CALIFORNIA ENVIRONMENTAL PROTECTION AGENCY
DEPARTMENT OF PESTICIDE REGULATION
MEDICAL TOXICOLOGY BRANCH

SUMMARY OF TOXICOLOGY DATA

Diflufenzopyr

Chemical Code # 5750, 5751, Tolerance # 52843

7/13/00

I. DATA GAP STATUS

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

Toxicology one-liners are attached.

All record numbers through 173142 were examined.
** indicates an acceptable study.
Bold face indicates a possible adverse effect.
indicates a study on file but not yet reviewed.
File name: T181734
Vidair, 7/13/00

II. TOXICOLOGY ONE-LINERS AND CONCLUSIONS

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

COMBINED, RAT

52843-023; 173142; "SAN 835 H TC Combined Chronic Toxicity/Carcinogenicity Feeding Study in Rats" (Doubovetzky, M., Novartis Crop Protection AG, Department of Toxicology/B.881, CH-4132 Muttenz/Switzerland, Study No. 540R, 4/23/97). Fifty two Wistar rats (Hannover-derived, outbred, SPF) per sex per dose level were administered test article SAN 835 H TC (Lot No. 5904-4, 97.1-99.6% pure) in their feed at 0, 500, 1500, 5000 and 10000 ppm until final sacrifice at 104 weeks. Additional satellite groups of 20 males and 20 females were included at each dose level for interim sacrifice at 52 weeks. A "Sentinel" group consisting of 12 males and 12 females was fed a control diet and used for health surveillance. A final "Batch Health Check" group (10 males and 10 females) was sacrificed shortly after animal arrival to check the health status of the animals for this study. Mean test article intake over 104 weeks in mg/kg/day was: males 0/22.2/68.5/235.5/517.6, females 0/29.2/93.2/323.4/697.0 for 0/500/1500/5000/10000 ppm. Main group animal mortality for 104 weeks was not affected by the test article: male mortality was 22/18/17/12/16 and female mortality was 17/21/24/21/25. Both high dose males and females exhibited a higher incidence of hunched posture and emaciation compared to controls. Mean bodyweights of high dose animals were significantly ($p<0.01$) lower than controls at both 52 weeks (males 85%, females 89% of controls) and 104 weeks (males 77%, females 79% of controls). Such was also true for males fed 5000 ppm (94% and 85% of controls at weeks 52 and 104, $p<0.05$). Mean bodyweight changes (in grams) over the 104 week period were: males 474/451/425*/401/325**, females 254/269/228/218*/174** for 0/500/1500/5000/10000 ppm (*$p<0.05$, **$p<.01$). The test article did not inhibit food consumption significantly, suggesting that the decreased bodyweights were due to decreased utilization of ingested food. Clinical chemistry detected decreased serum triglycerides in males at the two highest dose levels on week 13 ($p<0.05$), week 25 ($p<0.05$), week 51 ($p<0.01$) and week 77 ($p<0.01$, highest dose only). Females were unaffected. Acidic urine, relative to controls, was measured in high dose males on weeks 13, 77 and 103 ($p<0.05$). Once again, females were unaffected. Red blood cell morphology was analyzed in weeks 13, 25, 51, 77 and 103. Treated females exhibited some increased anisocytosis relative to controls (statistical tests not applied); however, it is stated that these levels fell within the expected ranges. Males were unaffected. At necropsy on both week 52 and weeks 104-106, treated animals' organ weights that were significantly different from controls were generally decreased in absolute terms and increased when normalized to bodyweights, suggesting that reduced bodyweight in treated animals was the cause. At terminal sacrifice after 104 weeks, non-neoplastic microscopic alterations in treated animals included: males with focal myodegeneration of the heart at 0/0/4/6**/13**, females with increased hemosiderin in the spleen at 3/9/12**/14**/12**, and females with increased lymphoid cell infiltration of the Harderian gland at 3/3/8/11*/13** (all for 0/500/1500/5000/10000 ppm, with 52 animals examined per dose level, *$p>0.05$, **$p<0.01$, Fischer's Exact test applied by the reviewer). There were no test article effects on benign or malignant neoplasms, including the total percentage of animals with neoplasms (76.9-96.2%), the number of animals with more than one primary neoplasm (25-77%), and the number of animals with metastases (0-3.9%). **No adverse effects indicated. Chronic NOEL (M) = 500 ppm** (22.2 mg/kg/day based on decreased mean bodyweights and decreased bodyweight changes in animals fed 1500 ppm), **(F) = 500 ppm** (29.2 mg/kg/day based on increased incidence of

hemosiderin in the spleen and lymphoid cell infiltration of the Harderian gland in animals fed 1500 ppm). **Study acceptable** (Vidair 4/13/00).

