**CALIFORNIA ENVIRONMENTAL PROTECTION AGENCY**
**DEPARTMENT OF PESTICIDE REGULATION**
**MEDICAL TOXICOLOGY BRANCH**

# SUMMARY OF TOXICOLOGY DATA

Flumioxazin

**Chemical Code #** 5802, **Tolerance #** 52894
**SB 950 #** New A.I.

Original Date: 10/9/02
Revised: 1/31/03

## I. DATA GAP STATUS

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|---|---|
| **Chronic toxicity, rat:** | No data gap, possible adverse effect |
| **Chronic toxicity, dog:** | No data gap, no adverse effect |
| **Oncogenicity, rat:** | No data gap, no adverse effect |
| **Oncogenicity, mouse:** | No data gap, no adverse effect |
| **Reproduction, rat:** | No data gap, possible adverse effect |
| **Teratology, rat:** | No data gap, possible 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:** | Study not submitted or required. |

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Toxicology one-liners are attached.

All record numbers through 202106 were examined.
** indicates an acceptable study.
Bold face indicates a possible adverse effect.
## indicates a study on file but not yet reviewed.
File name: T191861A
Revised by T. Moore, 1/31/03

## II. TOXICOLOGY ONE-LINERS AND CONCLUSIONS

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

### COMBINED, RAT

*** 075; 184706**; "Combined Chronic Toxicity and Oncogenicity Study of S-53482 by Dietary Administration in Rats"; (Takaki Seki; Environmental Health Science Laboratory, Sumitomo Chemical Co., Ltd., Osaka, Japan; Project ID 1835; 3/15/93); Fifty Crj:CD(SD) rats/sex/group were treated in the diet with 0, 50, 500 or 1000 ppm of S-53482 (lot no. PYG-89021-M, purity: 94.8%) for up to 2 years in the main study ((M) 0, 1.8, 18.0, 36.5 mg/kg/day, (F) 0, 2.2, 21.8, 43.6 mg/kg/day). An additional 24 animals/sex/group were dosed in the same manner for up to 79 weeks as satellite animals. These animals were used for interim hematology, blood biochemstry, urinalysis, and pathology studies. There was no treatment-related effect on the survival of the animals. The mean body weights and food consumption were not affected by the treatment over the course of the study. No treatment-related effects were noted in the urinalysis, ophthalmological or clinical chemistry examinations. In the hematology, hemoglobin content, MCV and MCH were reduced for the males in the 1000 ppm group at 14 weeks ($p<0.05$ or 0.01). Thereafter, the MCH was reduced at 27 and 53 weeks ($p<0.01$). For the females, the mean rbc count was increased for the 1000 ppm group at 14, 27 and 53 weeks ($p<0.01$). The hemoglobin content, hematocrit, MCV, MCH and MCHC were reduced for the 500 and 1000 ppm groups at 14 weeks ($p<0.05$ or 0.01). The hemoglobin content was reduced for the 1000 ppm group throughout the study ($p<0.01$ at 27, 53 and 79 weeks). The hematocrit was lower for this group ($p<0.01$ at 27 and 79 weeks). The lower MCV and MCH values for the 1000 ppm group persisted throughout the study ($p<0.05$ or 0.01). At various times the values for the 500 ppm group were also lower ($p<0.05$ or 0.01). The MCHC values were also less for the 1000 ppm group at 27, 53 and 79 weeks ($p<0.05$ or 0.01). The percentage of reticulocytes was increased for the 500 and 1000 ppm females at 14 weeks ($p<0.05$ or 0.01). The myeloid/erythroid ratio in the bone marrow was reduced at 53 weeks for the 1000 ppm females ($p<0.05$). In the histological examination, increased splenic extramedullary hematopoiesis was noted for the 500 and 1000 ppm males at 105 weeks ($p<0.05$). For the 1000 ppm females, an increased incidence and severity of bile duct hyperplasia and sclerosis were evident at 53 weeks ($p<0.05$) but not at 105 weeks. **Adverse Effect:** hypochromic, microcytic anemia; **Chronic NOEL:** 50 ppm ((M) 1.8 mg/kg/day, (F) 2.2 mg/kg/day) (based upon greater incidence of increased extramedullary hematopoiesis in the spleen of the 500 ppm males and hypochromic microcytic anemia in the 500 ppm females); No treatment-related **oncogenicity** observed. **Study acceptable.** (Moore, 8/7/02)

