

CALIFORNIA ENVIRONMENTAL PROTECTION AGENCY
DEPARTMENT OF PESTICIDE REGULATION
MEDICAL TOXICOLOGY BRANCH

SUMMARY OF TOXICOLOGY DATA
CLOTHIANIDIN

Chemical Code # 5792, Tolerance # 52884
SB 950 # N/A
4/28/03

I. DATA GAP STATUS

Chronic toxicity, rat:	No data gap, no adverse effects
Chronic toxicity, dog:	No data gap, no adverse effects
Oncogenicity, rat:	No data gap, no adverse effects
Oncogenicity, mouse:	No data gap, no adverse effects
Reproduction, rat:	No data gap, no adverse effects
Teratology, rat:	No data gap, no adverse effects
Teratology, rabbit:	No data gap, no adverse effects
Gene mutation:	No data gap, possible adverse effect (2 of several were positive studies)
Chromosome effects:	No data gap, possible adverse effect (one of a series was a positive study)
DNA damage:	No data gap, no adverse effects
Neurotoxicity:	Not required at this time. Developmental neurotoxicity study does not indicate adverse effects.

Toxicology one-liners are attached.

All record numbers for the above study types through 202270 (Document No. 52884-0050) were examined. This includes all relevant studies indexed by DPR as of 4/22/03.

In the 1-liners below:

** indicates an acceptable study.

Bold face indicates a possible adverse effect.

File name: t20030428.wpd
Revised by Aldous, 4/28/03

II. TOXICOLOGY ONE-LINERS AND CONCLUSIONS

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

COMBINED, RAT

52884-030 186361 Biegel, L. B., "104-Week Dietary Combined Chronic Toxicity and Carcinogenicity Study with TI 435 in Rats," Covance Laboratories Inc., Madison, Wisconsin, 4/11/00. Covance Study # 6155-108. Bayer Corp. Agric. Div. Report No. 109841. Sixty rats/sex/group were dosed in diet for 2 years with TI-435 (clothianidin), 95.2% purity, at 0, 150, 500, 1500, or 3000 ppm in a combined study design. Mean estimated dosages in respective groups were 0, 8, 27, 82, and 157 mg/kg/day (M) and 0, 10, 32, 98, and 193 mg/kg/day (F). Twenty additional rats/sex/group constituted a 1-year interim study. The main study also included an FOB series, wherein 10 rats/sex/group were evaluated during weeks 66-67. NOEL = 150 ppm (10 mg/kg/day) in females and 500 ppm (27 mg/kg/day) in males, based on body weight and food consumption decrements. Also observed at the respective LOEL's were mottled liver (males, no corresponding histopathology), and ovarian interstitial gland hyperplasia. The latter was only observed in primary study females, hence was a function of treatment plus aging. Incidence of the latter was dose-related, but degree of response was not. Survival was progressively increased in both sexes at the highest two dose levels, which probably influenced the ovarian finding. Major observations at 3000 ppm in kidneys included transitional cell hyperplasia (M), pelvic mineralization (M), tubular ectasia (F), and pelvic angiectasis (F). Liver eosinophilic foci were elevated in main study M and F rats. Lesions of the glandular stomach included modest erosion (F), hemorrhage (M), and/or edema (M and F). Uterine congestion was limited to 5 high dose females of the primary study. Treatment did not elicit tumors, and none of the observations are designated as "possible adverse effects," considering the types and degrees of responses, and relationships to the MTD. **Acceptable. C. Aldous, 12/5/02.

CHRONIC TOXICITY, RAT

(See Combined, Rat)

CHRONIC TOXICITY, DOG

**52884-029 186359 Bernier, L., "52-week dietary chronic toxicity study with TI 435 in dogs," Covance Laboratories, Inc., 3/22/00. Laboratory Study #: Covance 6155-113. Bayer Corp. Agric. Div. Report No. 109831. Four purebred beagles/sex/group were dosed in diet with TI 435 (clothianidin, purity 95.2%) at 0, 325, 650, 1500, or 2000 ppm in a 1 year chronic study. Estimated achieved dose levels were 7.8, 17, 36, and 46 mg/kg/day for males, and 8.5, 15, 40, and 53 mg/kg/day for females. NOEL = 325 ppm, based on dose-related decreases in alanine aminotransferase (ALT) in both sexes. This finding is not in the direction of primary diagnostic significance and was not associated with evidence of pathology to liver or other organs. There was no definitive chronic toxicity, however erythema in the ears was more prevalent in 1500 to

2000 ppm females than in respective controls (a possible response to topical exposure to treated diet), and 2000 ppm females had reductions in RBC parameters (Hb, HCT, hematocrit) at 1 year (but not at earlier times). Acceptable, with no adverse effects. Aldous, 12/5/02.

ONCOGENICITY, RAT

(See Combined, Rat)