CHRONIC TOXICITY, DOG

52843-018; 173134; "SAN 835 H Technical 52 Week Feeding Study in Dogs" (Carpy, S., Novartis Crop Protection Inc., Dept. of Toxicology/B.881/CH-4002 Basel/Switzerland, Study No. 555D, 1/31/97). Four Beagle dogs/sex/dose level were administered test article SAN 835 H Technical (Lot No. 5904-4, 98.1% pure) in their feed for 52 weeks at 0, 750, 7500 and 15000 ppm, yielding mean daily intakes of 0/26/299/529 (males) and 0/28/301/538 mg/kg/day (females). There were no mortalities, test article-induced clinical signs, or effects on food consumption. Mean bodyweights of males were also unaffected, while those of high dose females were less than control values beginning at about 6 weeks and continuing to study's end (92% of control at 52 weeks; difference not significant). The major hematological effect of the test article was to elevate the reticulocyte counts of 7500 and 15000 ppm males ($p<0.05$ at weeks 13 and 51), 7500 ppm females ($p<0.05$ at week 13), and 15000 ppm females ($p<0.01$ at week 51), all relative to controls. Correspondingly, the mean corpuscular volume (MCV) was increased in high dose males at week 13 ($p<0.05$), and the mean corpuscular haemoglobin concentration (MCHC) was decreased in 7500 and 15000 ppm males for weeks 13 and 51 ($p<0.05$) and in high dose females for week 26 ($p<0.01$). Blood chemistry and urinalysis did not identify any other convincing toxic effects of the test article. Gross pathology identified reddish discoloration of the diaphysis of the femur in 0/0/2/3 males and 0/1/0/1 females, corresponding to 0/750/7500/15000 ppm. The corresponding histopathology showed erythroid hyperplasia of the bone marrow of the femur and sternum for all animals in the two highest dosage groups, and no animals at 750 or 0 ppm. In addition, grading of hemosiderosis in the liver, kidneys and spleen detected a general test article-induced increase in severity for the 7500 and 15000 ppm groups, relative to controls. These data suggest that the test article induced erythroid hyperplasia in all animals of the two highest dosage groups, with some of these animals exhibiting increased hemosiderosis of the kidneys, spleen and/or liver. These changes suggest increased erythrocyte turnover at the 2 highest dose levels, consistent with the observed reticulocytosis. The authors suggest the above response is due to a low level toxic effect of the test article on erythrocytes, in the absence of any signs of anemia. **No adverse effects indicated. NOEL (M/F) = 750 ppm (26/28 mg/kg/day, based on reticulocytosis and erythroid hyperplasia in males and females fed 7500 ppm). **Study acceptable** (Vidair 4/11/00).

ONCOGENICITY, MOUSE

**52843-019; 173135; "SAN 835 H TC Carcinogenicity Feeding Study in Mice" (Carpy, S., Novartis Crop Protection Inc., Department of Toxicology/B.881, CH-4002 Basel/Switzerland, Study No. 549M, 3/14/97). Fifty CD-1 mice per sex per dose level were administered test article SAN 835 H TC (Lot No. 5904-4, 98.1% pure) in their feed at 0, 700, 3500 and 7000 ppm until final sacrifice at week 78. Additional satellite groups of 10 males and 10 females were included at each dose level until interim sacrifice at week 52. A sentinel group consisting of 10 males and 10 females was fed a control diet and used for health surveillance. A final "Batch Health Check" group (10 males, 10 females) was sacrificed shortly after animal arrival to check the health status of the animals for this study. Test article intake over 78 weeks in mg/kg/day was: males 0/100.2/516.9/1036.6, females 0/97.9/499.6/1003.9 for 0/700/3500/7000 ppm. Main group plus satellite group mortality was not affected by the test article: males 23/32/25/28 and females 26/24/27/30, all for 60 males and 60 females per dose level. In addition, the causes of death

