

CALIFORNIA ENVIRONMENTAL PROTECTION AGENCY
DEPARTMENT OF PESTICIDE REGULATION
MEDICAL TOXICOLOGY BRANCH

SUMMARY OF TOXICOLOGY DATA
THIAMETHOXAM

Chemical Code # 5598, Tolerance # 52691
SB 950: not assigned

Original date 10/22/99
Revised 8/23/00, 1/11/06, and Sept. 4, 2008

I. DATA GAP STATUS

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

Toxicology one-liners are attached.

All record numbers for the above study types through 236206 (Document No. 52691-0357) were examined. This includes all relevant studies indexed by DPR as of Sept. 3, 2008.

** indicates an acceptable study.

Bold face indicates a possible adverse effect.

File name: t20080904.wpd

Previous revisions by Kellner, Corlett, and Moore. Present revision by Aldous on Sept. 4, 2008.

II. TOXICOLOGY ONE-LINERS AND CONCLUSIONS

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

COMBINED, RAT

****52691-080 167773** “24-Month Carcinogenicity and Chronic Toxicity Study in Rats” (Bachmann, M. 835-Novartis Crop Protection AG, Stein, Switzerland; Study # 942110, 7/27/98). CGA-293343 Technical (Batch # P.506006, purity 98.6%) was administered in the feed to 80 Tif: RAIf(SPF) rats/sex/dose at levels of 0, 10, 30, 500 or 1500 ppm in males and 0, 10, 30, 1000 or 3000 ppm in females for 24 months. A treatment-related decrease in body weight gain was noted in females only at 3000 ppm; dose-related clinical observations were limited to slightly increased incidence of hunched posture in this group. Dose-related hematology, clinical chemistry and urinalysis changes were not indicated. At the terminal sacrifice, mean carcass weight was slightly lower for high-dose males and females (reduced by 4% and 8%, respectively). Interim sacrifice males showed increased incidences of chronic tubular lesions, basophilic proliferation and lymphocytic infiltration of the kidneys at 500 (2/10 vs. 4/10, p=0.31) and 1500 ppm (2/10 vs. 6/10, p=0.08); lymphocytic infiltration of the renal pelves was also increased in males at 1500 ppm. **Possible Adverse Effect:** Increased incidence and severity of chronic nephropathy (30/50 vs. 42/50, p<0.05) and higher incidence of lymphocytic infiltration (10/50 vs. 17/50, p<0.05) in the kidneys of high-dose males at terminal sacrifice, possibly due to α -2-microglobulin accumulation. Females show a slight increase in severity of hemosiderosis of the spleen at the 3000 ppm dose level at interim sacrifice, but not at the final sacrifice. Females at this level did show an increased incidence of foci of cellular change in the liver at terminal sacrifice (10/50 vs. 26/50, p<0.05). There were no indications of compound-related neoplastic development. **Chronic NOEL(M)=500 ppm** (21.0 mg/kg/day, based on kidney lesions). **(F)=1000 ppm** (50.3 mg/kg/day, based on liver lesions). **Acceptable.** Kellner, 9/8/99.

CHRONIC TOXICITY, DOG

****52691-060 167757** “12-Month Chronic Dietary Toxicity Study in Beagle Dogs” (Altmann, B. 831-Novartis Crop Protection AG, Stein Switzerland; Study # 942108, 7/22/98). CGA-293343 (Batch # P.506006, purity 98.6%) was administered in the feed to 4 Beagle Dogs/sex/dose at levels of 0, 25, 150, 750, 1500 ppm for 52 weeks. There were no mortalities and no compound-related clinical signs reported. Body weight gain was reduced by 26% in high-dose males over the duration of the study; a slight reduction in body weight and transient reduction in food consumption was noted in high-dose females early in the study. Reduced prothrombin activities were noted in both sexes at 1500 ppm. Dose-related increases in plasma creatinine and urea levels were reported throughout the study at 750 and 1500 ppm (both sexes). Alanine aminotransferase activity was reduced in males at 750 and 1500 ppm and lower albumin levels were noted in high-dose females. Absolute and relative testes weights were slightly reduced in two males at 1500 ppm; microscopically, this change was associated with a slight increase in the incidence and severity of tubular atrophy; **Possible Adverse Effect:** Bilateral tubular atrophy was noted in the testes of two dogs in each of the 750 and 1500 ppm dose groups. **NOEL(M/F) = 150 ppm** (M: 4.05 mg/kg; F: 4.49 mg/kg) based on bilateral tubular atrophy in males and changes in blood chemistry of both sexes at 750 and 1500 ppm. **Acceptable.** Kellner, 8/24/99.

ONCOGENICITY, RAT

See Combined Toxicity, Rat.

ONCOGENICITY, MOUSE

****52691-061**, -056, -082, -083 **167758** 167751 167700 167701 “18-Month Carcinogenicity Study in Mice” (Bachmann, M. 832-Ciba-Geigy Limited, Stein, Switzerland; Study # 942109, 6/2/98). CGA-293343 Technical (Batch # P.506006, purity 98%) was administered in the feed to 60 Tif: MAGf (SPF) mice/sex/dose at levels of 0, 5, 20, 500, 1250 or 2500 ppm for 18 months (50 mice/sex/dose for oncogenicity, 10/sex/dose for hematology and 10 control and high-dose mice/sex for 9 month interim sacrifice). Reductions in body weight gain resulted in significant differences in body weight in 2500 ppm males at week 7 and from week 11 onwards and in females from week 38 onwards. Clinical observations included distended abdomen in females at 1250 ppm and in both sexes at 2500 ppm. Significantly higher mean corpuscular hemoglobin (MCH) was noted for high-dose males at week 53 and 78. At terminal sacrifice, mean carcass weights were reduced in high dose males and females (-9% and -7%, respectively); absolute and relative liver weights were increased in males at 1250 ppm and above and in females at 500 ppm and above. Gross necropsy revealed increased incidences of masses and nodules in the liver of mice at 500 ppm and above and thickening of the stomach in some of the high-dose males.

Possible Adverse Effect: significantly increased hepatocellular adenomas were noted in both sexes at 500 ppm and above. Neoplastic lesions also included increased hepatocellular adenocarcinomas in high-dose males and females and in females at 1250 ppm. Mice at 500 ppm and above also showed increased incidence of inflammatory cell infiltration, necrosis of single hepatocytes, hepatocellular hypertrophy, increased mitotic activity, deposition of pigments and hyperplasia of Kupffer cells. **NOEL (non-neoplastic lesions)=20 ppm** (M=2.63 mg/kg; F=3.68 mg/kg, based on liver pathology at 500 ppm and above). Supplementary liver studies indicated that CGA-293343 increased proliferative activity of hepatocytes at 500 and 2500 ppm, with an enzyme induction profile similar to that of the model inducer phenobarbital. **Acceptable.** Kellner, 8/11/99.

REPRODUCTION, RAT

****52691-0346 236194** Moxon, M. E., “Thiamethoxam: Two generation reproduction study in rats,” Central Toxicology Laboratory, Alderley Park, Macclesfield, Cheshire, UK, 2/19/04. CTL # RR0941. Twenty-six Tif:RAIf pairs per dose were administered 0, 20, 50, 1000, or 2500 ppm Thiamethoxam, purity 98.6%, in diet, continuously for 2 generations (1 litter/generation) in a modern guideline reproduction study. [This study was undertaken primarily to refine NOEL’s from a previous acceptable reproduction study: DPR Document No. 52691-068 and Record No. (Dobovetzky, M. 834- Novartis, Stein, Switzerland, Study#’s 942121 and 982015, 7/20/98)]. Respective mean pre-mating intakes for increasing dose groups were: F0 (M) 1.2, 3.0, 62, and 156 mg/kg/day; F0 (F) 1.7, 4.3, 84, and 209 mg/kg/day; F1 (M) 1.5, 3.7, 75, and 192 mg/kg/day; and F1 (F) 2.1, 5.6, 110, and 277 mg/kg/day. Parental systemic toxicity NOEL (M) = 50 ppm (3.7 mg/kg/day), based on increased hyaline droplet formation and hyaline eosinophilic casts in

F1 kidneys; and (F) = 2500 ppm (209 mg/kg/day), (F0 females highest dose tested). Parental reproductive effects NOEL (M) = 1000 ppm (75 mg/kg/day), based on “germ cell loss/disorganization and/or Sertoli cell vacuolation,” usually “minimal” degree, in F1 males; and (F) = 2500 ppm (highest dose tested). There were higher percentages of sperm with detached heads observed in 3 high dose males (two F0 males and one F1 male) than in other groups: historical control incidences may help evaluate this observation. Offspring viability and growth NOEL = 2500 ppm (209 mg/kg/day), (F0 females highest dose tested). **Acceptable**. Aldous, Sept. 4, 2008.

52691-068 167771 “CGA-293343 Technical: Rat Dietary Two-Generation Reproduction Study (including Effects on Sperm Cell Parameters)” (Dobovetzky, M. 834- Novartis Crop Protection AG, Stein, Switzerland, Study# 942121 and 982015, 7/20/98). CGA-293343 technical (Batch # P.506006, purity 98.6%) was administered in the diet at 0, 10, 30, 1000 and 2500 ppm (mg/kg equivalents for F0 females at study start was 0.9, 2.8, 83.4 and 225.7 mg/kg/day for test-compound dosing dams, respectively) to 30 Tif: RAI f (SPF) rats/sex/dose (F0 mating, with two matings per generation); 30 weanlings/sex/dose from the first litters of the F0 generation were selected for the F1 mating. F0 and F1 animals were treated for 10 weeks prior to mating, and then during mating, gestation and lactation. There were no treatment-related mortalities, clinical signs or necropsy findings in the F0 and F1 adults. Body weights were slightly reduced (-7%) compared to controls in the high-dose F0 males from the pre-mating period (weeks 5 through 9); Bodyweight of both male and female rats selected for the F1 generation were reduced at 2500 ppm. Relative organ weights for spleen, heart and liver were significantly increased in high dose group F0 males. In F1 males, increased relative spleen and liver weights were reported. Absolute and relative thymus weights were reduced at 1000 and 2500 ppm F1 females. In 1000 and 2500 ppm F0 and F1 males and one (1/30) F1 female, an increased incidence of minimal to marked hyaline change of renal tubules was noted; increased incidence of renal casts was seen in high-dose F0 males and in 1000 and 2500 ppm F1 males. **Parental NOEL(M)=30 ppm (approx. 1.3 to 4.3 mg/kg/day); **(F)=1000 ppm** (59.3 to 219.6 mg/kg/day), based on kidney lesions. In both the F0 and F1 generations, mating and viability parameters (i.e., mating, fertility, gestation, live birth, viability and lactation indices) were similar to control in all dosage groups. **No reproductive effects; Reproductive NOEL=2500 ppm** (approx. 226-515 mg/kg/day); F2 litter weight gain showed reductions at the 1000 and 2500 ppm levels. **Developmental NOEL= 30 ppm** (1.3 to 4.3 mg/kg/day; based on reduced pup weight). **Acceptable**, Kellner, 7/29/99.

52691-0348 236196 [Pathology Working Group review of testes slides from study 52691-068 167771, “CGA-293343 Technical: Rat Dietary Two-Generation Reproduction Study,” (Dobovetzky, M., Novartis Crop Protection AG, Stein, Switzerland, Study#’s 942121 and 982015, 7/20/98). “Pathology Working Group (PWG) peer review of the testes from a rat dietary two-generation reproduction study of CGA-293343 Technical,” Experimental Pathology Laboratories, Inc., Research Triangle Park, NC, 6/16/00. EPL No. 140-090, Syngenta No. T007717-04. The review was done to re-examine testicular tubular atrophy incidence and severity data, which initially found a significant increase in incidence or severity at 1000 ppm, but a lesser elevation was not significant at 2500 ppm. Due to lack of dose-response, this finding was not attributed to treatment in the original DPR review. The PWG evaluation procedure was consistent with EPA Pesticide Regulation Notice 94-5 (EPA, 8/24/94). The PWG examined slides of all available tissues, blinded to treatment group. They also examined controls from 5 reference studies, performed at the subject lab. Overall incidence was significantly elevated in 30, 1000, and 2500 ppm groups. Four cases at 30 ppm were grade 2, and no cases at 30 ppm exceeded grade 2. Cases of \geq grade 2 were observed in all 5 reference control sets. There were

sufficient cases in 1000 and 2500 ppm groups \geq grade 2 that the PWG considered both to represent treatment effects, whereas 30 ppm was considered an equivocal increase. These results were considered in designing a new complete guideline reproduction study, which employed satellite groups specifically for detailed histopathology examinations of testes, which study was reviewed by DPR in 2008 (as Record No. 236194). Aldous, Sept. 3, 2008.