### CHRONIC TOXICITY, RAT

See Combined, Rat

### CHRONIC TOXICITY, DOG

** 049; 184617; "One-year Oral Toxicity Study of S-53482 in Dogs"; (Minoru Nakano; Environmental Health Science Laboratory, Sumitomo Chemical Co., Ltd., Osaka, Japan; Project ID 2097; 6/25/92 (amended, 1/11/93)); Four beagle dogs/sex/group were dosed orally by capsule with 0, 10, 100 or 1000 mg/kg/day of S-53482 (lot no. PYG-89021-M, purity: 94.8%) for 52 weeks. No animals died during the study. No treatment-related clinical signs were evident. No treatment-related effects were noted on body weight gain, food consumption, ophthalmology, or hematology. In the clinical chemistry, the $\alpha_2$-globulin percentage was increased for the 1000 mg/kg males at 26, 39 and 52 weeks of treatment ($p<0.01$). The total cholesterol and phospholipid concentrations and alkaline phosphatase activity were increased for the 1000 mg/kg males at 13, 26, 39 and 52 weeks ($p<0.01$). For the 1000 mg/kg females, the total cholesterol ($p<0.01$) and phospholipid ($p<0.05$) concentrations was increased at 13 weeks. The alkaline phosphatase activity for the 1000 mg/kg females was increased at 13 ($p<0.05$), 26 ($p<0.01$), 39 ($p<0.05$) and 52 weeks ($p<0.05$). The mean relative liver weight for the 1000 mg/kg males was greater than that of the controls ($p<0.01$). The mean absolute prostate weight was lower for the 1000 mg/kg males ($p<0.05$). No treatment-related lesions were noted on the histopathological examination. **No adverse effect indicated. (NOEL): (M/F)** 100 mg/kg/day (based upon the

effects noted in the clinical chemistry results for the 1000 mg/kg/day treatment group); **Study acceptable.** (Moore, 5/23/02)

## ONCOGENICITY, RAT

See Combined, Rat

## ONCOGENICITY, MOUSE

** 050; 184618; "Oncogenicity Study of S-53482 by Dietary Administration in Mice"; (T. Seki; Environmental Health Science Laboratory, Sumitomo Chemical Co., Ltd., Osaka, Japan; Project ID 1928; 9/24/93); Fifty one Crj:CD-1 (ICR) mice/sex/group were treated in the diet with 0, 300, 3000 or 7000 ppm of S-53482 (lot no. PYG-89021-M, purity: 94.8%) for 78 weeks ((M): 0, 31.1, 314.9, 754.1 mg/kg/day, (F) 0, 36.6, 346.4, 859.1 mg/kg/day). An additional 15 animals/sex/group were treated for 52 weeks (satellite). There was no treatment-related effect upon survivability, mean body weight or food consumption. The mean red blood cell count for the males in the 7000 ppm group satellite group was lower than that of the control ($p<0.01$). No effects on hematology were noted for the animals in the main study. Mean absolute and relative liver weights were increased for the 300 and 7000 ppm males at 52 weeks (satellite group) ($p<0.05$ or $p<0.01$). No effect upon the liver weight was noted for the animals in the main study. In the histopathology, an increase in the incidence of malignant lymphoma/leukemia was noted for the 3000 ppm males which died prior to the conclusion of the study (0:1/14 vs. 3000: 6/15) ($p<0.05$). However, there was no dose-response evident for this lesion. The incidence of pulmonary adenomas and adenocarcinomas was increased for the 3000 ppm females which survived to the termination of the study (0: 0/33 vs. 3000: 5/36) ($p<0.05$). However, there was no dose-response for the incidence of this lesion as well. For non-neoplastic lesions, the incidence of centrilobular hepatocellular hypertrophy for the 3000 ppm males which survived to the end of the study was increased over that of the controls (0: 6/37 vs. 3000: 14/36) ($p<0.05$). An increase in the incidences of diffuse hepatocellular hypertrophy (0: 1/33 vs. 3000: 7/36) ($p<0.05$) and single cell necrosis in the liver (0: 0/33 vs. 3000: 7/36) ($p<0.05$) were noted for the 3000 ppm females which survived to the conclusion of the study. However, there was no dose-response evident for the effects in the livers of either the males or females. An increased incidence of calcification in the brain was noted for the 3000 and 7000 ppm males which survived to the conclusion of the study (0: 3/37 vs. 3000: 11/36 ($p<0.05$), 7000: 13/38 ($p<0.01$). **No adverse effect indicated. Chronic (Non-Neoplastic) NOEL (M/F):** 300 ppm (( M): 31.1 mg/kg/day, (F): 36.6 mg/kg/day) (based upon the increased incidence of hepatocellular lesions in the 3000 ppm treatment group); **Oncogenicity not evident. Study acceptable.** (Moore, 5/31/02)