ONCOGENICITY, MOUSE

52884-030 186360 Biegel, L. B., "78-Week Dietary Carcinogenicity Study with TI-435 in Mice," Covance Laboratories Inc., Madison, Wisconsin, 3/27/00. Covance Study # 6155-109. Bayer Corp. Agric. Div. Report No. 109833. Fifty Crl:CD-1®(ICR)BR VAF/Plus® mice/sex/group were dosed in diet with 0, 100, 350, or 1250 ppm TI-435 (clothianidin), Lot No. 30037120, 95.2% pure, for 79 weeks [constituting groups 1, 2, 3, and 5, respectively]. An additional group received "700 ppm for Weeks 1 through 4; 2,000 ppm for Weeks 5 through 10; 2,500 ppm for Weeks 11 through 34; and 2,000 ppm for males and 1,800 ppm for females for Weeks 35 through termination." This variable dose group [Group 4] was designated as the "high dose" group in this report. Mean estimated dosages in groups 2-5 respectively were 14, 47, 252, and 171 mg/kg/day (M) and 17, 65, 281, and 216 mg/kg/day (F). These estimates for Group 4 were based on exposures to the dosages received since week 35, and were similar to overall mean weekly exposures throughout the entire dosing period. NOEL = 350 ppm, based on body weight decrements, especially in females, and on hepatocellular hypertrophy in both sexes. Group 4 toxicity proved excessive, based on mortality (18% at 26 weeks in females - much higher than any other groups) and on body weight decrements in survivors (15% lower body weights in Group 4 females compared to controls at Week 26). Body weights were decreased 17% in high dose males at week 26, but this group did not have statistically significantly increased mortality. These males had higher incidence of "labored breathing" than other male groups during weeks 20-40, reflecting a possible treatment response. The 1250 ppm females had appreciable body weight decrements throughout the study, and 1250 ppm males had modest decrements during the months 3-6. Food consumption was reduced significantly in "high dose" males and females, particularly prior to week 35. Hepatocellular hypertrophy incidences were 17, 34, 15, 40**, 27* in groups 1-5 males, respectively, and 5, 5, 3, 15**, and 11 in groups 1-5 females (* and ** meaning significant, $p < 0.05$ and $p < 0.01$, respectively). Considering the consistency of responses at 1250 ppm and above, these dose levels are considered to have responded to treatment. This reviewer does not consider the statistically significant finding at 100 ppm in males to show sufficient evidence of treatment-relationship to define an effect level, considering the lack of continuity at 350 ppm, (however investigators did so). Study is **acceptable, with no adverse effects**. Aldous, Jan. 3, 2003.

REPRODUCTION, RAT

**52884-0049 202267 Freshwater, K. J. and A. B. Astroff, "A two generation reproductive toxicity study with TI-435 in the Sprague-Dawley rat," Bayer Corporation, Stilwell, Kansas, 3/27/00. Laboratory Study #: 98-672-PF (Bayer Corp. Agriculture Division Report Number

109282). Thirty rats/sex/group were dosed continuously in diet with Clothianidin (TI-435), assayed purity range: 95.3 to 96.0%, for 2 generations (1 littering period/generation) in a standard reproduction study. Diet concentrations were 0, 150, 500, and 2500 ppm. Mean estimated achieved dose levels during pre-mating periods of both generations were 10.2, 32.7, and 180 mg/kg/day in treated males, and 11.8, 37.9, and 213 mg/kg/day in females. Evaluations included reproduction and litter data, sperm analyses, vaginal patency and preputial separation in F1 pups to evaluate pup maturation, and ano-genital distance measurements in F2 pups on lactation day 0. Estrous cycle determination was performed for 3 weeks prior to mating (vaginal smears), and just prior to termination of females. Mating was 1:1 for a period of up to 14 days. Parental NOEL = 150 ppm (24 mg/kg/day), based on body weight decrement in 500 ppm females (74 mg/kg/day), found significant at lactation day 14 only. Parental subchronic NOEL = 500 ppm (33 mg/kg/day) based on body weight decrements during virtually all phases of life of both parental generations at 2500 ppm. Sperm motility and progressive motility were also reduced in F1 males at that high dose, but sperm counts and morphology were unaffected. Maternal or maternal-fetal toxicity NOEL = 33 mg/kg/day, based on reduced birth weights of F1 pups, with an associated LOEL of 172 mg/kg/day. In addition to marked pup body weight decrements during and after the lactation period, there were several other measures of delayed maturation. These include significant weanling brain weight decrements in both generations (7% and 4% in F1 and F2 weanlings, respectively), as well as appreciable delays in developmental landmarks of preputial separation (6.7 days) and vaginal patency (2.3 days) in F1 pups. These do not appear to represent specific neurological or reproductive effects, but were consistent with marked growth delays associated with delayed body weight gain. Reproductive parameters were not affected (reproductive NOEL = 2500 ppm). The study is **acceptable, with no adverse effects**. Aldous, 4/25/03.

52884-0049 202268 Historical data for Record No. 202267, contained on the same CD as that record. Record No. 202268 is Bayer Report No. 109282-1, EPA MRID 45422804, addendum date April 6, 2001.

52884-028 186358 Astroff, A. B., "A pilot reproductive toxicity study with TI-435 in the Sprague-Dawley rat," Bayer Corp., Stilwell, KS, 2/15/00. Twenty Sprague-Dawley rats/sex were dosed with 0, 50, 100, 500, or 1000 ppm of clothianidin in diet continuously for an 8-wk pre-mating period, and through weaning of F1 pups. This study found no evidence of parental or developmental toxicity. Investigators recommended that the high dose for the subsequent primary reproduction study be greater than 1000 ppm. No DPR worksheet is necessary for this pilot study. Aldous, 10/15/02.

TERATOLOGY, RAT

**52884-027 186357 York, R. G., "Oral (gavage) developmental toxicity study of TI-435 in rats," Argus Research Laboratories, Inc., 4/16/98. Laboratory Project ID: Argus 1120-001. Bayer Corp. Agric. Div. Report No. 109971. Twenty-five CrI:CD®(SD) BR VAF/Plus® rats/group were dosed by gavage with clothianidin (95.5% purity) at 0, 10, 40, or 125 mg/kg/day (vehicle: 0.5% aq. methylcellulose, 10 ml/kg) on gestation days 6-19 in a standard developmental toxicity study. Maternal NOEL = 10 mg/kg/day (transient food consumption and

body weight decrements). Food consumption was decreased through most of the dosing period at 125 mg/kg/day, leading to persistent body weight decrement during the treatment period. Developmental toxicity NOEL = 125 mg/kg/day (no change at highest dose tested). Study is **acceptable, with no adverse effects**. Aldous, 1/6/03.

