effect |
| **Oncogenicity, rat:** | No data gap, no adverse effect |
| **Oncogenicity, mouse:** | No data gap, no adverse effect |
| **Reproduction, rat:** | No data gap, no adverse effect |
| **Developmental toxicity, rat:** | No data gap, no adverse effect |
| **Developmental toxicity, rabbit:** | No data gap, no adverse effect |
| **Gene mutation:** | No data gap, possible adverse effect |
| **Chromosome effects:** | No data gap, possible adverse effect |
| **DNA damage:** | No data gap, possible adverse effect |
| **Neurotoxicity:** | No data gap, no adverse effect |

Toxicology one-liners are attached.

All record numbers for the above study types through 286998 (Document No. 300-0351) were examined. This includes all relevant studies indexed by DPR as of 10/22/15.

In the 1-liners below:
indicates an acceptable study.
**Bold face** indicates a possible adverse effect.
## indicates a study on file but not yet reviewed.

File name: t20160107
Revised by [redacted] and [redacted], Jan. 13, 2016

NOTE: The following symbols may be used in the Table of Contents which follows:
** = data adequately address FIFRA requirement
† = study(ies) flagged as “possible adverse effect”
N/A = study type not currently required

This record contains summaries of studies. Individual worksheets may be useful for detailed assessment.

## Table of Contents

## METABOLISM AND PHARMACOKINETICS **

** 101; 106; 95536; 98088; "14C-Ethephon: Absorption, Distribution, Metabolism and Excretion in the Rat" (Hazleton UK, North Yorkshire, UK, Lab. Report No. 6525-68/103, 11/7/90); 851; Ethephon [2-chloro(U-14C)ethyl phosphonic acid] (46 mCi/mmol, >96% purity); single oral (50, 1000 mg/kg), i.v. (50 mg/kg) to 5 rats/sex/dose; multiple oral dosing: 50 mg/kg to 5 rats/sex qd @ 14 days; rapid absorption after oral administration; regardless of sex or dose regimen, excretion of radioactivity was essentially completed by 24 hrs post-dose resulting in low tissue residues at 120 hr; 85.8 - 90.7% of total radioactivity recovered after dosing; renal is primary route of excretion (47.4 - 71.3%); expired air (8.6 - 21.5%) and fecal route (0.89 - 6.49%) accounted for a smaller amount of radioactivity recovered; highest levels of radioactivity located in bone, and organs of metabolism and elimination (liver, kidney, and lungs); at least 4 radioactive regions resolved by TLC in urine and fecal extracts; the major component (U5+ETH+U7, Rf = 0.40) co-chromatographed with ethephon and accounted for 50% of the administered dose; study originally reviewed as unacceptable but possibly upgradeable with submission of analyses of dosing formulations for actual concentrations; (Leung, 1/31/91); study re-reviewed with submitted data regarding the actual dosage of the test article administered; **acceptable**; (upgraded, [redacted] 9/4/91).

00300-0325 227382 Odin-Fuertet, M., "Ethephon: Tissue metabolism study in the rat," Bayer CropScience, Sophia Antipolis, France, Sept. 2, 2002. Laboratory Study # SA 01411. This was a limited-scope metabolic disposition study using male Wistar rats dosed once by gavage with lactate-buffered (pH 3-4) saline solution targeting 50 mg/kg of U-$^{14}$C-labeled ethephon. Investigators reported percent of administered radioactivity in whole blood, plasma, liver, and kidney 1 hr after dosing. Results were 0.679% for whole blood, 0.495% for plasma, 1.123% for liver, and 1.792% for kidney. Aqueous kidney extracts upon HPLC separation and radiodetection were composed of 87% ethephon and 13% 2-hydroxyethephon (HEPA). Liver aqueous extracts contained 58% ethephon, 37% HEPA, with about 5% of label in a broad peak intermediate between the two identified peaks. Useful supplementary information. [redacted], Jan. 11, 2016.

## 300-0003 987999 "Preliminary metabolic studies on Ethrel 2-chloroethylphosphonic acid-1,2-$^{14}$C (ethephon) in rats - final report," 02/01/1971. This study has not been reviewed.

## 300-0003 988000 “Radiotracer Ethrel metabolism study, Phase I (2-chloroethyl) phosphonic acid-1,2-$^{14}$C in rats,” Hazleton Laboratories Inc. Falls Church, VA, 02/01/1971. This study has not been reviewed.

## 300-0003 988001 “Radiotracer Ethrel metabolism study, Phase II (2-chloroethyl) phosphonic acid-1,2-$^{14}$C in rats,” Hazelton Laboratories Inc., Falls Church, VA, 05/01/1971. This study has not been reviewed.

## GUIDELINE ACUTE STUDIES ON ACTIVE INGREDIENT

### Acute oral toxicity, rat **

**300-134; 143285; Acute Oral Toxicity Study; 811; Rat; Stillmeadow, Inc. Sugar Land, TX; Study No. 1430-94; 1/9/95; Ethephon Concentrate (GX-409006); 5 animals/sex/group; Doses: 1000 (F only), 2000, 3500, 5050 mg/kg; Mortality: 1000 (F:0/5), 2000 (M:1/5, F:3/5), 3500 (M:2/5, F:5/5), 5050 (M/F:5/5); Clinical Observations: decreased activity, diarrhea, emaciation, hypersensitivity, nasal discharge, ocular discharge, piloerection, polyuria, ptosis, red urine, respiratory gurgle, salivation; Necropsy: stomach filled with clear brown liquid or brown slurry; LD50 (95% confidence limits): (M) 3122 (1925 to 5063) mg/kg, (F) 1886 (1428 to 2491) mg/kg; Toxicity Category III; Study acceptable. ([redacted], 3/27/96)

### Acute dermal toxicity **

**300-134; 143286; Acute Dermal Toxicity Study; 812; Rabbit; Stillmeadow, Inc. Sugar Land, TX; Study No. 1431-94; 1/9/95; Ethephon Concentrate (GX-409006); 5 animals/sex/group; Doses: 1000, 2000, 5050 mg/kg, 24 hour exposure, occlusive wrap; Mortality: 1000 (M/F:0/5), 2020 (M:3/5, F:2/5), 5050 (M/F:5/5); Clinical Observations: decreased activity, ataxia, congestion, constricted pupils, decreased defecation, diarrhea, exposed nictitating membrane, hunching, lacrimation, muscle tremors, nasal discharge, piloerection, polyuria, tachycardia, bluish iris, body tremors, cyanosis, hypothermia, red urine, respiratory gurgle, salivation; Necropsy: fluid in the pleural cavity, discoloration of intestines, kidneys, mesentery, liver, lungs, pancreas, and stomach, swollen kidneys and lungs; LD50 (95% confidence limits): (M) 1913 (1469 to 2492) mg/kg, (F) 2181 (1522 to 3125) mg/kg; Toxicity Category II; Study acceptable. ([redacted], 3/27/96)

300-0076 55413 “Percutaneous toxicity with rabbits - Prep AXL-1317,” Union Carbide Corp. Bushy Run Research Center, Export, PA, 4/1/1992. This was a 24-hour dermal exposure to intact, closely clipped skin, designed to derive a dermal $LD_{50}$. Estimated $LD_{50's}$ were 1620 mg/kg in male and 1870 in females, with clinical signs similar to those reported in Record No. 143286 above in rats (largely indicative of cholinergic symptoms. As there is an accepted study in this category with responses similar to the present study, this study was not further reviewed by DPR. [redacted], Jan. 7, 2016.

### Acute inhalation toxicity, rat **

**300-134; 143287; Acute Inhalation Toxicity Study; 813; Stillmeadow, Inc. Sugar Land, TX; Study No.1432-94; 1/4/95; Ethephon Concentrate (GX-409006); 5 animals/sex; Exposure Concentration (analytical): 2.28 mg/l, mean MMAD (GSD): 1.87 (2.5) µm, 4 hour, whole body exposure; No mortality; Clinical Observations: piloerection, activity decrease, ptosis, nasal discharge, salivation, polyuria; Necropsy: lungs mottled red and slightly swollen; LC50 (M/F) > 2.28 mg/l; Toxicity Category III; Study acceptable. ([redacted], 3/27/96)

### Primary eye irritation, rabbit ** † (based on end-use product)

300-331 237595; Primary Eye Irritation Study; 814; Rabbit; Product Safety Laboratories, Dayton, NJ; PSL study no. 19642; 11/14/06; Ethephon 3#, Lot #06-104-01, PSL Reference No. 060420-6H, light amber liquid, (27% Ethephon); 3 animals (2M, 1F); Dose: 0.1 ml into conjunctival sac of the right eye; Clinical observations: Corneal opacity: score- 4 in one animal at 1 hour; score- 3 in 1/3 at 1 hour in 2/3 at 24 hours; score-2 lasting in one animal till 21 days (study termination day), in one animal till 17 days; score-1 lasting in one animal till 21 days; Iritis: score- 2 in 1/3 at 1 and 24 hours; score 1 lasting in one animal till 21 days; Conjunctivitis: (redness) persisting till 21 days; (chemosis): persisting till 10 days; Toxicity Category I; Study acceptable ([redacted], 3/3/08*)*.

### Primary dermal irritation **

**300-134; 143288; Primary Dermal Irritation Study; 815; Rabbits; Stillmeadow, Inc. Sugar Land, TX; Study No. 1433-94; 1/9/95; Ethephon Concentrate (GX-409006); 6 animals; Dose: 0.5 ml/site, one site/animal, 4 hour exposure, semi-occlusive wrap; Observations: erythema-grades 2 (2/6) and 1 (3/6), grades 3 (2/6) and 2 (2/6) at 48 hours, grades 3 (1/6) and 2 (3/6) at 72 hours, grades 3 (2/6), 2 (2/6) and 1 (2/6) at 4 days, gradually diminishing to grades 2 (1/6) and 1 (1/6) at 11 days and grade 1 (1/6) at 14 days, edema-grade 1 (2/6) at 24 hours, grade 1 (3/6) at 4 days, persisting as grade 1 (1/6) through 11 days; Toxicity Category III; Study acceptable. ([redacted], 3/27/96)

### Dermal sensitization ** (based on end-use products)

Although one of the studies following indicates a positive response, that study had a flawed design and un-interpretable responses. The suggested positive response that study does not overrule the designation as a non-sensitizer, based on acceptable studies ([redacted], 1/7/16).

