

CALIFORNIA ENVIRONMENTAL PROTECTION AGENCY
DEPARTMENT OF PESTICIDE REGULATION
MEDICAL TOXICOLOGY BRANCH

SUMMARY OF TOXICOLOGY DATA
EPTC

Chemical Code # 000264, Tolerance # 00117
SB 950 # 049

August 8, 1986

10/9/86, 8/4/87, 11/8/88, 3/5/90, 10/29/92, 7/13/93, 4/22/94, 8/12/94, 11/24/97, and 10/22/99

I. DATA GAP STATUS

Chronic toxicity, rat: No data gap, possible adverse effect
Chronic toxicity, dog: No data gap, possible 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
Teratology, rat: No data gap, possible adverse effect
Teratology, 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, no adverse effect
Hen Neurotoxicity: No data gap, no adverse effect*

*Although the acute delayed neurotoxicity study in **hens** indicated negative results, **rat** neurotoxicity studies did show possible adverse effects (rat neurotoxicity data follow the section on hen neurotoxicity in this Summary of Toxicology Data).

Toxicology one-liners are attached.

All relevant record numbers for the above study types through 169801 (Document No. 117-127) were examined. This includes all relevant studies indexed by DPR as of 10/22/99. Several older studies have record numbers > 900,000.

In the 1-liners below:

** indicates an acceptable study.

Bold face indicates a possible adverse effect.

File name: T991022

Revised by G. Chernoff, 3/5/90; Gee, 10/29/92 and 7/13/93; Kellner, 4/22/94 and 8/12/94; Aldous, 11/24/97 and 10/22/99.

II. TOXICOLOGY ONE-LINERS AND CONCLUSIONS

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

NOTE: DPR has on file 024 270, "Guidance for the Reregistration of Pesticide Products Containing EPTC as the Active Ingredient." (USEPA, September 30, 1983)

COMBINED, RAT

****117-032 026949**, "Two Year Oral Toxicity/Oncogenicity Study in Rats", (9/30/83, IRDC). EPTC (lot CHK-0601, 98.6% by weight) was fed to 60/sex/group at 0, 5, 25 or 125 mg/kg in diet over two years; diets analyzed periodically; several compound related effects at 125 mg/kg and to a lesser extent at 25 and 5 mg/kg; NOEL: < 5 mg/kg/day in males for muscle atrophy/degeneration and 5 mg/kg/day in females; adverse effects include cataracts, neuromuscular atrophy and degeneration, external testes and possibly chronic myocarditis; ACCEPTABLE with a **possible adverse effect**. Gee, 10/7/85.

106 127054, Addendum to the final report (032:026949); contains data from the re-examination of heart slides, confirming the incidence of heart lesions (atrophy, chronic myocarditis, endocarditis, fibrosis, pericardial chronic inflammation, mineralization, pigmentation and thrombosis) noted in the final report. The author reported a NOEL for the heart lesions at 25 mg/kg/day (mid-dose). The males may have shown a slight increase in chronic myocarditis at this level, so a more appropriate male NOEL may be 5 mg/kg/day. No Worksheet. Kellner, 4/25/94.

037-039, 034467-034469, Addenda (individual data) to 026949.

018 935176, Interim report for 026949.

****117-069 055491**, (10 volumes), "Two Year Oral Feeding Study of the Oncogenicity and Chronic Toxicity of EPTC in Rats", (Hazleton Labs America, 3/20/87, Study No. 6100-106). EPTC, technical, Lot No. 518-996, 98.4%; fed in the diet at 0, 9, 18, 36 or 72 mg/kg/day with periodic adjustments in the ppm to maintain the nominal doses; 90/sex/group of CrI:CD(SD)BR rats; NOEL in males not established (hindquarters muscle syndrome at 9 mg/kg/day, first observed in this group near to termination of study), NOEL in females = 9 mg/kg/day (decreased body weight gain, skeletal muscle atrophy and nerve changes especially in hind quarters, slight increase in degenerative cardiomyopathy; possible adverse effect (hindquarters muscle syndrome). The incidence of HMS appeared in males at earlier times as the dose increased and the incidence was dose related in both males and females. The occurrence of cataracts in treatment groups versus controls was not as clear. ACCEPTABLE. Marovich, 5/4/87 and Gee, 6/4/87.

030 017644 interim report for Document No. 117-069 (above). No Worksheet.

CHRONIC, DOG

**117-053-054, 046264-046265, "One-year Oral Feeding Study of the Chronic Toxicity of EPTC in Dogs", (6/10/86, Hazleton). EPTC, 98.4% (lot 518-996); fed in the diet at 0, 200, 600 or 1800 ppm for one year; 6/sex/group; NOEL not clearly established but \geq 1800 ppm; no adverse effect. No findings in the heart were reported upon necropsy or microscopic exam. Emesis was slightly increased in severity at the high dose over the test period. This study was initially reviewed as unacceptable because the selection of doses was not justified and the subchronic study, Record No. 50734, was not on file. With the submission of the subchronic, the study is upgraded to ACCEPTABLE with the comment that the high dose was marginal in showing any adverse effect. Gee, 10/7/86 and 8/3/87.