52884-0048 202264 Pilot study (Protocol 1120-001P) for Record No. 186357, above. Pilot study found 1000 mg/kg/day to be uniformly lethal, and 500 mg/kg/day to be highly toxic, based on numerous clinical signs and sharp body weight decrements. Lesser body weight decrements were also observed at 250 mg/kg/day, where clinical signs were limited to “scant feces” in all dams. Except for minor body weight decrements, 125 mg/kg/day appeared to be well tolerated. Dose levels selected for the primary study were justified, based on this pilot study. (No DPR worksheet for pilot study). Aldous, 4/14/03.

TERATOLOGY, RABBIT

52884-0049 202266 York, R. G., “Oral (stomach tube) developmental toxicity study of TI-435 in rabbits,” Argus Research Laboratories, Inc., Horsham, PA, 4/16/98. Bayer Corporation Agriculture Division Report No. 109970. Groups of 23 naturally-mated Hra:(NZW)SPF rabbits were dosed by gavage in 10 ml/kg volume of 0.5% aq. methylcellulose at 0, 10, 25, 75, and 100 mg/kg/day clothianidin (95.5%) on gestation days 6-28 in a standard developmental toxicity study. Maternal NOEL = 10 mg/kg/day (clinical signs of “scant feces in pan” and “orange urine”). Both findings were sharply increased at 75 and 100 mg/kg/day. Maternal toxicity at 75 and 100 mg/kg/day was indicated by increased mortalities (FD and/or KE incidences of 0, 0, 0, 2, and 3 in controls through high dose groups, respectively), abortions (corresponding incidences of 3, 0, 0, 1, and 6), and premature deliveries (incidences of 0, 0, 0, 2, and 2). Food consumption, which ranged from 140-145 g/day in controls through 25 mg/kg/day groups, was sharply reduced at 75 and 100 mg/kg/day (119 and 90 g/day, respectively). Developmental toxicity NOEL = 25 mg/kg/day (intermediate lung lobe absent in 3 and 4 study term litters at 75 and 100 mg/kg/day, plus one more case in premature delivery litters in each of those groups). There was also a modest indication of ossification delays at those dose levels indicated by fewer sternal centers at these doses. At 100 mg/kg/day there were increased resorptions, and a significant decrease in mean pup weights by about 7 g compared to controls. Patterns of developmental findings are not considered to be “adverse” when maternal toxicity is considered. **Acceptable. Aldous, 4/14/03.

52884-0048 202265 Pilot study (Protocol 1120-002P) for Record No. 202266, above. Pilot study found 125 mg/kg/day and above to cause abortions or deaths of all does. The next lower dose, 62.5 mg/kg/day, was associated with dark urine in all 5 does and with one premature delivery on day 29, but otherwise well tolerated. Dose levels selected for the primary study were justified, based on this pilot study. (No DPR worksheet for pilot study). Aldous, 4/14/03.

GENE MUTATION

** 52884-031 186362 Thompson, P. W., “TI-435: Reverse mutation assay “Ames Test” using *Salmonella typhimurium* and *Escherichia coli*,” Safeparm Laboratories Ltd., 3/8/00.

Laboratory Study # SPL Project No. 178/110. Bayer Corp. Agric. Div. Report No. 109953. Test strains were *Salmonella typhimurium* strains TA1535, TA1537, TA98, and TA100 and *Escherichia coli* strain WP2uvrA⁻ in standard reverse mutation assay design. There were three reps per concentration in each of 2 independent tests. Treatment levels were 0, 50, 150, 500, 1500, and 5000 : g/plate of clothianidin (with or without S9). Test was positive for mutagenicity, based on the modest but consistent responses of strain TA1535. The responses were statistically significant in all cases at 5000 : g/plate, as well as at 1500 : g/plate with and without S9 in the first test. Response with S9 activation was typically slightly stronger than without S9. **Acceptable, with a “possible adverse effect”**. Aldous, 12/5/02.

52884-031 186363 Otsuka, M., “Bacterial reverse mutation test of TIR-435,” Hita Research Laboratories, Japan, 4/23/90. Study Code No. K01-0820. Bayer Corp. Agric. Div. Report No. 109975. Test strains were *Salmonella typhimurium* strains TA1535, TA1537, TA98, and TA100 and *Escherichia coli* strain WP2uvrA⁻ in a reverse mutation assay (plate incorporation method). TI-435 (clothianidin), >99% purity, was evaluated in duplicate plates in the primary trial, with dose levels of 0 (DMSO), 313, 625, 1250, 2500, and 5000 : g/plate (with or without S9). The test was uniformly negative, with functional positive controls. A range-finding test utilized dose levels of 0 (DMSO), 100, 500, 1000, 2000, and 5000 : g/plate, likewise with duplicate plates and positive controls. That test was also negative. The study is **unacceptable**, the only serious concern being that this test article may not be comparable to the modern active ingredient of lower purity (purity about 96 %, as in the March 2000 study at Safepharm Laboratories Ltd., Record No. 186362). **No adverse effects were indicated**. Aldous, 12/5/02.

52884-031 186364 Herbold, B., “TI 435: Salmonella/microsome test: plate incorporation and preincubation method,” Bayer AG, Wuppertal, 6/14/99. Bayer AG Study # T 8053884. Bayer Corp. Agric. Div. Report No. 109990. *Salmonella typhimurium* strains TA 1535, TA 1537, TA 98, TA 100, and TA 102 were evaluated in three reps per concentration in each of 2 independent tests (the first test by plate incorporation, and the second test using a 20-min preincubation before plating). Treatment levels were 0, 16, 50, 158, 500, 1581, and 5000 : g/plate of clothianidin (95.2% purity, from Takeda Chemical Industries, Ltd.) with or without S-9. No significant increases in revertants were observed. Slightly reduced titers at 5000 : g/plate indicated cytotoxicity at that dose level. Study is **acceptable, with no adverse effects. Aldous, 12/5/02.