## REPRODUCTION, RAT

**** 074; 184705**; "Reproductive Effects of S-53482 Administered Orally in Feed to Crl:CD®BR VAF/Plus® Rats"; (A.M. Hoberman; Argus Research Laboratories, Inc., Horsham, PA; Project ID 1119-015; 9/21/92); Thirty rats/sex/group were dosed in the diet with 0, 50, 100, 200 or 300 ppm of S-53482 (lot no. PYG-89021-M; purity: 94.8%) for two generations. The treatment period for the P1 parents included 83 days prior to mating, the mating period, 3 weeks of gestation and 3 weeks of lactation. At that time, 30 F1 animals/sex/group were selected as parents and treated for a minimum of 86 days in the premating period, the mating period, and 3 weeks both for the gestation and lactation periods. For the P1 generation, no animals died as a result of the treatment. Only at the end of the gestation period was the mean maternal body weight of the 300 ppm treatment group less than that of the control ($p<0.01$). Mean food intake for the 300 ppm females was less than that of the controls during the lactation period ($p<0.01$). For the F1 generation, one male and 4 females in the 300 ppm treatment group died as a consequence of the treatment. Clinical signs manifested by these animals included pale appearance, decreased motor activity, ataxia, hypothermia and reduced body weight gain and food consumption. The mean body weight for the 300 ppm males at the end of the premating period was less than that of the control ($p<0.05$). The mean body weight of the 300 ppm females was less than that of the controls at the end of lactation ($p<0.01$). The mean food intake of these females was less over the lactation period ($p<0.01$). There was no treatment-related effect upon organ weights. The histopathological examination did not reveal any treatment-related lesions in the reproductive organs. For the reproductive parameters, the gestation index for the P1 dams in the 300 ppm group was less than that of the control ($p<0.01$). The mean litter size for the 300 ppm group of

both generations was less than that of the control ($p<0.01$). The pup viability index was reduced for the 100 ($p<0.05$), 200 ($p<0.01$) and 300 ppm ($p<0.01$) groups of the F1 generation and for the 300 ppm ($p<0.01$) group of the F2 generation. The mean pup weights for the 300 ppm group in both generations were less than those of the control for the first 7 days of lactation in the F1 generation and the first 14 days of the F2 generation ($p<0.01$ or $p<0.05$). The mean pup weight of the neonates in the 300 ppm group of the F1 generation was also less ($p<0.01$). **Possible adverse reproductive effects:** lower pup viability and mean pup weight**. Parental NOEL:** 200 ppm (based upon the lower mean body weight for males in the 300 F1 treatment group, mortality for the 300 ppm F1 females) ((M) 10.2 to 32.5 mg/kg/day, (F) 12.1 to 32.3 mg/kg/day), **Reproductive NOEL:** 200 ppm (based upon reduced gestation index for the dams in the 300 ppm treatment groups) ( (F) 12.1 to 32.3 mg/kg/day), **Developmental NOEL:** 50 ppm (based upon lower pup viability index for the 100 ppm F1 pups) (F): 3.0 to 8.3 mg/kg/day); **Study acceptable.** (Moore, 6/24/02)