**300-0336 237600; Skin Sensitization Study; 816; Guinea Pigs; Product Safety Laboratories (PSL), Dayton, New Jersey; PSL study no. 19644; 11/14/06; Ethephon 3#, Lot #06-104-01, PSL Reference No. 060420-6H, light amber liquid, (27% Ethephon); Guinea Pigs 20 (M) test chemical induced plus 10 (M) naïve controls; Method of Buehler; Dermal application was achieved by placing 0.4 ml of test article (100%) for the induction phase onto the exposed skin via 25 mm Hilltop chamber with occlusive patch; 6 hours per exposure; once each week for three weeks; Twenty seven days after the first induction dose, the challenge phase consisted of a single 0.4 ml dose of test article (50% in distilled water) applied to a second exposed area for a 6 hour exposure; scoring for irritation was performed at 24 and 48 hours post application; No positive scores in the study group; naïve control group exhibited no positive scores; positive control group showed sensitization reaction in 7/10 animals. Body weight gain was reported in all test animals at the end of study; Toxicity Category: Not a Sensitizer; Study acceptable. ([redacted], 3/3/08).

**300-302; 168240; “A Dermal Sensitization Study in Guinea Pigs with EXP 31648A, Modified Buehler Design”; (G.A. Douds; Springborn Laboratories, Inc. (SLI), Spencerville, OH; Study No. 3147.262; 8/13/98); The skin of 5 Hartley guinea pigs/sex was treated with 0.3 ml of 75% (w/v) EXP 31648A in deionized water topically applied for 6 hours (1$^{st}$ induction dose) or 100% test material (2$^{nd}$ and 3$^{rd}$ induction doses), once per week for 3 weeks in the induction phase. After a two week interlude, the skin of the treated animals and 10 naïve controls was treated with 0.3 ml of the 75% (w/v) EXP 31648A in deionized water. By the third induction dose, 7 of the treated animals exhibited an irritation score of 1 or 2 at 24 hours post application. In the challenge, none of the animals demonstrated a positive response at either 24 or 48 hours post-application. The mean scores for severity of response for the treated and control animals were 0.3 and 0.3 and 0.3 and 0.1 at 24 and 48 hours post-application, respectively. The test material

is not a dermal sensitizer in the modified Buehler test. The positive control is functional. **Study acceptable**. ([redacted], 4/16/03) (Note: the above contained 51% ethephon and 6% cyclanilide ).

**00300-0076 055414 Myers, R. C. and S. M. Christopher, "Prep AXF-1317: Dermal sensitization study in the guinea pig," Union Carbide, Bushy Run Research Center, Feb. 4, 1985. Project Report 48-5. Test article was reported to be 54.9% active ingredient. A preliminary test found that a 25% dilution in 0.25% aqueous methyl cellulose elicited slight patchy erythema, and was the basis of dose selection. In the primary study, groups of 5 Hartley guinea pigs/sex were given three weekly dermal induction treatments of 6 hours each, followed by challenge 2 weeks later. Treatments were a uniform 0.3 ml volume per treatment. Negative controls received 0.25% aqueous methyl cellulose. A 10% dilution of Prep AXF-1317 was used for induction and challenge treatments. Positive control (2,4-dinitro-1-chlorobenzene or DNCB) was administered at 0.5% strength for induction, and 0.1% for challenge. Negative control and Prep AXF-1317 animals showed no skin responses in either induction or in challenge phases. Positive control elicited progressively increasing skin responses during the induction phase, with 7 of 10 animals showing signs of necrosis following the third induction dose. All DNCB animals showed positive responses at 24 hours and at 48 hours, with moderate erythema being most commonly reported. Thus Prep AXF-1317 is negative for sensitization in this test system. Study is acceptable. [redacted], Jan. 5, 2016.

**00300 0134 143289** Kuhn, J. O., "Ethephon (Final Report): Dermal sensitization study in guinea pigs," Stillmeadow Inc., Jan. 9, 1995. Laboratory Study No. 1434-94. This study used 5 animals/sex in naïve and in ethephon-induced groups. Ethephon was the concentrate (GX-409006), at 59% purity of the active ingredient. All treatment volumes were 0.4 ml, diluted (where indicated) in deionized water. Ethephon concentrations during day 1 and day 8 induction treatments were undiluted, with a reduction for the day 15 induction to 50% dilution. Day 29 challenge was a 40% solution. Due to an ambiguous response at the day 29 challenge treatment, a second challenge was undertaken at 50% solution to previously untreated skin at day 36. There was no concurrent positive control group. A range-finding study had found that 100% test article elicited a strong (grade 3) erythema response (with or without edema). That range-finding study also found moderate erythema in one of four animals at 50% dilution. It is thus unclear why the investigator did not dilute the test article for the primary study. On the day 29 challenge with the test article, 8/10 ethephon animals showed a response (most commonly of "very faint" degree). Upon the second challenge, 10/10 animals displayed strong erythema. Naïve controls showed no response to the day 29 challenge with 40% concentration, but all controls responded to the second challenge at 50% concentration, with a median response of "moderate" grade. Both the investigator and the DPR reviewer (Bireley, 1996) considered this study to represent a "positive" sensitization response (see DPR review enclosed in the volume). Although nominally indicative of a sensitization response, this study is unacceptable due to the use of dose levels in induction and challenge phases which were moderately to markedly irritating, to failure to include a positive control, and due to the unexplained and atypical responses of both ethephon-treated and naïve animals upon challenge. This summary, without a new worksheet, is by [redacted], Jan. 7, 2016.

## SUBCHRONIC STUDIES

### Rat Subchronic Oral Toxicity

300-0017 987980 Holsing, G. C. (submitter), "Three-Month Dietary Administration - Albino Rats: Amchem 68-250 (Ethrel)," Hazelton Laboratories Inc., Falls Church, VA, 11/25/69. This is a 14-pg report with only 1 table, and not suitable for a DPR worksheet. The study pre-dated

GLP guidelines, and is supplementary by nature. Fifteen CD rats/sex/group were dosed in diet for 3 months at 0, 200, 1000, or 7500 ppm Amchem 68-250 (39.5% ethephon). Investigators reported no treatment effects in body weight, food consumption, clinical signs, hematology, clinical chemistry, or pathology. Dose-related inhibition of RBC and plasma cholinesterase (ChE) was observed at 1000 and 7500 ppm, respectively by sex as follows: RBC (M) averaging about 15% and 50%; RBC (F) about 20% and 55%; plasma (M) 19% at 4 weeks (unaffected at 8 wk and 13 wk assays) at 1000 ppm, and 22-45% inhibition at 7500 ppm; plasma (F) 37-57% at 1000 ppm, and 60-76% at 7500 ppm. Brain ChE was stated to be unaffected. There were no consistent ChE responses at 200 ppm. ChE inhibition is a "possible adverse effect". [redacted], Dec. 4, 2015.

### Mouse Subchronic Dietary Toxicity

52093-0042 145937 "A 6-week oral toxicity study of RPA 90946 in the mouse via dietary administration," Bio/Dynamics Inc. East Millstone, NJ, 04/01/1992. This study is not of a type required under FIFRA, and has not been reviewed by DPR ([redacted], Jan. 7, 2016).

### Dermal toxicity, 21/28-day or 90-day: †

300-0017 987976 Report by Stiehl; histopathology by W. M. Busey, "Three-week repeated dermal application - rabbits: Amchem 68-250 (Ethrel)," Hazelton Laboratories Inc. Falls Church, VA, 05/23/1969. Adult NZW rabbits, 5/sex (control) or 10/sex (300 or 600 mg/kg/day), were dosed for 5 days/week for 3 weeks, each exposure for 6-8 hours. Application was to the shaved abdominal skin. Test sites for about half of the rabbits per sex and group were abraded. Treatment sites were covered by gauze, with collars to prevent site disturbance. In-life parameters included clinical signs, body weight, and test site irritation. Rabbits were terminated 5 days after the last dose, following blood sampling for limited hematology and urinalysis of one-half of the animals. All animals were necropsied, and one half of rabbits per dose were examined microscopically for liver, kidneys, gross lesions, and dermal test site. This study pre-dated modern GLP guidelines, and is considered to provide useful supplementary data. There were no treatment-related deaths or changes in body weight. Hematology was uneventful. Urinalysis showed no definitive effects, although qualitative protein level was elevated in 4/10 high dose rabbits. Kidney and liver showed no pathology. There were several treatment site skin findings which showed dose-response over the two treatment groups. Acanthosis and sub-epidermal fibrosis were increased, dose-related, in treated rabbits. Hyperkeratosis was equally elevated in both treated groups. Ulceration was observed in skin of 2/5 high dose males. Loss of skin adnexa (such as hair follicles) was commonly observed both treated groups. Skin abrasion had no apparent effect. Although this study did not show a NOEL and did not meet current design and conduct standards, it provides confirmation that tested dose levels elicit skin lesions which persist for at least 5 days after cessation of dosing. [redacted], Dec. 4, 2015.

## CHRONIC STUDIES

### Combined (chronic and oncogenicity), rat **

** 300-091 074763, "Lifetime Dietary Combined Chronic Toxicity and Oncogenicity Study with Ethephon in Albino Rats," (Bushy Run Research Center, Laboratory Project I.D. 51-501, May 16, 1989). Ethephon from various lots with purity ranging from 70.6% to 72.1% was administered in the feed at concentrations of 0, 300, 3000, 10000, or 30000 ppm to groups of 30 and 50 Sprague-Dawley CD® rats/sex/treatment rate for 97 (males) and 104 (females) weeks. No adverse effect. Systemic NOEL = 3000 ppm (Dose-related decrease in urine pH at $\geq$ 10000 ppm; decreased body weight gain at 30000 ppm). ChE NOEL = 300 ppm (plasma and RBC cholinesterase inhibition at $\geq$ 3000 ppm). Ethephon was not oncogenic in this study. ACCEPTABLE. ([redacted] & [redacted], 11/21/89).

