I. DATA GAP STATUS

Combined, rat: No data gap, possible adverse effect (oncogenicity)
Chronic toxicity, dog: No data gap, no adverse effect
Oncogenicity, mouse: No data gap, no adverse effect
Reproduction, rat: No data gap, no adverse effect
Teratology, rat: No data gap, no adverse effect
Teratology, rabbit: No data gap, no adverse effect
Gene mutation: Data gap, inadequate study, no adverse effect indicated
Chromosome effects: No data gap, no adverse effect
DNA damage: No data gap, no adverse effect
Neurotoxicity: Not required at this time.

Toxicology one-liners are attached.

All record numbers through 167295 (Document 208-081) were examined. This includes all records indexed by DPR as of 4/18/00.

** indicates an acceptable study.

**Bold face** indicates a possible adverse effect.

File name: T2000421.wpd

These pages contain summaries only. Individual worksheets may contain additional effects.

II. TOXICOLOGY ONE-LINERS AND CONCLUSIONS
**208-079 167288**, “Benefin: Two-Year Dietary Chronic Toxicity/Oncogenicity Study in Fischer 344 Rats”, (Michael R. Moore, Corning Hazleton Inc, Vienna, VA., Laboratory ID # CHV 174-133, Sponsor Report # DR-0097-3397-005, 1 July 1996). Fifty CDF® (F-344)CrI BR rats per sex per group received benefin in the diet at 0, 10, 100, 2500, and 5000 ppm for 2 yr. An additional 10 per sex per group at the same dose levels were designated for 1-yr interim sacrifice. Estimated achieved dosages were 0, 0.5, 5.4, 136, and 275 mg/kg/day in males, and 0, 0.7, 6.8, 168, and 331 mg/kg/day in females, respectively over weeks 1-104. Chronic NOEL = 10 ppm (0.5 and 0.7 mg/kg/day in M and F), based on increased hyaline droplets in kidneys of both sexes at 100 ppm and above, increased and tubular cell karyomegaly and transitional cell hyperplasia in kidneys of males, hepatocellular hypertrophy and increased hepatocellular pigmentation in females, and calculus of renal pelvis in females. Substantial toxicity prompted investigators to determine that the two highest dose levels “exceeded the maximum tolerated dose and therefore should not be used for risk assessment”. The study design, with no dose levels between 100 and 2500 ppm, gave no opportunity to characterize dose-response in this range of primary interest. These were major changes at the highest two dose levels (in both sexes, unless indicated), in addition to the findings for the NOEL (above) applying to both sexes. Body weights were reduced over time (in males, primarily in last few weeks of the study), so that at termination the deficits of 2500 and 5000 ppm groups compared to controls were 8 and 17% in males, and 18 and 28% in females, respectively. In females only, this was accompanied by approximately 10% food consumption reductions at each of the higher dose levels. There were reductions in the main hematology parameters (RBC counts, HCT, and Hb levels). Incidence and/or degree of chronic progressive nephropathy was increased. The majority of these rats had “slight” to “minimal” degeneration of sciatic nerve and skeletal muscle (thigh). Males had elevated incidence of single cell necrosis in liver. Chronic inflammation of the lungs was seen in the majority of these rats, whereas low incidences of congestion of the abdominal cavity, and urinary bladder hyperplasia were limited to these dose levels. Numerous clinical chemistry changes mirrored the above pathology in kidneys, liver, and perhaps other tissues at the higher two dose levels. Tumor incidence: thyroid follicular tumors (adenomas and carcinomas) increased in males (incidences of 1, 1, 1, 7, and 8 in controls through high dose, respectively) and females (incidences of 0, 0, 1, 5, and 4). Hepatocellular tumors (primarily adenomas) were increased in males (incidences of 2, 2, 1, 5, and 11 in controls through high dose, respectively). There were no statistically significant increases in epithelial cell tumors in kidneys nor urinary bladder, however the low incidences of transitional cell papilloma and tubule cell adenoma or carcinoma were predominantly found in 2500 and 5000 ppm groups (compare to the congener, trifluralin). Study is acceptable. Tumors are possible adverse effects, which should be evaluated in perspective of the many indications of excessive exposures at effective dose levels. Green and Aldous, 4/11/00.