073; 184704; "Dosage-Range Finding of S-53482 Administered Orally in the Diet to Crl:CD®BR VAF/Plus® Rats (Pilot Study)"; (A.M. Hoberman; Argus Research Laboratories, Inc., Horsham, PA; Project ID 1119-018P; 2/4/91); Eight Crl:CD®BR VAF/Plus rats/sex/group were treated in the diet with 0, 100, 200, 300, 400 or 500 ppm of S-53482 (lot no. PYG-89021-M, purity: 94.8%) for 4 weeks of premating, up to 7 days of mating, and 3 weeks each of gestation and lactation ((M), premating: 0, 6.5, 13.2, 20.0, 26.6, 33.0 mg/kg/day, (F) premating: 0, 6.9, 13.6, 19.7, 27.1, 32.3 mg/kg/day, gestation: 0, 7.4, 14.0, 20.7, 28.6, 35.0 mg/kg/day, lactation: 0, 12.3, 25.0, 30.8, NA, NA due to no live offspring). No animals died during the treatment period. The mean body weights were not affected by the treatment during the premating period. The mean body weights for the dams in the 300 ppm and above treatment groups during the gestation period were lower than that of the controls ($p<0.05$ or $p<0.01$) due to the fewer number of viable offspring in those treatment groups. Mean food consumption was not affected during the premating, gestation or lactation periods. The fertility index was reduced for the 500 ppm treatment group. The mean litter size of the 300 ppm treatment group was less than that of the control group. No offspring survived from the 400 and 500 ppm treatment groups. **Parental NOEL:** 500 ppm (based upon the lack of treatment-related effects upon the highest dose tested) ((M): 33.0 mg/kg/day, (F): 32.3 to 35.0 mg/kg/kg); **Reproductive NOEL:** 200 ppm (based upon reduced pup viability for the 300 ppm treatment group); **Developemental NOEL:** 300 ppm (based upon the lack of surviving pups in the 400 ppm treatment group); **Study supplemental** (non-guideline study). (Moore, 6/25/02)

## TERATOLOGY, RAT

**** 069, 071, 072; 184637, 184702, 184703**; "Teratology Study of S-53482 Administered Orally to Rats (Amendment Included)"; (S. Kawamura; Sumitomo Chemical Co., Ltd., Biochemistry and Toxicology Laboratory, Osaka, Japan; Project ID 1759; 8/28/90, (addendum) 12/26/95); Twenty two mated Slc:SD® female rats were treated by oral gavage with 0, 1, 3, 10, or 30 mg/kg/day of S-53482 (lot no. PYG-89021-M, purity: 94.8%) from day 6 through day 15 of gestation. No deaths resulted from the treatment. No effect on body weight gain or food consumption was noted during the treatment period. No treatment-related clinical signs were evident. Developmental effects on the fetuses were reduced body weight (30 mg/kg, $p<0.01$) and a reduced number of live fetuses/litter (30 mg/kg, $p<0.05$) with a concomitant increase in embryonic death. There was an increased incidence/litter of cardiac (0:2/22 vs. 10:6/22, 30:12/18 ($p<0.01$)) and skeletal (0:0/22 vs. 30:4/18) malformations. The predominant cardiac and skeletal malformations were ventricular septal defect and double aortic arch and curvature of the scapula and ulna. There was also an increased incidence/litter of the minor skeletal anomaly, wavy ribs, in the 30 mg/kg group (0: 1/22 vs. 30: 12/18, $p<0.01$). **Adverse effect indicated:** malformations in cardiac and skeletal development; **Maternal NOEL:** 30 mg/kg/day (based upon a lack of treatment-related effects at the highest dose tested); **Developmental NOEL:** 3 mg/kg/day (based upon the increased incidence of cardiac malformations in the fetuses of the 10 mg/kg treatment group); **Study acceptable.** (Moore, 6/5/02)