52884-031 186365 Herbold, B., “TI 435: Salmonella/microsome test using Salmonella typhimurium TA 1535: plate incorporation and preincubation method,” Bayer AG, Wuppertal, 1/28/99. Laboratory Study # T 9053830. Bayer Corp. Agric. Div. Report No. 109989. *Salmonella typhimurium* strain TA 1535 was evaluated in three reps per concentration in each of 2 independent tests, one by plate incorporation and one using a 20-min. preincubation step, with clothianidin from two different sources [Bayer AG Batch No. NLL 6100-3 (98.6% purity), and Takeda Chemical Industries, Ltd. Lot 30034708 (96.0% purity)]. High dose levels ranged from 5000 to 8000 : g/plate of clothianidin with or without S-9 in all cases. No significant increases in revertants were observed. Toxicity was evidenced by reduced titers at 5000 : g/plate and above in all trials. Valid **supplemental data** (one strain only was tested). Aldous, 12/5/02.

**52884-034 186376 Durwood, R., “TI-435: L5178Y TK +/- mouse lymphoma assay,”

Safepharma Laboratories Ltd. (Derby, UK), 3/8/00. SPL Project No. # 178/112. Bayer Corp. Agric. Div. Report No. 109830. Cells were incubated 3 hours with test article, maintained 2 days with daily subculturing for expression, then plated for 10-14 days in medium containing trifluorothymidine (TFT) medium to visualize resistant colonies. Test article was Clothianidin (TI-435), 96% purity, at dose levels from 312.5 to 2500 : g/ml in Experiment 1 and 600 to 2400 : g/ml (more closely spaced intervals) in Experiment 2, with duplicate cultures. For mutant selection, there were four microtiter plates (each containing 96 wells) at each dose level in each experiment. At about 2000 cells/well, there were about 770,000 cells initially plated for each dose level, and twice that many for respective controls. Positive controls were functional. Mutation frequencies were significantly elevated at 1600 and 2000 : g/ml in the second experiment, and were non-significantly elevated in the first experiment, thus the test is considered positive. The responsive dose range was cytotoxic, as evidenced by decreased growth and survival. The proportion of "small" colonies was generally elevated at the higher dose levels, suggesting a clastogenic effect. **Acceptable, with a possible adverse effect.** Aldous, 1/6/03.

52884-034 186377 Brendler-Schwaab, S., "TI 435: Mutagenicity study for the detection of induced forward mutations in the V79-HPRT assay *in vitro*," Bayer AG, Wuppertal, 6/8/99. Laboratory Study # T 1053841. Bayer Corp. Agric. Div. Report No. 109992. Treatments were 0 (untreated), 0 (vehicle = DMSO, 1%), 156, 313, 625, 1250, 2500, or 5000 : g/ml Clothianidin (TI-435: purity 95.2%) for 5 hours using Chinese hamster lung cells (V79). There were two independent tests, with and without S-9, with duplicate cultures per concentration. Negative controls were either untreated or treated with vehicle. Positive controls were EMS or DMBA (both functional). There was no reproducible indication of treatment effects with or without S-9, in the presence of functional positive controls. **Acceptable. Aldous, 1/6/03.

Gene Mutation Studies on Clothianidin Metabolites, Degradation Products, or Contaminants:

52884-031 186367 Herbold, B., "N-methylnitroguanidin: Salmonella/microsome test: plate incorporation and preincubation method," Bayer AG, Wuppertal, 2/12/01. Bayer AG Study # T0069528. Bayer Corp. Agric. Div. Report No. 110246. *Salmonella typhimurium* strains TA 1535, TA 1537, TA 98, TA 100, and TA 102 were evaluated in three reps per concentration in each of 2 independent tests. Treatment levels were 0, 50, 158, 500, 1581, and 5000 : g/plate of 97.3% N-methylnitroguanidin with or without S-9. Study design was virtually identical to that of 52884-031 186364, which was performed in the same facility and by the same investigator on the active ingredient, clothianidin. No significant increases in revertants were observed. Study is supplemental by nature (test article not being the active ingredient). As with Record No. 186364, this report lacks analysis of dosing solutions. There was no evidence of toxicity to test bacteria with either of the methods employed. **Supplemental data**, no DPR worksheet. Aldous, 12/5/02.

52884-032 186368 Dawkes, N., "BN0335E2: Reverse mutation in five histidine-requiring strains of *Salmonella typhimurium*," Covance Laboratories, Ltd., Harrogate, Eng., 9/19/00. Covance Report No. # 586/225-D5140, Bayer Corp. Agric. Div. Report No. 110238. *Salmonella typhimurium* strains TA 1535, TA 1537, TA 98, TA 100, and TA 102 were treated in a standard reverse-mutation assay with BN0335E2 (99% purity), triplicate reps for test article and positive

controls with and without S-9, 5 vehicle reps per experiment with and without S-9. There were 2 experiments: one by plate incorporation design and one by preincubation. Both experiments employed 5000 : g/plate as the high exposure, without evidence of cytotoxicity. Treatments did not increase revertants. Useful **supplemental data** (lack of dosing solution assay was the only notable deficiency). Aldous, 1/6/03.