(amyloidosis) were similar for the different dose levels. Mean bodyweights of high dose males were significantly lower ($p<0.05$) than controls over 12 of the first 21 weeks of test article administration. From week 22 onwards, there were no significant differences. Female mean bodyweights were unaffected. Mean bodyweight change over 78 weeks was not affected by test article administration to high dose males, whereas high dose females were significantly lower ($p<0.05$) than controls. Food consumption was significantly lower ($p<0.05$) in high dose males relative to controls for 18 weeks out of the first 51 weeks of the study. Females were unaffected throughout. There were no significant effects of test article administration on organ weights, differential white cell counts or red cell morphology, either at 51-52 or 78 weeks. Likewise, histopathology performed at these times did not detect any effects on non-neoplastic or neoplastic lesions, including the number of animals with benign neoplasms or the number of animals with malignant neoplasms. **No adverse effects indicated. Chronic NOEL (M/F) = 3500 ppm** (517/500 mg/kg/day, based on lower mean bodyweights and lower mean food consumption in males and a lower mean bodyweight change over 78 weeks in females fed 7000 ppm). **Study acceptable** (Vidair 4/17/00).

REPRODUCTION, RAT

52843-022; 173139; "SAN 835 H Technical Two Generation Reproduction Study in Rats" (Eschbach, B., Sandoz Agro Ltd., Department of Toxicology/B.881, CH-4002 Basel/Switzerland, Study No. 550R, 11/6/96). Twenty six Wistar rats per sex per dose level were administered SAN 835 H Technical (Lot No. 5904-4, 98.1% pure) in their feed at 0, 500, 2000 and 8000 ppm for 32 weeks. These animals, called the F0 generation, were mated twice during this time (first mating after 70 days of test article consumption), producing F1A and F1B litters. Twenty six weaned F1A animals per sex per dose level were selected and continued on feed containing the test article. After 84 days on the test diet they were mated and allowed to produce litters called F2A. Parents and nonselected pups were sacrificed on day 21 post-partum and subjected to necropsy. Control and high dose parents (F0 and F1) were processed for histopathology. Mean bodyweights of high dose parents were significantly lower than controls ($p<0.05$, $P<0.01$) throughout, including premating, gestation and lactation periods. In addition, F0 parental males fed 2000 ppm had a significantly lower bodyweight change ($p<0.05$) relative to controls over weeks 0-32. Food consumption was significantly increased ($p<0.05$, $p<0.01$) relative to controls in males during premating and in females during premating and gestation, at 2000 and 8000 ppm. Delivery and litter data from F1A dams (F2A pups) show increased incidence of the following: stillborn 5/3/5/17*, pre-perinatal loss 31/35/36/65, pup loss during lactation days 1-4 6/7/4/28**, and pup loss during lactation days 1-21 8/17/10/34** (all for 0/500/2000/5000 ppm, *$p<0.05$, **$p<0.01$). However, the total number of liveborn pups was not decreased significantly at 8000 ppm. Mean pup bodyweights at day 21 of lactation were significantly lower than controls for high dose F1A pups only (40.9/42.8/39.6/35.5** grams, **$p<0.01$). Gross necropsy of male parents revealed a consistent increase in mean seminal vesicle weight in both 8000 ppm F0 males (relative to bodyweight and relative to brain weight, $p<0.05$) and 2000 and 8000 ppm F1 males (absolute, relative to bodyweight, and relative to brain weight, $p<0.05$). There were no microscopic correlates in the seminal vesicles. There was also no test article induction of any non-neoplastic or neoplastic microscopic lesions in parental animals. Gross necropsy of high dose pups detected an increased incidence ($p<0.01$) of runts (undersized animals) in F1A and F1B litters, increased ($p<0.01$) autolysis in F2A litters, and an increased incidence ($p<0.01$) of "no milk in stomach" in F2A litters. A few pup organ weights in the high dose group were significantly lower than controls: the thymus in F1B males (both absolute and relative to bodyweight, $p<0.05$) and F2A

females (relative to bodyweight, $p<0.05$); the kidneys in F1B females (both absolute and relative to bodyweight, $p<0.05$) and F2A females (relative to bodyweight, $p<0.05$). **No adverse effects indicated. Parental NOEL (M): 500 ppm** (based on a lower mean bodyweight gain during weeks 0-32 in F0 males fed 2000 ppm); **(F): 2000 ppm** (based on lower mean bodyweights in females fed 8000 ppm). **Reproductive NOEL (F): 2000 ppm** (based on an increased incidence of stillborn pups and pre-perinatal loss in F1A dams fed 8000 ppm). **Developmental NOEL (M/F): 2000 ppm** (based on decreased pup survival [F2A], decreased mean pup bodyweights on day 21 [F1A], increased incidence of runts [F1A and F1B], increased autolysis [F2A] and increased incidence of "no milk in stomach" [F2A], decreased thymus weight [F1B males and F2A females] and decreased kidney weight [F1B females and F2A females], all in pups from dams fed 8000 ppm). **Study acceptable** (Vidair 4/18/00).