066 050734, "A Three Month Subchronic Oral Toxicity Study of EPTC in Beagle Dogs", (Bio/Dynamics, Inc., 2/15/85, Project No. 83-2781). EPTC, 98.4%; fed in the diet to 6/sex/group at 0, 200, 600 or 1800 ppm for 3 months; no adverse effect reported and NOEL \geq 1800 ppm. Submitted as justification for the selection of doses in the one-year study, Record numbers 46264 and 46265. Marovich, 5/5/87 and Gee, 6/3/87.

**117-077 065928, "One-Year Oral Toxicity Study with Eptam Technical in Dogs; Final Report", (Stauffer Chemical, CT, 9/8/87, T-12712). Eptam technical, 97.6% by weight; tested at 0, 1, 8 or 60 mg/kg/day by capsule with 5 beagle dogs/sex in control and high dose groups and 4/sex in low- and mid-dose groups; NOEL = 8 mg/kg/day (body weight, cholinesterase inhibition, neuropathology at 60 mg/kg with 1 male sacrificed day 84 due to toxicity). Neuropathology described as Wallerian degeneration of the spinal cord (multiple sites) and peripheral nerves with males more affected than females. In addition, bile stasis was increased in incidence at 60 mg/kg and was considered potentially treatment-related. ACCEPTABLE. Gee, 7/20/88,

SUMMARY: Both studies in the dog were evaluated as acceptable but with different conclusions about adverse effects. Although both studies used the oral route, the study at Hazleton fed the EPTC in the diet with a maximum of 1800 ppm which was marginally toxic. The study by Stauffer gave the EPTC by capsule and used 60 mg/kg/day as the high dose - estimated as approximately 2400 ppm based on 1 mg/kg/day equivalent to 40 ppm in the diet. Since this dose was higher than that used by Hazleton and was administered all at one time, the conclusion is that EPTC treatment resulted in a **possible adverse effect**. Gee, 11/8/88.

ONCOGENICITY, RAT

See under combined rat.

ONCOGENICITY, MOUSE

018 935172, "Lifetime Oral Study in Mice", (12/22/78, IRDC). EPTC technical grade, three lots; fed to 60/sex/group, CD-1 mice, at 0, 5, 20, or 80 mg/kg in the diet for two years; NOEL not clearly established because high dose showed marginal effect on blood parameters with no MTD; no oncogenic effects reported; UNACCEPTABLE (test material not described, doses not justified, muscle and sciatic nerve were not examined microscopically), possibly upgradeable.

Note that this report was judged as acceptable 3/20/85 but re-review found the study was missing some necessary data. Gee, 3/20/85 and 10/7/85.

040 034470, Addendum (individual data) for 935172.

**117-049-052, 045704-07, "Oncogenicity Study in Mice with EPTC, Study no. 6100-104", (5/15/86, Hazleton). EPTC, lot 518-996, 98.4%; fed to 60/sex/group, CRL:CD-1 mice, at 0, 200, 600 or 1800 ppm for 73 weeks; NOEL = 200 ppm based on lower body weights and lower food intake; no oncogenic effect; no adverse effect reported; no findings in muscle or sciatic nerve; ACCEPTABLE. Gee, 10/8/86.

Comment on long-term feeding studies in rats, dogs and mice: The adverse effects on nerve and muscle in the rats in two studies conducted for two years was confirmed in one dog study conducted over a one-year period but not in the mouse at 73 weeks. In the rat, the effect did not appear until late in the study. In the dog study by Stauffer, degeneration of the spinal cord and peripheral nerves and skeletal muscles was reported. In the second mouse study, Record # 935172, which lasted two years, the skeletal muscle and sciatic nerve were not included in the list of tissues examined histologically but no behavioral effect was reported. Gee, 11/8/88.

REPRODUCTION, RAT

018 935174, (5/12/75, Woodard Research Corp.). Eptam technical, five male and 15 female Sprague-Dawley rats were fed 32 mg/kg/day starting 3 days before mating. Not by standard protocol, UNACCEPTABLE, not upgradeable. Insufficient information to evaluate. Gee, 3/21/85.

**117-018 935175, "A Two-Generation Rat Reproduction Study with Eptam Technical", (10/8/82, Stauffer, T-10123). EPTC (98.6%) tested at 0, 40, 200 and 1000 ppm in the diet; two generations with two litters per generation; 15 males and 30 females per group; NOEL = 200 ppm (parental body weight and pup weights); no reproduction effects reported; necropsy on all parental animals. ACCEPTABLE with 046266-71 below. Gee, 10/7/85

EPA one-liner: core grade of Supplementary. NOEL = 200 ppm based on decreased parental body weights and food intake at 1000 ppm (HDT). No histopathology data on reproductive tissues or target organs. [Review date of EPA is not clear.]