52884-032 186370 Dawkes, N., "TZMU: Reverse mutation in five histidine-requiring strains of *Salmonella typhimurium*," Covance Laboratories, Ltd., Harrogate, Eng., 2/10/99. Covance Report No. # 586/150-D5140, Bayer Corp. Agric. Div. Report No. 110202. This is one of seven reports of the same study design as 52884-032 186368, testing several chemicals associated with clothianidin. Summary tables show no increases in revertants. Useful **supplemental data**. No DPR worksheet. Aldous, 11/22/02.

52884-032 186371 Dawkes, N., "Methyl guanidine: Reverse mutation in five histidine-requiring strains of *Salmonella typhimurium*," Covance Laboratories, Ltd., Harrogate, Eng., 2/10/99. Covance Report No. # 586/151-D5140, Bayer Corp. Agric. Div. Report No. 110201. This is one of seven reports of the same study design as 52884-032 186368, testing several chemicals associated with clothianidin. Summary tables show no increases in revertants. Useful **supplemental data**. No DPR worksheet. Aldous, 11/22/02.

52884-032 186372 Dawkes, N., "TZNG: Reverse mutation in five histidine-requiring strains of *Salmonella typhimurium*," Covance Laboratories, Ltd., Harrogate, Eng., 6/3/99. Covance Report No. # 586/165-D5140, Bayer Corp. Agric. Div. Report No. 110204. This is one of seven reports of the same study design as 52884-032 186368, testing several chemicals associated with clothianidin. Summary tables show no increases in revertants. Useful **supplemental data**. No DPR worksheet. Aldous, 11/22/02.

52884-032 186373 Dawkes, N., "TMG: Reverse mutation in five histidine-requiring strains of *Salmonella typhimurium*," Covance Laboratories, Ltd., Harrogate, Eng., 6/3/99. Covance Report No. # 586/166-D5140, Bayer Corp. Agric. Div. Report No. 110203. This is one of seven reports of the same study design as 52884-032 186368, testing several chemicals associated with clothianidin. Summary tables show no increases in revertants. Useful **supplemental data**. No DPR worksheet. Aldous, 11/22/02.

52884-033 186374 Dawkes, N., "BN0230M: Reverse mutation in five histidine-requiring strains of *Salmonella typhimurium*," Covance Laboratories, Ltd., Harrogate, Eng., 9/19/00. Covance Report No. # 586/224-D5140, Bayer Corp. Agric. Div. Report No. 110239. This is one of seven reports of the same study design as 52884-032 186368, testing several chemicals associated with clothianidin. Summary tables show no remarkable increases in revertants except for 5000 : g/plate TA98 in the pre-incubation assay design without S-9 (control value 27 ± 2 vs. high dose value of 42 ± 1 ; p. 39), which was statistically elevated ($p < 0.005$) over concurrent controls. TA98 was negative in the plate incorporation assay. The other frameshift mutant, TA1537, was negative in all cases (except for the one cited value, both TA98 and TA1537 were negative with or w/o S-9 in both independent tests). The single significant positive value was close to the historical mean value for the strain (p. 51). Investigators considered this to be a negative test. In the context of the other findings of the test, this was a reasonable judgement. Useful **supplemental data**. No DPR worksheet. Aldous, 11/22/02.

52884-033 186375 Dawkes, N., "MAI: Reverse mutation in five histidine-requiring strains of

Salmonella typhimurium,” Covance Laboratories, Ltd., Harrogate, Eng., 9/6/99. Covance Report No. # 586/177-D5140, Bayer Corp. Agric. Div. Report No. 110205. This is one of seven reports of the same study design as 52884-032 186368, testing several chemicals associated with clothianidin. Summary tables show no increases in revertants. Useful **supplemental data**. No DPR worksheet. Aldous, 11/22/02.

CHROMOSOME EFFECTS

[See also Record No. (52884-034) 186376, in the gene mutation section, above. That study is considered to have indicated clastogenic findings].

****52884-031 186366** Wright, N. P., “TI-435: chromosomal aberration test in CHL cells in vitro,” Safeparm Laboratories Ltd., 3/8/00. SPL Project No. 178/111. Bayer Corp. Agric. Div. Report No. 110232. A Chinese hamster lung cell line (original isolation by Koyama, cloned by Ishidate and Sofuni) with a natural generation time of about 11 hr was used to assess chromosomal aberrations after varied lengths of exposure with and without S-9 activation. Duplicate flasks/treatment in each test contained $(1 \text{ to } 4.5) \times 10^5$ cells, depending on exposure duration: 100 metaphase spreads were prepared and read per flask. Doses of Clothianidin (TI-435: 96.0% purity) were utilized to limits of toxicity, determined mainly by numbers of scorable metaphases. With the longest exposure time (48 hr), the highest functional dose level was 625 : g/ml (w/o S-9): other groups used doses as high as 1875 : g/ml (6-hr exposure, with S-9). Positive controls (Mitomycin C and cyclophosphamide, w/o and with S-9, respectively) were functional, except for the 12-hr harvest interval, which was generally too short to elicit a positive response. Exposures at the highest tolerated levels of clothianidin with S-9 reliably increased chromosomal aberrations following at least 24 hr incubation. Marginal, sometimes statistically significant, elevations were also seen without S-9 at the highest and/or second highest dose of clothianidin. Investigators justifiably considered test article as a clastogen under conditions of the study. **Acceptable, with a possible adverse effect**. Aldous, 12/5/02.