TERATOLOGY, RAT

52843-021; 173137; "Developmental Toxicity (Embryo-Fetal Toxicity and Teratogenic Potential) Study of SAN 835 H Administered Orally via Gavage to Crl:CD BR VAF/Plus Presumed Pregnant Rats" (Sharper, V., Argus Research Laboratories, Inc., Horsham, PA, Project ID No. 1819-001, Doc. No. 95/5226, 2/28/95). Twenty five mated female rats (Crl:CD BR VAF/Plus) were administered a suspension of SAN 835 H (Lot No. 5904-4, 98.1% pure) via oral intubation at 0 (0.5% methylcellulose), 100, 300 and 1000 mg/kg/day during days 6-15 of presumed gestation until sacrifice and Caesarean-section on day 20. There were no maternal deaths. The only maternal effect was that of reduced food consumption (both absolute and relative to bodyweight) during gestation in high dose animals ($p<0.01$). Necropsy of dams was also normal. Fetal examination detected the following in high dose animals: reduced male fetal bodyweight ($p<0.05$), increased fetal incidence of incompletely or unossified sternal centra ($p<0.01$), and reduced ossification sites per fetus per litter for caudal vertebrae ($p<0.01$) and forelimb metacarpals ($p<0.05$). These delays in ossification were called reversible by the study's author, and the mean numbers of ossification sites per fetus per litter at each above-mentioned location all fell within the ranges of historical controls. **No adverse effects indicated. Maternal NOEL = 300 mg/kg/day (based on decreased absolute and relative food consumption during gestation in dams fed 1000 mg/kg/day). **Developmental NOEL = 300 mg/kg/day** (based on reduced male fetal bodyweights in litters from dams fed 1000 mg/kg/day). **Study acceptable** (Vidair 4/27/00).

TERATOLOGY, RABBIT

52843-020; 173136; "Developmental Toxicity (Embryo-Fetal Toxicity and Teratogenic Potential) Study of SAN 835 H Administered Orally via Stomach Tube to New Zealand White Rabbits" (Sharper, V., Argus Research Laboratories, Inc., Horsham, PA, Project ID No. 1819-008, 2/23/95). Twenty pregnant New Zealand White rabbits (strain Hra:[NZW]SPF) were administered a suspension of SAN 835 H (Lot No. 5904-4, 98.1% pure) via oral intubation at 0 (0.5% methylcellulose), 30, 100 and 300 mg/kg/day during days 6-19 of gestation, until sacrifice and Caesarean-section on day 29. Mortality was 1/2/1/4 for 0/30/100/300 mg/kg/day, with 0/0/0/3 deaths considered to have been caused by the test article. All 3 high dose deaths followed weight loss or failure to gain weight, and 2/3 animals had a trichobezoar in their stomach. The incidences of abortion were 0/0/1/5 (** $p<0.01$), with all high dose animals losing or failing to gain weight and 4/5 animals with a trichobezoar in their stomachs. Clinical signs included soft or liquid feces (number of rabbits exhibiting this sign: 0/1/4*/6**, * $p<0.05$, ** $p<0.01$), no feces (2/0/2/9**), and

mucoid feces (0/0/0/3**). Mean maternal bodyweights were not significantly lower in treated animals relative to controls. However, high dose animals exhibited a lower mean bodyweight change ($p<0.05$) over days 12-15 and a higher mean change over days 24-29 ($p<0.01$), all relative to controls (the increase considered to have been a rebound effect, common to this type of study). Mean maternal food consumption (in g/kg/day) was lower ($p<0.05$) than controls in high dose animals for days 7-8. Examination of ovaries and uteri from does sacrificed on day 29 detected no effects of the test article on corpora lutea, implantations, litter sizes, resorptions or % of does with live fetuses. There were also no effects on the mean numbers of live fetuses, sex ratios or fetal bodyweights. The incidences of fetuses with malformations plus variations (external, soft tissue and skeletal) were not affected by test article administration (34.6%/26.1%/27.6%/23.8%). The incidences of supernumerary thoracic ribs (12.47/12.36/12.61/12.74**), thoracic vertebrae (12.54/12.42/12.67/12.79*) and lumbar vertebrae (6.45/6.56/6.32/6.20*, * $p<0.05$, ** $p<0.01$) were altered in high dose fetuses. However, the small magnitude of these changes, and the prior findings that these variations commonly occur at maternally toxic doses, cast doubt on their biological significance as developmental defects. **No adverse effects indicated. Maternal NOEL = 30 mg/kg/day** (based on abortions in does administered 100 and 300 mg/kg/day). **Developmental NOEL = 300 mg/kg/day** (based on similar incidences of malformations and variations in fetuses from control does and does administered 300 mg/kg/day). **Study acceptable** (Vidair 4/20/00).