036 034465, Addendum (individual data) to 935175.

036 034466, Duplicate of 935175.

**117-055 to 060, 046266-71, "Two-Generation Reproduction Study with EPTC in Rats", (6/9/86, Hazleton, Study 6100-108). EPTC, lot. 518-996, 98.4% purity; fed at 0, 50, 200 or 800 ppm in the diet to 30/sex/group, for 10 weeks before mating for 2 generations; no adverse reproductive effect on parental animals; significantly reduced pup weights at 800 ppm in F₁a pups and F₂a pups; parental body weights were also reduced at these doses; all F₀ parents given a gross and microscopic exam of gonads and selected tissues; Reproductive NOEL = 800 ppm (HDT); systemic NOEL = 50 ppm. Report states NOEL to be 200 ppm. Note: Although not a reproductive effect, the incidence of cardiomyopathy was increased in F₁A adults at termination in a dose-dependent manner with a NOEL of 50 ppm. ACCEPTABLE. Gee, 10/8/86.

TERATOGENICITY, RAT

****117-035 034463**, "A Teratology Study in Rats with Eptam", (11/3/83, WIL Research, WIL-27013). EPTC (considered 100% pure, 98.6% by weight, lot #CHK 0601) was administered by gavage to groups of 25 COBS-CD rats at doses of 0 (corn oil vehicle), 30, 100, or 300 mg/kg/day on days 6-15 of gestation. Maternal mortality and toxicity, and decreased fetal weight was observed at 300 mg/kg; increased resorptions were noted at 100 and 300 mg/kg. Maternal NOEL = 100 mg/kg (toxicity and mortality); Developmental NOEL = 30 mg/kg (resorptions) and a **POSSIBLE ADVERSE HEALTH EFFECT** is noted. The study was initially evaluated as acceptable (J. Gee, 10/4/85) but then downgraded to unacceptable for lack of dosing solution analyses (J. Gee and J. Parker, 8/87). Supplemental data on the dosing solution (CDFA record no. 090111) are adequate to complete the study and upgrade the status to ACCEPTABLE (G. Chernoff, 3/2/90).

071 060524, Supplement to 034463. Characterization of Technical Eptam Lot CHK 0601. This document identifies the impurities in the technical grade. CDFA needed analyses of dosing solutions or a retrospective analysis accompanied by copies of the laboratory notebooks showing the correct weights of active ingredient. No worksheet prepared. No change in study status. Parker 11-8-88.

031 26944 Partial duplicate of -035:034463.

089 090111, "A Retrospective Analysis of EPTC (EPTAM) Technical Lot No. CHK 0601: Addendum to Teratology Study in Rats with EPTAM, T-11753", (ICI Americas Inc., T-13536, 7/14/89). Supplementary to 034463.

044 039607, "Effect of EPTC on Pregnancy of the Rat", (11/6/85, Huntingdon Res. Centre). EPTC (98.4%), batch no. 518996; Crl:COBS CD (SD) BR rats; tested at 0, 30, 100 and 300 mg/kg in 1% methyl cellulose by gavage days 6-15 of gestation to 25/group; analyses of dosing solutions and stability included; maternal and developmental NOEL: > 300 mg/kg; UNACCEPTABLE (MTD not achieved), not upgradeable. Parker, 4/25/86.

066 051092, Supplement to 039607. Summary table of maternal findings and rebuttal. No worksheet prepared. No change in study status. Gee and Parker, 8/4/87.

SUMMARY: The two rat teratology studies arrived at very different NOEL's, and gave contradictory results as regards possible adverse health effects. As noted by Gee and Parker (8/4/87 and 11/9/88), these discrepancies could be attributed to differences in: the source of animals; the purity and source of test compound; and the control vehicle used. After consideration of the merits of both studies, CDFA has decided to use the findings in study 034463, where clear signs of maternal toxicity were observed at the high dose tested. Based on these findings, the Maternal NOEL is set at 100 mg/kg, and the Developmental NOEL is set at 30 mg/kg with a possible adverse health effect (increased resorptions) noted (G. Chernoff, 3/5/90).

TERATOGENICITY, RABBIT

****117-045 039608**, "Effect of EPTC on Pregnancy of the Rabbit", (10/10/85, Huntingdon Res. Centre). EPTC (98.5%), batch 518996; tested at 0 (1% methylcellulose), 30, 100 and 300 mg/kg by gavage to 16-18 per group on days 6 through 18 with day of mating = day 0; New Zealand White rabbits; maternal toxicity NOEL = 300 mg/kg, MTD not reached - however, a preliminary study had cholinergic signs at 350 mg/kg/day; developmental toxicity NOEL: < 30 mg/kg, malformations noted in all treated groups; ACCEPTABLE. Parker, 4/25/86. The incidence of malformations was, per total litters, 1/15, 4/15, 5/13 and 5/15. These occurred in 1/1, 4/3, 5/4 and 5/5 actual litters. No pattern of malformations was reported. The authors did not consider EPTC to cause developmental effects based on the lack of specific types of malformations, distribution of malformations to "occasional" bucks and no associated findings with death or increases in anomalies or variants. Gee, 7/9/93.