52884-035 186378 Durwood, R., “TI-435: Micronucleus test in the mouse,” Safeparm Laboratories Ltd., 3/8/00. SPL Project No. 178/113; Bayer Report No. 109969. Five CD-1 mice/sex/treatment/time period were dosed by gavage with 0 [arachis (peanut) oil (10 ml/kg)], 25, 50, and 100 mg/kg clothianidin (96% purity) at 24, 48, and 72 hr before sacrifice. Positive controls (5/sex) received cyclophosphamide at 5 mg/kg at the 24-hr pre-sacrifice time only. Femur bone marrow smears were prepared, stained, and evaluated for PCE/NCE ratios and for # micronuclei per 1000 PCE's. Neither of the latter parameters were affected by clothianidin. Positive controls were functional. This study had technical problems which cannot be entirely remedied: the high dose assayed levels were 66% of nominal, although other indicators suggested that this reading may have been an analytical error rather than a problem in actual exposure. Further, the pilot studies indicated that a higher maximum dose may have been tolerated: perhaps in the range of 200 mg/kg. This study is **unacceptable and not upgradeable**. Since **no adverse effects were indicated** and since other studies fill this study type requirement, there is no requirement to repeat this study. Aldous, 12/2/02.

DNA DAMAGE

****52884-036 186380** Brendler-Schwaab, S., “TI 435: test on unscheduled DNA synthesis with rat liver cells *in vivo*,” Bayer AG, Wuppertal, 6/8/99. Bayer AG Report No. # 28850; Bayer AG

Study No. T 0053840/T 9053902; Bayer Corp. Agric. Div. Report No. 109991. Four male Wistar rats/group/time interval were dosed by gavage with clothianidin (purity 95.2% to 96.2%) at 0, 2500, or 5000 mg/kg 4 hr and 16 hr prior to respective sacrifices. Hepatocytes were placed in culture and exposed to ³H-Thymidine for an unspecified time. Fifty viable cells (excluding cells in S-phase) per slide (3 per animal) were evaluated for UDS. Net grain counts (NNG) were calculated by subtraction of 3 nucleus-sized cytoplasmic regions per cell. Treatments did not cause UDS. Positive controls [2-AAF (100 mg/kg) for the 4-hr pre-treatment, and N,N'-dimethylhydrazine (DMH: 40 mg/kg) for the 16-hr pre-treatment] were functional. Study is **acceptable** with one deficiency (no concurrent assays of dosing suspensions). This does not invalidate the study, since this laboratory had shown assayed clothianidin to be close to nominal in the stability study, and clinical signs of high dose rats demonstrated effective exposure. **No adverse effects.** Aldous, 1/6/03.

52884-036 186379 Otsuka, M., "DNA repair test of TIR-435 in *Bacillus subtilis*," Hita Research Laboratories, Japan, 4/23/90. Study Code No. K02-0096. Bayer Corp. Report No. 109978. *Bacillus subtilis* strains M45 and H17 were tested for DNA repair (these strains being with and w/o responsiveness to positive controls, respectively). There was a 20 hour exposure from addition of the paper disk containing test article to the dish containing about 2 million spores in 10 ml agar until reading of the inhibition zone. Test article, TIR-435 (clothianidin) was of 99.7% purity (hence a much higher purity than more current technical material). Doses of clothianidin were 0 (DMSO), 375, 750, 1500, 3000, and 6000 : g/disk. Positive controls were AF-2 [2-(2-furyl)-3-(5-nitro-2-furyl) acrylamide, 1 ng/disk] w/o S-9 and 2-aminoanthracene (5 : g/disk) with S-9 activation. As a negative control, kanamycin, an antibiotic protein inhibitor, 10 : g/disk, was used to show an inhibitory zone without S-9 only. There was only one dish per combination of strain/activation/treatment. Clothianidin was negative at all levels tested. Study is **unacceptable and not upgradeable** due to multiple deficiencies: possibly inappropriate test article, lack of dosing solution analyses, limitation to one replicate and one trial, and insufficient justification of dosing levels. **No adverse effects were indicated.** Aldous, 12/4/02.

NEUROTOXICITY

Not required at this time.

DEVELOPMENTAL NEUROTOXICITY

**52884-0049 202269 Hoberman, A. M., "Developmental neurotoxicity study of TI 435 administered orally via diet to CRL:CD® presumed pregnant rats," Argus Research Laboratories, Inc., Horsham, PA. Bayer Corporation Agriculture Division Report No. 110218, 10/20/00. TI-435 (Clothianidin, purity 95.5%) was administered in diets of 25 Crl:CD®(SD)IGS BR VAF/Plus® dams/group at 0, 150, 500, or 1750 ppm from gestation day 0 through lactation day 22, with estimated mean exposures of 0, 13, 43, and 142 mg/kg/day during gestation and 0, 27, 90, and 299 mg/kg/day during lactation. Pups were not intentionally dosed directly, however appreciable solid diet consumption was expected during the last days of maternal treatment. Pups from 20 randomly selected litters per dose were studied for developmental neurotoxicity. One to four subsets of 1 pup/sex/litter were used to evaluate "signs of autonomic dysfunction, abnormal postures, abnormal movements or abnormal behavior patterns and unusual appearance," food consumption and body weight change, maturation (pinna unfolding, eye

opening, preputial separation, vaginal opening, basic sensory and proprioceptive reflexes), motor activity, learning and memory tests, and auditory startle habituation. Brain weights, morphometric measurements, and neurohistopathology were evaluated in 10/sex/dose/interval at days 12 and 85 (after immersion fixation in the latter case). Developmental toxicity NOEL = 150 ppm (F) and 500 ppm (M). The NOEL in females was based on very slight body weight decrement (2.3 g) at day 22, and slight (transient) decrease in auditory startle response at day 23 at 500 ppm (both findings only at days 22-23). Five 1750 ppm pups (2 M, 3 F) died between days 25 and 27 of age. These decedents had all suffered from severe body weight decrements for several days prior to death, so that maternal toxicity, exposure in milk, and/or direct diet consumption may have led to pup deaths. Day 22 high dose pups (excluding those which died shortly thereafter) had treatment-related 5-6 g body weight decrements (significant, $p < 0.01$), but soon achieved normal growth and development. Maternal NOEL = 500 ppm (reduced food consumption and body weight, especially during early gestation). No findings indicated specific developmental neurotoxicity. **Acceptable, with no adverse effects.** Aldous, 4/28/03.