52691-070, -071 167775, 167777; “CGA-293343 Technical: Rangefinding Rat Dietary Reproduction Study” (Winkler, G., Short/Long-term Toxicology, Novartis Crop Protection, Basle, Switzerland). CGA-293343 Technical (Batch # P.506006, purity 98%) was administered in the feed to 15 Tif: RAI f (SPF) rats/sex/dose at doses of 0, 1000, 2000 and 4000 ppm beginning two weeks before mating and continuing through two weeks postpartum. There were no treatment-related mortalities or clinical signs. Body weight and body weight gain of high-dose males was reduced during pre-mating. In females, body weight gain was reduced at all dose levels during pre-mating; body weight was reduced during lactation in the high-dose females. Food consumption was reduced in high-dose males and mid- and high-dose females during pre-mating. There were no effects on male and female mating and fertility indices and no changes reported for gestation and parturition indices or duration of gestation. The only compound-related change in litters was reduced litter weights (day 14) and litter weight gain between days 0 and 14 in the high dose group. Supplemental study. The addendum to the final report (-071:167777) was a copy of the protocol for the range-finding study. Kellner, 10/4/99 .

176; 171348; “CGA-293343 Technical: Rat Dietary Two-Generation Reproduction Study (including Effects on Sperm Cell Parameters), Amendments 3 and 4”; (Dobovetzky, M.; Novartis Crop Protection AG, Stein, Switzerland; Study# 942121 and 982015; 1/7/99 (Amendment 3), 7/26/99 (Amendment 4). The original reported incidence of testicular tubular atrophy in F1 males was 6/30, 8/30, 15/30, 24/30, and 14/30 (for 0, 10, 30, 1000, and 2500 ppm, respectively). In this submission, testes in all F0 and F1 males were reexamined to distinguish between minute focal tubular changes and diffuse tubular atrophy. The primary conclusion was that the difference in the 1000 ppm group remained: the incidence of diffuse tubular atrophy was 0/30, 1/30, 1/30, 7/30** ($p \leq 0.01$), and 3/30 (for 0, 10, 30, 1000, and 2500 ppm, respectively). Historical control incidence ranged up to 10%. This is not considered a treatment-related effect because of the lack of a dose response at 2500 ppm. **Supplemental.** (Duncan, 8/22/00)

52691-0344 236192 [Overview of two complete reproduction studies: DPR designations 52691-0346 236194 and 52691-068 167771, regarding NOEL's], Lloyd, S., “Thiamethoxam: overview of multigeneration reproduction studies,” date of the overview: 4/8/04. Syngenta No. T009017-04. The author noted that the first study (Record No. 167771) had technical problems with sperm motility assessment, and left considerable uncertainty about the appropriate NOEL for testicular histopathology. The second study used the same rat strain, incorporated the most recent study design, and took great care to provide a robust evaluation of sperm parameters and testicular pathology. Combined evaluation of testicular histopathology from both studies supports a NOEL of 50 ppm for reproductive effects in males, whereas females showed no reproductive effects up to the highest dose of 2500 ppm. The author concluded that the NOEL for general systemic parental toxicity should be 50 ppm in males (due to kidney histopathology, elsewhere shown to be related to a male rat-specific $\alpha_{2\mu}$ -globulin effect) and 2500 ppm in females. She considered the NOEL for offspring to be 1000 ppm, due to reduced pup weights by late lactation. Aldous, 6/18/08.

52691-0356 236204 [Overview of two complete reproduction studies: DPR designations 52691-0346 236194 and 52691-068 167771 regarding NOEL's]. Peffer, R. C., "Thiamethoxam - external pathologists' assessment and supplemental data regarding testis pathology in two multigeneration reproduction studies (MRID 46402904, Guideline 870.3800 and MRID 44718707, Guideline 83-4)," Two conducting laboratories (see Summary of Toxicology Data). Date of this assessment: 8/18/06. Report # T005308-06. This record consists of a brief narrative by Dr. Peffer, supported by subsequent statements by Drs. J. F. Hardisty and D. M. Creasy, both of whom participated in the Pathology Working Group (PWG) of the first reproduction study, and each directed comments at the findings of the combined reproduction studies. Peffer noted that Central Toxicology Laboratory, which performed the most recent study, conducted two additional control studies to assess background incidences in Tif:RAIf rats. Peffer and Hardisty clarified that the findings of "equivocal" testicular pathology at 30 ppm in the first reproduction study should be considered to be superseded by the much more rigorous later study, which found no testicular histopathology below 2500 ppm. This latter study protocol called for careful sectioning the testes to include the rete testis, to provide 4 serial sections per testis, to strictly define pathological changes, and to provide 14 males/group in addition to mainstream animals for bilateral histopathological examination of testes. The findings defining the NOEL for testicular pathology of 1000 ppm in the second study were noted to be small in scope (numbers of tubules affected) and in degree for affected tubules. For this reason, the findings in the second study were argued not to be considered "adverse." Dr. Creasy observed that there is extensive control variability in incidence of tubular atrophy, but considered that the high incidence observed in the older reproduction study at 1000 ppm was a plausible treatment response, even though no response was noted in the newer study. She echoed that 50 ppm was a clear NOEL for testicular toxicity. Page 32 notes that testicular germ cell loss/disorganization and/or Sertoli cell vacuolation comprised typically only about 0.4% of tubular cross-sections (one exceptionally high case was 1.1% of cross-sections). This was too small a scope to have significant functional importance. Aldous, 6/18/08.

TERATOLOGY, RAT

52691-065, -066, -067 167767, 167768, 167770 "CGA-293343 Technical Rat Oral Teratogenicity" (Winkler, G. 833-Novartis Crop Protection, AG, Basel, Switzerland, Study #942118, 8/7/96). CGA-293343 Technical was administered via oral gavage to 24 pregnant Tif : RAI f (SPF) rats/dose at levels of 0, 5, 30, 200 or 750 mg/kg/day on days 6 to 15 of gestation. All surviving dams underwent cesarean sectioning on day 21 of gestation and the uteri were examined for the number and distribution of implantation sites, total resorptions and live and dead fetuses; the ovaries were examined for the number of corpora lutea. One high-dose dam was killed for humane reasons and all other dams survived to study termination; 17 other high-dose dams had dose-related hypoactive behavior, piloerection and regurgitation of test material. Mean maternal body weight gain and food consumption was significantly decreased in the mid- and high-dose groups. **Maternal NOEL =30 mg/kg/day (based on body weight effects). No significant differences in mean number of corpora lutea, implants, litter size, sex ratio or number of resorbed fetuses were seen. Mean body weights of male and female fetuses were significantly reduced in the high dose group. Compound-related skeletal anomalies in the high-dose group consisted of increased incidence of asymmetrically shaped sternebra-6 and increased irregular ossification of the occipital bone. Fetal skeletal variations (probably related to delayed ossification) consisted of increased incidences of irregular, poor or absent ossification of cranial bones, sternebra, metatarsals and phalanges and shortened ribs in the high dose group. **No**

Adverse Effect: . **Developmental NOEL=200 mg/kg** (based on decreased fetal body weight and skeletal variations at 750 mg/kg). Acceptable. Kellner, 7/7/99.

TERATOLOGY, RABBIT

52691-062, -063, -064 167762, 167763, 167765 “CGA-293343 Technical: Rabbit Oral Teratogenicity” (Winkler, G. 833- Novartis Crop Protection AG, Basle, Switzerland. Study #942119, 8/13/96). CGA-293343 technical (Batch # P.506006, purity 98.6%) was administered via oral gavage to 19 artificially inseminated Russian, Chbb:HM Rabbits/dose at levels of 0, 5, 15, 50 and 150 mg/kg/day on days 7 through 19 of gestation. Cesarean sectioning occurred on day 29 of pregnancy. One high-dose dam had vaginal bloody discharge on day 19 of gestation and was found dead the next day; another in this group had vaginal bloody discharge on day 18 and 19 and was sacrificed moribund on day 19. Bloody discharge in the perineal area was noted in a high-dose dam on day 22 and this dam was sacrificed the same day for severe weight loss. Body weight gain was reduced at 50 and 150 mg/kg. Food consumption was significantly reduced in these groups during the treatment period (days 7 to 12 and 12 to 16) and from day 16 to 20 in the high-dose group. Findings after necropsy included hemorrhagic contents in the uterus and vaginal hemorrhage in the dam that was found dead. The two dams that were sacrificed on days 19 and 22 also had hemorrhagic contents in the uteri **Maternal NOEL =15 mg/kg/day (based on reduced body weight gain). Dose-related increases in postimplantation losses resulting from increased early resorptions were noted in the 150 mg/kg dose group and mean fetal weights were significantly reduced at this level. Fetal skeletal evaluations revealed significant increases in fused sternebrae 3 and 4 (anomalies) at the high dose level. An increase in variations, namely absent ossification of the medial phalanx of anterior digit-5, was noted in 4 high-dose fetuses. **No Adverse Effects. Developmental NOEL=50 mg/kg** (based on reduced fetal bodyweight at 150 mg/kg). Acceptable. Kellner, 7/13/99.

GENE MUTATION

52691-073 167691 “Salmonella and Escherichia/Mammalian-Microsome Mutagenicity Test” (Hertner, Th., 842-Genetic Toxicology, Novartis Crop Protection AG, Basle, Switzerland Study #952014, 11/2/95). CGA-293343 Technical (Batch # P.506006, purity 98.6%) was tested for mutagenic potential in the Salmonella and Escherichia coli/Mammalian-Microsome Mutagenicity Assay at levels of 0, 312.5, 625, 1250, 2500 and 5000 ug/plate (triplicate plating) using strains TA98, TA100, TA 102, TA1535, TA1537 (*S. typhimurium*) and WP2uvrA (*E. coli*) with and without metabolic activation (Aroclor 1254-induced male rat liver S-9 fraction) using triplicate plating in two separate trials. Positive controls were functional. **No Adverse Effects: The test article was negative for mutagenic potential under the conditions tested. **Acceptable.** Kellner, 9/22/99.

**52691-074 167692 “Gene Mutation Test with Chinese Hamster Cells V79” (Ogorek, B., 842-Genetic Toxicology, Novartis Crop Protection AG, Basle, Switzerland, Study # 952015, 1/12/96). CGA-293343 Technical (Batch # P.506006, purity 98.6%) was tested for mutagenic potential in Chinese hamster V79 cells using the V79/HGPRT mutation assay with and without metabolic activation (Aroclor 1254-induced rat liver S-9 fraction) at dose levels in the original trial ranging from 123.33 to 3330.0 µg/ml (with S-9) and 61.67 to 1665.0 µg/ml (without S-9); levels in the confirmatory assay ranged from 416.25 to 3330.0 µg/ml (with S-9) and 277.5 to 2220.0 µg/ml (without S-9). Incubation times with CGA-293343 or control with S-9 were 5 hr;

without S-9: 21 hr. **No Adverse Effects:** there was no significant increase in mutant frequency at any level of CGA-293343 in the original or confirmatory trial. The test article was negative for mutagenicity under the conditions tested. **Acceptable.** Kellner, 9/23/99.

186; 172708; "*Salmonella*/Mammalian-Microsome Mutagenicity Test"; (E. Deparade; Genetic Toxicology, Novartis Crop Protection AG, Basle, Switzerland; Study No. 992020, Novartis No. 1170-99; 10/21/99); *S. typhimurium* strains TA98, TA100, TA102, TA1535, and TA1537 were treated with 312.5-5000 ug/plate CGA-293343 (thiamethoxam; purity = 98.6%; Batch No. P.506006)/DMSO with metabolic activation by mouse liver S-9 fraction from untreated mice, mice fed 50 ppm, 500 ppm, or 2500 ppm CGA-293343 in the diet for 20 d, or mice injected IP with Aroclor 1254 five days prior to sacrifice; triplicate plates/treatment, 30 mins. preincubation, one trial; **no adverse effects;** no increase in reversion rates; positive controls were functional; **Supplemental.** (Duncan, 7/31/00)

CHROMOSOME EFFECTS

52691-072 167690 "Cytogenetic Test on Chinese Hamster Cells *In Vitro*" (Zeugin, S., 843-Genetic Toxicology, Novartis Crop Protection, Basle, Switzerland, Study #952016, 6/18/96). CGA 293343 Technical (Batch # P.506006, purity 98.6%) was tested for clastogenic potential in Chinese hamster ovary cells (CCL 61) at concentrations ranging from 35.47 to 4540 ug/ml with and without metabolic activation (Aroclor 1254 induced rat liver microsomal enzyme) in two trials. In the absence of S-9 activation, cells were exposed for 21 or 45 hours (3 groups); in the presence of S-9 activation cells were exposed for 3 hours (3 groups). One-hundred metaphase spreads/duplicate flask were scored for chromosomal aberrations. Five of the six groups showed no significant compound-related increase in chromosome aberrations. In the experiment performed with metabolic activation after 3 hours treatment/42 hours recovery, the percentage of specific chromosomal aberrations was 1.0%, 1.5% and 0% at the 2270, 3405 and 4540 µg/ml concentrations, respectively. The percent specific aberrations obtained in the 3405 ug/ml dose group was statistically significantly different from the 0% value seen in the negative control group using Chi-square analysis. This probably did not represent compound-related effect (i.e., this was an isolated positive outcome in which the negative control value was unusually low). **No Adverse Effects: the test compound is negative for chromosome aberrations under the conditions tested. **Acceptable.** Kellner, 9/22/99.