**** 070, 072; 184638, 184703**; "Teratology Study of S-53482 Administered Dermally to Rats"; (S. Kawamura; Environmental Health Science Laboratory, Sumitomo Chemical Co., Ltd., Osaka, Japan; Project ID 2018; 3/14/91); The skin of twenty four mated Slc:SD® female rats was treated with 0, 30 or 100 mg/kg/day of S-53482 (lot no. PYG-89021-M, purity: 94.8%) for 6 hours/day from day 6 through day 15 of gestation. An additional group of 25 females were treated in the same manner with 300 mg/kg/day of the test material. No deaths resulted from the treatment. One, 2, 2, and 2 dams in groups 0, 30, 100, and 300 mg/kg, respectively, were removed from the study due to wounds at the site of application. Another female in the 300 mg/kg group was removed prior to the beginning of treatment. The mean body weight gain for the 300 mg/kg treatment group from day 6 to day 15 of gestation was less than that of the control ($p<0.05$). Developmental effects on the fetuses were reduced body weight (males, 300 mg/kg, $p<0.05$) and a reduced number of live fetuses (300 mg/kg, $p<0.01$) with a concomitant increase in embryonic death. There was an increased incidence/litter of cardiac malformations (0:1/23 vs. 300:9/17). The predominant cardiac malformation was a ventricular septal defect. There was an increased incidence of a minor skeletal anomaly of wavy ribs for the 300 mg/kg group (0:0/23 vs. 10/17). Among the visceral variations noted for the 300 mg/kg group, there was an increased incidence/litter of persistent right azygous vein (0: 1/23 vs. 300: 7/17) and supernumerary coronary orifice in the heart (0:0/23 vs. 300: 3/17). **Indicated adverse effect:** increased incidence of a ventricular septal defect in the heart; **Maternal NOEL:** 100 mg/kg/day (based upon decreased weight gain noted for the 300 mg/kg treatment group); **Developmental NOEL:** 30 mg/kg/day (based upon the increased incidence of cardiovascular variations experienced by the 100 mg/kg treatment group); **Study acceptable.** (Moore, 6/7/02)

**064; 184632**; "Preliminary Teratology Study of SB-1297, SB-1335 or SB-1855 Administered Orally to Rats"; (S. Kawamura; Environmental Health Science Laboratory, Sumitomo Chemical Co. Ltd, Osaka, Japan; Project ID 599; 1/9/89); Six mated female SPF Slc:SD rats/group were dosed by oral gavage with 0, 30, 100, 200, or 500 mg/kg of SB-1855 (lot no. OK-86-01, purity: 98.2%, also identified as S-53482 in vol. 52894-082) from gestation day 6 through day 15. No maternal deaths resulted from the treatment. Vaginal bleeding was evident late in the gestation period for the 100 and 200 mg/kg treatment group. The initial mean body weights were too different between the control and treated groups to make a valid evaluation of that parameter. Food consumption was not significantly affected for the 30 mg/kg treatment group. At the other treatment levels, food consumption was intermittently affected during the treatment period. The developing fetuses were adversely affected at all of the treatment levels. There were no surviving fetuses in the 200 and 500 mg/kg treatment groups. Excessive death was noted for both the 30 and 100 mg/kg groups. The mean fetal body weights of the 30 mg/kg group were less than those of the control ($p<0.05$). Teratologic abnormalities for the 30 mg/kg group included ventricular septal defects in the heart (0:0/38 vs. 30:11/25, $p<0.01$)), persistent left umbilical artery (0:0/38 vs. 30:3/25), and wavy ribs (0:0/42 vs. 30:9/28). These data indicate that, even at the 30 mg/kg treatment level, significant developmental defects occurred. **Possible adverse effect:** ventricular septal defects in the heart. **Maternal NOEL:** not determinable. **Developmental NOEL:** < 30 mg/kg/day (based upon the incidence of developmental defects in the 30 mg/kg treatment group). **Study supplemental** (non-guideline study). (Moore, 7/29/02)