GENE MUTATION

52843-025; 173046; "*Salmonella*/Mammalian-Microsome Plate Incorporation Mutagenicity Assay (Ames Test), Test Article 320-220, Batch No. 1054-01" (Lawlor, T. and Wagner, V., Microbiological Associates, Inc., Rockville, MD, Study No. T6010.501, Doc. No. 88/5456, 3/3/88). Test article 320-220 (Batch No. 1054-01, purity not indicated) was evaluated for its ability to induce reversion to histidine prototrophy in *Salmonella typhimurium* tester strains TA98, TA1537 and TA1538, reverted by frameshift mutations, and tester strains TA100 and TA1535, reverted by base substitutions. Concentrations of test article, from 0 (DMSO only) to 10000 ug/plate, were evaluated in triplicate plates, both in the presence and absence of an S9 microsomal activating fraction. Exposure to the test article was for 48 hrs at 37°C. The test article did not cause any bacterial toxicity. The only concentration of test article that produced a 2-fold increase in mean revertants per plate relative to the vehicle control was 10000 ug/plate in TA1537 in the absence of S9 (mean of 4 revertants in control [4,3,6], 8 in 10000 ug/plate [10,6,9]). However, since there was no dose-response, the test was considered negative. In contrast, positive controls were functional. Therefore, the test article was judged nonmutagenic in the assay. **No adverse effects indicated. Study unacceptable but possibly upgradeable with submission of test article purity** (Vidair 5/3/00).

**52843-024; 173050; "Mutagenicity Test on SAN 835H in the *Salmonella*/Mammalian-Microsome Reverse Mutation Assay" (Lawlor, M. and Valentine, D., Hazleton Laboratories America, Inc., Kensington, MD, Project ID No. 11030-0-401, Doc. No. 89/5249, 11/17/89). Test article SAN 835H (Lot No. 1086-23, 97.1% pure) was evaluated for its ability to induce reversion to histidine-prototrophy in *Salmonella typhimurium* tester strains TA98, TA1537 and TA1538, reverted by frameshift mutations, and tester strains TA100 and TA 1535, reverted by base substitutions. Concentrations of test article, from 0 (DMSO only) to 10,000 ug/plate, were evaluated in triplicate plates, both in the presence and absence of an S9 microsomal activating fraction. Exposure to the test article was for 48 hrs at 37°C. In the absence of S9, the two highest

concentrations of test article caused small reductions in the background bacterial lawn and the number of revertant colonies, indicating some toxicity. There were no significant increases in the mean numbers of revertant colonies per plate relative to negative controls, either in the presence or absence of S9. In contrast, positive controls were functional. Therefore, the test article was judged nonmutagenic in this assay. **No adverse effects indicated. Study acceptable** (Vidair 4/21/00).

52843-024; 173052; "Mutagenicity Test on SAN 835H in the L5178Y TK+/- Mouse Lymphoma Forward Mutation Assay with Independent Repeat" (Young, R., Hazleton Laboratories America, Inc., Kensington, MD, Project ID No. 11030-0-431, Doc. No. 96/5368, 2/22/90). Test article SAN 835H (Lot No. 1086-23, 97.1% pure) was evaluated for its ability to induce a mutation in the mouse L5178Y cell line, changing the genotype from TK+/- to TK-/-, thereby conferring resistance to the toxic thymidine analogue, 5-triflurothymidine (TFT). Suspension cultures were exposed to concentrations of test article ranging from 0 (DMSO) to 3 mg/ml for 4 hrs at 37^{o}C, in the presence or absence of an S9 microsomal fraction, followed by washing, 2 days of subculture for expression of mutations, and final plating into triplicate plates containing TFT for growth of TK -/- mutant colonies over 10-14 days. Two independent trials were performed without S9 and two trials with S9. Significant toxicity, measured as an inhibition of growth relative to the control, was often observed at the higher concentrations (a precipitate formed above 0.5 mg/ml). Increases above the background mutation frequency were measured at the higher test article concentrations. However, applying the consensus guidelines from the 1994 mouse lymphoma workshop (Environmental and Molecular Mutagenesis, 25, pp. 165-168, 1995), no concentration tested gave a positive response, since either relative growth was less than 10% of the negative control, or the increase in mutant colonies per 10^6 cells was less than 100 above that of the negative control. In contrast, positive controls were functional. A single concentration (1.8 mg/ml) tested in the absence of S9 gave an equivocal response in one trial. **No adverse effects indicated. Study acceptable (Vidair 4/21/00).