52691-075 167693 "Micronucleus Test, Mouse (OECD Conform)" (Hertner, Th., 843-Genetic Toxicology, Novartis Crop Protection, Basle, Switzerland, Project #A952018, 12/15/95). CGA 293343 Technical (Batch # P.506006, purity 98.6%) was tested *in vivo* for clastogenic activity in polychromatic erythrocytes from bone marrow in two parts: Part 1 had 13 female Tif: MAGf(SPF) mice that were administered the test compound (oral gavage) with a single dose (1250 mg/kg) at two sampling times (24 and 48 hours). In part 2, the test was performed with three doses (312.5, 625 and 1000 mg/kg) with groups at 1000 mg/kg sampled at 16, 24 and 48 hours and the remaining dose groups sampled at 24 hours only; 2000 polychromatic erythrocytes/animal were scored for the presence of micronuclei. The positive control group received cyclophosphamide (64 mg/kg). In part 1, 7 of 13 mice were found dead after 4 to 5 hours; three females died after 48 h of treatment at 1250 mg/kg, two died after 24 h treatment and two females died in the 1250 mg/kg reserve group. Because of high female losses, dose levels for females in part 2 at the 16 hour sampling time and males at all sampling times were reduced to 1000 mg/kg. At this level, half of the mice had symptoms indicating compound-related toxicity; reduced motor activity was seen as low as 625 mg/kg. **No Adverse Effects: In

groups treated with CGA-293343, there were no dose-related increases in the number of micronucleated polychromatic erythrocytes in bone marrow cells compared to control. Acceptable. Kellner, 9/20/99.

DNA DAMAGE

52691-076 167694 “Autoradiographic DNA Repair Test on Rat Hepatocytes (OECD Conform) *in Vitro*” (Ogorek, B., 844; Genetic Toxicology, Novartis Crop Protection AG, Basle, Switzerland, Study # 952017, 1/29/96). CGA-293343 Technical (Batch # P.506006, purity 98.6%) was tested for potential DNA damage (UDS) *in vitro* at concentrations of 0.0, 13.01, 52.04, 208.13, 416.25, 832.5 and 1665 µg/ml for 16-18 hours in two trials using primary rat hepatocyte from male Tif.RAIf(SPF) rats. The mean net nuclear grain counts for the 416.25, 832.5 and 1665 µg/ml exposure levels were 0.5, 0.5 and 0.3, respectively, in the initial trial and 0.7, 0.6 and 0.8, respectively, in the second (confirmatory) trial. **No Adverse Effects: mean net nuclear grain counts in both trials were comparable to the mean net control value of 0.5. **Acceptable.** Kellner, 9/16/99.

NEUROTOXICITY

035, 036, 170; 167679, 167680, 170881; “Acute Neurotoxicity Study of Orally Administered CGA-293343 Tech in Rats” (Minnema, D.J., Covance Laboratories Inc., Vienna, VA, Study No. 6117-364, Novartis Nexus No. 507-96, 9/23/97). 818. CGA-293343 Technical (Batch No. 9600110, purity=98.7%), prepared in 0.5% aqueous methylcellulose, was administered by gavage in a single dose at dose levels of 0 (vehicle), 100, 500, and 1500 mg/kg to 10 Sprague-Dawley Crl:CD⁷Br rats per sex per dose level. 3 female animals at 1500 mg/kg died during the first 2 days of the study. During FOB assessments, a treatment-related decrease in mean body temperature and treatment-related incidences of uncoordinated landing in the righting reflex test in both sexes at 500 and 1500 mg/kg approximately 2 hours post-dose were observed. Decreased locomotor activity was observed during the first 15 to 20 minutes of the 2 hours post-dose assessment in both sexes at 500 and 1500 mg/kg. FOB and locomotor activity assessments conducted 1 week and 2 weeks post-dose revealed no treatment-related effects. Microscopic examination revealed no treatment-related effects. **No adverse effects.** NOEL (M/F)=100 mg/kg (based on treatment-related decreases locomotor activity and body temperature, and uncoordinated landing in righting reflex test). **Acceptable.** (Corlett and Leung, 9/29/99)

059; 167755; “13-Week Subchronic Neurotoxicity Study with CGA-293343 Tech. in Rats” (Minnema, D.J., Covance Laboratories Inc., Vienna, VA, Study No. 6117-363, Novartis Nexus No. 772-97, 6/23/98). 827. CGA-293343 Technical (Batch No. 9600110, purity=98.7%) was admixed to the feed at dose levels of 0, 10, 30, 500, or 1500 ppm for males (0, 0.7, 1.9, 31.8 or 95.4 mg/kg/day, respectively) and 0, 10, 30, 1000, or 3000 ppm for females (0, 0.7, 2.1, 73.2, or 216.4 mg/kg/day, respectively) and fed to 10 Sprague-Dawley Crl:CD7BR rats per sex per dose level continuously for a period of at least 13 weeks. No animals died. No treatment-related clinical signs were observed. No treatment-related effects were observed during FOB and locomotor activity assessments. No treatment-related effects were observed at gross necropsy or microscopic examination. **No adverse effects.** NOEL (M)=95.4 mg/kg/day (1500 ppm) and (F)=216.4 mg/kg/day (3000 ppm) (based on no treatment-related effects at HDT). **Acceptable.** (Corlett and Leung, 9/29/99)

52691-0263; 215498; "Thiamethoxam: Preliminary Developmental Neurotoxicity Study in Rats"; (A. Brammer; Central Toxicology Laboratory, Alderley Park, Macclesfield, Cheshire, UK; Study No. RR0935; 5/22/03); Ten time-mated female Alpk:AP_rSD (Wistar-derived) rats/group received 0, 1000, 2500 or 5000 ppm of thiamethoxam (batch no. P 506006, purity: 98.8%) from day 7 of gestation through day 22 *post-partum* (gestation: 0, 92.3, 212.5, 362.1 mg/kg/day, lactation: 0, 156.5, 395.8, 740.6 mg/kg/day). The mean body weights of the 2500 and 5000 ppm females were less than that of the controls during the gestation period ($p < 0.01$). The mean body weight of the 5000 ppm females were lower than that of the control during the lactation period ($p < 0.05$ or 0.01). The mean food consumption of 5000 ppm females was lower than that of the control throughout the treatment period ($p < 0.01$). There was no apparent treatment-related effect on the reproductive performance of the animals. The mean body weights of the male pups in the 2500 ppm group and both sexes in the 5000 ppm group were less than those of the control pups on day 1 *post-partum* ($p < 0.01$). The mean body weight gain of the 2500 ppm male pups and both sexes in the 5000 ppm group was less than that of the controls over the lactation period ($p < 0.05$ or 0.01). **No adverse effect indicated. Study supplemental** (non-guideline dose range-finding study). (Moore, 8/9/05)

52691-0264; 215499; "Thiamethoxam: Developmental Neurotoxicity Study in Rats"; (A. Brammer; Central Toxicology Laboratory, Alderley Park, Macclesfield, Cheshire, UK; Study No. RR0936; 5/29/03); Thirty time-mated Alpk:AP_rSD (Wistar-derived) females/group received 0, 50, 400 or 4000 ppm of Thiamethoxam (batch no. P 506006, purity: 98.8%) in the diet from day 7 of gestation through day 22 *post-partum* (gestation: 0, 4.3, 34.5, 298.7 mg/kg/day, *post partum*: 0, 8.0, 64.0, 593.5 mg/kg/day). The F1 offspring were culled on day 5 *post partum*. At least 10 pups/sex/group were examined in the Functional Observational Battery on days 5, 12, 22, 36, 46 and 61 *post partum*. One pup/sex/litter/group was examined for motor activity on days 14, 18, 22 and 60 *post partum*. One pup/sex/litter/group was examined in the auditory startle response assessment on days 23 and 61 *post partum*. One pup/sex/litter/group was assigned to the learning and memory test. The learning phase was performed on days 21 and 59 *post partum*. The memory test was performed on days 24 and 62. On day 12 *post partum*, one pup/sex/litter/group was euthanized. The brain weights were recorded and the brain examined histologically. On day 63 *post partum*, one animal/sex/litter/group was euthanized and the brain weights were recorded. An additional 10 animals/sex/group were euthanized by perfusion fixation. The brain weights were recorded as well. The appropriate neuronal and muscle tissues were examined histologically and the brains was assessed morphometrically. No treatment-related clinical signs were evident. The mean body weight of the 4000 ppm dams was less than that of the control dams throughout the gestation and *post partum* period ($p < 0.01$). The mean food consumption for these dams was less than that of the controls throughout this period as well ($p < 0.01$). The reproductive performance of these dams was not affected by the treatment. The mean body weight of the F1 offspring in the 4000 ppm group was less than that of the controls throughout the *post partum* period ($p < 0.01$). The mean time to preputial separation was delayed for the 4000 ppm male offspring ($p < 0.01$). There were no apparent treatment-related effects in the Functional Observational Battery, motor activity, startle response or learning/memory assessments over the course of the study. The absolute brain weights of the F1 offspring at 12 and 63 days *post-partum* were less than those of the controls ($p < 0.05$). The relative brain weights were not affected by the treatment. The brain morphometry indicated certain regions of the brain for both sexes in the 4000 ppm F1 offspring to be smaller than that of the control ($p < 0.05$ or 0.01). However, the lack of an effect on the relative brain weights for these animals and the lack of any histological or clinical neurological effects give evidence that variations in the brain morphometry are the consequence of non-specific effects on the development of the F1 offspring and are not a direct neurological effect. **No adverse effect indicated.** Reported

Developmental Neurotoxic NOEL: 4000 ppm (298.7 mg/kg/day) (based upon the lack of neurological effects at the highest dose tested); **Study unacceptable**, possibly upgradeable with the submission of positive control data). (Moore, 8/16/05) NOTE: Acceptable validation studies have been found, and this study is now considered acceptable (see paragraph at end of this section preceded by **, by Aldous, 6/25/08.).

52691-0355 236203 Peffer, R. C., and S. Chivers [Supplemental to 52691-0264 215499] “Thiamethoxam: a response to EPA review (DP Barcode: D294153) of preliminary and main OPPTS 870.6300 developmental neurotoxicity studies in rats” (addendum to MRID Numbers 46028201 and 46028202). Rebuttal date: 8/18/06, Report No. T005307-06. The original U.S. EPA DER commented that the body weight decrements in 4000 ppm male offspring (6.8%) cannot account for the observed morphometric changes in brain dimensions. Authors responded that differences in body weight are indeed correlated with brain dimensions, hence an analysis of covariance is relevant. Appendix 2 of this record presents morphometric data blocked by body weight from historical controls of 10 studies, showing usefulness of covariance analysis. Data from this appendix will be addressed in the DPR review for Record No. 236205. The DER requested morphometric analysis of all brain regions for both sexes at the low and mid dose groups to ascertain the possible effects at the lower doses. Authors responded that intermediate groups were evaluated whenever relevant body-weight-adjusted high dose group measures indicated, according to the professional judgement of the study pathologist. Useful supplementary information. Aldous, 6/23/08.

52691-0357 236205 Brammer, A., [Supplemental to 52691-0264 215499], “Thiamethoxam: supplement to developmental neurotoxicity study in rats (supplemental to MRID Number 46028202),” Central Toxicology Laboratory, Alderley Park, UK. This report, No. RR0936-REG-S1, is dated 1/15/07. This report presents morphometric measurements for intermediate groups at PND 12 and PND 63, which data were requested by U.S. EPA in a review dated 10/24/05 (included in Record No. 52691-0355 236203). These measurements were presented with and without adjustment for historical differences in these parameters associated with pup body weight. NOEL for developmental neurotoxicity = 400 ppm (associated with maternal exposures of 34.5 and 64 mg/kg/day during gestation and lactation, respectively). LEL for developmental neurotoxicity of 4000 ppm corresponds to maternal exposures of 299 and 594 mg/kg/day during gestation and lactation, respectively. At 4000 ppm, there was no change in morphometric measures in PND 12 pups after adjustment for expected effects of reduced body weights. At that dose in PND 63 rats, eleven different morphometric measures at PND 63 were significantly reduced in males, and six in females. Adjustment for body weight-associated effects had minimal effect on statistical significance of these parameters. These morphometric differences were small, and not indicative of targeted effects on particular brain regions. There were no observed functional nor histopathological changes in offspring. This reviewer concludes that the brain morphometric differences be considered as developmental neurotoxicity endpoints (a change from the previous DPR data review). This 4000 ppm dose had markedly reduced the food consumption of the nurturing dams and reduced their body weights as well as the body weights of pups from PND 1 through termination at PND 63. Considering general maternal and offspring body weight and growth effects of treatment at 4000 ppm, no uniquely developmental “adverse” effects are indicated by morphometric changes. Data support a NOAEL of 4000 ppm for developmental neurotoxicity. Useful supplementary data. Aldous, Sept. 4, 2008.

52691-0357 236206 This is the U.S. EPA Review of the primary developmental neurotoxicity study (52691-0264; 215499) as amended by the new data in 52691-0357 236205, above. At this

stage in evaluation, the study was accepted (usable for regulatory purposes), but not considered to satisfy guideline requirements for this study type, “pending comprehensive review of positive control data.”