066 051091, Historical control data for 039608 and rebuttal. No change - the possible adverse effect remained. Gee and Parker, 8-4-87.

****117-076 065927**, "A Teratology Study in Rabbits with Eptam Technical", (Stauffer Chemical Company, CT, 8-12-87, T-12982). Eptam Technical, 97.6%, administered by gavage at 0 (corn oil), 5, 40, or 300 mg/kg/day to 16-18 mated NZW rabbits/dose level on days 7-19 of presumed gestation. Maternal NOEL = 40 mg/kg/day (decreased body weight gain, cholinergic signs and decreased serum and RBC cholinesterase). Developmental NOEL = 40 mg/kg/day (decreased fetal weight). ACCEPTABLE. No Adverse Effect. Parker 11/7/88.

SUMMARY; The fetal malformations seen in the HRC study (039608) were not confirmed in the subsequent study conducted at Stauffer (065927). Differences may be due to different vehicles and source of animals. In looking at the collective data, the evidence for a developmental effect is weak. Since in the Stauffer study there was no indication of fetal changes similar to those seen in the HRC study, and maternal cholinesterase was decreased both statistically and biologically at 300 mg/kg/day, CDFA considers there to be no indication of an adverse effect. Thus the rabbit teratology data gap is filled and no adverse effect is indicated. Parker 11/8/88. The overall NOEL is considered to be 40 mg/kg, based on 065927. Gee, 7/13/93.

071 060523 Supplemental to study 076:065927. Contains analyses of technical EPTC and dosing solutions. No worksheet.

TERATOGENICITY, MOUSE

018 935173, (4/6/67, Woodard Research Corp.). EPTC, 97.8%; UNACCEPTABLE (major variances from guidelines), not upgradeable. Twenty females were fed 0, 8 or 24 mg/kg/day of 97.8% EPTC. No justification of dose or evidence of toxicity. Gee, 3/21/85

EPA one-liner: Core grade of invalid. Non-gavage study and data on actual intake of test material. Only 7, 8 and 6 pregnant animals. Only 2 doses with data under reported.

035 034464, Duplicate of 935173.

GENE MUTATION

****117-095 113165** "EPTC - An Evaluation of Mutagenic Potential using S. typhimurium and E. coli." (R. D. Callander, ICI Central Toxicology Laboratory, UK, Report No. CTL/P/3585, 2/6/92) EPTC, 98.6%, Batch P4, D7534/10, was tested with Salmonella typhimurium strains TA1535, TA1537, TA98 and TA100 and with Escherichia coli strains WP2P and WP2P uvrA with and without activation with Aroclor-induced male rat liver S9. Concentrations used were 0 (DMSO), 100, 200, 500, 1000, 2500 and 5000 µg/plate. Evidence of cytotoxicity was noted at 1000 µg/plate and above by effects on the background lawn and reduced number of spontaneous revertants. There were triplicate plates per strain/concentration/trial. In the first trial, the plate incorporation method was used. In the second trial with S9, a pre-incubation of 60 minutes was used before plating; without S9, the plate incorporation method was used. Positive controls were functional. No evidence for the induction of revertants was reported. No adverse effect. ACCEPTABLE. Gee, 10/28/92.

018 935178, 031727, "Mutagenicity Evaluation of Eptam Tech 3905-35, Final Report", (Litton Bionetics, 10/77, project no. 20838). EPTC, purity not stated; tested in Salmonella at 0, .001, .01, .1, 1.0 and 5.0 ug/plate with and without activation; TA1535, TA1537, TA1538, TA98, and TA100, single plate per strain; UNACCEPTABLE (no replicates and no good evidence for activity of S9 fraction), not upgradeable. Also includes Saccharomyces D4 (# 31727). No increase in reversion rate reported. Gee, 3/21/85.

018 031728-031729, "Mutagenicity Testing on EPTC in Microbial Systems", (7/24/78, Institute of Environmental Toxicology). EPTC (97.2%) tested at 0, 10, 50, 100, 500, 1000, and 5000 ug/plate in Salmonella strains TA1535, TA1537, TA1538, TA98, and TA100 with and without metabolic activation; UNACCEPTABLE (no replicate trial); not upgradeable. No increase in reversion rate reported. Also tested E. coli WP2 hcr strain. Gee, 3/21/85.

018 935180, "Evaluation of Herbicides for Possible Mutagenic Properties", (1972, publication in J. Agr. Food Chem. 20: 649). 110 Pesticides including EPTC; "-" for activity in Salmonella; UNACCEPTABLE with insufficient information. Gee, 3/21/85.