METABOLISM

**52884-0050 202270 Weber, E., "[Nitroimino-¹⁴C]-and [Thiazolyl-2-¹⁴C] TI-435 toxicokinetic behaviour and metabolism in the rat including whole body autoradiography," Bayer AG, Leverkusen, FRG, Oct. 11, 2000. Bayer Study # M01819065. Bayer Report #110270. This was a standard metabolism study, with gavage dosing of CD rats using suspensions in aq. 0.5% tragacanth in all cases. The majority of tests employed nitroimino-¹⁴C label in clothianidin (purity of the a.i. 99.8%: radiopurity of both labeled materials > 99%). With the exception of the autoradiographic studies requiring serial sacrifices of 6 males administered 5 mg/kg nitroimino-¹⁴C, all groups contained 4 rats, and all sacrifices were 72 hr after dosing. Most other doses were 2.5 mg/kg. Exceptions were the high dose group (250 mg/kg), and a repeat dose group (14 daily doses of unlabeled clothianidin, followed by a single treatment with nitroimino-¹⁴C clothianidin, each treatment with 25 mg/kg). One set of females was used to assess sex differences. Primary separation of metabolites was by HPLC with reverse-phase columns, assessing elution profiles by UV absorbance and detection of ¹⁴C label, using authentic reference standards for comparison. Four key elution peaks from fecal samples were further characterized by mass spectrometry, followed by MS/MS of molecular ions. Very polar fractions obtained under HPLC were further analyzed by TLC. Urinary and fecal sample extracts were assayed by HPLC without hydrolysis or other sample preparation. Conjugation products were not common, and most radioactive eluates were identified. Since the pilot test found only about 0.02% of radiolabel in exhaled CO₂, subsequent testing did not sample exhaled air. Clothianidin was well absorbed, based on findings of 89% to 95% radioactivity in urine, compared to 6-9% in feces of males and 3% in feces of females, at 72 hr after oral dosing. This was consistent with autoradiography studies, which showed rapid perfusion into the body, followed by rapid clearance. By 72 hr, highest remaining organ concentrations were in the liver, at less than 1% of levels in liver during the first few hours. Relating to the rapid excretion observed, there was no compelling evidence of a sex effect, a high dose effect, nor of an effect of repeated dosing. Unaltered clothianidin in urine comprised 55-73% of administered label. Primary urinary metabolites and associated percentages of administered dose were (1) the N-demethylated product (designated "TZNG," 7-12%), (2) the cleavage product separating the methylene carbon on the thiazolyl group from the adjacent nitrogen of the nitroguanidine group (designated "MNG", 8-13%), and (3) the product of both events, producing a demethylated product of MNG, (designated "NTG," 1-4%). The methylene carbon on the thiazolyl ring, part of the complementary cleavage product to MNG (metabolite #2 above) was quickly oxidized to the

carboxylic acid (designated CTCA, 1%). Subsequent conjugation to displace the chlorine moiety, followed by cleavage of that conjugate provided a more abundant metabolite than the initial CTCA, produced the methylthioether analog (designated MTCA, 10%). In addition to about 10 to 11% of parent being metabolized between the methylene carbon on the thiazolyl group to produce primarily MNG, CTCA, and MTCA, one additional noteworthy metabolite was the product of cleavage between the nitroimino-labeled carbon and the secondary amino group attached to the methylene carbon. This product, designated ACT, constituted 1% of administered label. No other individual urinary excretion product characterized constituted as much as 1% of label. Only about 2-7% of urinary metabolic residues were not characterized in these studies. Fecal residues included about equal parts of (1) parent clothianidin and (2) parent without the nitro group (designated TMG): about 1% to 2% of either substance. No other compound extracted from feces comprised as much as 1% of dose administered. This study is **acceptable, with no adverse effect**. Aldous, 4/28/03.

NOTE: Record No. 186381 Document Number:52884-0037 contains references to several studies not indexed by DPR, as follows:

Subchronic studies, such as the 90-day rat (p. 96).

Neurotoxicity studies: acute and subchronic, described on pp. 161-174.

These are not required at this time for SB-950 data review. Aldous, 4/22/03.

SUBCHRONIC STUDIES

(Oral)

051; 202271; "TI-435: Toxicity to Rats by Dietary Administration for 4 Weeks" (Chambers, P.R., Huntingdon Life Sciences Ltd., Huntingdon, Cambridgeshire, England, Laboratory Project ID TDA 179/960496, 2/19/97). TI-435 (Lot No. 30033623, purity = 97.5%) was admixed to the feed and fed to 5 CrI: CD BR rats per sex per dose at dose levels of 0 (diet only), 1250, 2500, 5000, or 7500 ppm (0, 120, 249, 475, 602 mg/kg/day, respectively for males and 0, 137, 228, 454, 689 mg/kg/day, respectively for females) for 28 days. No mortalities occurred. Treatment-related half closed eyes were observed in all animals at 5000 and 7500 ppm. A treatment-related decrease in mean body weight gain was observed in both sexes at 2500, 5000, and 7500 ppm during the study interval. Treatment-related increases in mean red blood cell, hemoglobin, and hematocrit levels were observed in both sexes at 2500, 5000, and 7500 ppm. Microscopic examination revealed treatment-related spleen with reduced cellularity of the white pulp (minimal) in males at 7500 ppm. **No adverse effects**. NOEL (M) = 120 mg/kg/day (1250 ppm) and NOEL (F) = 137 mg/kg/day (1250 ppm) (based on a decrease in body weight gain and increases red blood cell, hemoglobin, and hematocrit levels). **Supplemental** (because 1) only 5 animals per sex per dose were used, 2) no ophthalmological examinations were performed, and 3) the animals were treated for only 28 days. (Corlett, 1/23/03)