Supplementary information for 52691-0264 215499: DPR had requested positive control data as the sole needed information for upgrading the primary record to acceptable status. To date, the registrant had not sent such data in support of the present active ingredient, nor identified where such data might exist in the DPR database. There was a contemporary study sponsored by Syngenta, which was accepted by DPR on 2/23/05. That study was DPR Document No. 50907-0215; Record No. 216612; performed at the Alderley Park, Macclesfield, Cheshire, UK facility; Study ID No. CTL No. RR0969; dated 11/3/04. The DPR review for that study found that: “Neurotoxicity assessment studies were performed by the laboratory with trimethyl tin (1/6/97 to 1/24/97, vol. no. 50907-0209, rec. no. 216606), chlorpromazine and amphetamine (6/8/00 to 7/12/00, vol. no. 50907-0210, rec. no. 216607) and morphine sulfate (6/9/00 to 7/18/00, vol. no. 50907-0211, rec. no. 216608); the results were sufficient to document the capability of the laboratory to assess neurotoxic effects.” The present reviewer suggests that validation study data sufficient for a study completed on 11/3/04 should be considered sufficient for the present study, for which the original report was completed on 5/29/03. This being the only barrier noted for **acceptable status, the thiamethoxam developmental neurotoxicity study should be re-classified as **acceptable**. Aldous, 6/25/08.

SUBCHRONIC STUDIES

(Oral)

054; 167749; “3-Month Oral Toxicity Study in Rats (Administration in Food)” (Bachmann, M., Novartis Crop Protection AG, Short/Long-term Toxicology, Basle, Switzerland, Study No. 942089, Novartis Nexus No. 471-94, 1/23/96). 821. CGA 293343 tech. (Batch No. KI-4654/18, purity=98.4%) was admixed to the pelleted food at dose levels of 0, 25, 250, 1250, 2500, or 5000 ppm (0, 1.74, 17.6, 84.9, 167.8, and 328.8 mg/kg/day, respectively, for males and 0, 1.88, 19.2, 92.5, 182.1, and 359.1 mg/kg/day, respectively, for females) and fed to 10 Tif:RAIf (SPF), hybrids of RII/1 x RII/2 (Sprague-Dawley derived) rats per sex per dose level for a period of 90 consecutive days. No treatment-related deaths occurred. No treatment-related clinical signs were observed. Statistically significant increases in mean relative heart, liver, kidney, spleen, and adrenal weights in males at 5000 ppm were observed. Microscopic examination of males revealed treatment-related incidences of hepatocyte hypertrophy beginning at 2500 ppm, acute tubular lesions in the kidney beginning at 1250 ppm, and hyaline change in the renal tubular epithelium and chronic tubular lesions in the kidney beginning at 250 ppm. Microscopic examination of females revealed treatment-related hepatocyte hypertrophy and Kupffer cell pigmentation at 5000 ppm, and increases in severity of hemosiderosis in the spleen and nephrocalcinosis in the kidneys and treatment-related cortical fatty change in the adrenal glands and hepatocyte necrosis beginning at 2500 ppm. **Possible adverse effect:** treatment-related hyaline change in the renal tubular epithelium. NOEL (M)=1.74 mg/kg/day (25 ppm) and (F)=92.5 mg/kg/day (1250 ppm) both based on microscopic findings. **Acceptable**. (Corlett, 9/10/99)

056; 167751; “3-Month Range Finding Study in Mice (Administration in Food)” (Bachmann, M., Novartis Crop Protection AG, Short/Long-term Toxicology, Basle, Switzerland, Study No. 942105, Novartis Nexus No. 704-95, 8/13/96). CGA 293343 tech. (Batch No. KI-4654/18,

purity=98.4%) was admixed to the pelleted food at dose levels of 0 (pelleted food), 10, 100, 1250, 3500, or 7000 ppm (0, 1.41, 14.3, 176, 543, and 1335 mg/kg/day, respectively for males and 0, 2.01, 19.2, 231, 626, and 1163 mg/kg/day, respectively for females) and fed to 10 Tif:MAGf (SPF) hybrids of NIH x MAG mice per sex per dose level for 3 months. No treatment-related mortalities occurred. Treatment-related respiratory sounds occurred in males at 3500 and 7000 ppm and in females at 7000 ppm. A treatment-related decrease in mean body weight was observed in males at 7000 ppm. Treatment-related increases in mean relative liver weight in both sexes at 3500 and 7000 ppm and in mean relative testis (both) weight at 7000 ppm, and a treatment-related decrease in mean relative ovary (both) weight at 3500 and 7000 ppm were observed. Microscopic examination revealed a treatment-related increase in incidence and severity of hepatocyte hypertrophy in males beginning at 100 ppm and in females beginning at 1250 ppm and a treatment-related reduction in the number of corpora lutea in the ovaries at 3500 and 7000 ppm. **No adverse effects.** NOEL (M)=1.41 mg/kg/day (10 ppm) and (F)=19.2 mg/kg/day (100 ppm), based on hepatocyte hypertrophy. **Supplemental study** (no clinical biochemistry and no ophthalmological examinations conducted). (Corlett, 9/15/99).

053; 167746; “3-Month Subchronic Dietary Toxicity Study in Beagle Dogs” (Altmann, B., Novartis Crop Protection AG, Short/Long-term Toxicology, Basle, Switzerland, Study No. 942107, Novartis Nexus No. 705-95, 10/15/98). 821. CGA 293343 tech. (Batch No. P. 506006, purity=98.6%) was admixed to the pelleted food at dose levels of 0, 50, 250, 1000, and 2500/2000 ppm (0, 1.58, 8.23, 32.04, and 54.81 mg/kg/day, respectively, for males and 0, 1.80, 9.27, 33.87, and 50.45 mg/kg/day, respectively, for females) and fed to 4 beagle dogs per sex per dose level for a period of 13 weeks. No deaths occurred. No treatment-related clinical signs were observed. Treatment-related decreases in mean body weight and mean food consumption in females at 2500/2000 ppm were observed. Treatment-related decreases in mean cell hemoglobin and mean alanine aminotransferase levels in both sexes at 1000 and 2500/2000 ppm were observed. A statistically significant decrease in mean relative testes weight at 2500/2000 ppm was observed. Microscopic examination revealed moderate bilateral tubular atrophy of the testes in one animal at 2500/2000 ppm, and treatment-related reduced spermatogenesis (bilateral) in the testes, the presence of spermatid giant cells (bilateral) in spermatogenic epithelium of the testes, and immature ovaries (bilateral) and uteri at 2500/2000 ppm. **Possible adverse effect:** decrease in mean relative testes weight and bilateral tubular atrophy, presence of spermatid giant cells, and reduced spermatogenesis in the testes. NOEL (M)= 32.04 mg/kg/day (1000 ppm, reduced spermatogenesis and testicular atrophy) and (F)= 33.87 mg/kg/day (1000 ppm, immature ovaries and uteri). **Acceptable.** (Corlett, 9/23/99)

052; 167747; “28-Day Exploratory Toxicity Study in Male Rats (Gavage)” (Bachmann, M., Novartis Crop Protection, Inc., Short/long-term Toxicology, Stein, Switzerland, Study No. 932107, Novartis Nexus No. 638-93, 4/25/94). CGA 293343 tech. (Batch No. MF-1846/4, purity > 95%), prepared in 0.5% carboxymethylcellulose and 0.1% Tween 80, was administered 5 times per week for 4 weeks by gavage at daily doses of 0, 100, 300, and 1000 mg/kg to 5 male PRT GAV 01M rats per dose level. One animal at 100 mg/kg died accidentally on day 29. A treatment-related decrease in mean body weight was observed at 1000 mg/kg. Blood chemistry analysis revealed treatment-related increases in mean alkaline phosphatase, aspartate aminotransferase, and gamma-glutamyl transpeptidase levels at 1000 mg/kg were observed. Treatment-related increases in mean relative liver and kidney weights at 300 and 1000 mg/kg were observed. Macroscopic examination revealed treatment-related increased incidences of large livers and renal pelvis dilatation at 1000 mg/kg. Microscopic examination revealed treatment-related increased incidences of hepatocyte hypertrophy at 300 and 1000 mg/kg, hyaline change in the renal tubule at 100 and 300 mg/kg/day, and renal pelvis dilatation and

adrenal cortex fatty change at 1000 mg/kg. **No adverse effects.** NOEL (M)<100 mg/kg/day (based on a treatment-related increased incidence of renal tubular hyaline change).

Supplemental study (no ophthalmological examinations conducted, only male animals used, and animals treated for only 28 days). (Corlett, 8/30/99).

055; 167750; “28-Days Range Finding Study in Rats (Administration in Food)” (Bachmann, M., Novartis Crop Protection, Inc., Short/Long-term Toxicology, Stein, Switzerland, Study No. 942088, Novartis Nexus No. 470-94, 5/5/95). CGA 293343 tech. (Batch No. KGL4654/12, purity> 95%) was admixed to the pelleted food at dose levels of 0 (pelleted food), 100, 1000, 2500, or 10000 ppm (0, 8.04, 81.7, 199, and 711 mg/kg/day, respectively for males and 0, 8.69, 89.3, 211, and 763 mg/kg/day, respectively for females) and fed to 5 Tif:RAIf (SPF) hybrids of RII/1 x RII/2 (Sprague-Dawley derived) rats per sex per dose level for 4 weeks. No animals died. No treatment-related clinical signs were observed. A treatment-related decrease in mean body weight was observed in males at 10000 ppm. Blood chemistry analysis revealed treatment-related increases in mean cholesterol (in males beginning at 1000 ppm and in females at 10000 ppm), urea (in both sexes at 10000 ppm), and aspartate aminotransferase (in males at 10000 ppm) levels. Treatment-related increases in mean relative liver (in both sexes) and kidney (in females only) weights at 10000 ppm were observed. Microscopic examination revealed treatment-related increased incidences of hepatocyte hypertrophy and thyroid follicular epithelium hypertrophy (in males at 2500 and 10000 ppm and in females at 10000), a decrease in hepatocyte glycogen deposition and renal pelvis dilatation (in males at 10000 ppm), renal tubular hyaline change (in males at 1000 and 2500 ppm), and adrenal cortex fatty change (in both sexes at 10000 ppm). **No adverse effects.** NOEL (M)=8.04 mg/kg/day (100 ppm, based on a treatment-related increased incidence of renal tubular hyaline change), (F)=211 mg/kg/day (2500 ppm, based on treatment-related increases in mean relative kidney and liver weights and increased incidence of hepatocyte hypertrophy). **Supplemental study** (no ophthalmological examinations conducted, only 5 animals per sex per dose used, and animals treated for only 28 days). (Corlett, 9/2/99)

52691-081 167699 “Assessment of Replicative DNA Synthesis in the Course of a 28-Day Oral (Feeding) Toxicity Study in Male Rats” (Persohn, E., Toxicology Services/Cell Biology, Novartis Crop Protection, Basle, Switzerland). In a previous 28-day toxicity study in male rats (DPR# 52691-052:167747; Novartis study # 932107), increased liver weights in rats treated with 300 and 1000 mg/kg of test chemical accompanied by minimal to moderate hepatocellular hypertrophy and moderate proliferation of smooth endoplasmic reticulum was observed. One high-dose animal also showed pronounced nuclear alterations in hepatocytes (vesicular nuclei and prominent, large nucleoli) as well as increased mitotic activity. Liver sections from rats administered CGA-293343 Technical (Batch # P.506006, purity 98%) in the feed for 28 days at doses of 0, 100, 1000, 2500 and 10000 ppm (DPR# 52691-055:167750; Novartis study # 942088) were used to assess a possible effect of CGA 293343 on replicative DNA synthesis. hepatocellular proliferation was tested using four liver tissue blocks (fixed and embedded in paraffin) from each animal; initially, two liver blocks from each animal of the control and 10000 ppm dose group were processed using immunohistochemical staining to detect proliferating cell nuclear antigen (PCNA). Three sections per block were incubated with monoclonal anti-PCNA antibody clone PC10 and further processed with an Avidin Biotin Alkaline Phosphatase Detection Kit. Cells in S-phase of the cell cycle were identified by uniform dark red nuclear staining for PCNA; there was no indication for a treatment-related increase in the fraction of DNA synthesizing hepatocytes in S-phase. It was concluded that CGA 293343 did not stimulate hepatocyte cell proliferation in male rats at dietary levels of 10000 ppm (711 mg/kg b. wt.) for 28 days. Supplemental study. Kellner, 10/4/99.

057; 167752; “28-Day Range Finding Study in Beagle Dogs” (Altmann, B., Novartis Crop Protection AG, Short/Long-term Toxicology, Basle, Switzerland, Study No. 942106, Novartis Nexus No. 473-94, 6/19/96). CGA 293343 tech. (Batch No. KI-4654/18, purity=98.4%) was admixed to the pelleted food at dose levels of 0 (pelleted food), 300, 1000, or 3000 ppm (0, 10.0, 31.6, and 47.7 mg/kg/day, respectively, for males and 0, 10.7, 32.6, and 43.0 mg/kg/day, respectively, for females) and fed to 2 beagle dogs per sex per dose level for 28 consecutive days. One male at 3000 ppm was found dead on day 15. No treatment-related clinical signs were observed. Treatment-related decreases in mean body weight and mean food consumption in both sexes at 3000 ppm were observed. A treatment-related decrease in mean relative thymus weight in both sexes at 3000 ppm was observed. Microscopic examination revealed treatment-related pigmentation in the Kupffer cells, and atrophy of the thymus and of the marginal zone of the splenic white pulp. **No adverse effects.** NOEL (M)=31.8 mg/kg/day (1000 ppm) and (F)=32.6 mg/kg/day (1000 ppm), based on decreased body weight and food consumption, and microscopic findings. **Supplemental study** (only 2 animals per sex per dose used and animals treated for only 28 days). (Corlett, 9/16/99).