****117-032 026946**, "Mutagenicity Evaluation in Mouse Lymphoma Multiple Endpoint Test: Forward Mutation Assay", (9/17/84, Stauffer). EPTC (98.6%, lot 4921-4-10) tested at 0, 0.005, 0.01, 0.02, 0.04 and 0.06 ul/ml on mouse lymphoma (L5178Y) cell + rat liver S9; 0.0125, 0.025, 0.05, 0.1 and 0.15 without S9; in presence of S9, test article induced moderate increase in mutation frequency; two trials. At 0.06 ul/ml, mutation frequency per 10^6 was 105 in trial 1 and 158 in trial 2 compared with 31 for solvent controls. ACCEPTABLE. Gee, 10/8/85.

047 042779, "Mouse Lymphoma Cell Mutagenesis Assay (TK+/- to TK-/-) of EPTC", (2/18/86, SRI International). EPTC (98.5%, lot 518-996), in mouse lymphoma (L5178Y) cells, tested at 0, 42, 60, 86, 123, 175, and 250 ug/ml with rat liver activation and 0, 118, 131, 145, 162, 180 and 200 ug/ml without activation; 2 or 3 cultures for each concentration; one trial only; no increase in mutation frequency (MF) without activation; with S9 activation, MF increased to 487 and 521 at 175 and 250 ug/ml compared with 86 for solvent control. UNACCEPTABLE (single trial). Gee, 10/6/86.

Summary: The data gap for gene mutation is filled. Studies with bacteria were negative for mutagenicity. Comparison of the two studies in mammalian cells (036946 and 042779) shows that EPTC treatment gave comparable results -- no observable increase without activation and about a 3-fold increase with activation. Concentrations used in the second study were somewhat higher than in the first. **The data gap is filled with a possible adverse effect.** Gee, 11/8/88 and 10/28/92.

CHROMOSOME EFFECTS

****117-032 026947**, "Mutagenicity Evaluation in Mouse Lymphoma Multiple Endpoint Test: Cytogenetic Assay", (9/17/84, Stauffer). EPTC (98.6%) tested at 0, 0.005, 0.01, 0.02, 0.04 and 0.06 ul/ml on mouse lymphoma (L5178Y TK+/-) cells with activation and 0.0125, 0.025, 0.05, 0.10 and 0.15 ul/ml without activation; exposed 4 hours, resuspended in Budr for 20 hours; 50 cells per culture were scored. Increased aberrations at 0.025 ul/ml and increased aneuploidy at 0.01 ul/ml without activation -- neither finding was concentration dependent; initially reviewed as unacceptable based on no repeat experiment to confirm the possible adverse effect and not upgradeable. Reconsideration upgrades the study to ACCEPTABLE status as a confirming repeat is not essential with this test type. Gee, 10/7/85 and 8/4/87.

032 026948, "Mutagenicity Evaluation in Bone Marrow Micronucleus", (11/28/84, Stauffer). EPTC (98.6%, Lot 4921-4-10) tested at 0, 250, 500, and 1000 mg/kg (trial 1, single dose) or 1000, 1200 and 1400 g/Kg (trial 2, two doses at 24 hours) by gavage in a micronucleus assay using CD-1 mice (both male and female); animals were sacrificed at 24, 48 and 72 hours; increase in micronuclei/1000 PCE in the first, but not the second trial; UNACCEPTABLE (need individual data on micronuclei/1000 cells, CP control questionable, vehicle controls for females are high compared to males in trial 1 and for both males and females in trial 2, high mortality in trial 2, results of trial 2 vary considerably from those of trial 1), possibly upgradeable. Gee, 10/7/85.

097 115687, "EPTC: An Evaluation in the Mouse Micronucleus Test", (V. Randall and J. M. Mackay, ICI Central Toxicology Laboratory, Report No. CTL/P/3724, Study No. SM0612, 6/3/92). EPTC, purity 98.6%, a single oral dose at concentrations of 0 (corn oil) or 800 mg/kg was administered to 5 (C57BL/6JfBL10/Alpk) mice/sex/group/sacrifice interval. The selection for MTD (800 mg/kg) was based on results obtained from a range-finding study. Bone marrow erythrocytes were sampled at 24 and 48 hours after dosing. **Possible adverse effect:** A slight but statistically significant increase in micronucleated polychromatic erythrocytes was observed at 24 hours. This was reported as biologically not significant by the authors. Because of the deficiency of not having a third sampling time, the study is considered to suggest an adverse effect. UNACCEPTABLE. Not upgradeable (study lacks 3 sampling intervals for bone marrow erythrocytes and/or additional dose levels). (Kishiyama and Gee, 10/28/92)

****117-047, 048 042777**, "Clastogenic Evaluation of EPTC Technical, 518-996, BR85-40, in an in vitro Cytogenetic Assay Measuring Chromosomal Aberration Frequencies in Chinese Hamster Ovary (CHO) Cells", (11/85, Litton Bionetics). EPTC (lot 518-996, 98.4%) was tested in duplicate in Chinese hamster ovary (CHO) cells at 0, 15, 30, 75 or 150 ug/ml with rat liver activation (2 hours) and at 0, 30, 60, 90 or 120 ug/ml without activation (17.5 hours); no increase in chromosome aberrations is reported; ACCEPTABLE. Gee, 10/6/86.