052; 202272; "TI-435: Toxicity to Mice by Dietary Administration for 4 Weeks" (Chambers, P.R., Huntingdon Life Sciences Ltd., Huntingdon, Cambridgeshire, England, Laboratory Project ID TDA 180/960497, 2/19/97). TI-435 (Lot No. 30033623, purity = 97.5%) was admixed to the feed and fed to 6 CrI: CD-1(ICR) BR mice per sex per dose at dose levels of 0 (diet only), 500, 1000, 2000, or 4000 ppm (0, 90, 190, 383, 683 mg/kg/day, respectively for males and 0, 122, 248, 491, 619 mg/kg/day, respectively for females) for 28 days. 6 females and 4 males at 4000 ppm were found dead or were sacrificed due to moribund condition; no other treatment-related mortalities occurred. Body tremors were observed in 5/6 males and in 6/6 females at 4000

ppm. Treatment-related hunched posture and lethargy were observed in both sexes at 2000 and 4000 ppm. A treatment-related decrease in mean body weight gain was observed in both sexes at 1000, 2000, and 4000 ppm during the study interval. A treatment-related increase in mean relative brain weight in males at 2000 and 4000 ppm and in females at 2000 ppm was observed. Microscopic examination revealed spleens with treatment-related reduced cellularity of the white pulp and atrophy of the red pulp in males at 4000 ppm and in females at 2000 and 4000 ppm. **Possible adverse effect:** body tremors at 4000 ppm. NOEL (M) = 90 mg/kg/day (500 ppm) and NOEL (F) = 122 mg/kg/day (500 ppm) (based on a decrease in body weight gain). **Supplemental** because 1) only 5 animals per sex per dose were used, 2) no ophthalmological examinations were performed, and 3) the animals were treated for only 28 days. (Corlett, 1/30/03)

053; 202273; "Palatability Study for Dietary Concentrations of TI 435 in Dogs" (Moore, M.R., Covance Laboratories Inc., Vienna, VA, Laboratory Project ID: Covance 6155-107, 5/1/98). TI-435 (Lot No. 30037120, purity = 95.2%) was admixed to the feed and fed to 2 female pure-bred beagle dogs for 11 days at concentrations of 3000 ppm (Days 1 through 3), 4000 ppm (Days 4 through 8), and 5000 ppm (Days 9 through 11). 2 female pure-bred beagle dogs were fed basal diet only. No animals died. Red ears were observed in one treated animal on Day 11. Treatment group animals lost weight during the study interval while the control animals gained weight. Mean food consumption of the treated animals was 65% of the control animals at 3000 ppm, 43% at 4000 ppm, and 37% at 5000 ppm. **No adverse effects.** NOEL cannot be determined from the data presented in this study. **Supplemental** (this study is not a guideline study). (Corlett, 1/31/03)

055; 202277; "13-Week Dietary Toxicity Study with TI 435 in Dogs" (Bernier, L., Covance Laboratories Inc., Vienna, VA, Laboratory Project ID: Covance 6155-11, 3/14/00). 870.3150. TI-435 (Lot No. 30037120, purity = 95.2%) was admixed to the feed and fed to 4 purebred beagle dogs per sex per dose at dose levels of 0 (basal diet only), 325, 650, 1500, or 2250 ppm (0, 9.2, 19.3, 40.9, 58.2 mg/kg/day, respectively, for males and 0, 9.6, 21.2, 42.1, 61.8 mg/kg/day, respectively, for females) 7 days per week for 13 weeks. No animals died during the study interval. Treatment-related thin appearance was observed in males at 1500 and 2250 ppm and in females at 2250 ppm. Treatment-related decreased mean body weight gain was observed in males at 1500 and 2250 ppm and in females at 2250 ppm. A treatment related decrease in mean serum alanine aminotransferase in males at 2250 ppm and in females at 1500 and 2250 ppm was observed. Macroscopic and microscopic examinations on the animals revealed no treatment-related abnormalities. **No adverse effects.** NOEL (M) = 40.9 mg/kg/day (650 ppm) and NOEL (F) = 42.1 mg/kg/day (650 ppm) based on thin appearance, decreased mean body weight gain, and decreased mean serum alanine aminotransferase level. **Acceptable.** (Corlett, 2/10/03)

(Dermal)

047; 202263; "28-Day Dermal Toxicity Study with TI-435 in Rats" (Weiler, M.S., Covance Laboratories Inc., Madison, WI, Laboratory Project ID: Covance 6155-120, 10/13/00). 870.32. TI-435 (Lot No. 30037120, purity = 95.2%) was applied to the clipped skin of 10 CRL:CD®(SD)IGS BR rats per sex per dose at dose levels of 0 (reverse osmosis water only), 100, 300, or 1000 mg/kg/day for 6 to 6.5 hours per day for 28 consecutive days. No treatment-related mortalities occurred. No treatment-related skin effects at the test site or treatment-related systemic clinical signs were observed. Body weight and organ weight determinations along with hematology and serum chemistry revealed no treatment-related effects. Macroscopic and microscopic examinations on the animals revealed no treatment-related abnormalities. **No adverse effects.** NOEL (M/F, systemic and skin) = 1000 mg/kg/day based on no effects at the highest dose tested. **Unacceptable but possibly upgradable** with documentation that moistening the test site on the test animal with reverse osmosis water, and

then applying the test article, a powder, to this test site and covering with a moistened gauze sufficiently moistened the test article to ensure good contact with the skin. (Corlett, 2/18/03)