(Dermal)

058; 167754; “28-Day Repeated-Dose Dermal Toxicity Study in Rats” (Gerspach, R., Novartis Crop Protection AG, Short/long-term Toxicology, Basle, Switzerland, Study Number 942116, Novartis Nexus No. 511-96, 10/8/96). 822. CGA 293343 tech. (Batch No. P. 506006, purity=98.6%), suspended in 0.5% (w/v) carboxymethylcellulose in 0.1% (w/v) aqueous polysorbate 80, was applied to the clipped skin of 5 Tif:RAIf (SPF) hybrids of RII/1 x RII/2 (Sprague-Dawley derived) rats per sex per dose at dose levels of 0 (vehicle only), 20, 60, 250, or 1000 mg/kg/day for 6 hours per day 5 days per week for 4 weeks using an occlusive dressing. No animals died. No treatment-related clinical signs or signs of local irritation were observed. Dose-related increases in mean serum glucose and mean serum triglycerides in females at 250 and 1000 mg/kg/day were observed. Also, a treatment-related increase in mean serum alkaline phosphatase level was observed in females at 250 and 1000 mg/kg. Microscopic examination revealed treatment-related hyaline change in renal tubules of males at 1000 mg/kg/day and treatment-related minimal-moderate inflammatory cell infiltration in the liver of females at 60, 250, and 1000 mg/kg/day. **Possible adverse effect:** treatment-related hyaline change in the renal tubules of high dose males. NOEL (systemic, M)=250 mg/kg/day based on hyaline change in renal tubules, NOEL (systemic, F)=60 mg/kg/day based on elevated alkaline phosphatase and abnormal liver histology, NOEL (dermal, M/F)=1000 mg/kg/day based on no signs at HDT. **Acceptable.** (Corlett, 9/27/99).

METABOLISM STUDIES

**52691-144, -077, -145 167788, 167695, 167789 “Absorption, Distribution and Excretion of [Thiazol-2-¹⁴C] and [Oxadiazin-4-¹⁴C] CGA-293343 in the Rat” (Muller, T., 851-Animal Metabolism, Novartis Crop Protection, Basle, Switzerland, Project # 027AM01, 027AM09, 027AM02, Report 11/96, 8/15/96). Non-radiolabeled: CGA 293343 (Batch # KI-4654-18 and AMS 780/101, purity >98% and >99%, respectively); Radiolabeled: [Thiazol-2-¹⁴C] CGA-293343 (Batch #Ko-73.1A and Ko-73.2A-1, specific activity 68.9 and 57.3 μCi/mg, respectively, purity of >97%) and [Oxadiazin-4-¹⁴C] CGA-293343 (Batch Ko-75.2A-2 and Ko-75.2A-3, spec. act. of 87.0 and 84.6 μCi/mg, purity >96%) were administered to 4 or 5 Tif:RAIf (SPF) rats/sex/dose at 0.5 mg/kg, to 5 rats/sex at 0.5 mg/kg (after 14 days of unlabeled CGA 293343) and to 5 rats/sex at 0.5 or 100 mg/kg by oral gavage or i.v. Supplemental study (-077:167695) used 3 groups of 4 male Tiflbm:MAG (SPF) mice receiving [Thiazol-2-¹⁴C] CGA

293343 for 14 days at 118 mg/kg to determine excretion and metabolic fate in mice. In rats, the dose was rapidly absorbed from the G.I. tract into the general circulation with maximum blood levels ($t_{c_{max}}$ [h]) achieved 1 to 4 hours independent of the radiolabel site, dose level or sex. C_{max} ranged from 0.17 to 0.20 ppm (low dose) and 33 to 43 ppm (high dose) and levels declined rapidly ($t_{c_{max}/2}$ about 8 hours). Bioavailability 0.6 to 0.8 (males) and 0.7 to 0.9 (females) indicated sizable oral absorption. Absorbed material was primarily excreted via the urine (approximately 90%) compared to about 4% in feces within 24 hours. The preponderance of fecal elimination originated from biliary excretion. Half-lives in all tissues ranged from 2 to 6 hours. Comparison of metabolite patterns in mice and rats indicated that the major metabolic pathways were similar. Biotransformation in mice was 30-60%, with CGA-322704 the major urinary metabolite. The formation of polar metabolites in urine was greater in mice (4.7-8% of dose) than rats (0.3% of daily dose). **Acceptable.** (Kellner, 9/30/99).

52691-0276 215595 Mewes, K. E., "Absorption, metabolism and excretion of [Oxadiazin-4-¹⁴C] CGA 293343 after dietary administration of CGA 293343 at four dose levels in the mouse," Novartis Crop Protection AG, Basel, Switzerland, 9/15/00. Basel No. 027AM10. Fifteen Tiflbm: MAG (SPF) mice/group were dosed with non-labeled thiamethoxam in diet for 29 days at 0, 100, 500, or 2500 ppm. Labeled thiamethoxam (10 mg/kg b.w.) was given by gavage to all groups on day 30, and again 72 hours later (non-labeled dietary treatments continued until termination). Mice were killed 6 hours after the second radio-labeled treatment. Investigators evaluated urine, feces, liver, plasma, and bile for radiolabel and metabolites. Regardless of dose, 58-76% of the first dose was found in urine and 24-36% of first dose in feces within 72 hours (accounting for 94-102% of administered dose). Six hours after the 2nd dose, liver contained about 0.9 to 1.5% of that dose, pooled bile from the gall bladders contained only 0.01 to 0.22% of that dose, and plasma contained 0.3 to 0.4% of that dose (no effect of pre-treatment for liver, bile, or plasma). Excreta and other samples showed no influence of dose on metabolite patterns. Primary urinary metabolites as % of administered dose were thiamethoxam (36-44 %), CGA 322704 (10-15%), and CGA 265307 (8-11%). Primary fecal metabolites as % of administered dose were thiamethoxam (8-12 %), CGA 322704 (4-6%), and CGA 265307 (3-7%). Mouse liver fractions contained only small amounts of the above 3 species: major metabolites were products of glutathione addition at the chloro- position or, to a lesser extent, downstream products after cleavage of the oxadiazine ring. Glutathione derivatives were likewise significant proportions of bile contents, in addition to several polar metabolites which were not routinely characterized, plus probably less than 20% of thiamethoxam, CGA 322704, and CGA 265307 combined. Plasma label (as % of label in plasma) was primarily CGA 265307 (43-54%), CGA 322704 (20-26%), and thiamethoxam (17-26%). Useful supplementary data. Aldous, Sept. 4, 2008.

52691-0269 215588 Briswalter, C., "Absorption, metabolism and excretion of [Oxadiazin-4-¹⁴C] CGA 293343 in the mouse after oral administration," Syngenta Crop Protection AG, Basel, Switzerland, Oct. 1, 2002. Basel No. 027AM15. Four Tiflbm: MAG (SPF) mice/sex/group were dosed once by gavage with labeled thiamethoxam at 0.5 or at 100 mg/kg. Mice were killed after 3 days, with collection of urine and feces, and radioactivity analyses of tissues at termination. Individual metabolites were not isolated. Most label was recovered in urine (72% and 73% of administered dose in 0.5 mg/kg M and F: 82% and 90% in 100 mg/kg M and F). Corresponding fecal excretion was 20% and 14% in 0.5 mg/kg M and F: 11% and 15% in 100 mg/kg M and F). Total tissue residues constituted about 1% of administered dose. Residues in liver were typically about 10x higher than other tissues. There was no clear sex or dose difference in disposition of thiamethoxam. Useful supplementary data. No DPR worksheet (similar or complementary information is available in other sources, such as the above record). Aldous, 8/26/08.

52691-0266 215585 Briswalter, C., "The metabolism of [Oxadiazin-4-¹⁴C] CGA 293343 in the mouse after oral administration," Syngenta Crop Protection AG, Basel, Switzerland, 11/19/02. Basel No. 027AM16. This is the metabolite study utilizing samples from the mice in study 52691-0269 215588 (Basel Study No. 027AM15). Average percentages of administered dose in urine for both sexes at the two dose levels (0.5 or at 100 mg/kg) were 35% thiamethoxam, 13% CGA 322704, 16% CGA 265307, 6% N-formyl-N'-hydroxy-urea, and 2% N-formyl-N'-hydroxymethyl-urea. CGA 330050 was observed at much lower levels than any of these. It appeared that metabolism was slightly greater in females than in males, and data suggested higher urinary excretion of the high dose level than at the low dose, however with only N = 4, it is unclear whether there was any sex or dose effect. No DPR worksheet. Aldous, 9/2/08.

52691-0268 215587 Briswalter, C., "Blood kinetics of CGA 293343 and its metabolites in male mice after oral administration of [Oxadiazin-4-¹⁴C] CGA 293343," Syngenta Crop Protection AG, Basel, Switzerland, 8/27/03. Basel No. 027AM14. Six male Tiflbn: MAG (SPF) mice/group were dosed once with 100 mg/kg [Oxadiazin-4-¹⁴C] CGA 293343 by gavage. Sacrifices were 0.5, 1, 2, 4, 6, 8, or 24 hrs after dosing. Blood was collected to assess total residues and to identify major metabolites. A TLC radiochromatogram of whole blood extracts taken 1 hr post-dosing revealed 3 strong peaks, with very little label outside those areas. One of the peaks represented 2 constituents, so that the HPLC radiochromatogram revealed 4 perceptible peaks. These were thiamethoxam (dominant peak), and three metabolites: CGA 322704, CGA 265307, and CGA 330050. During the first hour, about 1.5% to 2.9% of label was non-extractable, whereas residues other than thiamethoxam and the above metabolites were below levels of detection. Kinetics parameters for $t_{c_{max}}$ [hr] 0.5 for thiamethoxam, and 2 for the three metabolites, and estimated $t_{1/2}$ [hr] were 3 hr (for thiamethoxam and all metabolites). During the period from 4-8 hrs post-dosing, "other" residues constituted about 5% of extractable label. At 8 and 24 hours, respectively, total residues in blood were only 30% and 1% of the peak [0.5 hr] levels. Metabolic profile (as % of total radioactive residues of a given sampling time) for thiamethoxam, CGA 322704, CGA 265307, and CGA 330050, respectively were 77.5, 11.2, 3.2, and 6.6 at 0.5 hrs; 60.0, 15.7, 9.8, and 11.6 at 1 hr; and 39.5, 12.7, 30.4, and 9.0 at 8 hrs. This experiment confirmed high absorption, rapid clearance, and a simple metabolic profile. Useful supplementary data. Aldous, 8/27/08.

52691-0267 215586 Briswalter, C., "Blood kinetics of CGA 293343 and its metabolites in male rats after oral administration of [Oxadiazin-4-¹⁴C] CGA 293343," Syngenta Crop Protection AG, Basel, Switzerland, 8/26/03. Basel No. 027AM13. [Compare with analogous mouse study in 52691-0268 215587]. Two male Tiflbn: RAI (SPF) rats/group were dosed once with 100 mg/kg [Oxadiazin-4-¹⁴C] CGA 293343 by gavage. Sacrifices were 0.5, 1, 2, 4, 6, 8, or 24 hrs after dosing. Blood was collected to assess total residues and to identify major metabolites. A TLC radiochromatogram of whole blood extracts taken 4 hrs post-dosing revealed 1 strong peak, one much lesser peak, and very little label outside those areas. The corresponding HPLC radiochromatogram revealed 2 perceptible peaks: thiamethoxam and CGA 322704. At peak levels of thiamethoxam (6 hrs after dosing), 99.8% of radiolabel was extractable. Extractable residues other than thiamethoxam and two metabolites were 1.74% of label. Maximum concentrations were at 6 hr for thiamethoxam and its metabolites. Estimated $t_{1/2}$ were 2 hrs for thiamethoxam, 4 hrs for CGA 322704, and 8 hrs for CGA 265307. During the period from 0.5 to 8 hrs post-dosing, "other" residues graduated from 0.3% to 2.2% of extractable label. At 24 hours, total residues in blood were only 2% of the peak [6 hr] levels. Metabolic profile (as % of total radioactive residues of a given sampling time) for thiamethoxam, CGA 322704, and CGA 265307, respectively were 94.6%, 5.0%, and (below quantifiable levels) at 1 hr; 81.9%, 15.0%, and 1.2% at 6 hrs; and 15.5%, 30.6%, and 17.6% at 24 hrs. CGA 330050,

a significant metabolite in mice, was not detectable. This experiment confirmed moderate rate of absorption (appreciably lower rate than mice), rapid clearance, and a simple metabolic profile. Useful supplementary data. Aldous, 8/27/08.