300-064 037712, "Two-year Dietary Administration Rats, Ethrel Final Report," (Hazleton , Report No. 141-205, 6/6/72). Ethephon 39.5%; fed to 60/sex/control group at 0 and 30/sex/group at 100, 300 or 1000/12,500 ppm over two years; NOEL: 100 ppm (ChE). Sacrifices of 10/sex/control and 5/sex/test group at 6 and 12 months. High dose at 1000 ppm, weeks 0-31, at 7500 ppm, weeks 32-36, at 10,000 ppm, weeks 37-41 and at 12,500 ppm, weeks 42-103 - no explanation. Clinical observations suggest intercurrent respiratory disease. No chronic effect related to test article; UNACCEPTABLE (incomplete description of test article, no justification of doses and why changed high dose 4 times, no analysis of diet, historical control data, or individual weights, no food intake, time to death, histopathology on all survivors at termination, inadequate number of animals at start, poor accountability of test animals. ([redacted], 5/8/86).

EPA 1-liner: SUPPLEMENTARY. Sys NOEL = 100 ppm, ChE NOEL = 100 ppm.

300-018 987986 (1971, Hazleton) Pfeifer, 7/11/85. Six-month progress report for 300-064 037712.

300-003 046983. This document contains a brief summary of the six month progress report of a two year dietary study (probably the study at 300-064 037712) in rat using exposure levels of 100, 300, or 1000 ppm. No adverse effect was reported. No worksheet was done ([redacted], 8/8/91).

300-064 037713, "104-Week Chronic Toxicity Study in Rats, Ethrel," (Hazleton, Report No. 141-263, 8/4/78). Ethephon source A 75.6%, source B 73.6%; 55/sex/group were fed 0, 30, 300 or 3000 ppm in the diet for two years from source A and a separate group of 30/sex from source B were fed 300 ppm; NOEL: 30 ppm (ChE); 3000 ppm (histopathology); No oncogenicity or chronic effect attributable to the test article. UNACCEPTABLE. Need justification of doses, composition of other 25% of test article; analysis of diet over test period; husbandry conditions, individual clinical observations; individual body weights and food consumption. Hematology and clinical chemistry at week 13, 26, 53, 78 and 104 on 5/sex/group, same animals where possible. Plasma, RBC and brain cholinesterase were measured. Bone marrow cellularity varied but was comparable between control and test groups. Upgradeable. ([redacted], 5/8/86).

300-067 037716,
300-068 037722, Supplemental to 300-064 037713.
EPA 1-liner: MINIMUM. ChE NOEL = 30 ppm; histopathology NOEL = 3000 ppm; sys NOEL (body weight) = 300 ppm.

300-052 027146. This document contains a summary of 300-064 037713. No worksheet was done ([redacted], 8/7/91).

300-039 033587, "Long Term Toxicity and Carcinogenicity of Ethephon Formulation of 75% Technical Ethephon to Rats," (No date or lab given). Very brief summary with unspecified number of animals given up to 3000 ppm in the diet with no reported adverse effects. ([redacted], 7/5/85).

300-015 047068. This document contains a brief summary of "long term" study (probably the study at 300-064 037713) in rat at "levels up to 3000 ppm." No adverse effect was reported. No worksheet was done ([redacted], 8/7/91).

### Chronic, dog **

** 300-090 074653, "One Year Oral Toxicity in Dogs," (Hazleton Laboratories America Inc., HLA study no. 400-722, 5/30/89). Ethephon®, purity 71.4%, administered in the feed at concentrations of 0, 100, 300, 1000, or 2000 ppm and fed to 5 Beagle dog/sex/group for 1 year. No adverse effect. Spleen weight is reduced for the high dose groups, NOEL = 1000 ppm. NOAEL ≥ 2000 ppm (HTD). ACCEPTABLE. ([redacted] & [redacted], 11/21/89).

**300-061 037585**, "Two-year Dietary Feeding - Dogs Ethrel (39.5% AI)," (Hazleton, Report No. 141-206, 5/31/72). Ethephon, 39.5% Ethrel; 4/sex/group were fed 0, 50, 75, 100 or 250/7500/5000/6000 ppm in the diet over two years with 50 ppm being comparable to 1.5-3 mg/kg bw/day; NOEL: cannot be determined for chronic effects because the 100 and 75 ppm groups were terminated after 16 weeks; NOEL < 50 ppm (ChE); decreased cellularity of bone marrow and thickening of the duodenal wall at high dose; UNACCEPTABLE. Not Upgradeable. Deficiencies: Problem with dose selection resulting in substantial changes over the 2 years; inadequate histopathology in terms of tissues taken and findings reported; poor randomization at start in terms of clinical chemistry, especially cholinesterase; justification for using test article at 39.5% - need description of remainder of material; no analysis of diet; no age of dogs is given; no individual clinical observations. ([redacted], 5/8/86).
NOTE: The above study was considered by the original Medical Toxicology Branch reviewers to indicate "possible adverse effects" due to "decreased cellularity of bone marrow and thickening of the duodenal wall at high dose," which occurred in high dose dogs. This high dose level varied from 250 to 7500 ppm, making this study uninterpretable for dose-response analysis. The later (1989) study supersedes the present study, and no overall "adverse" effect is attributed to this study type. [redacted], 11/25/15.

EPA 1-liner: SUPPLEMENTARY. ChE NOEL <50 ppm; sys NOEL (body weight) 50ppm.

300-017 987987. (1971, Hazleton). One year interim report for 300-061 037585. [redacted], 7/8/85).

**399-062 037586**, "104-Week Dietary Administration-Dogs, Ethrel (Formulated - 39.5% AI) Final Report," (Hazleton, Project No. 141-219, 9/28/72). Ethephon 39.5%, lot 68-250 (Ethrel formulation); 4/sex/group were fed 0, 25, or 10/2500 ppm for two years; NOEL = 25 ppm; < 25 ppm for ChE; decreased femoral marrow cellularity (in females) and thickening of the duodenal wall at the high dose; UNACCEPTABLE. Not upgradeable. Deficiencies: No justification of dose and why the low dose was drastically increased well into the study, inadequate description of the test article and the composition of the remaining 60%, no diet analysis over 2 years for content of active ingredient, insufficient number of animals at termination (3), no individual clinical observations. The low dose of 10 ppm was increased to 2500 at week 30. Hematology at weeks 4, 13, 26, 52, 78, and 103. Clinical chemistry at the same intervals. One per sex per group was sacrificed at 52 weeks. The report discusses the finding in the bone marrow of the femur in relation to the normal marrow found in the ribs and the negative peripheral blood differences suggesting that there may have been a problem in preparing the samples as well as questioning the significance. This finding was also reported in 037585 above. The finding of the thickening (hypertrophy) of the duodenal wall at 2500 ppm was also reported by Hazleton at 6000 ppm -see above. [redacted], 5/8/86).

EPA 1-liner: SUPPLEMENTARY. NOEL (sys) = 25 ppm; ChE NOEL < 25 ppm.

**300-063 037711**, "Two-year Dietary Study in Dogs - Ethrel Final Report," (Hazleton, Report No. 141-260, 11/17/77). Ethephon, (source A: 75.6%, B: 73.6% purity); 6/sex/group were fed 0, 30, 300 (A and B) or 3000/2000/1000/1500 ppm for two years; NOEL: 30 ppm (duodenal wall hypertrophy); cholinesterase inhibition at all doses; GI smooth muscle hypertrophy in 300 and

1500 ppm, source "A," but not source "B"; UNACCEPTABLE, possibly upgradeable. Deficiencies: no justification of dose selection and why level was changed; needs complete description of test article; needs complete histopathology for all tissues preserved; no analysis of diet; age of dogs at start is not given. ([redacted], 5/8/86)

EPA 1-liner: MINIMUM. RBC ChE NOEL = 30 ppm; Histopathology NOEL = 300 ppm.

300-052 027145. This document contains a summary of 300-063 037711. No worksheet was done (S. Morris, 8/7/91).

Two earlier chronic studies in dogs (see above) reported decreased cellularity in femoral bone marrow. One of them discounted this finding based on normal rib marrow and other hematological parameters. This report, 037711, indicates no remarkable findings. Taken all together, this effect may have been due to preparation of the marrow for examination as suggested in 037586. The other finding, however, of hypertrophy of the smooth muscle is supported by this study as well as the two earlier ones. ([redacted], 5/8/86).

Conclusion: Based upon the latest study, performed according to FIFRA Guidelines, there were no adverse effects observed in beagle dogs, due to ethephon, over the required 1 year exposure period. There were questionable and inconsistent findings in the bone marrow and certain hematological parameters, however when the study was repeated (074653) according to the FIFRA Guidelines, these phenomena were not observed. ([redacted], 12/89).

### Oncogenicity, mouse **

** 300-087 072846, "Lifetime Dietary Oncogenicity Study with Ethephon in Albino Mice," (Bushy Run Research Center, Project I.D. 51-502, 11/14/88). Ethephon, from various lots with purity ranging from 70.6% - 72.1%, administered in the feed at concentrations of 0, 100, 1000, or 10000 ppm to groups of 20 and 50 CD®-1 albino mice/sex/group for 52 and 78 weeks, respectively. No adverse effects. NOEL = 1000 ppm (decreased body weight and body weight gain in females; decreased urine pH in males). ChE NOEL = 100 ppm (significant plasma and erythrocyte inhibition was achieved at $\geq$ 1000 ppm). NOAEL = 10000 ppm (HTD). An oncogenic effect was not observed with ethephon. ACCEPTABLE. [redacted] & [redacted] 11/22/89).

300-069 037717, "78-Week Oncogenic Evaluation in Swiss Albino Mice," (1981 and 8/85 for amended report, Food and Drug Research Labs, Report No. 5754). Ethephon, 75%; 85/sex/group were fed 0, 30, 300, or 1000 ppm in the diet, 78 weeks; NOEL: 30 ppm (ChE); no compound-related chronic or oncogenicity effect could be identified; UNACCEPTABLE, possibly upgradeable. Deficiencies: husbandry problems with 7 mis-sexed, 18 found dead in 1st fourteen weeks with no explanation, mite infestation, wet feeders, parasites; no description of the other 25% of the test article; no analysis of diet during test period; no justification of dose selection and no evidence MTD was reached for other than ChE depression (40-60% for plasma, 20-50% for RBC); no clinical observations or individual body weights; poor randomization with group 3 females being statistically heavier at start of study. ([redacted], 5/9/86).

