COPPER NAPHTHENATE and ZINC NAPHTHENATE

Chemical Code # 000153 and 1111, Tolerance # 50168 and 50390

December 20, 2000

I. DATA GAP STATUS

Chronic toxicity, rat: Data gap, no study submitted (A 90-day dermal study with copper is on file with a possible adverse effect to skin)

Chronic toxicity, dog: Data gap, no study submitted

Oncogenicity, rat: Data gap, no study submitted

Oncogenicity, mouse: Data gap, no study submitted

Reproduction, rat: Data gap, no study submitted

Teratology, rat: No data gap, no adverse effect (copper); no adverse effect indicated (zinc)

Teratology, rabbit: Data gap, no study submitted

Gene mutation: No data gap, possible adverse effect (copper and zinc)

Chromosome effects: No data gap, no adverse effect (copper); no data gap, possible adverse effect (zinc)

DNA damage: Data gap, inadequate studies, no adverse effect indicated (copper and zinc)

Neurotoxicity Not required at this time

Toxicology one-liners are attached.

All record numbers through 133015 with copper naphthenate and 116032 with zinc naphthenate were examined.

** indicates an acceptable study.

Bold face indicates a possible adverse effect.

File name: T001220 in the 50168 Directory

Original: Kishiyama and Gee, 12/20/2000

Copper (153) and zinc (1111) naphthenates have been grouped for toxicological testing.
II. TOXICOLOGY ONE-LINERS AND CONCLUSIONS

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

COPPER NAPHTHENATE

CHRONIC TOXICITY, RAT

No study submitted.

SUBCHRONIC RAT

** 50168-030 133015 Tompkins, E. C. "90-Day Dermal Study in Rats with Copper Naphthenate". (WIL Research Laboratories, WIL-153012, October 26, 1990.) Copper Naphthenate, 9.5% copper, was administered dermally 6 hours/day, 5 days/week for a minimum of 13 weeks at concentrations of 0 (mineral oil), 100, 300 and 1000 mg/kg/day to the clipped dorsal skin of 10 Crl:CD7BR rats/sex/group under wrapping using 20 - 25% of the body surface. Dermal NOEL <100 mg/kg/day. Copper naphthenate at all dose levels caused dermal irritation (erythema and edema), which was dose related. These effects were more apparent early in the study. There were no effects on survival, body weight, food consumption, hematology or clinical chemistry. Histopathology revealed a higher incidence of dermal irritation for mid and high dose groups (dermal hyperplasia, suppurative inflammation and hyperkeratosis). Stomach lesions for the high dose group (5/20) were reported as related to the test material but not toxicologically significant. Systemic NOEL = 300 mg/kg (based on stomach pathology). Testicular effects (small testes, aspermatogenesis) for two (20%) high dose males were reported most likely not treatment related, due to lack of evidence for progression of changes and the age (8 weeks) at beginning of dosing. ACCEPTABLE with a possible adverse effect on skin. (Kishiyama and Gee, 12/29/98).

No study submitted.

CHRONIC TOXICITY, DOG

No study submitted.

ONCOGENICITY, RAT

No study submitted.

ONCOGENICITY, MOUSE

No study submitted.

REPRODUCTION, RAT

No study submitted.

TERATOLOGY, RAT

** 50168-025 115616, "A Developmental Toxicity Study of Copper Naphthenate in Rats", (M. D. Nemec, WIL Research Laboratories, Inc., Lab Study Number WIL-153002, 8/20/90). Copper Naphthenate, 9.5% copper, was administered via gastric intubation at nominal doses of 0 (Mazola7 corn oil), 30, 100 or 300 mg/kg/day to 25 mated Sprague-Dawley Crl:CD7BR rats/group from gestation days 6 through 15. The low dose averaged 29% lower than nominal (30 mg/kg); therefore, Maternal NOEL = 21 mg/kg/day (reduced food consumption, body weight gain). Mean post implantation loss at 300 mg/kg/day (1.6) exceeded WIL historical range (1.4) but was not statistically significant and was due primarily to one female with total litter resorption.
Developmental NOEL = 100 mg/kg. ACCEPTABLE. (Kishiyama and Gee, 12/21/98).

**GENE MUTATION**

** 50168-025 11561 "L5178Y TK+/- Mouse Lymphoma Mutagenesis Assay with Confirmation", (J. W. Harbell, Microbiological Associates, Inc., MBA Study Number T9037.701, 1/22/90). Copper naphthenate, 9.5% copper, at concentrations of 0, 4.2, 5.6, 7.5, 10, 13, 18, 24, 32, 42 and 56 ug/ml without metabolic activation and at 0, 3.2, 4.2, 5.6, 7.5, 10, 13, 18, 24, 32 and 42 ug/ml with metabolic activation (S-9 Mix) was evaluated for mutagenic potential using the L5178Y TK+/- mouse lymphoma mutagenesis assay. In the repeat assay, the concentrations ranged from 7.5 to 36 ug/ml. Adverse effect: A repeat test confirmed the increase of mutant frequencies with copper naphthenate in the presence of metabolic activation (S-9). ACCEPTABLE. (Kishiyama and Gee, 12/21/98)