## TERATOLOGY, RABBIT

** 068; 184636; "Teratology Study in Rabbits with S-53482"; (A. M. Hoberman; Argus Research Laboratories, Inc., Horsham, PA; Project ID 1119-014; Twenty artificially-inseminated female New Zealand white rabbits/group were dosed by oral gavage with 0, 300, 1000 or 3000 mg/kg/day of S-53482 (lot no. PYG-89021, purity: 94.8%) from day 7 through day 19 of gestation. No deaths resulted from the treatment. The 3000 mg/kg group demonstrated a lower mean body weight gain during the treatment period than did the controls ($p<0.01$). The mean food consumption during the treatment period was also less than that of the controls ($p<0.05$). There were no treatment-related effects upon fetal development. **No adverse effect indicated. Maternal NOEL:** 1000 mg/kg/day (based upon reduced body weight gain and food consumption for the 3000 mg/kg/day does); **Developmental NOEL:** 3000 mg/kg/day (based upon the lack of treatment-related effects on the fetuses of the highest dose tested); **Study acceptable.** (Moore, 6/3/02)

## GENE MUTATION

** 078; 184709; "Reverse Mutation Test of S-53482 in *Salmonella typhimurium* and *Escherichia coli*"; (S. Kogiso; Sumitomo Chemical Co., Ltd., Biochemistry and Toxicology Laboratory, Osaka, Japan; Project ID 1806; 10/18/89); *S. typhimurium* strains TA98, TA100, TA1535, TA1537 and TA1538 and *E. coli* strain WP2uvrA were treated with S-53482 (lot no. PYG-89021-M; purity: 94.8%) at concentrations ranging from 0 to 2000 ug/plate with a preincubation of 20 minutes and an incubation with plate incorporation for 65 hours at 37$^{O}$ C under conditions of activation and non-activation. Two trials were performed with duplicate samples for each treatment level. A Kanechlor-400-induced rat liver S9 fraction was used to metabolize the test material. There was no treatment-related increase in the incidence of reverse mutation. **No adverse effect indicated.** The positive controls were functional. **Study acceptable.** (Moore, 6/27/02)

## CHROMOSOME EFFECTS

080; 184711; "Micronucleus Test of S-23031 and S-53482"; (M. Hara and S. Kogiso; Biochemistry and Toxicology Laboratory, Sumitomo Chemical Co. Ltd., Osaka, Japan; Project IDs 1168, 1169; 8/19/88); Four male mice/group were treated by intraperitoneal injection with 0 (corn oil), 300, 1000 or 5000 mg/kg of S-23031 (lot no. LF-71001, purity: 99.3%) or S-53482 (lot no. OK-8751, purity: 98.4%) and euthanized 24 hours post-dose. Another group of 4 mice as a positive control was treated ip with 80 mg/kg of cyclophosphamide. The incidence of micronucleated polychromatic erythrocyctes (PCE) in 1000 PCEs and the ratio of PCEs to the total number of erythrocytes were reported. There was no treatment-related increase in the percentage of micronucleated PCEs. The ratio of PCEs to the total erythrocyte count was less than that of the controls for the animals treated with 1000 and 5000 mg/kg of S-53482. **No adverse effect indicated.** Positive control was functional. **Study unacceptable,** not upgradeable (number of animals fewer than that recommended in the guidelines). (Moore, 6/28/02)