Summary: The two in vitro chromosomal aberration studies have conflicting results -- reconsideration of the results with mouse lymphoma, in view of the later study, make the biological significance questionable. The study did not show a dose-related response for either aberrations or aneuploidy. The micronucleus effect, however, is still unresolved for a possible adverse effect. **The data gap is filled with a possible adverse effect.** Gee, 11/8/88. A second micronucleus study has been submitted and suggests a slight increase in micronuclei at the 24 hour harvest time. The study is, however, unacceptable - see above for record no. 115687. The possible adverse effect remains. Gee, 10/28/92.

DNA DAMAGE/OTHER

018 935179, "Mutagenicity Testing on EPTC in Microbial Systems", (7/24/78, Institute of Environmental Toxicology). EPTC (97.2%) tested at 0, 1, 5, 10, 25, 50, and 100 % V/V in B. subtilis (strains M 45 and H17); disk assay; UNACCEPTABLE (no repeat experiment, no activation included and other major variances from guidelines), not upgradeable. Gee, 3/21/85.

030 026945, "Effects of EPTC on Human Fibroblast DNA", (9/19/84, Stauffer). EPTC (98.6%, lot 4921-4-10) tested on human skin fibroblast DNA; no effects reported; UNACCEPTABLE (insufficient protocol for evaluation), Two tests were run: 1) cells were treated 30 min and the DNA sized on alkaline sucrose gradients and 2) DNA was nick translated with E. coli pol I and radioactive dCTP with DNA collected on filters. Gee, 10/8/85.

**117-047, 048 042778, "Evaluation of the Potential of Ethyl-(N,N-dipropyl) thiocarbamate to Induce Unscheduled DNA Synthesis in Primary Rat Hepatocyte Cultures", (1/86, SRI International). EPTC, lot 518-996, 98.5%; primary rat hepatocytes tested for unscheduled DNA synthesis at 0, 0.1, 0.5, 1.0, 3.0, 5.0, 30, 50, 100, 250, 500, 1000 or 5000 ug/ml, 19-21 hours with ³H-thymidine; no increase in UDS is reported; ACCEPTABLE. Gee, 16/6/86.

NEUROTOXICITY, HEN

031 026950, "Acute Delayed Neurotoxicity Study with Technical EPTAM in adult Hens", (2/5/81, Stauffer). EPTC (98.6%, lot CHK 0601) treatment on days 1 and 22 at 7200 mg/kg; 12 hens in negative and positive control groups, 16 in treatment group; test article caused mortality of 6/16; 4/10 survivors showed some bilateral degeneration of the sciatic nerve, but no brain or spinal cord lesions different from controls; Marek's disease in all; UNACCEPTABLE (no individual data), upgradeable. A more recent study (see below) does not report an adverse finding but the dose was lower by 35%. Study 026949 in rats (see above) indicated neuromuscular atrophy and degeneration due to EPTC. Gee, 10/9/85.

**117-034 032736, "Acute Delayed Neurotoxicity Study with EPTC Technical in the Domestic Hen", (12/28/84, Huntingdon Research Centre). EPTC, 98.4%; forty hens dosed at 4674 mg/kg, 10 hens in both positive and negative control groups; 4674 mg/kg the calculated LD₅₀; no evidence of acute delayed neurotoxicity; initially reviewed as unacceptable (not all hens subjected to histopathology (10/31 selected), no atropine protection and in view of data in record 026950, dose may not be high enough) but possibly upgradeable. With submission of the rebuttal in 117-066, the question of how the hens were selected for histopathology has been

answered. Also, as pointed out, the dose of 4674 mg/kg is close to the limit test of 5000 mg/kg. The study has been upgraded to ACCEPTABLE status. Gee, 10/4/85 and 8/3/87.

EPA: Guideline.

Note: The previous EPTC Toxicology Summary (T930713) contained an adverse effect flag for neurotoxicity based on neuromuscular atrophy and degeneration seen in two-year chronic toxicity/oncogenicity studies in rats (-032:026949 and -069: 055491). Subsequent to the review of these studies, an acute delayed neurotoxicity study in hens (-034:032736) was upgraded to acceptable by DPR. No adverse effect with respect to delayed neurotoxicity was indicated, so the adverse effect flag was removed from the neurotoxicity category in the current summary to reflect the acceptable (and negative) hen study. The neuromuscular findings in rats will still be indicated by the possible adverse effects flag seen in the combined rat category.