**CHROMOSOME EFFECTS**

** 50168 025 115614, "Chromosome Aberrations in Chinese Hamster Ovary (CHO) Cells", (D. L. Putman and M. J. Morris, Microbiological Associates, Inc., Lab Study Number T9037.337, 2/21/90). Copper Naphthenate, 9.5% copper, at concentrations of 4, 8, 15, 30 and 60 ug/ml without metabolic activation and at 2, 4, 8, 15 and 30 ug/ml with Aroclor-induced rat liver S-9 mix was tested in the first trial in a chromosome aberration assay using Chinese hamster ovary cells. Concentrations in the repeat trial -S9 were 40, 80, 120 and 160 ug/ml and +S9 were 20, 30, 40, 50 or 60 ug/ml. Total incubation was 16 hours -S9 and 12 hours (with a 2 hour exposure to treatment) +S9. Times were chosen to ensure cells were in the first metaphase, based on a preliminary cytotoxicity study. The mitotic index was decreased at 120 ug/ml with no mitotic cells at 160 ug/ml without activation. No effect on the mitotic index was seen at any concentration with activation. There were no significant reproducible increases in CHO cells with aberrations for any of the copper naphthenate doses. ACCEPTABLE. (Kishiyama and Gee, 12/18/98).

**DNA DAMAGE**

50168-025 115613, "Unscheduled DNA Synthesis in Rat Primary Hepatocytes", (R. D. Curren, Microbiological Associates, Inc., Lab. Study Number T9037.380, 12/20/89). Copper naphthenate, 9.5% copper, at concentrations of 0 (acetone), 0.15, 0.5, 1.5, 5.0 and 15 ug/ml was evaluated in the unscheduled DNA synthesis test using rat primary hepatocytes and autoradiography. Cytotoxicity was determined by the release of lactic acid dehydrogenase (LDH) into the medium following treatment. The release with solvent (acetone) + 1% Triton was considered 100% and with acetone alone, 0% cytotoxicity. The initial viability before plating was not reported. Net nuclear grains were calculated by subtracting the average of the counts in three nuclear-size cytoplasmic areas from the nuclear counts. The mean number of net nuclear grains did not increase significantly at any dose level over the solvent control. UNACCEPTABLE (initial viability was not reported). Upgradeable with submission of viability data. (Kishiyama and Gee, 12/17/98).

**NEUROTOXICITY**

Not required at this time.
ZINC NAPHTHENATE

CHRONIC, RAT
No study on file.

CHRONIC TOXICITY, DOG
No study on file.

ONCOGENICITY, RAT
No study on file.

ONCOGENICITY, MOUSE
No study on file.

REPRODUCTION, RAT
No study on file.

TERATOLOGY, RAT
50390-022  116025  A developmental toxicity study of zinc naphthenate in rats. (M. D. Nemec, WIL Research Laboratories, WIL-153004, 8/20/90) Zinc naphthenate (14.5% zinc) was given by oral gavage to Sprague Dawley Crl:CD\#BR rats, 25 per group, at 0 (corn oil), 50, 250 or 500 mg/kg nominal doses. The actual doses were somewhat less, being 82.1%, 89.1% and 96.5% by analytical determination of zinc content by atomic absorption. Dams were treated days 6 through 15 of gestation. There was no effect on body weight, food consumption, or developmental parameters at any dose. The only finding of maternal toxicity were clinical signs appearing in the hour following dosing on multiple occasions with an increasing frequency at 250 and, especially, at 500 mg/kg/day. These signs consisted of dried yellow staining anogenital area, dried red material around the nose, wet yellow staining anogenital area and dried light red or clear staining around the mouth. On the basis of these effects, the maternal NOEL = 50 mg/kg/day. Since these signs were seen at only about an hour after dosing but not at 2 or 4 hours, the response appeared to be an acute one. NOEL for any other consideration would be >500 mg/kg/day. Developmental NOEL = 500 mg/kg/day. There were two unusual findings at 50 mg/kg/day in the external exam of fetuses, mandibular micrognathia and macroglossia, one fetus each in separate litters. These effects were not seen at the significantly higher doses and were probably spontaneous in origin. The study has been evaluated as showing no adverse effects but UNACCEPTABLE but upgradeable based on the lack of justification of the doses. Submission of the range-finding study, WIL 153003, is needed for a possible upgrade. (Gee, 12/30/98).

TERATOLOGY, RABBIT
No study on file.