52691-0270 215589 Green, T., "Thiamethoxam: comparative metabolism in mice and rats *in vivo*, and in mouse, rat and human liver fractions *in vitro*," Central Toxicology Laboratory, Alderley Park, UK, CTL No. 024607, 11/21/02. In the *in vivo* study compared rat and mouse plasma metabolite levels after 1-week or 10-week dietary exposures of 3000 ppm in rats and 2500 ppm in mice (N = 5). Plasma thiamethoxam levels were 12 and 4 µg/ml in 1-wk and 10-wk mice, and 7 and 19 µg/ml in respective rats. In mice, it appeared that metabolic induction was progressing over that interval, as CGA 265307 (downstream metabolite of both CGA 322704 and CGA 330050) increased from 2 to 5 µg/ml. CGA 322704 levels in mice stayed about the same and CGA 330050 levels were marginally reduced during this interval. In rats, CGA 322704 ranged from 1.0 to 0.6 µg/ml. Other metabolite levels were exceedingly low in rats: CGA 265307 at 0.05 to 0.09 µg/ml, and CGA 330050 at 0.10 to 0.14 µg/ml. Liver microsomal fractions were prepared from mice, rats, and humans for *in vitro* studies of metabolism of thiamethoxam to metabolites. In all cases, mice had the most rapid metabolic rates (i.e. for metabolism of thiamethoxam to CGA 322704, thiamethoxam to CGA 330050, CGA 322704 to CGA 265307, and CGA 330050 to CGA 265307). Rats had slightly higher metabolic rates than humans for these reactions. Useful summary data, insufficient detail for a DPR worksheet. Aldous, 9/3/08.

52691-0271 215590 Green, T., "Thiamethoxam (CGA 293343): metabolism in mice and rats during dietary feeding studies," Central Toxicology Laboratory, Alderley Park, UK, CTL No. 024606, 9/3/08. A focus of this brief report was the difference in internal dose of thiamethoxam metabolites (specifically CGA 265307, CGA 330050, and CGA 322704) between mice and rats in the context of long-term studies. There was close correlation between concentrations of thiamethoxam and of these 3 metabolites between liver and plasma in male Tif:MAG mice after 10 weeks of feeding at 2500 ppm, indicating that plasma would be a good surrogate for liver as the target organ for mouse oncogenicity. Plasma concentrations of thiamethoxam were quite variable over time (sampling at weeks 1, 10, and 20) in two mouse strains (Tif:MAG and CD-1), but neither strain had consistently higher levels than the other. In the same study, plasma levels of the three metabolites were quite comparable. These inter-strain mouse data are of particular interest because CGA 322704 (= clothianidin: see DPR DPN#52884) showed no liver tumors in CD-1 mice. Investigators consider a key difference between thiamethoxam and clothianidin is that the latter does not form metabolite CGA 330050, and the present data indicate that the difference is unlikely due to selection of mouse strain. When either mouse strain was fed CGA 322704 for up to 20 weeks, there were close correlations in levels of plasma CGA 265307, as well as close correlations in levels of test article CGA 322704 except for week 20, at which time CD-1 mice had appreciable lower concentrations of CGA 322704 than did Tif:MAG mice. When CGA 265307 was fed for up to 20 weeks to these mouse strains, plasma CGA 265307 levels were comparable between strains at all intervals. Tif:MAG male mouse dietary levels of 500 to 2500 ppm thiamethoxam showed no dose effect on relative levels of thiamethoxam nor of its metabolites. A comparison of high dose thiamethoxam levels (2500 ppm in Tif:MAG male mice vs. 3000 ppm in female Tif:RAIf rats: plasma assayed at 1, 10, and 50 weeks) found comparable plasma levels of thiamethoxam (10-15 µg/ml in mice vs. 7-19 µg/ml in rats). In this study, CGA 322704 ranged from 2.5 to 5.3 µg/ml in mice and 0.6 to 1.2 µg/ml in rats, CGA 265307 ranged from 2.0 to 7.0 µg/ml in mice to only 0.05 to 0.09 µg/ml in rats, and CGA 330050 ranged from 0.9 to 1.5 µg/ml in mice to only 0.10 to 0.14 µg/ml in rats. Thus it appears that species differences in internal dose of metabolites (such as CGA 330050) could account for

differences in tumor outcomes between mice and rats due to internal dose in liver. Useful summary data, insufficient detail for a DPR worksheet. Aldous, 9/3/08.

SUPPLEMENTAL STUDIES

187; 172709; "Liver Tumor Formation in Mice by Thiamethoxam (CGA-293343) - Implications for Human Risk Assessment "; (E. Weber, *et al*; Novartis Crop Protection AG, Basle, Switzerland; Novartis No. 1199-99; 12/7/92). This report provides a risk assessment of liver tumors in Tif: MAGf (SPF) mice fed for 18 months with 5, 20, 500, 1250 or 2500 ppm thiamethoxam (CGA-293343 Technical, 98% purity), arguing that the mechanisms involved with their formation are species specific and threshold in nature, and therefore justify the use of margin of exposure methods for risk assessment rather than linear low-dose extrapolation. The key points of the argument are: thiamethoxam shows a lack of mutagenicity in standard assays; carcinogenicity was observed only in the mouse (not in rat or dog); and the postulated mechanism of action involves hepatocellular apoptosis and necrosis followed by regenerative hepatocyte proliferation, which is unlikely to occur in humans. A risk assessment method is proposed based on the LED10 for hepatocellular hypertrophy or single cell necrosis and using a margin of exposure of 300. The calculated RfD was 0.0017 mg/kg/day based on 0.5 mg/kg/day human LED10. **Supplemental.** (Duncan, 7/31/00)

52691-0345 236193 Lloyd, S. and R. Peffer, "Thiamethoxam: assessment of potential endocrine-related effects in mammals," (analysis: not a laboratory report), 4/8/04. Authors evaluated thiamethoxam studies reporting changes of hormone secreting or hormone-receptive organs or tissues, and determined that none of these changes suggested an endocrine-driven mechanism. No DPR worksheet. Aldous 8/18/08.

52691-0353 236201 Sturgess, N. C., "Thiamethoxam: receptor binding studies with thiamethoxam, imidacloprid and nicotine: interaction with mammalian $\alpha 4\beta 2$ and $\alpha 7$ -type nicotinic acetylcholine receptors," Central Toxicology Laboratory, Alderley Park, UK, 7/14/04. Thiamethoxam, imidacloprid and nicotine were evaluated for their abilities to displace ligands with strong affinities for the above two binding sites in rat forebrain homogenate preparations. Thiamethoxam bound very weakly compared to nicotine (i.e. minimal nicotinic response would be expected in mammals). Imidacloprid bound to an intermediate extent, but still much less avidly than nicotine in both cases. Useful supplementary data. Not a mandated study. No DPR worksheet. Aldous, 6/18/08.

- Kidney histopathology and $\alpha_{2\mu}$ -globulin:

52691-0349 236197 Weber, E., "Immunohistochemical assessment of $\alpha_{2\mu}$ -globulin in the rat kidney upon administration of CGA 293343 for 28 days," Novartis Crop Protection AG, Basel, Switzerland, July 3, 2000, Novartis Study No. CB 00/16. Investigators evaluated kidney tissues from DPR Document No. 52691-0055, Record No. 167750 to determine whether $\alpha_{2\mu}$ -globulin is the characteristic material in regions of hyaline change. They used a monoclonal antibody to $\alpha_{2\mu}$ -globulin to show that this protein was characteristically present in males, but absent in females. They determined that the amount of staining was greatly increased in males administered 1000 ppm and particularly at 2500 ppm thiamethoxam. In contrast, there was no remarkable change in staining at 10000 ppm thiamethoxam: the latter lack of response considered to reflect greatly diminished liver synthesis of $\alpha_{2\mu}$ -globulin. Staining was strongest in cortical regions, corresponding to the P₂ segments of the proximal convoluted tubules, and also

appreciable in the outer stripe of the outer medulla (mainly as granular casts, considered to be in lumina of the straight portions of the proximal tubules). Cortical areas showed about a 30-fold increase of discrete particles with strong staining for $\alpha_{2\mu}$ -globulin in 2500 ppm males compared with controls. Average particle size was increased about 26%. Useful supplementary data. Aldous, 6/19/08.

52691-0350 236198 Weber, E., "Immunohistochemical assessment of $\alpha_{2\mu}$ -globulin in the rat kidney upon administration of CGA 293343 for 3 months," Novartis Crop Protection AG, Basel, Switzerland, July 3, 2000, Novartis Study No. CB 99/55. This study is analogous to 52691-0349 236197, above. Kidney tissues came from 52691-0054; 167749; "3-Month Oral Toxicity Study in Rats (Administration in Food)" (Bachmann, M., Novartis Crop Protection AG, Short/Long-term Toxicology, Basle, Switzerland, Study No. 942089, Novartis Nexus No. 471-94, 1/23/96). In that study, thiamethoxam was administered in diet at dose levels of 0, 25, 250, 1250, 2500, or 5000 ppm for 3 months. Results for these $\alpha_{2\mu}$ -globulin studies were qualitatively like Record No. 236197, however the strongest response in this study was at 5000 ppm. At that dose level, the increase in numbers of $\alpha_{2\mu}$ -globulin particles in cortical slices was about 5-fold over controls. Useful supplementary data. No DPR worksheet. Aldous, 6/19/08.

52691-0351 236199 Weber, E., "Immunohistochemical assessment of $\alpha_{2\mu}$ -globulin in the rat kidney upon administration of CGA 293343 for 12 months," Novartis Crop Protection AG, Basel, Switzerland, July 3, 2000, Novartis Study No. CB 00/14. This study is analogous to 52691-0349 236197, above. Kidney tissues came from interim sacrifice rats of study 52691-080 167773, "24-Month Carcinogenicity and Chronic Toxicity Study in Rats" (Bachmann, M. 835-Novartis Crop Protection AG, Stein, Switzerland; Study # 942110, 7/27/98). CGA-293343 Technical (Batch # P.506006, purity 98.6%). In that study, thiamethoxam was administered in diet at dose levels of 0, 10, 30, 500 or 1500 ppm in males and 0, 10, 30, 1000 or 3000 ppm in females. Tissues for the present $\alpha_{2\mu}$ -globulin immunohistochemical assessment derived from 1-yr interim sacrifice rats. Cortical areas showed about a 14-fold increase of discrete particles with strong staining for $\alpha_{2\mu}$ -globulin in 1500 ppm males compared to controls. Average particle size was increased about 42%. Useful supplementary data. No DPR worksheet. Aldous, 6/20/08.

52691-0352 236200 Weber, E., "Immunohistochemical assessment of $\alpha_{2\mu}$ -globulin in the rat kidney upon administration of CGA 293343 for 24 months," Novartis Crop Protection AG, Basel, Switzerland, July 3, 2000, Novartis Study No. CB 00/15. This study is analogous to 52691-0349 236197, above. Kidney tissues came from 52691-080 167773, "24-Month Carcinogenicity and Chronic Toxicity Study in Rats" (Bachmann, M. 835-Novartis Crop Protection AG, Stein, Switzerland; Study # 942110, 7/27/98). CGA-293343 Technical (Batch # P.506006, purity 98.6%). Tissues evaluated in this immunohistochemical assessment came from rats treated with thiamethoxam for 2 years in diet at dose levels of 0, 10, 30, 500 or 1500 ppm in males and 0, 10, 30, 1000 or 3000 ppm in females. Unlike the conspicuous staining of $\alpha_{2\mu}$ -globulin particles in the kidney cortex of young untreated males as was shown in Record No. 236197, untreated males aged over 2 years no longer displayed such staining. At 1500 ppm there was a modest staining of $\alpha_{2\mu}$ -globulin particles. Useful supplementary data. No DPR worksheet. Aldous, 6/20/08.

NOTE: Control male immunohistochemical staining in Record Nos. 236197-236200, above, was strong in younger rats, but diminished as rats aged. For purposes of comparison, the number of objects staining for $\alpha_{2\mu}$ -globulin per mm² of cortical slides averaged 4.5, 15.3, and 0.5 in the 28-day, 3-month, and 12-month groups, respectively. As noted above, the 24-month sacrifice control males showed no specific $\alpha_{2\mu}$ -globulin staining. Aldous, 6/20/08.