RAT NEUROTOXICITY

117-108 127756 Brammer, A. "EPTC: Acute Neurotoxicity Study in Rats" (Zeneca Central Toxicology Laboratory, Alderley Park, UK; Zeneca report # CTL/P/4092, 10/18/93). EPTC technical (batch # P4/D7534/10, 98.4% purity) was administered in a single oral dose to 10 Alpk:APfSD (Wistar-derived) rats/sex/dose group at levels of 0, 200, 1000 or 2000 mg/kg. Compound-related deaths included 2 males and 1 female at 2000 mg/kg, plus 1 female at 1000 mg/kg. Clinical signs in these groups were lacrimation, salivation, upward spinal curvature and decreased activity. Body weights and food consumption were lower than control in the 1000 and 2000 mg/kg during week 1. **Possible Adverse Effects:** Neuronal cell necrosis of the pyriform/entorhinal cortex and the dentate gyrus; brain weight reductions. Original report indicated no NOEL in males (neuronal cell necrosis at all dose levels). Blind re-reading of slides by two pathologists (see Document No. 117-122, below) justifies 200 mg/kg as a NOEL, since there was no pattern of dead cells in those brain areas attributable to treatment at that dose. Unacceptable, with useful information: inadequate positive control data and test compound analyses and missing FOB observations (i.e. incomplete reflex/physiologic data and no home cage, handling, or open field observations). Not upgradeable, but useful information. Some additional data, mainly FOB and clinical observations, were presented (see Document No. 117-123, below). These data do **not** confirm that a formal FOB was undertaken according to U.S. EPA Guidelines (i.e. systematic set of observations beginning in the home cage, with subsequent observations progressing from least interactive with investigators, to most interactive), although most recommended parameters were examined. Kellner and Gee, 3/4/94; Aldous, 11/24/97.

117-123 157379 Brammer, A., "First revision to EPTC: Acute neurotoxicity study in rats", [Addendum to Document # 117-108, Record # 127756], Report revision date: 12/1/95. Part 1 contains summary tables, including titles of "Clinical Observations, Total", "Home Cage/Open Field Observations", and "Manipulative/In Hand Observations". Most of the specific parameters which would be expected to be reported in an FOB were found among the entries of these summary tables. Individual "Clinical Observations" data, which comprised most of Part 2 of this record, contain an entry for each of the listed parameters for each animal at each of the scheduled FOB evaluation days, plus additional notations for any findings noted during clinical observations made between FOB days. Because of limitations of the positive control data (see 1994 DPR review), it is not possible to evaluate the capability of technicians to identify subtle

FOB findings. The neuronal cell necrosis and the several identified clinical signs define the apparent NOEL for this study. Study remains unacceptable, with useful data. Aldous, 11/24/97.

117-110 131203 Tinston, D. "EPTC: Subchronic Neurotoxicity Study in Rats" (Zeneca Central Toxicology Laboratory, Alderley Park, UK; Zeneca report # CTL/P/3930, 4/29/94). EPTC technical (batch # P4/D7534/10, 98.4% purity) was administered in the feed to 12 Alpk:APfSD (Wistar-derived) rats/sex/dose group at levels of 0, 100, 500 or 2500 ppm for 13 weeks. Dose-related clinical signs in the high-dose rats were limited to increased incidence of urinary incontinence in females. Body weights and food consumption were less than control in the 500 and 2500 ppm dose groups. **Possible Adverse Effects:** Neuronal cell necrosis of the pyriform cortex and part of the hippocampal formation of the forebrain. NOEL (for neuronal cell necrosis) = 500 ppm (review based on original readings had suggested a NOEL of 100 ppm, however see re-readings in 117-122 157378, below). Brain weight reductions were noted in high-dose males and females and mid-dose females. NOEL (brain weight) = 100 ppm. UNACCEPTABLE. Inadequate positive control data and dose-level justification; missing FOB observations (i.e. incomplete reflex/physiologic data and no home cage, handling, or open field observations). Some additional data relating to FOB and clinical observations were presented (see Document No. 117-124, below). These data do **not** confirm that a formal FOB was undertaken according to U.S. EPA Guidelines (i.e. systematic set of observations beginning in the home cage, with subsequent observations progressing from least interactive with investigators, to most interactive), although most recommended parameters were examined. Not upgradeable, but useful information. Kellner and Gee, 8/3/94; Aldous, 11/24/97.

117-124 157380 Tinston, D. J., "First revision to EPTC: Subchronic neurotoxicity study in rats", [Addendum to Document # 117-110, Record # 131203], Report revision date: 11/20/95. This record bears the same relationship to its primary study that Record No. 157379 bears to Record No. 127756 (see review for Record No. 157379). One difference in the content of the clinical observations data in Part 2 of this record is that findings are presented by week instead of by day. There are no new findings. The same limitations noted for the acute neurotoxicity study apply to this study as amended. Study remains unacceptable, with useful data. Aldous, 11/24/97.