300-071 037718
300-070 037719
300-072 037720
300-073 037721, Supplemental to 300-069 037717.

EPA 1-liner: SUPPLEMENTARY as a cholinesterase study; invalid as an oncogenicity study based on inconsistencies in the histopathology data -- the report was amended and submitted as 037717-21 with correction of the discrepancies but there is no updated EPA 1-liner.

300-065 037714
300-066 037715. Original report of 300-071 037717 to 300-073 037721, reviewed by [redacted], 5/9/86.

300-052 027156. This document contains a summary of 300-069 037717. No worksheet was done ([redacted] 8/7/91).

## GENOTOXICITY

### Bacterial (and Yeast) Reverse Mutation Assay ** †

**** 300-087 073138**, " Mutagenicity Test on Ethephon Base 250: In Ames Salmonella/Microsome Reverse Mutation Assay," (Hazleton Laboratories America, HLA study no. 10065-0-401, 10/12/87). Ethephon, purity 72.3%, exposure concentrations at 0 (deionized water), 0.1, 0.5, 1.0, 2.5, 5.0, 10.0, 25.0, or 50.0 µl/plate using Salmonella typhimurium strains TA-1535, TA-1537, TA-1538, TA-98 and TA-100, with and without Aroclor 1254 rat liver (S9-Mix) activation, for 48-72 hours (3 plates/dose & 2 trials). **An increase in his+ revertant colonies was observed with TA1535 (with and without S9) in both initial and repeated assays. Acceptable.** ([redacted] & [redacted], 11/22/89).

300-058 037701, "Ames Salmonella typhimurium/Microsome Plate Ethrel," (Pharmakon, PH 301-UC-001-80, 6/18/80). Salmonella, five strains; ethephon liquid, no purity stated ; bacteria exposed with and without rat liver S-9 to 50, 166, 500, 1666 or 5000 µg/plate, in triplicate, one trial; no increase in reversion rate reported. UNACCEPTABLE. Need description of test article and how dilutions were made; no individual plate counts, lacks some positive controls for some strains; no repeat trial. ([redacted] 5/1/86).

EPA 1-liner: ACCEPTABLE. Negative for TA1537, 1538, 98 and 100. Marginal results with 1535 with and without activation.

300-052 027150. This document contains a summary of 300-058 037701. No worksheet was done ([redacted], 8/7/91).

**300-058 037702**, "Eukaryotic Reverse Mutation Saccharomyces cerevisiae - Ethrel," (Pharmakon, PH 303-UC-001-80 and 80A, 7/5/80). Ethephon, no purity stated.; Saccharomyces cerevisiae D7 - ilv I-92/I-92 exposed to 0, 0.42, 1.4, 4.2, 14 or 42 mg/ml, trial one, or 15, 30 or 45 mg/ml, trial two; no activation, only one hour, 20 plates each conc.; increase in revertants/ $10^7$ at 42 and 45. Incomplete (missing data), UNACCEPTABLE - no description of test article, no activation, no rationale for 1-hour exposure instead of, e.g., 3-16 hours. ([redacted], 5/1/86).

No EPA 1-liner.

300-052 027149. This document contains a summary of 300-058 037702. No worksheet was done ([redacted], 8/7/91).

Summary: Ethephon was negative for gene mutation in mammalian cells (CHO) (3 studies: 073138, 037697 & 037699) but positive for increasing revertants in Saccharomyces and Salmonella TA1535 in 073139 both with and without metabolic activation (marginal results with TA1535 in 037701, according to the EPA). A positive effect without activation was also found in

increased mitotic crossing-over and mitotic gene conversion in the same organism while negative findings were reported for other genotoxicity tests. Taken altogether, the Saccharomyces and Salmonella may be especially sensitive to this chemical. Therefore, ethephon can be considered to have a mutagenic effect in bacteria and yeast. [redacted] 12/89.

### Mutagenicity: In vitro mammalian cell assay ** †

** 300-087 073139, "Mutagenicity Test on Ethephon Base 250: CHO/HGPRT Forward Mutation Assay," (Hazleton Laboratories America Inc., HLA Study No. 10065-0 -435, 1/19/88). Ethephon (presumed purity = 72.3%, EPA Est #NC264-01, Ref. #10-LJH-44) exposure concentrations (12 plates/concentration for mutation frequency) at 0, 0.5, 1.0, 2.0, 2.5, 3.0, 3.5, 4.0, or 5.0 mg/ml were tested with CHO-K1-$BH_4$ Chinese Hamster ovary (CHO) cell line, with and without Aroclor 1254 rat liver (S9-mix) activation, for 4 hours. A similar trial followed with Ethephon concentrations at 0, 0.5, 1.0, 2.0, 2.2, 2.4, 2.6, 2.8, or 3.0 mg/ml. An increase in mutation frequency observed for the 2.4/2.5 mg/ml dose was not confirmed in a second trial, therefore, ethephon is not considered to induce gene mutations in cultured Chinese hamster ovary cells. **Acceptable.** ([redacted] & [redacted], 11/22/89).

300-058 037697, "CHO/HGPRT Mammalian Cell Forward Gene Mutation Assay Ethephon/Base 250," (Pharmakon, PH 314-UC-003-83, 12/29/83). Ethephon, 75% purity in water (Ethephon/Base 250) CHO exposed for 5 hours with and without activation to 0, 500, 1500, 1750, 2000 or 2500 µg/ml; no increase in mutation frequency is reported; duplicate cultures; ACCEPTABLE, with 037699. Retest at 2000 and 2500 µg/ml only +S9. Initially reviewed as unacceptable due to lack of test article. This has been submitted in 079 051086. ([redacted], 51/86 and 6/1/87).

No EPA 1-liner.

300-079 051086. This document contains a description of the test article for 300-058 037697.

**300-058 037699, "CHO/HGPRT Mammalian Cell Forward Gene Mutation Assay Ethephon Base 250," (Pharmakon Research Int'l, PH 314-UC-001-84, 6/14/84). Ethephon Base 250 lot #A-41213; CHO were exposed for five hours with and without activation to 0, 166, 333, 1666, 3333, or 5000 µg/ml; cytotoxic at 5000 (12-14% survival); no increased mutation frequency noted in two trials. ACCEPTABLE with 037697. Initially reviewed as unacceptable due to lack of description of test article. This has been submitted in 079 051086. [redacted], 5/1/86 and 6/1/87).

No EPA 1-liner.

### Mutagenicity: In vivo cytogenetics ** †

** 300-087 073140, "Mutagenicity Test on Ethephon Base 250: In an In Vitro Cytogenetic Assay Measuring Chromosomal Aberration Frequencies in Chinese hamster Ovary (CHO) Cells," (Hazleton Laboratories America Inc., HLA Study No.10065-0-437, 2/18/88). Ethephon, purity 71.3% by weight, at exposure concentrations of 0 (McCoy's 5a medium), 753, 1000, 1510, or 2010 µg/ml without metabolic activation for 7.25 hours and at 0, (McCoy's 5a medium), 502, 1000, 1510, or 2010 µg/ml with Aroclor 1254-induced rat liver (S9-mix) for 2 hours to Chinese Hamster (CHO-WBL) ovary cells (duplicate cultures, 100 cells scored/culture). There was no significant increase in chromosome aberrations. ACCEPTABLE. ([redacted] & [redacted], 11/27/89).

300-059 037694, "Evaluation of Ethrel on Spermatogenesis of Mice, Using the Dominant Lethal Test, Final Report," (Affiliated Medical Enterprises, Inc., 1/7/72). Ethephon technical, no purity stated; 20 males given 0, 100 or 1000 mg/kg by oral gavage and mated 1:1 over 5 periods (6-9, 13-16, 22-25, 36-39, and 42-45 days). UNACCEPTABLE protocol. Deficiencies: No description of test article; no individual data; low % pregnancies (30% to 74% over 5 breeding periods; no clinical observations and no evidence of toxicity in main study; no husbandry information; no concurrent positive control. ([redacted], 5/1/86).

300-059 037695, "Dominant Lethal Study (Ethephon-56375) - Male Rats," (Pharmakon, 4/11/79). Ethephon Lot# 56375, no purity stated; ten males were given 0, 250, 500 or 1000 mg/kg for five doses by oral gavage then mated 1:2 over eight weekly periods; no dose-related dominant lethal effect is reported. UNACCEPTABLE (no purity of test article; inadequate number of pregnant females per period). TEM as positive control. ([redacted], 5/1/86).

EPA 1-liner: No grade. Negative.

300-039 033588. Very brief summary of 300-059 037695 (No date or lab given). ([redacted], 7/5/85).

300-052 027152. This document contains a summary of 300-059 037695. No worksheet was done ([redacted], 8/7/91).

300-015 047069. This document contains a brief summary of a study (probably the study at 300-059 037695) that reported a dominant lethal effect was not detected in male rats exposed to 250, 500, or 1000 mg/kg. No worksheet was done ([redacted], 8/8/91).

300-058 037696, "Genetic Toxicology Micronucleus Test (MNT) Ethephon - Mice," (Pharmakon, PH 309A-UC-001-81, 4/6/81). Ethephon technical, no purity stated; four/sex/group were given 200 mg/kg i.p. - once or twice and sacrifice at 30 or 48 hours after one dose, 48 or 72 hours after two doses; no increase in MN's reported. UNACCEPTABLE, upgradeable. Deficiencies: No PCE/NCE for each animal; no purity for test article. This study used CD-1 mice. The other two used BS-1 strain. Also, the toxicity seems greater in this report in terms of clinical observations. Since none of the three reports describes the purity or lot number of the ethephon, that aspect cannot be evaluated. Also, the MN/1000 is much lower in controls and treated alike. Controls: #037708, 23.75; #037707, 15.5 and #037696, 1.5 MN/1000 cells. ([redacted], 5/1/86).