- Supplementary studies relating to mouse liver response to thiamethoxam:

52691-0277 215596 Noakes, J. P., "CGA 293343 (Thiamethoxam), CGA 322704 and CGA 265307: comparative toxicity in the liver of male Tif:MAGf and CD-1 mice," Central Toxicology Laboratory, Alderley Park, Macclesfield, Cheshire, UK, 10/21/03. CTL No. XM7081. This study was conducted to assess (1) strain differences between male Tif:MAGf and CD-1 mice respecting liver toxicity, and (2) relative responses to thiamethoxam compared to two of its major metabolites. Thiamethoxam (2500 ppm), CGA 322704 (2000 ppm), and CGA 265307 (500 ppm) were administered in diet to 17 mice/strain/interval for 1, 10, or 20 weeks. At termination, 12/strain/interval were allocated for histopathology of livers, blood clinical chemistry, BrdU immunohistochemistry of livers, and TUNEL histochemistry/image assay to assess increase in apoptosis. Five/strain/interval were used for hematology. All surviving mice were used for clinical signs, body weight, food consumption, and organ weight evaluations. Clinical signs of vocalizations and chirruping were common in CD-1 mice administered CGA 322704. Ten of 17 mice in this group were killed *in extremis* shortly after the scheduled week 10 sacrifice. There were small (occasionally statistically significant) decrements in body weights of Tif:MAGf mice administered CGA 322704. CD-1 mice fed CGA 322704 had more severe body weight effects (6.7 g decrement at week 10). Tif:MAGf mice fed CGA 322704 had modestly reduced food consumption (about 10% reduction during weeks 1 to 6), whereas corresponding CD-1 mice commonly had food consumption decrements of over 20%. Only thiamethoxam mice showed definitive liver histopathology: at least 50% of either strain displayed hepatocyte apoptosis, hepatocellular hypertrophy, and hepatocellular necrosis. Also livers of 20-week thiamethoxam Tif:MAGf mice and of 10- and 20-week CD-1 mice routinely showed inflammatory infiltration and pigmentation of centrilobular hepatocytes. Investigators found increased BrdU labeling indices of thiamethoxam groups suggesting increased DNA synthesis: statistically significant in 20-week Tif:MAGf mice and in 10- and 20-week CD-1 mice. TUNEL-histochemistry did not confirm apoptosis in any groups. Thiamethoxam was the only material in this study to influence clinical chemistry parameters examined, including a marked reduction of plasma cholesterol and a modest reduction in plasma protein levels in both strains. It thus appears that thiamethoxam and not its major metabolites is the cause of characteristic hepatotoxicity in either strain. Useful supplementary data. Aldous, Sept. 4, 2008.

52691-0279 215598 Sagelsdorff, P., "Determination of parameters indicative for oxidative stress in male mice following subchronic treatment for up to 60 days," Novartis Crop Protection AG, Basel, Switzerland, 12/22/00. Basel No. CB 00/21. Ten young adult male Tif:MAG (SPF) mice/group/interval were dosed in diet with 0, 2500 or 5000 ppm thiamethoxam (98.6%) for 7, 14, 28, or 60 days. Oxidative stress parameters evaluated were hepatic concentration of 8-isoprostane $F_{2\alpha}$, plasma concentration of free 8-isoprostane $F_{2\alpha}$, and hepatic concentration of malondialdehyde. To assess cellular antioxidant capacity, investigators evaluated hepatic concentration of α -tocopherol and hepatic glutathione (GSH: reduced and oxidized). Mean daily dose levels were estimated to have been 448 and 976 mg/kg/day. Body weight was reduced in 5000 ppm mice (7.2 g decrement at 60 days), but not at 2500 ppm. There were no apparent clinical signs. There were no systematic changes in total 8-isoprostane $F_{2\alpha}$ in liver or in free 8-isoprostane $F_{2\alpha}$ in plasma. Liver concentration of malondialdehyde was unaffected. Liver concentration of α -tocopherol was unchanged. Concentration of reduced liver GSH was highly significantly elevated, dose-related, at 2500 (16% to 33% increase) and 5000 ppm (26% to 48% increase). There was no effect on liver concentration of oxidized GSH. Thus study parameters do not indicate oxidative stress, while the increase in reduced liver GSH indicates liver induction. Useful supplementary information. Aldous, 7/24/08.

52691-0281 215600 Sagelsdorff, P., "Determination of parameters indicative for oxidative stress in male mice upon treatment with CGA 293343 tech. for up to 50 weeks," Novartis Crop Protection AG, Basel, Switzerland, June 6, 2003. Basel No. CB 00/09. Ten young adult male Tif:MAG (SPF) mice/group/interval were dosed in diet with 0, 2500 or 5000 ppm thiamethoxam (98.6%) for 10, 20, 30, 40, or 50 weeks. Oxidative stress parameters evaluated were hepatic concentration of 8-isoprostane $F_{2\alpha}$ and plasma concentration of free 8-isoprostane $F_{2\alpha}$. To assess cellular antioxidant capacity, investigators evaluated hepatic concentration of α -tocopherol and hepatic glutathione (GSH: reduced and oxidized). Also, investigators measured liver activities of γ -glutamylcysteine synthetase and glutathione S-transferase. Liver tissues were examined at 50 weeks for histopathology. Mean daily dose levels were estimated to have been 318 and 693 mg/kg/day. This study neither sought nor found a NOEL. Body weights were reduced in treated groups (at wk 40 sacrifice: 3.9 g decrement at 2500 ppm and 9.4 g at 5000 ppm). There were no apparent clinical signs. Livers commonly showed "accentuated lobular pattern" at 2500 and 5000 ppm. Livers were examined microscopically, only at week 50 sacrifice, with 10, 10, and 9 mice examined, respectively, at 0, 2500, and 5000 ppm. Histopathology found the following non-neoplastic hepatocellular incidences: hypertrophy (0, 6, and 8), necrosis (0, 9, and 9), and apoptosis (0, 4, and 5). Other respective non-neoplastic liver incidences included inflammatory cell infiltration (4, 8, and 8) and pigmentation (0, 6, and 7). Hepatocellular neoplasia incidences overall were 1, 1, and 4 for respective treatment groups. Most affected mice showed both hepatocellular adenoma plus carcinoma (adenoma plus carcinoma incidences of 0, 1, and 3, respectively). There were no systematic changes in free 8-isoprostane $F_{2\alpha}$ in plasma, whereas there was typically a statistically significant ($p < 0.05$) decrease in total 8-isoprostane $F_{2\alpha}$ in liver at 5000 ppm only. Liver concentration of α -tocopherol was unchanged. Level of reduced liver GSH was highly significantly elevated, dose-related, at 2500 (16% to 33% increase) and 5000 ppm (26% to 48% increase). There was no treatment effect on liver concentration of oxidized GSH. Liver activity of γ -glutamylcysteine synthetase was elevated 21-58% over controls at 2500 ppm, and 71-89% at 5000 ppm. Liver activity of glutathione S-transferase was elevated 55-125% at 2500 ppm and 117-195% at 5000 ppm. Thus study parameters do not indicate oxidative stress, while there is strong indication of liver metabolic induction. Useful supplementary information. Aldous, Sept. 4, 2008.

52691-0285 215604 Weber, E., "Assessment of hepatic cell proliferation and apoptosis in male mice upon treatment with CGA 293343 tech. for up to fifty weeks," Syngenta Crop Protection AG, Basel, Switzerland, 6/29/03. Basel No. CB 00/12. Groups of 15 Tif: MAGf (SPF) male mice/dose/interval were dosed for 10, 20, 30, 40, or 50 weeks with Thiamethoxam, 98.6% purity, at 0, 50, 200, 500, 1250, 2500, or 5000 ppm (about 6.3, 25, 62, 151, 314, and 684 mg/kg/day, respectively). Primary parameters were histopathology, cellular proliferation, and apoptosis in the liver: these processes relate to the primary mouse oncogenicity study (Ciba-Geigy Study # 942109, dated 6/2/98, DPR Record Nos. 167758, 167751, 167700, and 167701). Body weights were significantly reduced at 2500 and 5000 ppm (e.g. 5% and 17% reductions, respectively, at 30 weeks). Absolute liver weights were unaffected, but relative liver weights were elevated at 2500 ppm (significant at 2/5 intervals, but generally elevated) and 5000 ppm (significant at all 5 intervals, up to 29% increase). Plasma alanine aminotransferase showed a dose-related increase over 1250 to 5000 ppm, with a trend toward increased response over time. A lesser but statistically significant response was observed in this range for aspartate aminotransferase, but alkaline phosphatase was unaffected. Gross findings of liver "accentuated lobular pattern" were slightly elevated at 500 ppm, and sharply elevated at 1250 and 2500 ppm, but not at 5000 ppm (a dose-response despite the high dose discontinuity). Livers showed a consistent pattern of fatty change, hepatocellular apoptosis, hepatocellular necrosis, and inflammatory cell infiltration at 500 ppm and above. Hepatocellular hypertrophy was

significantly elevated only at 2500 and 5000 ppm, but this showed a strong dose-response. Hepatocellular pigmentation was significantly elevated at 1250 to 5000 ppm, also with a strong dose-response. BrdU labeling index was indicative of cell proliferation at 1250 ppm and above, without systematic differences over time. TUNEL analysis indicated increased apoptosis at 500 ppm and above, with increases at 500 ppm beginning at 30 weeks. TUNEL responses appeared to increase progressively over time. Both of these latter tests showed definitive dose-responses at 2500 to 5000 ppm, with changes evident at 10 weeks at the highest dose level. Thus male mice demonstrated a consistent pattern of liver pathology including altered functional behavior, cell losses through necrosis and apoptosis, and evidences of heightened compensatory or repair activity from 500 ppm and upward. Data are consistent with the concept that hepatocellular damage and repair characterized over time and dose in this study are associated with, and are likely pre-disposing conditions to, hepatocellular tumors. Useful supplementary information. Aldous, Aug. 5, 2008.

52691-0282 215601 Soames, T., "Thiamethoxam (CGA 293343 Tech.): Sublobular assessment of hepatic cell proliferation after 40 weeks," Central Toxicology Laboratory, Alderley Park, UK, 9/19/03. CTL No. 03R026. Tissue sections evaluated in this report derived from a Syngenta study (52691-0285 215604 Weber, E., 6/29/03, Basel No. CB 00/12). The latter study found BrdU labeling indices consistent with cell proliferation at 1250 ppm and above. The present study focused strictly on centrilobular regions for BrdU analysis, and re-evaluated the 40-week interval sacrifice livers at 0, 200, 500, and 1250 ppm. This re-analysis found statistically significant increases in labeling indices at 500 and 1250 ppm, placing the NOEL for this effect one dose lower (200 ppm) than had been obtained in the previous study by evaluation without respect for lobular regions. Useful supplementary data. Aldous, 8/12/08.

52691-0284 215603 Weber, E., "Histochemical assessment of hepatic apoptosis upon treatment of male mice with CGA 293343 Tech. (Thiamethoxam) for up to 9 months," Novartis Crop Protection AG, Basel, Switzerland, Dec. 6, 1999. Basel No. CB 99/57. Liver sections from paraffin blocks were evaluated from two earlier studies: (1) Bachmann, M. (6/2/98), Ciba-Geigy Study # 942109, DPR Record Nos. 167758, 167751, 167700, and 167701 [the primary mouse oncogenicity study] [only control and 2500 ppm males were evaluated here for apoptosis], and (2) Weber, E. (1998), Study No. CB 98/12, "Assessment of hepatic cell proliferation in mice," [dose levels of 0, 100, 500, and 2500 ppm at study durations of 3, 7, 13, 27, and 59 days]. Apoptosis was assessed by TUNEL histochemistry as an indicator of associated DNA fragments, coupled with Eosin staining to obtain a measurement of hepatic tissue area. Apoptosis was increased only after 59 days in the Weber study, with dose-related and significant increases at 500 and 2500 ppm, while no effects were seen at 100 ppm on or before 59 days. Apoptosis in the primary mouse oncogenicity study was only quantified at 2500 ppm, but was highly significantly elevated at that dose level (about 10 x over controls). Useful supplementary data. Aldous, Sept. 4, 2008.

52691-0280 215599 Sagelsdorff, P., "CGA 293343 Tech.: Determination of selected enzymes known to be involved in the biosynthesis and modulation of glutathione in the liver of mice following subchronic treatment for up to 60 days," Syngenta Crop Protection AG, Basel, Switzerland, 1/28/03. Basel No. CB 00/67. Investigators assayed γ -glutamylcysteine synthetase, glutathione reductase, glucose-6-phosphate dehydrogenase, and glutathione S-transferase. Ten male Tif: MAGf (SPF) mice/duration/dose received thiamethoxam (CGA 293343, 98.6%) in diet at 0, 2500, or 5000 ppm for 7, 14, 28, or 60 days. There was an increase in γ -glutamylcysteine synthetase, noted to be inducible and rate-limiting for GSH biosynthesis. This was significant in 3/4 intervals at 2500 ppm and highly significant at all intervals at 5000

ppm (typically about twice control levels at 5000 ppm). This increased activity was comparable at all treatment intervals. Glutathione S-transferase activity was highly significantly elevated at both dose levels and at all time intervals, with a modest dose-response in the treatment range. Continued treatment had little or no influence on treatment response after 14-28 weeks. Other assayed parameters were unaltered. Useful supplementary data. Aldous, 8/12/08.