**CHRONIC TOXICITY, RAT**

019 045313, "A Study of the Effects on Rats from the Ingestion of Benefin for Two Years", (H. M. Worth et al., The Lilly Toxicological Research Laboratories, Study R-0295, February 1973). Benefin, technical, purity 95.6% was administered in the feed at concentrations of 0, 1,000, 5,000, or 10,000 ppm/day to 24-26 Harlan rats/sex/group for 2 years. UNACCEPTABLE. Not upgradeable: excessive mortality, disease problems; deficient in ophthalmological, urinalysis, dosing material analysis and food consumption data; insufficient information on hematological, serum chemistry, histopathological evaluation and body weights. NOEL not determined due to insufficient information. Authors consider 1000 ppm to be the NOEL. (Kishiyama, J., and P. Iyer, 4/24/98).
CHRONIC TOXICITY, DOG

**208-081 167295, "Benefin: One-Year Oral Chronic Toxicity Study in Beagle Dogs", (M. D. Walker, Corning Hazleton Incorporated, Vienna, VA., Project # CHV 174-143, Report # DR-0097-3309-004, 26 April 1995). Four beagle dogs per sex per group received benefin (95.8% purity) orally in gelatin capsules (neat) at 0, 5, 25, and 125 mg/kg/day for 52 weeks in a guideline chronic study. Chronic NOEL = 5 mg/kg/day [modest, but statistically significant increases in liver-to-body weight values, and decreases in alanine aminotransferase (ALT) levels at the mid and high dose]. These findings were somewhat more pronounced at 125 mg/kg/day. The high dose also elicited a general increase in liver sinusoidal cell pigments (possibly hemosiderin). There were no observable changes in hematology, clinical chemistry, urinalysis, nor ophthalmology. Acceptable (H. Green, and C. Aldous, 4/17/2000).

Subchronic Dog (range-finding study for 208-081 167295, above):

**208-061 128806, "Benefin: 13-Week Oral Toxicity Study in Beagle Dogs", (D.W. Dalgard, Hazleton Washington, HWA 174-135, 12/28/93). Benefin, purity 95.8%, administered via capsule at concentrations of 0, 5, 25, 125 mg/kg/day to 4 Beagle dogs/sex/group for 13 weeks. Emesis (compound colored) was slightly increased at all dose levels for females and for mid and high dose males. Liver weights was increased for high dose males; and the incidence of hepatocellular hypertrophy was increased for high dose males and females. \textit{Increased incidence of hemosiderin pigment was observed in the liver at the high dose and the spleen at mid and high dose levels}. NOAEL = 5 mg/kg/day. Acceptable (Kishiyama, J., and P. Iyer, 5/12/98).

019 045312, "Two Year Study of Benefin Administered Orally to Beagles", (G. F. Kiplinger et al., The Lilly Toxicological Research Laboratories, November 1972). Technical benefin, purity 95.6% administered orally in gelatin capsules at concentrations of 0, 5, 25, or 125 mg/kg/day to 4 Beagle dogs/sex/group for 2 years. Controls included 5 males and 3 female beagle dogs. Red blood cell count depressed, NOEL = 5 mg/kg. \textbf{UNACCEPTABLE, Not upgradeable.} Some of the animals were in poor condition (mite/mange) and a few were of very low body weight at start; not all serum chemistry parameters for liver function were tested. Only gross eye examinations were conducted (no ophthalmology or histopathology). A number of deficiencies in the conduct of the study, including age of animals, incomplete histopathology, clinical chemistry and reporting of data. (Kishiyama, J., and P. Iyer, 4/20/98).