CHROMOSOME EFFECTS

52843-024; 173054; "Mutagenicity Test on SAN 835H In Vivo Mouse Micronucleus Assay" (Ivett, J., Hazleton Laboratories America, Inc., Kensington, MD, Project ID No. 11030-0-455, Doc. No. 90/5168, 1/18/90). Test article SAN 835H (Lot No. 1086-23, 97.1% pure) was evaluated for its ability to induce micronuclei in mice (ICR) following administration by single-dose oral gavage at 0 (corn oil), 500, 1667 and 5000 mg/kg. Five males and five females receiving test article were sacrificed at 24, 48 and 72 hours after dosing, their bone marrow isolated, and slides prepared for scoring of polychromatic erythrocytes (PCE), normochromatic erythrocytes (NCE), and micronucleated PCEs. At least 1000 PCEs were scored per animal. No clinical signs were observed in treated animals. There were no effects of the test article on either the frequency of micronucleated PCEs (0.10 and 0.14% for negative control males and females, respectively) or the PCE/NCE ratio. In contrast, animals treated with cyclophosphamide exhibited a significant ($p<0.05$) increase in the frequency of micronucleated PCEs. It was concluded that up to the limit dose of 5000 mg/kg, the test article was not clastogenic in the assay. **No adverse effects indicated. Study acceptable (Vidair 4/21/00).

DNA DAMAGE

**52843-024; 173056; "Mutagenicity Test on SAN 835H Technical in the IN VITRO Rat Primary Hepatocyte Unscheduled DNA Synthesis Assay" (McKeon, M., Hazleton Laboratories America, Inc., Kensington, MD, Project ID No. 11030-0-447, Doc. No. 90/5180, 9/7/90). Primary

hepatocytes, freshly isolated from rat liver, were incubated for 19.1 hrs at 37°C with test article SAN 835H technical (Lot No. 1086-23, 97.1% pure) at concentrations ranging from 0 (DMSO) to 250 ug/ml, with 10 uCi/ml of ^{3}H-thymidine. Three replicate cultures per concentration were processed for autoradiography and two for cytotoxicity. A single trial was run, with at least 150 cells per concentration scored for mean net nuclear grain counts and the percentages of cells with $\geq$ 5 net nuclear grains. The highest dose tested produced approximately 50% cellular survival (trypan blue dye exclusion) relative to the negative control. No concentration of test article caused an increase in net nuclear grain counts of $\geq$ 5 above that of the negative control. Although the percentages of cells with mean net nuclear grain counts of $\geq$ 5 were higher in all treated samples compared to the negative control (0%), the percentages were less than the average percentage for 24 historical control trials (6.9%) and no dose-response was indicated. Thus, the test article was judged negative in this assay of DNA damage and repair. **No adverse effects indicated. Study acceptable** (Vidair 4/25/00).

NEUROTOXICITY

012, 059, 060, 061, 062, 063; 173114, 175276, 175277, 175279, 175280, 175281; "SAN 835 H Neurotoxicity to Rats by Acute Oral Administration" (Hughes, E.W., Huntingdon life Sciences Ltd., Huntingdon, Cambridgeshire, England, Project Identity SNC/186, BASF Reg Doc No. 96/5313, 5/30/96). 818. SAN 835 H (Batch No. 6500-19, purity=96.4%), suspended in 1% methylcellulose, was administered by gavage in a single dose to 10 Crl: CD BR rats per sex per dose at dose levels of 0, 125, 500, and 2000 mg/kg. No mortalities occurred. Treatment-related slight brown nasal staining was observed in both males and females (during FOB) at 2000 mg/kg on Day 0 (about 3 hours post-dosing) with this sign clearing in all animals by Day 7. Motor activity assessments revealed no treatment-related effects. Microscopic examination of the nervous system revealed no treatment-related abnormalities. **No adverse effects. NOEL (M/F)=500 mg/kg (based on the slight brown nasal staining on Day 0). **Acceptable**. (Corlett and Leung, 6/15/00)