52691-0275 215594 Green, T., "Thiamethoxam (CGA 293343): the role of nitric oxide in the development of hepatotoxicity in mice," Central Toxicology Laboratory, Alderley Park, UK, Sept. 8, 2003. CTL No. 024607-001. Authors noted that chemical toxicity and eventual tumor formation in liver can be enhanced through the release of tumor necrosis factor α (TNF- α), which is normally controlled by inhibitory effects nitric oxide, produced from arginine by an inducible nitric oxide synthase (iNOS). A metabolite of thiamethoxam, CGA265307, resembles a known iNOS inhibitor, nitro-L-arginine methyl ester (termed L-NAME in this report). Toxicity of hepatotoxins such as CCl_4 is decreased in TNF- α knockout mice, and increased in iNOS knockout mice. In the present study, investigators evaluated the iNOS-dependent inhibition of L-arginine to L-citrulline (which reaction releases nitric oxide) *in vitro* in the presence of thiamethoxam and of its metabolites, CGA265307, CGA322704, CGA330050, NOA421276, NOA412275, and NOA404617, all at 1 mM. CGA265307 inhibited iNOS (to 39% of control activity), as did the known inhibitor, L-NAME (11% of control activity). Of other test articles, only NOA421276 indicated a marginal effect. Groups of 5 male mice were dosed once with 10 $\mu\text{l/kg}$ CCl_4 (ip, 16 hours before sacrifice) [this dose previously shown to be mildly hepatotoxic, based on AST and ALT plasma levels], or 7 days with 2000 ppm of CGA265307 [yielding plasma CGA265307 concentrations similar to that following 2500 ppm dietary thiamethoxam], or a combination of CGA265307 treatment followed by a single ip dose of 10 $\mu\text{l/kg}$ CCl_4 , vs. untreated controls. Combined treatment with CGA265307 and CCl_4 appeared to enhance liver histopathology such as increased incidence of microvesicular vacuolation, subcapsular necrosis, and possibly hepatocyte necrosis and hydropic degeneration over either treatment alone. Investigators concluded that thiamethoxam might elicit liver toxicity including liver tumors by CGA265307 inhibition of nitric oxide synthases. Useful supplementary data. Aldous, 8/13/08.

52691-0272 215591 Green, T., "Thiamethoxam (CGA 293343): changes in plasma cholesterol levels during dietary feeding studies," Central Toxicology Laboratory, Alderley Park, UK, Sept. 8, 2003. CTL No. 024607-002. This report evaluated cholesterol parameters, primarily in mice. Some data in this report reflect studies that have been submitted separately. Tif:MAG mice on thiamethoxam diets for up to 50 weeks showed significant decreases in plasma cholesterol at 500 ppm and above (NOEL = 200 ppm). Tif:MAG mice dosed with 350 mg/kg/day thiamethoxam for 1 day showed non-significant decreases in cholesterol, and significant decreases in cholesterol, LDL, and HDL were observed after 4 or 7 days of treatment. Tif:MAG and CD-1 mice fed 2500 ppm thiamethoxam in diet for 1, 10, or 20 weeks showed significant (about 20%) decrements in plasma cholesterol at 1 week, and further (30-40%) decrements at 10-20 weeks. Thiamethoxam metabolite CGA330050 at 0, 500, or 1000 ppm likewise reduced cholesterol in Tif:MAG mice after 1, 4 or 10 weeks, with an apparent progressive response over time at 1000 ppm. In contrast, other thiamethoxam metabolites (CGA322704 and CGA265307) did NOT decrease cholesterol levels after 1, 10, or 20 weeks at 2000 ppm and 500 ppm, respectively. Mice fed 5000 ppm thiamethoxam for 1 week prior to a single injection of ^3H -mevalonolactone (a precursor to squalene and hence eventually to cholesterol and related molecules) led to a 4-fold increase in squalene in liver lipid fractions, with no associated change in cholesterol. Investigators indicated that the squalene increase may be due to inhibition of squalene monooxygenase, on the cholesterol biosynthesis pathway. In contrast to above cholesterol-

related responses in mice, Tif:RAIF rats administered 1000 or 3000 ppm thiamethoxam for 1 to 50 weeks showed no alterations in plasma cholesterol levels. A recovery study in Tif:MAG mice administered 2500 ppm thiamethoxam for 4 weeks, followed by up to 4 weeks of recovery, found a return to normal cholesterol levels after 2 and 4 weeks of recovery. Liver microsomal fractions were exposed to thiamethoxam, CGA330050, or CGA265307 (300 μ M in each case for *in vitro* studies) prior to evaluation for HMGCoA reductase activity (a rate-controlling enzyme on the pathway to cholesterol synthesis, hence a target for statin pharmaceuticals, which investigators noted to include several rodent carcinogens). None of these compounds significantly altered HMGCoA reductase activity. An *in vivo* exposure to thiamethoxam for 20 weeks at 2500 ppm followed by evaluation of liver microsomes for HMGCoA reductase activity likewise proved negative. The latter experiments suggest that thiamethoxam does not have a mechanism similar to statins. Useful supplementary data. Aldous, Sept. 4, 2008.

52691-0273 215592 Green, T., "Thiamethoxam (CGA 293343): comparative hepatotoxicity in weanling and adult mice," Central Toxicology Laboratory, Alderley Park, UK, Sept. 8, 2003. CTL No. 024607-003. Investigators evaluated male Tif:MAG mice administered 0, 500, 1250, or 2500 ppm thiamethoxam for 7 days, beginning at either 21 days of age or at 15-17 weeks of age. LOEL's for significant reductions in cholesterol were < 500 ppm in adults and 1250 ppm in weanlings (NOEL of 500 ppm). Plasma concentrations of thiamethoxam, and of the 3 assayed metabolites (CGA322704, CGA265307, and CGA330050) were uniformly higher in weanlings than in adults (typically 1.5 to 2-fold), regardless of dose. There were no changes in liver weights, nor in plasma ALT nor AST activities in either weanlings or adults administered 0 to 2500 ppm thiamethoxam. Histopathology did not show definitive changes: there appeared to be a slight increase in "reduced eosinophilic staining of centrilobular hepatocytes" in adults at 1250 and 2500 ppm, and possibly in weanlings at 2500 ppm. In all features evaluated, adults were more sensitive than weanlings to hepatotoxicity. Aldous, useful supplementary data, 8/20/08.

52691-0274 215593 Green, T., "Thiamethoxam (CGA 293343): hepatotoxicity of metabolites"

Central Toxicology Laboratory, Alderley Park, UK, 9/16/03. CTL No. 024607-004. Test articles were thiamethoxam and 3 metabolites (CGA322704, CGA265307, and CGA330050). Test animals were Tif:MAG mice and Tif:RaIf rats. There were 3 in-life experiments cited:

(1) Investigators cited study 52691-0277 215596, "CGA 293343 (Thiamethoxam), CGA 322704 and CGA 265307: comparative toxicity in the liver of male Tif:MAGf and CD-1 mice," Noakes, J. P., 10/21/03. CTL No. XM7081. The present report is confined to male Tif:MAGf mice respecting liver toxicity and relative responses to thiamethoxam compared to two major metabolites (see Record No. 215596, showing similar results with CD-1 mice). Thiamethoxam (2500 ppm), CGA 322704 (2000 ppm), and CGA 265307 (500 ppm) were administered in diet to 17 mice/interval for 1, 10, or 20 weeks. Only thiamethoxam mice showed definitive liver histopathology: at least 50% displayed hepatocyte apoptosis, hepatocellular hypertrophy, and hepatocellular necrosis at weeks 10 and 20. Thiamethoxam also reduced cholesterol levels: evident from weeks 1 through 20 in this study. Thiamethoxam also significantly elevated plasma ALT activity at weeks 10 to 20. Thiamethoxam increased BrdU labeling indices of thiamethoxam groups, suggesting increased DNA synthesis: statistically significant at week 20. In this study, TUNEL-histochemistry did not confirm apoptosis in any groups (positive trend, but not statistically significant).

(2) This report included data on CGA330050 exposure to Tif:MAG mice. The full report is in 52691-0272 215591 Green, T., "Thiamethoxam (CGA 293343): changes in plasma cholesterol levels during dietary feeding studies," Central Toxicology Laboratory, Alderley Park, UK, Sept. 8, 2003. CTL No. 024607-002. The present report includes histopathology data from that study.

Plasma cholesterol in this study was reduced after 1, 4, and 10 weeks of dosing with CGA330050 at 500 ppm (81-85% of controls) and at 1000 ppm (83% of controls at 1 wk, progressing to 69% of controls at 10 weeks). Hepatocellular hypertrophy was observed in 11/12 mice at 1000 ppm (but in none at 500 ppm): typically minimal in degree, observed at week 10. BrdU labeling was significantly elevated in this study at 1000 ppm CGA330050 at 10 weeks. Significantly elevated apoptosis (by TUNEL-histochemistry) was reported at 500 ppm but not at 1000 ppm in this study, weakly suggestive of a treatment response.

(3) Female Tif:Ralf rats were dosed with CGA330050 at 0, 500, or 1000 ppm for one week, then killed after taking blood and liver samples. There was marginal reduction of plasma cholesterol in this study [to 91% of control at 500 ppm ($p < 0.05$), and to 92% of control at 1000 ppm (not significant)]. These reductions were considered by investigators to reflect a treatment response. At 1 week in this study, plasma ALT and AST activities were modestly but statistically significantly reduced, indicative of altered liver function (but not losses in cellular viability).

Investigators concluded that the general correlation between responses of mice to thiamethoxam and CGA330050 (but not to metabolites CGA322704 or CGA265307) suggested a common mode of action, which may be associated with CGA330050 alone. Hepatotoxicity was less evident in rats. CGA330050 is a very minor metabolite in rats. This study suggests that CGA 330050 is a key metabolite responsible for changes leading to the development of liver tumors in mice treated with diets containing thiamethoxam. Useful supplementary data. Aldous, September 4, 2008.

52691-0283 215602 Waechter, F., "CGA 293343 Tech.: effects on selected biochemical parameters in the liver following dietary administration to female rats for 1 and 10 weeks," Syngenta Crop Protection AG, Basel, Switzerland, June 10, 2003. Basel No. CB 02/34. Thirty metabolizing enzymes were assayed from female rat liver samples. Ten enzymes had slightly elevated activities (up to 151% of controls). Five enzymes had slightly reduced activities (as low as 66% of controls). Fifteen enzymes appeared unaffected. In the judgement of the investigator, none of the observed activities indicated remarkable changes, which contrasts with responses of a comparable study in mice[†]. Useful supplementary data. Aldous, 8/21/08.

[†] The cited study is: DPR Document No. 52691-0083, Record No. 167701; Trendelenburg, C., "CGA 293343 Tech.: Effects on biochemical parameters in the liver following administration to male and female mice," (Project No. CB 98/11), 9/15/98. The Medical Toxicology Branch evaluation for that study is incorporated into the review for the primary mouse oncogenicity study (DPR Document No. 52691-061, Record No. 167758).

52691-0265 215584 Green, T., T. Pastoor, S. Lloyd, R. Peffer, and P. Rose, "Weight of evidence for a mode of action for thiamethoxam-related mouse liver tumors," Syngenta No. T003075-03. This report summarizes evidence that the tumors found in mouse livers arise from perturbed cholesterol synthesis, hepatotoxicity, cell death, and tumors associated with increased cell replication. Unique metabolic patterns in mice predispose this species to pathology which would not be expected in rats (which do not acquire liver tumors). Effects would be expected to be limited to comparatively high dose levels. This report is primarily a risk assessment document, summarizing findings noted in many reports found in this summary, and does not contain unique reviewable material. No DPR worksheet. Aldous, Sept. 3, 2008.

- Supplementary studies relating to female rat liver response to thiamethoxam:

52691-0278 215597 Noakes, J. P., "CGA 293343 (Thiamethoxam): assessment of hepatic cell proliferation and apoptosis in female rats upon treatment for up to fifty weeks," Central Toxicology Laboratory, Alderley Park, UK, Nov. 3, 2003. CTL No. XR7068. Groups of 15 female Tif:RAIf (SPF) rats/interval were dosed with thiamethoxam (98.8%) in diet at 0, 1000, or 3000 ppm (0, 59, or 181 mg/kg/day) for 1, 10, 20, 30, 40, or 50 weeks. Primary focus was liver toxicity. Rats were subcutaneously implanted with osmotic pumps 3 days before respective termination days for infusion of BrdU, to assess increased liver DNA synthesis as evidence of cell proliferation. TUNEL-histochemistry was conducted to assess apoptosis. Satellite groups of 10/dose were used for urine and blood sampling at the above intervals, the latter for cholesterol, total protein, and for 3 liver-associated enzymes. Premature deaths were slightly elevated in 3000 ppm groups (2/100 controls vs. 9/100 at 3000 ppm, $p \approx 0.029$ with all rats considered at risk). Investigators considered survival differences to be incidental, since 4 of the 9 high dose deaths occurred in one set of 15 rats between weeks 9 and 15. Since mortality was unaffected in the combined rat study at 3000 ppm (Record No. 167773), this reviewer does not attribute the incidences in this study to treatment. Investigators considered the body weight decrements at 3000 ppm as incidental (typically 12-15 g, significant at $p < 0.01$). Since the combined study found body weight decrements in 3000 ppm females (27 g at 53 weeks), this reviewer considers the present study to confirm a body weight effect. Food consumption was also marginally reduced at 3000 ppm (although usually not statistically significant). Clinical chemistry and urinalysis were negative, as were organ weights. Livers histopathology was negative for all treatment groups. Hepatocyte proliferation and apoptosis assessments were negative. Useful non-guideline study, with no adverse effects. Aldous, 7/23/08.