EPA 1-liner: ACCEPTABLE. Negative.

300-052 027157. This document contains a summary of 300-058 037696. No worksheet was done ([redacted], 8/7/91).

**300-058 037707**, "Genetic Toxicology Micronucleus Test Ethrel - Mice," (Pharmakon, PH 309-UC-002-80, 8/27/80). Ethephon, no purity stated; four/sex/group given 0, 400 or 600 mg/kg i.p., twice and sacrificed at six hours, increase in micronuclei over controls is reported. UNACCEPTABLE (protocol). Deficiencies: no purity for test article; single time of sacrifice at 6 hours only; inadequate number of animals (should be 5/sex/group) especially important in view of the scattering among animals; no PCE/NCE or MI for cytotoxicity evaluation of marrow. MN/1000 PCE's was 15.5 (control), 27.9 (400 mg/kg) and 26.6 (600 mg/kg bw). ([redacted], 5/1/86).

**300-058 037708**, "Genetic Toxicology Micronucleus Test Ethrel - Mice," (Pharmakon, PH 309-UC-001-80, 8/27/80). Ethephon, no purity stated; four/sex/group were given 0, 400 or 800 mg/kg twice by i.p. injection and sacrificed at six hours after second dose; an increase in MN

seen at 400 mg. UNACCEPTABLE (protocol). Deficiencies: No purity of test article; single time of sacrifice following two dosings; insufficient number of animals, no comment on PCE/NCE or cytotoxicity noted to indicate an adequate dose was used. Dose selection was based on a preliminary study. Since there were no deaths at 1 gm/kg, a higher dose than 800 mg/kg could have been used. MN/PCE's: 23.75 (control), 42.125 (400 mg/kg) and 30.75 (800 mg/kg). ([redacted], 5/1/86).

Summary: Although only a single study is acceptable (others are flawed in design or report), when all are considered collectively, CDPR concludes there are sufficient data to determine that ethephon has a potential for clastogenic effect, at least in one strain (BDS-1) of mouse.

### DNA damage or miscellaneous effects ** †

** 300-087 073141, "Mutagenicity Test on Ethephon: In Rat Hepatocyte Unscheduled DNA Synthesis Assay," (Hazleton Laboratories America Inc., HLA Study No. 10065-0-447, 2/17/88). Ethephon (purity 71.3%), in exposure concentrations of 0 (WME), 25, 50, 250, or 1,000 µg/ml in one trial and 0 (WME), 500, 1,000, or 2,000 µg/ml in a second trial was used on rat primary hepatocytes (19 hour exposure). The test was performed by autoradiography and 150 cells were scored from triplicate cover slips/concentration. There was no indication of Ethephon induced DNA damage to in vitro rat hepatocytes. ACCEPTABLE**.** ([redacted] & [redacted], 11/27/89).

** 300-058 037698, "Rat Hepatocyte Primary Culture/DNA Repair Test, Ethephon Base 250," (Pharmakon, PH 311-UC-002-84, 6/13/84). Ethephon lot A-41213, 75% purity in water; hepatocytes were exposed 18-20 hours to 0, 0.3, 1.0, 10, 33, 100, 333, 1000, 3333 or 10,000 µg/well in 2 ml medium; no evidence of UDS; cytotoxic at 3333 and 10,000. ACCEPTABLE. Table I in text on analytical data needs an explanation. Initially reviewed as unacceptable due to lack of description of test article. This has been submitted in 079 051086. ([redacted], 5/1/86 and 6/2/87).

300-079 051086. This document contains a description of the test article for 300-058 037698.

300-058 037700, "DNA Polymerase Deficient Assay; Escherichia coli Ethrel," (Pharmakon, PH 305-UC-001-80, 5/23/80). Escherichia coli strains W3110 and P3478; ethephon, no purity stated; bacteria were exposed with and without S-9 to 0, 0.1112, 1.112, 11.12, or 1112.0 mg/ml (20 µl without S-9, 50 µl with rat liver S-9) with no differential effect on growths. UNACCEPTABLE, upgradeable with active ingredient description. ([redacted], 5/1/86).

EPA 1-liner: ACCEPTABLE. Negative for DNA damage.

300-052 027151. This document contains a summary of 300-058 037700. No worksheet was done ([redacted], 8/7/91).

**300-058 037703**, "Mitotic Crossing-over Saccharomyces cerevisiae Ethrel," (Pharmakon, PH 302-UC-001-80, 7/15/80). Ethephon liquid, no purity stated; strain D7 heteroallelic diploid ade2-40/ade2-119; 0, 0.42, 1.4, 4.2, 14 or 42 mg/ml, one hour, no activation only, 40 plates, one trial; suggestion of an increase at 42 mg/ml in mitotic crossing-over-gene conversion. UNACCEPTABLE (protocol). Deficiencies: Use of DMSO as solvent; no activation system included; high concentration should have been higher from cytotoxicity in preliminary trial especially since there is a suggestion of an effect; rationale for 1 hour treatment rather than usual 3-16 hours. Percent twin-sectored colonies: Control - 0.05; 0.42 - 0.06; 1.4- 0.05; 4.2 - 0.04; 14 - 0.04 and 42 - 0.16. ([redacted], 5/5/86).

EPA 1-liner: UNACCEPTABLE. Negative for mitotic crossing over. Not tested with metabolic activation or past 50% survival rate.

300-052 027148. This document contains a summary of 300-058 037703. No worksheet was done ([redacted], 8/7/91).

**300-058 037704**, "Mitotic Gene Conversion, Saccharomyces cerevisiae, Ethrel," (Pharmakon, PH 304-UC-001-80 and -80A, 1980). Ethephon, no purity stated; Strain D7 heteroallelic diploid trp5-12/trp5-27; incubated with 0, 0.42, 1.4, 4.2, 14 or 42 mg/ml for one hour without S-9 (trial 1) or with 0, 15, 30 or 45 mg/ml without S-9 for one hour trial 2), 30 plates each; an increase in revertants to trp independence was reported for 42 and 45 mg/ml with a suggestion of a dose response. UNACCEPTABLE (no activation). Trial 1: Control - 1.17 revertants/$10^5$ cells, 14 - 1.88, 42 - 4.21. Trial 2: Control - 0.67, 30 - 1.09 and 45 - 2.19. ([redacted], 5/5/86).

EPA 1-liner: ACCEPTABLE. Produced an increase in reverse mutation in strain D7 at 42 and 45 mg/ml -S9. [Not clear from EPA if also positive for mitotic gene conversion - seems to be confusion with study numbers.]

300-052 021758. This document contains a summary of 300-058 037704. No worksheet was done [redacted], 8/7/91).

Conclusion: Saccharomyces appears to be sensitive to DNA damage by ethephon, where E. Coli and rat hepatocytes are not. Although the study with rat hepatocytes is negative, there are sufficient data from other studies to support the possibility that ethephon induces DNA damage.

## REPRODUCTIVE TOXICITY, RAT

** 300-094 088512, T. L. Neeper-Bradley and R. W. Tyl, "Two-Generation Reproduction Study in CD® Albino Rats Exposed to Ethephon by Dietary Inclusion," Bushy Run Research Center, Laboratory Project ID 51-539, 5/17/90. A 2 generation (F0, F1B), 2 litter / generation (F1A, F1B, F2A, F2B) reproduction study was conducted in which Ethephon (Base A-250, approximately 71% stated purity) was administered in the feed at 0, 300, 3000, or 30,000 ppm to 28 parental rats/sex/dose/generation for a 10 week pre-breeding period, mating, gestation, and lactation. Twenty-eight pups/sex/dose were chosen from F1B litters for the second generation. Significant treatment-related effects in both sexes at 30,000 ppm were decreases in adult body weight gain, birth weights, and pup body weight gain (NOEL = 3000 ppm). No adverse effect was indicated. The study was ACCEPTABLE ([redacted] and [redacted], 8/5/91).

300-059 037582, "Three-generation Reproduction Study - Rats, Experimental Ethrel Final Report (39.5% AI)," (Hazleton, Report No. 141-214, 4/11/72). Ethephon, 39.5% Lot 68-250 ("experimental" Ethrel); 10 males and 20 females per group were fed 0, 200, 750 or 1500 ppm, three generations, two litters; NOEL: not established - > 1500 ppm; UNACCEPTABLE. Deficiencies: Incomplete (missing info - no individual data; body weight and food consumption for 3 of 9 weeks only, no justification of dose levels with no evidence of MTD from toxicity; no necropsy or histopathology information at all; inadequate description of test article, no age of animals. Report states "small pups" in all groups, all generations. Evidence of respiratory disease in colony. [redacted], 5/6/86).

EPA 1-liner: GUIDELINE. Reproductive NOEL >1500 ppm.

300-017 987990 (Hazleton) Pfeifer 7/8/85 Summary of 300-059 037582

300-052 027147. This document contains a summary of 300-059 037582. No worksheet was done ([redacted], 7/8/91).

300-014 047070. This document contains a brief summary of a three generation study (probably the study at 300-059 037582) in rats in which "dosage up to 1500 ppm" produced no compound-related effects. No worksheet was done ([redacted], 8/8/91).

## DEVELOPMENTAL TOXICITY

### Rat **

300-089 074067, "Teratology Study with Ethephon Technical-Base 250 in Rats," (Hazleton Laboratories America Inc., Lab Project I.D. No. HLA 6224-125, 4/6/89). Ethephon technical, purity 71.7%, administered by gavage at concentrations of 0, 125, 250 or 500 mg/kg/day to 25 Crl:CD® (SD)Br female rats/group on day 6 thru 15 of gestation. No evidence of teratogenicity reported at dose levels in this study. No adverse effects**.** Maternal NOEL and embryo/fetal NOEL > 500 mg/kg. NOT ACCEPTABLE (no effects were observed at any dose level). ([redacted] & [redacted], 11/22/89).