117-122 157378 Chalmers, D. T., S. J. Duffell, and S. A. Horner, "Thiocarbamates: Selective re-examination of neuropathology", Addendum to acute and subchronic rat neurotoxicity data in Document #'s 117-108 and 117-110 (Record #'s 127756 and 131203). Addendum Report No. CTL/P/4618, dated 3/28/95. Two pathologists reviewed slides of the pyriform cortex and the ventral portion of the dentate gyrus for all rats included in acute and subchronic neurotoxicity studies on six thiocarbamates, including EPTC. Pathologists were unaware of individual rat identity until after reaching diagnoses on neuronal cell necrosis. Diagnostic criteria were designed to discriminate between the low background incidence of dead cells in the regions of interest, vs. distribution patterns or degree of incidence of dead cells suggestive of a treatment effect. Original and re-evaluation results for EPTC are presented in this review. Data support a NOEL of 200 mg/kg for acute exposure, and raise the NOEL to 500 ppm (44 mg/kg/day) for histopathology after subchronic exposure. Variances from guideline study design preclude upgrading the studies to acceptable status. Aldous, 10/31/97.

RAT METABOLISM

117-127 169799 Gledhill, A. J., "EPTC: Biotransformation in the rat", Zeneca Central Toxicology Laboratory, Alderley Park, 4/23/98. Laboratory Project ID # CTL/P/5101. This study characterized metabolites in bile duct-cannulated (2/sex) and non-cannulated (5/sex) rats administered single doses of EPTC in corn oil by gavage at 300 mg/kg. Non-labeled EPTC purity was 99.8%. Radioactive material was ¹⁴C-labeled on the n-propyl carbons adjacent to the nitrogen, and was > 98% radiopurity. Most of the label collected in bile duct-cannulated rats was eliminated within the first 24 hours after dosing. There were no remarkable sex differences in proportions of metabolites. Over half of recovered label was dipropylamine (primarily in urine). Non-cannulated rats excreted 16% to 20% of label in urine as the EPTC mercapturate. Several other metabolites identified evidently derived from glutathione conjugation products. Investigators determined that the probable main pathway of metabolism involved an initial sulfur oxidation of EPTC, followed by either hydrolysis to yield the dipropylamine (easily excreted in urine) or GSH conjugation, to be excreted initially through the bile. Thus 9% to 11% of administered label was found in bile as the glutathione conjugate in cannulated rats, this being (via reabsorption) the source of the urinary mercapturate noted above in intact rats. Comparatively small amounts of other metabolites resulted from O-glucuronide conjugation products, hydroxylation products, or de-alkylation of one of the N-propyl groups. Parent compound was not found in urine or bile. This is a valid component of the metabolism study series. Aldous, 10/21/99.

117-127 169800 Davies, D. J., "EPTC: Excretion and tissue retention of a single oral dose (300 mg/kg) in the rat", Zeneca Central Toxicology Laboratory, Alderley Park, 8/15/96. Report # CTL/P/4917. Five CD rats/sex were each given a single gavage dose of 300 mg/kg in corn oil. Non-labeled EPTC purity was 99.8%. Radioactive material was ¹⁴C-labeled on the N-propyl carbons adjacent to the nitrogen, and was > 98% radiopurity. Rats were maintained in metabolism cages, with periodic monitoring of radiolabel in urine, feces, and exhaled air. Rats were sacrificed after 96 hr, and major tissues were analyzed for radioactivity. From 82% to 84% of administered label was found in urine: most of this was collected within 24 hr of dosing. Excretion in feces or as CO₂ comprised 3 to 5% of dose. About 1.5% to 1.7% of label remained in tissues at 96 hr termination. Highest levels were found in blood, primarily in the cellular component. This is a valid component of the metabolism study series. Aldous, 10/21/99.

117-127 169801 Davies, D. J., "EPTC: Excretion and tissue retention of a single oral dose (3 mg/kg) in the rat", Zeneca Central Toxicology Laboratory, Alderley Park, 8/19/96. Report # CTL/P/4916. Five CD rats/sex were each given a single gavage dose of 3 mg/kg in corn oil. Non-labeled EPTC purity was 99.8%. Radioactive material was ¹⁴C-labeled on the N-propyl carbons adjacent to the nitrogen, and was > 98% radiopurity. Rats were maintained in metabolism cages, with periodic monitoring of radiolabel in urine, feces, and exhaled air. Rats were sacrificed after 96 hr, and major tissues were analyzed for radioactivity. From 74% to 81% of administered label was found in urine: most of this was collected within 24 hr of dosing. Excretion in feces or as CO₂ comprised 4 to 7% of dose. About 2.3% to 3.5% of label remained in tissues at 96 hr termination. Blood levels were not disproportionately high, which is somewhat at variance with results of 300 mg/kg dosing in the companion study (Record No. 169800). This is a valid component of the metabolism study series. Aldous, 10/21/99.