ONCOGENICITY, MOUSE

**208-051 114649, "A Chronic Toxicity and Oncogenicity Study in B6C3F1 Mice Given Benefin (EL-110, Compound 54521) in the Diet for Two Years", (G.R. Koenig and W.H. Jordan, Lilly Research Laboratories, Lab. Proj. ID: M02785 and M02885, 12/14/88). Benefin (EL-110, Compound 54521), purity 95.25% was admixed with the feed at concentrations 0, 0.005, 0.03 or 0.15% and given to 30 B6C3F1 mice/sex/group in each of the two replicates for two years. These dietary concentrations resulted in estimated average daily benefin doses of 6.0, 36.4 or 184.7 mg/kg for males and 6.9, 41.8 or 223.5 mg/kg for females. Body weight was slightly lowered (4-8%); \textbf{incidence of mouse urologic syndrome increased and renal tubular epithelial vacuolization decreased at mid and high dose levels} (Chronic NOEL = 0.005%). Liver weight, ALT, ALP, and the incidence of bright yellow urine and hepatocellular hyperplasia increased at the high dose level. The combined total of neoplastic tumors (adenomas + carcinomas) appears slightly (not statistically) elevated for the high dose female group, but reported as minimally biologically significant. The Oncogenicity portion of the study is ACCEPTABLE; however the chronic portion of the study \textbf{UNACCEPTABLE and not upgradeable}.}
(urinalysis and ophthalmological exam not performed; hematological, serum chemistry tests not complete). (Kishiyama, J., and Iyer, P., 6/12/98).

**REPRODUCTION, RAT**

**208-078  167287, A** Two-Generation Reproduction Study in Rats with Benefin@ (Janet A. Trutter, Hazleton Washington, Inc., Vienna, VA., Report # HWA 174-136, 9 February 1995). Thirty Sprague-Dawley Crl:CD®BR rats per sex per group received benefin (95.8% purity) in the diet at concentrations of 0, 100, 1000, and 5000 ppm through 2 generations with one litter per generation. Parental NOEL = 100 ppm (7.1 and 8.8 mg/kg/day for M and F, respectively, based on hepatocellular enlargement, enhanced extent of chronic progressive nephropathy in both sexes, and renal tubule hyaline droplets in males: also associated increased liver and kidney weights). Offspring NOEL = 100 ppm (reduced body weights of pups by day 7, continuing at least through weaning). The high dose caused pup weight reductions at weaning to about 60% of controls. Adult 5000 ppm F1 males (the more sensitive gender and generation of adults) weighed about 80% of controls after 10 to 19 weeks of treatment. Lesser but statistically significant decrements were seen in F0 males and in F0 and F1 females at 5000 ppm. Food consumption was correspondingly reduced in both sexes at this dose level. Offspring effects limited to 5000 ppm included significant reduction of mean live litter size at birth and a reduction of pup viability between day 4 cull and weaning (21% loss in 5000 ppm F2 pups). Acceptable, with no adverse effects. (H. Green and C. Aldous, 4/21/00).

021  045317, "A Multi-Generation Rat Reproduction Study with Benefin", (H. M. Worth et al., The Lilly Toxicological Research Laboratories, Study R-0305, R-0795, R-0316, R-0057 and R-0657, April 1973). Benefin (95.6%), administered in the feed at concentrations of 0, 0.1%, or 0.5% to Harlan rats during 7 and 2 matings for F0 and for F1, F2, F3 generations, respectively. No adverse effects reported. Decreased parental body weight gain; pup survival and growth were affected at the high dose: NOEL = 0.1%/day. UNACCEPTABLE. Not upgradeable (2 dose levels; too few pregnant females; pup body weight at birth not given; test substance not characterized, dosing material not analyzed). This is an older study and was not conducted according to FIFRA guidelines (Kishiyama, J., and P. Iyer, 5/7/98).

**TERATOLOGY, RAT**

**020  045316, "Rat Teratology Study with Benefin", (K.M. MacKenzie, Hazleton Research Laboratories, Study No. 6180-101, 6/18/85). Benefin, purity 97.3%, administered by gavage at concentrations of 0 (10% acacia in deionized water), 50, 225, 475, and 1,000 mg/10 mL/kg to 25 mated female Sprague Dawley (Crl:CD®BR) rats/group on days 6 through 15 of gestation. Body weight reduced slightly (4%-5%) but dose-related; corrected body weight and food consumption were also reduced for the 2 highest dose groups. Maternal NOEL = 225 mg/kg/day. Developmental NOEL = 475 mg/kg/day. ACCEPTABLE. (Kishiyama, J., and P. Iyer, 4/30/98).
**TERATOLOGY, RABBIT**

**208-077 167286, “Teratology Study in Rabbits with Benefin”, (M. D. Mercieca, Springborn Laboratories, Inc., Spencerville, OH, Report # 3130.9, 3 June 1991).** Twenty inseminated female New Zealand white rabbits per group received benefin (in 10% aqueous acacia) at concentrations of 0, 25, 50, 100, and 225 mg/kg/day on gestation days 6 through 18 in a guideline teratology study. Maternal NOEL = 50 mg/kg/day (increased incidence of does with few or no feces). Developmental NOEL = 225 mg/kg/day (highest dose tested). The highest dose was clearly maternally toxic, as indicated by significant (p < 0.01) reductions in food consumption and body weight gain, and by the presence of 3 aborted litters and one total litter resorption loss.