300-059 037583, "Teratology Study in Rats - Ethrel (Ethephon)," (IRDC, Report No. 369-042, 11/18/80). Ethephon technical (solid), no purity stated ; 25/group were given 0, 200, 600, or 1800 mg/kg/day, 6-19, by oral gavage. NOEL: 600 mg/kg maternal toxicity, at 1800 mg/kg, 14/25 died. No indication of a teratogenic effect at any dose is reported. UNACCEPTABLE but upgradeable. Major deficiencies: no dosing analysis, no food consumption; only 1/3 were subjected to visceral examination instead of 1/2. Despite the high mortality at the high dose, 130 viable fetuses were available for examination so some estimate of fetotoxicity-developmental toxicity could be made. The report does not indicate a teratogenic effect at 1800 mg/kg. ([redacted], 5/7/86).

EPA 1-liner: MINIMUM. Teratogenic NOEL >600 mg/kg; poor survival at 1800 precludes usefulness for teratogenic effects. Maternal NOEL = 600 mg/kg (necrotic hepatitis, death. Fetotoxic NOEL >1800 mg/kg (HDT).

300-039 033586. Summary of 300-059 037583 (No date or lab given). (Pfeifer, 7/5/85).

300-079 051085. This document contains individual clinical observations for 300-059 037583.

300-079 051086. This document contains a description of the test article for 300-059 037583.

300-052 027154. This document contains a summary of an IRDC study (11/11/80, probably the study at 300-059 037583) that indicated no adverse effects at 200 or 600 mg/kg/day in a rat teratology study. No worksheet was done [redacted], 8/7/91).

300-015 047066. This document contains a brief summary of a study (probably the study at 300-059 037583) in rats using 0, 200, 600, or 1800 mg/kg. No adverse effects were reported at $\leq$ 600 mg/kg. No worksheet was done ([redacted], 8/7/91).

Conclusion: Despite the deficiencies, it is evident in study 300-059 037583 that an MTD has been reached and there are no teratogenic effects due to ethephon. Study 300-089 074067 shows results consistent with that of 300-059 037583 with a similar level for maternal NOEL. Although neither study alone is adequate, when taken collectively, 300-089 074067 and 300-059 037583 are acceptable for filling the rat teratology data gap. [redacted], 2/9/90.

### Rabbit **

** 300-098 087118, S. M. Henwood, "Teratology Study with Ethephon Technical - Base 250 in Rabbits," Hazleton Laboratories America, HLA 6224-158, 6/27/90. Twenty-two, virgin female Hra:(NZW)SPF rabbits / dose were artificially-inseminated (gestation day 0); injected with human chorionic gonadotropin (100 USP units / kg); exposed to ethephon (technical - base 250, lot # 4022193, purity ≈ 72%, water vehicle) by oral gavage at 0, 62.5, 125, or 250 mg/kg on gestation days 7 through 19; and sacrificed on day 29. Maternal effects were behavioral abnormalities, reduced body weights, stomach lesions, and lethality (17/22) at 250 mg/kg/day. Except for death accompanying maternal lethality, no treatment-related fetal effects were reported. No adverse effect was indicated (maternal NOEL = fetal NOEL = 125 mg/kg/day). The study was acceptable ([redacted] and [redacted], 7/26/91).

300-059 037584, "Teratology Study in Rabbits, Technical Ethephon, Final Report," (Hazleton Labs, Report No. 400-635, 4/17/81). Ethephon technical, Lot aa, solid, 90% purity - 100% assumed; 17 does/group were given 0, 50, 100 or 250 mg/kg by oral gavage, days 6 through 19; high dose = 48% mortality; NOEL: 50 mg/kg (maternal mortality); no evidence of dev. toxicity without maternal toxicity. UNACCEPTABLE. Major deficiencies: no analysis of dosing solution for content or stability. Live fetuses were 71, 82, 47 and 25 at 0, 50, 100 and 250 respectively. ([redacted], 5/7/86).

EPA 1-liner: SUPPLEMENTARY. Teratogenic NOEL > 50 mg/kg/day. Litters at termination were insufficient to determine teratogenic effect at 100 and 150 (sic) mg/kg/day. Embryotoxic-fetotoxic NOEL = 50; Maternal tox NOEL = 100 mg/kg/day (body weight gain, food consumption, mortality.)

300-079 050993. Individual maternal clinical observations for 300-059 037584.

300-052 027153. This document contains a summary of 300-059 037584. No worksheet was done ([redacted], 8/7/91).

300-018 987989, "Segment II - Teratology - Rabbits Experimental Ethrel - Final Report," (Hazleton Labs, 10/21/70). Ethephon, 39.5% was fed in the diet to 12/per group at 0, 200 or 1000 ppm on days 8 -16 of gestation. No adverse effect indicated**.** NOEL (Maternal and fetal) > 1000 ppm. UNACCEPTABLE (Half of animals were sacrificed before term and half delivered; skeletal data for two groups should not be combined; no maternal body weight or food consumption; only 2 doses with no justification and no indication of maternal toxicity; no analysis of diet; no historical data; no sexing of fetuses/pups. Administration in the diet is not the preferred route for teratology study.) This study was originally reviewed with a potential adverse effect (Pfeifer 7/10/85). Reexamination of the data revealed no teratogenic effect due to ethephon since implantation occurs before dosing. In this study, however, there were too few does to evaluate fetal death. Therefore the study remains UNACCEPTABLE and not upgradeable. ([redacted] 6/6/86).

EPA 1-liner: SUPPLEMENTARY. Teratogenic NOEL >1000 ppm (HDT)

300-059 037581. (1970, Hazleton Labs). Exact duplicate of 018 987989.

300-015 047071. This document contains a brief summary of a rabbit teratology study (probably the study at 300-018 987989) that used levels "up to 1000 ppm." No adverse effect was reported. No worksheet was done ([redacted], 8/7/91).

300-003 987988. This document contains a brief summary of a rabbit teratology study (probably the study at 300-018 987989) that used dietary exposures of 100 or 1000 ppm on

gestation days 8 to 16. Increased fetal deaths were reported at 1000 ppm but too little data were given to ascertain the possibility of an adverse effect. No worksheet was done ([redacted], 8/8/91).

## NEUROTOXICITY

### Acute neurotoxicity, rat **

**00300-282 159933 Beyrouty, P., "An acute study of the potential effects of a single orally administered dose of Ethephon, Technical Grade, on behavior and neuromorphology in rats," Bio-Research Laboratories, Ltd., Senneville, Quebec, Canada, 4/19/96. Laboratory Project ID No. 97412. Groups of 12 Sprague-Dawley rats were dosed once by gavage with Base 250 Ethephon, Lot No. 4051511, 72.4% purity in an acute neurotoxicity study design. Test article was a clear liquid, designated as technical material. Single gavage doses were 0, 500, 1000, and 2000 mg/kg. NOEL = 500 mg/kg, based on reduced food consumption in females (days 0-7) and reduced motor activity in males (day 0) at 1000 mg/kg. A death of a 1000 mg/kg female on day 5 following prolonged clinical signs of distress was probably due to test article. Two 2000 mg/kg females died by day 2. Body weight and food consumption decrements were observed in both sexes at 2000 mg/kg. Day 0 motor activity counts were statistically reduced in both sexes at 2000 mg/kg. Clinical signs at 2000 mg/kg (limited primarily to a few females) included stained fur, labored breathing, body cold to touch, and decreased activity. Statistically significant FOB findings in 2000 mg/kg females included a marked increase in pinpoint pupils and a modest reduction in body temperature, both at day 0 only. A non-significant increase in pinpoint pupils in 2000 mg/kg males on day 0 was also plausibly treatment-related. No responses appeared to continue past the first week. There was no treatment-related neuropathology. Study is acceptable, with no adverse effects. [redacted], 11/18/15.

00300-281 159932 Beyrouty, P., "A time of peak effects study of a single orally administered dose of Ethephon in rats," Bio-Research Laboratories, Ltd., Senneville, Quebec, Canada, 4/19/96. Laboratory Project ID No. 97411. Groups of Sprague-Dawley rats were dosed once by gavage with Base 250 Ethephon, Lot No. 4051511, 72.4% purity, in a pilot study assessing effects of 0, 250, 500, 1000, and 2000 mg/kg test article. Major assessed endpoints were an abridged FOB (including a qualitative motor activity assessment) and cholinesterase (ChE) inhibition (RBC, plasma, and brain). Groups of 3/sex were tested repeatedly for the FOB measurements pre-test, and at 0.5, 1, 2, 4, 6, 8, and 24 hrs after dosing. Other groups of 3/sex were sacrificed at the above intervals for the ChE assays. FOB results did not indicate clear treatment effects, although wet muzzle or red discolored muzzle were seen in two of three 2000 mg/kg males, one female at 500, and one female at 2000 mg/kg. Plasma ChE activity was clearly reduced at all dose levels with a gradual dose-response. Time of peak plasma ChE response varied, but generally the period of 4 hrs to 8 hrs was at or near maximum response. This justifies the scheduling of FOB and motor activity assessments in the primary acute neurotoxicity study by this laboratory. [redacted], 11/18/15.

### 90-day neurotoxicity, rat **

** 300-271 154981 "A 13-week study of the potential effects of orally administered ethephon, technical grade base 250 on behavior, neurochemistry and neuromorphology" (P. Beyrouty, ClinTrials BioResearch Ltd., Canada, Project I.D. 97414, 4/28/97) Groups of 22/sex/dose Sprague-Dawley rats [Crl:CD®(SD)BR] were treated with 0 (deionized water), 75, 150 or 400-300 [reduced in week 10/11] mg/kg by gavage. Three males and 3 females died at the high dose before it was reduced. Body weights, food consumption, clinical signs, a FOB and motor activity were determined. Blood, plasma and brain cholinesterase activity were recorded.

Positive control data were submitted for a new active ingredient, cyclanilide [52093-081, -082 and -083]. Six per sex from the control and high dose groups were submitted to histopathology of the nervous system. Erythrocyte and plasma cholinesterase levels were depressed at all doses but brain cholinesterase was not affected. ChE NOEL < 75 mg/kg. Systemic NOEL = 150 mg/kg (mortality, clinical signs). No behavioral changes were observed and no treatment-related neuropathological lesions were found. ACCEPTABLE. ([redacted], 11/24/97).