017, 059, 060, 061, 062, 063; 173132, 175276, 175277, 175279, 175280, 175281; "SAN 835 H Neurotoxicity to Rats by Dietary Administration for 13 Weeks" (Hughes, E.W., Huntingdon Life Sciences Ltd., Huntingdon, Cambridgeshire, England, Project Identity SNC/187, BASF Reg. Doc. No. 97/5095, 11/5/96). 827. SAN 835 H (Batch No. 6500-19, purity=96.4%) was admixed to the diet at dose levels of 0 (untreated diet), 25, 75, or 1000 mg/kg/day and fed to 10 Crl: CD BR rats per sex per dose for 13 weeks. No mortalities occurred. A treatment-related decrease in mean body weight was observed in both sexes at 1000 mg/kg/day. Treatment-related decreases in mean activity and mean rearing in females during arena observations of FOB at 1000 mg/kg/day during the 4th week of exposure but not during the 8th and 13th weeks. Motor activity assessments revealed no treatment-related effects. Microscopic examination of the nervous system revealed no treatment-related abnormalities. **No adverse effects. NOEL (M/F)=75 mg/kg/day (based on decrease in body weight in both sexes and decreases in mean activity and rearing in females during FOB). **Acceptable**. (Corlett and Leung, 6/15/00)

SUBCHRONIC STUDIES

(90-day feeding study)

014; 173126; "SAN 835 H: 13-Week Feeding Study in Dogs" (Carpy, S.A., Sandoz Agro Ltd., Department of Toxicology, Muttenz, Switzerland, Study No. 554D, BASF Reg. Doc. No. 96/5352, 10/8/96). 821. SAN 835 H Technical (Lot No. 5904-4, purity=98.1%) was admixed to the diet at dose levels of 0 (untreated diet), 1500, 10000, or 30000 ppm (0, 58, 403, or 1131 mg/kg/day, respectively, for males and 0, 59, 424, or 1172 mg/kg/day, for females) and fed to 4 beagle dogs per sex per dose for 13 weeks. No mortalities occurred. Treatment-related decreases in mean red blood cell and hemoglobin levels in both sexes at 30000 ppm and treatment-related increases white blood cell and reticulocyte levels in females at 30000 ppm were observed. Treatment-related increases in mean relative spleen, kidney, and liver weights were observed in females at 30000 ppm. Macroscopic examination revealed a treatment-related absence of bone marrow in males at 10000 and 30000 ppm and in females at 30000 ppm. Microscopic examination revealed treatment-related erythroid hyperplasia in the femoral bone marrow, extramedullary hematopoiesis in the liver, and pigment deposits in the liver at 10000 and 30000 ppm in both sexes. **No adverse effects.** NOEL (M)=58 mg/kg/day (1500 ppm) and NOEL /F)=59 mg/kg/day (1500 ppm) (based on microscopic findings). **Acceptable**. (Corlett, 6/2/00)

015; 173127; "SAN 835 H: A 13-Week Dietary Toxicity Study in Rats with a 4-Week Recovery Period" (Simon, F.P.W. et al., Sandoz Agro Ltd., Department of Toxicology, Muttenz, Switzerland, Study No. 448-R, BASF Reg. Doc. No. 92/5244, 10/7/92). 821. SAN 835 H (Lot No. 5131-65C, purity=96%) was admixed to the diet at dose levels of 0 (untreated diet), 1000, 5000, 10000, or 20000 ppm (0, 65, 350, 720, or 1500 mg/kg/day, respectively, for males and 0, 70, 430, 890, or 1750 mg/kg/day, for females) and fed to 10 Wistar rats per sex per dose for 13 weeks (an additional 10 rats per sex per dose at the control and high dose levels were included to test recovery for 4 weeks following dosing). No mortalities occurred. A treatment-related decrease in mean body weight was observed in males at 10000 ppm and in both sexes at 20000 ppm persisting in recovery group males. A treatment-related increase in mean serum alanine aminotransferase level was observed in both sexes at 10000 and 20000 ppm but not in recovery group animals. Microscopic examination revealed treatment-related foam cells in the lungs in both sexes at 10000 and 20000 ppm and testicular atrophy at 20000 ppm with both conditions persisting in recovery group animals. **Possible adverse effect:** testicular atrophy at 20000 ppm (1500 mg/kg/day). NOEL (M)=350 mg/kg/day (5000 ppm) and (F)=430 mg/kg/day (5000 ppm) based on decreased body weight (in males), increased serum alanine aminotransferase levels (in both sexes), and foamy macrophages in the lungs (in both sexes). **Acceptable**. (Corlett and Leung, 5/11/00)