**Acceptable, with no adverse effects** (H. Green, and C. Aldous, 4/18/00).

**020 045314, "A Teratology Study on Benefin in the Rabbit", (H. M. Worth et al., The Lilly Toxicological Research Laboratories, Study B-0-7-68, April 1973).** Benefin, purity 96.8%, administered by gavage at 0 (10% feed slurry), 50 and 100 mg/kg/day to 45 artificially inseminated Dutch Belted rabbits on gestation days 6 through 18. Maternal and Developmental NOEL = > 100 mg/kg. UNACCEPTABLE. Not upgradeable, study contains only 2 dose levels; no MTD. Inappropriate dose level selection (Kishiyama, J., and P. Iyer, 4/28/98).

**MUTAGENICITY STUDIES: U.S. EPA PHASE 3 SUMMARIES**

The following six “Phase 3 Summaries”, each prepared for U.S. EPA in the spring of 1990, were submitted as part of Package ID 176338. All summaries relate to studies previously reviewed by DPR. Each summary certified GLP compliance, availability of raw data, and accuracy of summary data in the cited study. Each cited study was assessed according to EPA acceptance criteria. Major study design features and study outcomes were presented. No tabulated data were provided to affect acceptability of studies for DPR evaluation. All asserted that results did not indicate mutagenicity, consistent with original DPR reviews in all cases. Relationship of each “Summary” record to the corresponding record of the DPR report is given below. No DPR reviews of the summaries are needed, since there are no new data to examine. Aldous, 4/18/00.

**Associations of New Records to Cited Study DPR Record Numbers**

<table>
<thead>
<tr>
<th>Study Type</th>
<th>New Submission</th>
<th>Previous Submission</th>
<th>Study ID</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Record</td>
<td>Document</td>
<td>Record</td>
</tr>
<tr>
<td>Gene Mutation</td>
<td>167289</td>
<td>208-080</td>
<td>045311</td>
</tr>
<tr>
<td></td>
<td>167290</td>
<td>208-080</td>
<td>045307</td>
</tr>
<tr>
<td></td>
<td>167293</td>
<td>208-080</td>
<td>045309</td>
</tr>
<tr>
<td>Chromosome Effects</td>
<td>167291</td>
<td>208-080</td>
<td>075190</td>
</tr>
<tr>
<td></td>
<td>167292</td>
<td>208-080</td>
<td>045308</td>
</tr>
<tr>
<td>DNA Damage</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>


GENE MUTATION

018 045307, "The Effect of Benefin (EL-110, Compound 54521) on the Induction of Reverse Mutations in the Using the Ames Test", (G.S. Probst et al., Lilly Research Laboratories, Toxicology Division, Studies 850624AMS2598 and 850708AMS2598, August 1985). Benefin, purity 97.3%, at concentrations of 0 (DMSO), 62.5, 125, 250, 500, or 750 Fg/plate without metabolic activation and at 0 (DMSO), 25, 50, 100, 200, or 300 µg/plate with metabolic activation (rat liver - Aroclor 1254 induced) was evaluated for mutagenicity potential on S. Typhimurium strains TA1535, TA1537, TA1538, TA98, and TA100. The plates were exposed for 48 hours. There were no increases in the number of revertant colonies with benefin. UNACCEPTABLE. Upgradeable (no individual plate counts). (Kishiyama, J and P. Iyer, 3/11/98).

018 045311, "The Effect of Benefin (EL-110, Compound 054521) on the Induction of Bacterial Mutation Using A Modification of the Ames Test", (G.S. Probst et al., Lilly Research Laboratories, Toxicology Division, Study 850610GPA2598, July 1985). Benefin, purity 97.3%, tested (Gradient Plate Assay) at concentrations of 1 (1.0-0.1), 10 (10-1), 100 (100-10), or 1,000 (1,000-100) µg/ml with Salmonella typhimurium strains G46, TA1535, TA100, C3076, TA1537, D3052, TA1538, and TA98 and Escherichia coli strains WP2 and WP2uvrA- without and with metabolic activation (rat liver, Aroclor 1254 induced) for 48 hours. No adverse effect indicated. UNACCEPTABLE. Not upgradeable. Not a guideline study (Kishiyama, J., and P. Iyer, 4/8/98).