300-270 154980 "A 2-week range-finding toxicity study of orally administered ethephon technical grade base 250 in rats." (P. Beyrouty, Study Director, Bio-Research Laboratories, Canada, Project ID 97453, April 28, 1997) Six/sex/group of Sprague-Dawley Crl:CD®(SD)BR rats were given Ethephon technical base 250, 72.4%, lot no. 4051511, by gavage for 14 days. Doses were 0 (deionized water), 100, 300, 600 or 1000 mg/kg, corrected for purity with dosing solutions analyzed weekly. Animals were examined twice daily. The FOB was performed prior to dosing, on days 2, 8 and 15. Plasma and whole blood cholinesterase activity were measured prior to dosing on days 2, 8 and 15 after the FOB evaluation. Mortality: 2 males and 4 females at 600 mg/kg and all males and 5 females at 1000 mg/kg died or were sacrificed prior to scheduled termination. Clinical signs at these doses included fur staining, skin pallor, abnormal breathing, respiratory sounds, dehydration, cold to touch, decreased activity, weak appearance and abdominal distension. Minimal effects were seen at the lower doses. A few effects were noted in the FOB portion such as respiratory sounds, pinpoint pupils and impaired gait. No significant differences were detected in red blood cell cholinesterase while plasma cholinesterase levels were decreased at 300 mg/kg and higher. Only gross pathology was performed. SUPPLEMENTARY STUDY to 300-271, 154981. No worksheet. ([redacted], 11/20/97).

### Developmental neurotoxicity, rat

Study has not been submitted.

### Validation studies for neurotoxicity, rat

52093-079 156317 Beyrouty, P., "An inter-observer reliability (IOR) study for grip strength and hindlimb splay measurements in rats," Bio-Research Laboratories Ltd. Project ID #29539 (11/4/94). Six individuals performed limited procedures of typical rat neurotoxicity studies, such as forelimb and hindlimb grip strength, and hindlimb splay measurements. Measures were comparable between experimenters, except that one individual recorded somewhat a lower hindlimb grip strength mean value than the other 5 experimenters, such that the inter-experimenter variability was significant ($p < 0.05$), due primarily to the one individual (pp. 13, 18). Following subsequent training, this individual performed the same test two days later, and obtained mean values comparable to the other experimenters (p. 13). Investigators concluded that the concordance between experimenters was sufficient to allow the use of multiple trained experimenters, if necessary, in the course of an experiment. ([redacted], 10/3/97).

52093-080 156318 Beyrouty, P., "An acute neurotoxicity study of the effects of orally administered DDT and trimethyltin chloride in rats," Bio-Research Laboratories Ltd. Project ID #29537(11/4/94). These two known positive control materials were administered once to male Sprague-Dawley rats (12/group). Motor activity and FOB measurements were recorded on day 0 (6-6.5 hr after dosing), and days 7 and 14. On day 0 only, DDT (112.5 mg/kg) elicited tremors (pp. 41, 43), a tendency toward sitting or standing position in the home cage (compared with lying on side or curled up in most other rats), and a marked reduction in rearing behavior (p. 42). The same group tended to be hyper-reactive to sounds in the home cage, in the arena, and upon handling (p. 48). On day 7, the trimethyltin (TMT, 9 mg/kg) rats registered a significant increase in rearing behavior (p. 50), an increase in locomotor activity level (p. 52), and a

moderate reduction in auricular startle response (p. 55). Several grip strength and hindlimb splay values were significantly altered from concurrent controls, but without consistent temporal patterns (treatment effects had not been recorded earlier with these positive controls - see pp. 21, 63-66). DDT elevated body temperature shortly after dosing (day 0 - p. 68). Motor activity counts were substantially reduced with DDT on day 0 (p. 72). Motor activity counts were dramatically increased in TMT groups on days 7 and 14 (pp. 73-74). Central nervous system alterations in TMT rats included neuronal necrosis and gliosis (p. 75), whereas peripheral nervous system changes frequently included axonal degeneration or Schwann cell hypertrophy and/or hyperplasia. Collectively, these results indicated an effective evaluation capacity of the laboratory to perform neurotoxicity testing. ([redacted], 10/3/97).

52093-081 156319 Beyrouty, P., "A subchronic neurotoxicity study of the effects of orally administered acrylamide in rats," Bio-Research Laboratories Ltd. Project ID #29538. This test on positive control materials confirmed the investigators' capability to assess motor activity, FOB parameters, and neuropathology in neurotoxicity studies. Remarkable treatment effects included substantial reduction in mean activity counts, hypersensitive or aggressive behavior, abnormal (hypotonic, ataxic) gait, decreased arousal, flaccid abdominal tone, degeneration or necrosis of cerebellar Purkinje cells, and degeneration of peripheral nerve axons with associated Schwann cell hypertrophy or hyperplasia. This record was submitted in support of Record No. 45936, above. (No DPR worksheet ). [redacted] 10/8/97.

52093-082 156320 Beyrouty, P., "An inter-observer reliability (IOR) study for qualitative functional observational battery assessments in rats", Bio-Research Laboratories Ltd. Project ID #29540. The concordance in observational scoring between experimenters was generally satisfactory for conduct of neurotoxicity studies. Nevertheless, on occasion the six experimenters had a variety of responses for observation parameters (such as "body tone" or "abdominal tone" - see p. 41) for acrylamide rats, whereas the 14-day acrylamide FOB in Record No. 156319 (which employed technicians who were blind to treatment) yielded 100% "flaccid" responses in 12 treated rats, and 0% in 12 controls in evaluating these "tone" parameters (pp. 13 and 52 of that record). The variabilities of assessment in the present study were acknowledged, and after further technician training, the concordance between findings such as these for observations on a given animal were very good (pp. 75 and 76 of Record No. 156320). This record was submitted in support of Record No. 45936, above. [redacted], 10/8/97.

52093-083 156321 Beyrouty, P., "An acute neurotoxicity study of the effects of orally administered carbaryl and triadimefon in rats", Bio-Research Laboratories Ltd. Project ID #29546. This test on positive control materials confirmed the investigators' capability to assess motor activity and FOB parameters in neurotoxicity studies. Remarkable treatment effects included mean activity counts elevated by triadimefon (T) and reduced by carbaryl (C), tremors (C), rearing scores increased (T) or decreased (C), ataxic gait (C), vigorous locomotor activity (T), arousal levels increased (T) or decreased (C), pinpoint pupil (C), salivation (C), and low responsiveness to tail pinch or vibrissae touch (C). These findings were observed at day 0 only (evaluations about 1 hr after dosing). This record was submitted in support of Record No. 45936, above. (No DPR worksheet ). [redacted], 10/8/97.

### Delayed neurotoxicity, hen **

300-018 987973, "Neurotoxicity Study of Formulated Ethrel and Technical Ethrel on Hens - Final Report," (Hazleton, Report No. 141-218, 12/30/70). Ethrel formulated, purity not stated; 10 per group were given 0, 500 or 1000 mg/kg/day of technical or formulated active ingredient but schedule and rationale are unclear. Insufficient information to evaluate; UNACCEPTABLE.

No delayed neurotoxicity is reported but protocol and dosing are inadequate for test. (Pfeifer, 7/9/85).

300-060 037709. Exact duplicate of 300-018 987973 (1970, Hazleton).

300-052 027155. This document is a summary of 300-018 987973. No worksheet was done (S. Morris, 8/7/91).

300-015 047065. This document contains a brief summary of a neurotoxicity study (probably the study at 300-018 987973) in hen. No adverse effect was reported. No worksheet was done (S. Morris, 8/7/91).

300-003 987971. This document contains a brief summary of a neurotoxicity study (probably the study at 300-018 987973) in hen. No adverse effect was reported. No worksheet was done (S. Morris, 8/8/91).

** 300-060 037710, "42-Day Neurotoxicity Study with Ethephon: Base 250 in Mature White Leghorn Chickens," (Bio-Life Associates Report No. 83 DN 102, 12/15/83). Ethephon 70.75%; 30/treated group were given 3850 or 3160 mg/kg by gavage on day 0; survivors (approx. 20) were given 2370 mg/kg on day 21. Fifteen/vehicle and positive control. No evidence of delayed neurotoxicity. ACCEPTABLE. Justification for using hens 17-18 months old is in 300-079, Tab ([redacted], 5/7/86 and 6/1/87; [redacted], 6/1/87).

300-079 051086. This document contains a description of the test article for 300-060 037710.

## IMMUNOTOXICITY

Study has not been submitted.

## ENDOCRINE DISRUPTOR STUDIES

Study has not been submitted nor is required at this time.

## CHOLINESTERASE STUDIES †

300-0351; 286998; "A 90-Day Cholinesterase Inhibition Study via Dietary Administration in the Beagle Dog with Technical Ethephon"; (D.A. Eigenberg; Midwest Research Institute, Kansas City, MO, Bayer CropScience LP, Toxicology, Stilwell, KS, BioSTAT Consultants, Inc., Portage, MI; Study No. 04-S76-VW; 8/14/06); Four beagle dogs/sex/group received 0, 70, 140 or 525 ppm of Ethephon technical (batch no. 040201; purity: 71.4% (6/29/04), 71.9% (3/3/05)) in the diet for 91 days ((M) 0, 2, 4, 15 mg/kg/day, (F) 0, 2, 4, 18 mg/kg/day). No deaths resulted from the treatment. The mean body weights or food consumption were not affected by the treatment. No treatment-related clinical signs were evident. The mean plasma cholinesterase (ChE) activity levels of the 70 ppm group and above were less than that of the control group throughout the treatment period ($p<0.01$). The mean red blood cell ChE activity levels of both sexes in the 525 ppm group and the females in the 140 ppm group were less than that of the control group ($p<0.01$). The brain ChE activity levels of the females in the 70 ppm group and above were less than that of the control group ($p<0.05$). **Possible adverse effect:** significant red blood cell and brain ChE inhibition. **Rat Subchronic Dietary Toxicity NOEL:** <70 ppm ((M) <2 mg/kg/day, (F) <2 mg/kg/day) (based upon significant inhibition of plasma ChE for both sexes and of female brain ChE in the 70 ppm treatment group); **Study supplemental**. ([redacted], 10/16/15)

300-018 988004 Holsing, G. C., "Cholinesterase recovery study - dogs: Ethrel," Hazelton Laboratories Inc., Falls Church, VA, 8/26/70. Four beagles/sex were dosed in diet at either 0 or 2000 ppm of Ethrel (39.5% purity) for 2 weeks, followed by an 11-week recovery. Plasma and RBC ChE were assessed in all dogs initially (actually 3 days into the dosing period), at week 2 (the end of the dosing period), and at weeks 4, 6, 8, 10, and 13. Two treated dogs vomited on day 9, and two treated dogs lost ≥ 1.2 kg, both attributed to treatment. No other clinical signs were observed. Using concurrent controls for comparison, plasma ChE activities were at 26-30% of controls at the end of dosing (week 2), and recovered completely after about 4-6 weeks of recovery. RBC ChE activities were at 24-33% of controls at week 2, recovering to roughly 70 to 80% of concurrent controls at the end of the 11-week recovery period (study week 13). Useful supplementary data from a study which pre-dates modern guideline requirements. Insufficient information to warrant a DPR worksheet. [redacted], Dec. 28, 2015.