GENE MUTATION
** 50390-022 116026 ** A L5178Y TK +/- mouse lymphoma mutagenesis assay with confirmation. (J. W. Harbell, Microbiological Associates, T9036.701, January 22, 1990) Zinc naphthenate, batch 24044P [14.5% zinc from Record No. 116025], was tested with mouse lymphoma L5178Y TK +/- cells without and with rat liver activation (male Sprague Dawley, Aroclor 1254 induced) in two trials with duplicate cultures per concentration, 4 hours exposure. The expression time was two days followed by plating for trifluorothymidine (TFT) resistance. The concentrations used were, without activation: Trial 1: 0 (ethanol), 3.2, 4.2, 5.6, 7.5, 10, 13, 18, 24, 32, 42, 56 and 75 ug/ml; trial 2: 0 (ethanol), 7.5, 11, 15, 19, 23, 27, 30, 33, 36, 40, 44 and 48 ug/ml. With activation, the concentrations were: Trial 1: 0 (ethanol), 4.2, 5.6, 7.5, 10, 13, 18, 24, 32, 42, 56, 75 and 100 ug/ml; trial 2: 0 (ethanol), 23, 27, 30, 33, 36, 40, 44, 48, 52, 56, 65 and 75 ug/ml. Several of the higher concentrations per trial were too toxic to process. EMS and DMBA were positive controls and functional. Following the expression period, three plates per concentration were made each for mutation frequency with TFT and for viable colonies. After 10 - 12 days incubation, plates were scored for total colonies and the colony size distribution between 0.2 and 1.1 mm determined with an automatic colony counter. Possible adverse effect: The mutation frequency was increased in a concentration-dependent response both without and with activation. Also, there was a greater increase in small colonies versus large colonies with exposure to zinc naphthenate. ACCEPTABLE. (Gee, 1/4/99).

CHROMOSOMAL EFFECTS

** 50390-022 116026 ** Chromosome aberrations in Chinese hamster ovary (CHO) cells. (D. L. Putman and M. J. Morris, Microbiological Associates, T9036.337, 2/21/90) Zinc naphthenate, 14.5% zinc (from record 116025), was tested with CHO cells with and without Aroclor-induced rat liver S9. There were duplicate cultures per concentration and two trials. Concentrations were selected from a preliminary assay evaluating mitotic index as a measure of cell cycle delay with BrdU to determine sister chromatid exchanges for the first, second and third cell divisions. In the chromosomal assay, concentrations tested without activation were: First trial: 0, 5, 10, 20, 40 and 80 ug/ml and Second trial: 0, 80, 110, 140, 170 and 200 ug/ml. With activation: First trial: 0, 10, 20, 40,80 and 160 ug/ml and Second trial: 0, 60, 80, 100, 120 and 140 ug/ml. Exposure without activation was 18 hours followed by an additional 2 hours with colcemid. With activation, exposure was 2 hours followed by 18 hours, both for a total of 20 hours. When possible, 50 metaphases per culture for a total of 100 per concentration were evaluated for mitotic index, aberrations per cell (excluding gaps) and percentage of cells with aberrations. In the first trial with activation up to 80 ug/ml, the results were negative. In the repeat trial with results reported at 80, 110 and 140 ug/ml, positive results were found at all three concentrations. With activation, in the first trial, positive results were found at 80 ug/ml. In the repeat +S9 trial, positive results were reported at 60, 80 and 100 ug/ml. In both repeat trials, there was a concentration dependent increase. Possible adverse effect: increase in chromosomal aberrations with increasing concentrations of zinc naphthenate. ACCEPTABLE. (Gee, 12/31/98)

OTHER DNA EFFECTS

50390-022 116032 AUncropped DNA synthesis in rat primary hepatocytes: Test article zinc naphthenate, final report. (R. D. Curren, Microbiological Associates, T9036.380, December 20, 1989) Primary rat hepatocytes, isolated from male (Sprague Dawley) rat livers, were treated with zinc naphthenate, 14.5% zinc (see Record No. 116025), at 0 (ethanol), 0.15, 0.5, 1.5, 5.0, 15 or 35 ug/ml for 18 - 20 hours in the presence of 10 uCi/ml $^3$H-thymidine. There were 3 cultures for unscheduled DNA synthesis (UDS) and 2 cultures for the parallel determination of cytotoxicity by release of lactic acid dehydrogenase (LDH) into the medium. DMBA in DMSO was the positive control and was functional. UDS was determined by autoradiography. The net nuclear grain counts were calculated by subtracting the average of the counts of 3 nuclear-sized cytoplasmic areas from the nuclear count. Cytotoxicity was measured by comparing the release of LDH as a
function of zinc naphthenate concentration with the release of LDH in the presence of ethanol as 100%. At concentrations of 35 ug/ml and higher (from the preliminary toxicity assay), the zinc naphthenate apparently interfered with the LDH assay as the release of LDH with 1% Triton was less when zinc naphthenate was present. When the release with ethanol + Triton was considered as 100%, the release in the presence of 35 ug/ml + Triton was 75%. There was no evidence for the induction of UDS by zinc naphthenate to toxic doses. Toxicity was also evaluated microscopically by cellular morphology. Data were presented per culture; no individual cell data were included. Initial viability was not reported. UNACCEPTABLE, upgradeable with the submission of data regarding the initial viability at the time of seeding of the hepatocytes. No adverse effect under the conditions of the assay. (Gee, 1/5/99).

NEUROTOXICITY

Not required at this time.