018 045309, "The Effect of Benefin (EL-110, Compound 054521) on the Induction of Forward Mutation at the Thymidine Kinase Locus of L5178Y Mouse Lymphoma Cells", (G.S. Probst et al., Lilly Research Laboratories, Toxicology Division, Studies 850612MLA2598 and 850724MLA2598, October 1985). Benefin, purity 97.3%, was tested at concentrations of 0 (DMSO), 5, 10, 15, 20, 25, 30, 35, or 40 µg/ml without metabolic activation and at 0 (DMSO), 0.5, 1, 10, 20, 40, 60, 80, or 100 µg/ml with microsomal enzymes (liver of Fischer rats; Aroclor 1254 induced) for mutagenicity potential to TK+/- cells (TK3.7.2C), a subline of the mouse lymphoma cell L5178Y. No adverse effects were observed. Cell survival was less than 10% for 30 and 40 µg/ml and higher benefin dosages without and with activation, respectively. Benefin groups with greater than 10% cell survival gave no evidence of mutagenicity to L5178Y TK+/- cells. UNACCEPTABLE. Upgradeable (no individual plate counts). (Kishiyama, J. and P. Iyer, 3/17/98).

CHROMOSOME EFFECTS

** 041 075190, "The Effect of Benefin (EL-110, Compound 054521) on the In Vitro Induction of Chromosome Aberrations in Chinese Hamster Ovary Cells", (G.R. Koenig et al., Lilly Research Laboratories, Toxicology Division, Lab. Proj. I.D. 881026CTX2598, 881108CAB2598, 881109CAB2598, 12/20/88). Benefin, purity 96.63%, at concentrations of 0 (DMSO), 20, 30, or 40 µg/ml without metabolic activation and at 0 (DMSO), 0.5, 1, 10, 20, 40, 60, 80, or 100 µg/ml with microsomal enzymes (liver of Fischer rats; Aroclor 1245 induced) for mutagenicity potential to Chinese Hamster ovary cells for 4 hours. No evidence of chromosomal aberrations. ACCEPTABLE. (Kishiyama, J. and P. Iyer, 5/18/98).

018 045308, "The Effect of Benefin (EL-110, Compound 054521) on the In Vivo Induction of Sister Chromatid Exchange in Bone Marrow of Chinese Hamsters", (G.S. Probst et al., Lilly
Research Laboratories, Toxicology Division, Study 850722SCE2598, October 1985). Benefin, purity 97.3%, administered orally (single) at concentrations of 200, 300, 400 or 500 mg/kg to 3 female Chinese hamsters. Slight cytotoxicity (increase in metaphase figures in the first division) is indicated at the high dose. Benefin doses did not induce reciprocal interchanges in the bone marrow of female Chinese hamsters. UNACCEPTABLE and not upgradeable (no males; rationale for dose selection not given). (Kishiyama, J., and P. Iyer, 3/16/98).

DNA DAMAGE

**018 045310, “The Effect of Benefin (EL-110, Compound 054521) on the Induction of DNA Repair Synthesis in Primary Cultures of Rat Hepatocytes”, (G.S. Probst et al., Lilly Research Laboratories, Toxicology Division, Studies 850716UDS2598 and 850723UDS2598, October 1985). Benefin, purity 97.3%, at concentrations of 0 (DMSO), 0.5, 1, 5, 10, 50, 100, 500, or 1,000 µg/ml were tested for mutagenicity potential on cultures of rat hepatocytes (Fischer 344 males). Exposure time was 20 hours. The test was repeated in a second assay. Benefin was cytotoxic at doses 50 µg/ml and higher and precipitated at the 2 highest levels in both tests. No induction of UDS was observed with benefin in either test (0.5, 1, 5, or 10 µg/ml). ACCEPTABLE. (Kishiyama, J., and Iyer, P., 4/8/98).

NEUROTOXICITY

Not required at this time.