300-0018 988003 Teeters, W. R., "Effect of experimental Ethrel in an anesthetized dog preparation and on blood cholinesterase activity in this species," Hazelton Laboratories Inc., Falls Church, VA, 10/23/1970. The main experiment involved 2 female beagle dogs: both previous test subjects which had been at least 2 weeks off treatment. Each dog received 30 mg/kg pentobarbital sodium iv prior to recording, and at intervals where needed. Physiological responses were recorded with the aid of an endotracheal tube to record rate and pattern of respiration, and arterial cannulation to assess blood pressure. Electrodes were placed to assess contractile response of the anterior tibialis muscle (which did not elicit a consistent response). The first dog received progressively larger iv doses of Ethrel at 20 to 30-minute intervals, targeting 10, 20, 40, 80, 160, and 320 mg/kg of the active ingredient. The most dramatic change was a great increase in respiratory rate (from background of 24 breaths/minute pre-treatment to 42 at 10 mg/kg, with similar increases after dosing at 20 and 80 mg/kg. At 160 mg/kg, a series of changes included a profound initial drop in blood pressure, associated with a substantial increase in respiratory rate (72 breaths/min), but evidently the rapid breathing was not efficient for gas exchange. Blood pressure subsequently spiked, attributed to anoxia. Respiratory movements stopped, and the dog was given artificial respiration until it could breathe on its own. The next sequential dose of 320 mg/kg was lethal. In the above series, tremors were noted at 18 minutes after the second dose at 20 mg/kg, and occasionally with higher doses (usually delayed several minutes after any given treatment). A second anesthetized female was first assessed for responses to fixed iv dosing with acetylcholine (ACh); or to the nicotinic receptor agonist, dimethylphenylpiperazinium (DMPP); or to a 45-second bilateral carotid occlusion (BCO). Prior to administration of Ethrel, ACh caused reduced blood pressure, DMPP caused sharply increased blood pressure, and BCO elicited moderately increased blood pressure. Effects on blood pressure of Ethrel alone were essentially as with the first dog, with an initial drop in blood pressure, followed by a substantial increase. There was no perceptible effect of Ethrel on the subsequent response to ACh. There was an increase in DMPP blood pressure response at 40 to 80 mg/kg Ethrel, and a smaller increase in DMPP blood pressure response at 160 mg/kg Ethrel. BCO raised blood pressure by about the same amount without Ethrel or with Ethrel doses up to 80 mg/kg, whereas 160 mg/kg Ethrel elicited a smaller increase due to BCO. Respiratory rate in this dog was very slow at 10 mg/kg Ethrel and above, except that at 160 mg/kg, there was a marked increase in respiratory rate. This dog died following the next dose of 320 mg/kg. Two additional female beagles, also carried over from a previous study, were tested for plasma and RBC cholinesterase (ChE) activities after a single iv dose (assaying at 1, 4, 24, 48, and 72 hrs). Plasma ChE inhibition recovered from 84% at 1 hr to 39% at 72 hrs. RBC ChE inhibition did not recover perceptibly during the 72 hrs: inhibition varied stably in the range of 22% in one dog and about 29% in the other, with no consistent change during the assay interval. This is a supplementary study of an uncommon design,

unsuitable for contemporary dose-response analyses, and thus not relevant for a DPR worksheet. [redacted], 1/13/16.

## SUPPLEMENTAL STUDIES ON METABOLITES OR IMPURITIES SUCH AS HEPA

00300-0319 227376 Bigot, D., "HEPA (2-hydroxyethylphosphonic acid): Exploratory 15-day toxicity study in the rat by gavage," Bayer CropScience, Sophia Antipolis, France, 1/30/03, Laboratory Study # SA 02122. Test article is a plant metabolite of ethephon (95.9% purity). Groups of 5 Sprague-Dawley rats/sex were dosed by gavage with 0, 125, 250, or 500 mg/kg/day. There were no substantial changes indicated in the present study, and none of the statistically significant changes in the present study observed at 500 mg/kg/day showed effects in the subsequent 28-day study (which had larger group sizes than the present study). Thus the NOEL for this 15-day study is the highest dose tested: 500 mg/kg/day. This is a useful supplementary data, with no adverse effects. [redacted] 1/13/16.

00300-0320 227377 Bigot, D., "HEPA (2-hydroxy-ethylphosphonic acid): 28-day toxicity study in the rat by gavage," Bayer CropScience, Sophia Antipolis, France, 3/17/03. Laboratory Study #: SA 02184. Test article is a plant metabolite of ethephon (95.9% purity). Groups of 10 Sprague-Dawley rats/sex were initially dosed by gavage with 0, 125, 350, or 1000 mg/kg/day, however excessive toxicity at 1000 mg/kg/day prompted a reduction from day 5 onward to 700 mg/kg/day for that group. In addition to a basic protocol for a supplementary toxicity study, rats were assessed for reflex responses (grasping, righting, corneal, pupillary, auditory, head shaking in response to blowing on the ear) during the last study week. Dosing suspensions were acidic, with pH of 6.5, 1.8, 1.6, 1.4, and 1.2 for 0, 125, 350, 700, and 1000 mg/kg/day suspensions. Toxicity NOEL is 350 mg/kg/day in both sexes. Clinical signs at 700 mg/kg/day included noisy respiration, wasted appearance, soft feces, and increased scabs (males only). Body weight was reduced at 700 mg/kg/day in both sexes. The initial high dose of 1000 mg/kg/day led to death or moribund sacrifice in 1/10 males and 3/10 females within 4 days. A dose-related reduction in urinary crystal formation (compared to controls) in 350-700 mg/kg/day males was plausibly a direct consequence of the low pH of the dosing suspensions. Acidity of suspensions may have been the primary cause of other responses, however the study design does not address causality. Useful supplementary data, with no adverse effects. [redacted], Jan. 4, 2016.

00300-0321 227378 Denton, S. M., "HEPA: Acute oral toxicity study in the rat," Covance Laboratories Ltd, North Yorkshire, England, 12/20/01. Covance Report 2014/30-D6144. Three Wistar rats/sex were dosed by gavage once with HEPA (a plant metabolite of ethephon, 95.7% purity) at 2000 mg/kg (in 10 ml/kg purified water vehicle) in a basic acute oral study. All rats showed diarrhea on day 2. One male displayed lethargy during the first hour. There were no deaths. All were normal at day 15 necropsy. Study provides useful supplementary data, showing no adverse effects. No DPR worksheet is needed for this study on an ethephon metabolite indicating an $LD_{50}$ > 2000 mg/kg. [redacted], Jan. 4, 2016.

300-0322 227379 "Johnson, M., HEPA: Reverse mutation in five histidine requiring strains of *Salmonella typhimurium*," Covance, North Yorkshire, England, 01/29/2002. Test article was HEPA, 95.7% purity. Test article was acidic (1% aqueous solution was pH 1.7). This study was comprised of two independent experiments using strains TA98, TA100, TA1535, TA1537, and TA102: each with and without S-9. Each series spanned dose levels up to 5000 µg/plate, with triplicate samples at every dose. Other than slight thinning of the background lawn, limited primarily to strain TA102 at the highest dose level (with and without S9), there was no indication of treatment responses, and no mutagenicity. Positive controls were functional. Since the test article is not the active ingredient, there is no DPR worksheet at this time. [redacted], Jan. 7, 2016.

00300-0323 227380 Whitwell, J., "HEPA: Induction of chromosomal aberrations in cultured human peripheral blood lymphocytes," Covance Laboratories Inc., North Yorkshire, England, 2/13/2002. This study reports 2 independent experiments, each using pooled blood from 3 healthy male volunteers. Closely-spaced concentrations were evaluated in all cases, with maximum concentration of 1261 µg/ml (10 mM) HEPA, 95.7% purity. Incubation time with test article with S-9 was 3 hours, followed by 17 hours post-treatment recovery. Incubation time without S9 was 3 hours in the first experiment followed by 17 hours post-treatment recovery, or 20 hours continuous treatment. Mitotic inhibition at 1261 µg/ml was up to 49% without S9 and up to 48% with S9, indicating an appropriate dose range. Numbers of cells with aberrations were unaffected by ethephon treatment with or without S9 in either experiment. Positive controls were functional. Since the test article is not the active ingredient, there is no DPR worksheet at this time. [redacted], Jan. 7, 2016.

00300-0324 227381 Ballantyne, M., "HEPA: Mutation at the thymidine kinase (*tk*) locus of mouse lymphoma L5178Y cells (MLA) using the Microtitre® Fluctuation Technique," Covance Laboratories Inc., North Yorkshire, England, 2/13/2002. This study reports 2 independent experiments, each achieving a maximum concentration of 1260 µg/ml (10 mM) HEPA (95.7% purity). The first experiment used 2x steps between successive concentrations, whereas the second experiment using quite closely spaced concentrations near to 1260 µg/ml. Relative survival at the highest concentration ranged from 86-89% of controls. There were no increases in mutant frequencies evident with or without S9 in either experiment. Positive controls were functional. Since the test article is not the active ingredient, there is no DPR worksheet at this time. [redacted], Jan. 7, 2016.