(21-day dermal study)

016; 173130; "SAN 835 H Technical Twenty-One Day Dermal Toxicity Study in the Rabbit" (Allan, S.A., Huntingdon Life Sciences Ltd., Huntingdon, Cambridgeshire, England, HRC Project Identity SNC 199, BASF Reg. Doc. # 97/5094, 3/22/96). 822. SAN 835 H Technical (Batch No. 6500-19, purity=96.4%), moistened with distilled water, was placed on the clipped dorsal surface of 5 New Zealand White rabbits per sex per dose at dose levels of 0 (2 ml/kg distilled water), 100, 300, or 1000 mg/kg/day for 6 hours per day for 22 to 24 consecutive days covered with an impervious bandage. No mortalities occurred. No treatment-related skin irritation or clinical signs were observed. No treatment-related effects on body weight or serum chemistry were observed. Microscopic examination revealed no treatment-related abnormalities. **No adverse effects**. NOEL (M/F, systemic and skin)=1000 mg/kg/day both based on no treatment-related effects at HDT. **Acceptable**. (Corlett, 5/30/00)

METABOLISM STUDIES

52843-025; 173058; "Metabolism of SAN 835 H in Rats" (Yu, C., Thome, L. and Sen, P., Metabolism/Pharmokinetics Section, Sandoz Agro Inc., Des Plaines, Illinois, Project ID No. 414205, Doc. No. 97/5201, 4/17/97). Wistar rats were administered ^{14}C-labeled SAN 835 H (phenyl-labeled: Lot Nos. CFQ6737 and CFQ7939, radiochemical purity > 98%; pyridinyl-labeled: Lot No. CFQ7629, radiochemical purity > 98%; unlabeled test article purity = 99.4%) according to each of four protocols: 1) a single i.v. dose at 1 mg/kg, 2) a single oral dose at 10 mg/kg, 3) a single oral dose at 1000 mg/kg, 4) fourteen daily oral doses of unlabeled test article at 10 mg/kg followed by a single oral dose of labeled compound at 10 mg/kg. For protocols 1, 2 and 3, five males and five females were monitored according to each of 3 schedules: a) until sacrifice at 72 hrs post-dosing, b) until sacrifice at 24 hrs post-dosing, c) bile-duct cannulated animals followed until sacrifice at 48 hrs post-dosing. For the repeated dosing protocol, five males and five females were sacrificed at 24 hrs and 72 hrs after dosing with the labeled test article. Intravenous administration led to rapid elimination in the urine of between approximately 58 and 68% of both labeled forms by 7 hrs, and between 70 and 90% by 72 hrs. Of the approximate 7-20% excreted in the feces, almost all was a result of bilary excretion. Oral dosing at both 10 and 1000 mg/kg resulted in elimination of both labels mainly through the feces (approximately 55-80% by 72 hrs). Excretion in the urine accounted for approximately 20-39%, with approximately 3-10% excreted in the bile. Tissue retention at 72 hrs was low after either intravenous or oral dosing, being 0.37% or less for the phenyl-labeled compound (highest in blood) and 0.04% or less for the pyridinyl compound (highest in kidney and liver). The percent absorption for both labeled compounds, calculated by dividing the amount of labeled compound excreted in the urine after oral dosing by the amount excreted in the urine after i.v. dosing, was between 30 and 50%, with no difference between the 10 and 1000 mg/kg dose levels. Elimination rates followed a one compartmental model, yielding an average half-time of 6.3 hrs for all groups combined. Elimination rates were not different between dose levels, or between the two different labels. Metabolite analysis of the urine, bile and feces was conducted by TLC, HPLC, mass spectrometry and NMR spectroscopy. Unaltered test article was the major labeled compound in all excreta. Major metabolic reactions included cyclization, hydrolysis and hydroxylation, yielding 7 major metabolites (M1, M2, M5, M6, M9, M10 and M19). M19, M10 and M9 were also identified in excreta from goats and hens, and in corn silage. There were no significant differences in test article absorption, elimination, tissue distribution or metabolism by males versus females. Likewise, high and low dose levels gave similar results, suggesting no saturation for absorption or elimination. There was less tissue retention of the pyridinyl-labeled compound and greater retention of the phenyl-labeled compound in the blood. Lastly, single versus multiple dosing showed that the test article did not bioaccumulate or induce metabolizing enzymes. **Study acceptable** (Vidair 5/4/00).