** 077; 184708; "*In Vivo* Chromosomal Aberration Test of S-53482 in Rat Bone Marrow Cells"; (M. Hara; Sumitomo Chemical Co. Ltd, Biochemistry and Toxicology Laboratory, Osaka, Japan; Project ID 1841; 2/16/90); In a dose-response study, 5 Sprague-Dawley rats/sex/group were orally dosed with 0, 1250, 2500 or 5000 mg/kg of S-53482 (Lot no. PYG-89021-M; purity: 94.8%) and euthanized 24 hours post-dose. In a time course study, 20 rats/sex were dosed with 5000 (M) or 4400 (F) mg/kg and 5 animals/sex were euthanized at 6, 12, 24 and 48 hours post-dose. Five animals/sex/group were dosed with corn oil (negative control) or cyclophosphamide, 40 mg/kg (positive control) and euthanized at 24 hours post-dose. Two hours prior to euthanasia, each animal was injected intraperitoneally with 2 mg/kg of colchicine Bone marrow which was recovered from the shaft of the femur was analyzed for the incidence of chromosomal aberrations. The mitotic index and the ratio of polychromatic erthrocytes (PCE) to the total number of erythrocytes (both PCE and normochromatic) were determined. There was no treatment-related increase in the percentage of cells with chromosomal aberrations. The positive control was functional. **No adverse effect indicated. Study acceptable.** (Moore, 6/26/02)

## DNA DAMAGE

** 079; 184710; "*In Vivo*/*invitro* Unscheduled DNA Synthesis (UDS) Assay of S-53482 in Rat Hepatocytes"; (S. Kogiso; Sumitomo Chemical Co. Ltd, Biochemistry and Toxicology Laboratory, Osaka, Japan; Project ID 1879; 9/27/90); In a time-course study, 3 male Sprague-Dawley rats/time point were dosed orally by gavage with 5000 mg/kg of S-53482 (lot no. PYG-89021-M, purity: 94.8%) and euthanized at 3, 12 and 24 hours post-dose. Three males were treated with corn oil and euthanized at 12 hours. In a dose-response study, 3 males/group were dosed orally by gavage with 0 (corn oil), 1250, 2500 or 5000 mg/kg of the test material and euthanized at 12 hours post-dose. In both studies, as a positive control, 3 males each were dosed with 50 mg/kg of 2-acetylaminofluorene and euthanized at 12 hours. Upon recovery of the hepatocytes, a primary culture was established and the cells were exposed to $^{3}$H-thymidine (370 kBq/ml) for 4 hours. Unscheduled DNA synthesis was measured by counting nuclear grains and subtracting the number of grains in a comparably sized cytoplasmic area adjacent to the nucleus. Fifty nuclei/animal were examined. There was no treatment-related increase in the net nuclear grain

count. **No adverse effect indicated.** The positive control was functional. **Study acceptable.** (Moore, 6/28/02)

## NEUROTOXICITY

Study not submitted nor required.

## SUBCHRONIC STUDIES

52894-046; 184614; "Three-Month Subacute Toxicity Study of S-53482 by Dietary Administration in Rats"; (Haruhiko Adachi; Sumitomo Chemical Co., Environmental Health Science Laboratory,, Ltd., Osaka, Japan; Study No. 1760; 4/26/91); Sixteen Crj:CD (SD) rats/sex/group were treated in the diet with 0, 30, 300, 1000 or 3000 ppm of S-53482 (lot no. PYG-89021-M, purity: 94.8%). Six of these animals/sex/group were treated for 5 weeks. The remaining 10 animals/sex/group were treated for 13 weeks ((M): 0, 1.94, 19.4, 65.2, 197.3 mg/kg/day, (F) 0, 2.22, 22.4, 72.8, 218.4 mg/kg/day). One female in the 3000 ppm group died during week 12. The mean body weights for the 3000 ppm males and females were 95.3 and 90.2% of the mean values for the control animals, respectively at the termination of the study. Except for the first week of the study, food consumption was not affected by the treatment. The primary effect of the treatment was the induction of anemia. Mean hemoglobin levels were decreased for the 1000 and 3000 ppm males and for the 3000 ppm females ($p<0.01$) at both 5 weeks and 13 weeks. The mean hematocrit was reduced for the 3000 ppm males ($p<0.05$) and females ($p<0.01$) at 5 and 13 weeks and the 1000 ppm females ($p<0.01$) at 5 weeks. The mean MCV and MCH values were reduced for the 1000 and 3000 ppm males and females ($p<0.01$) at 5 and 13 weeks and the 300 ppm females at 13 weeks ($p<0.05$). The mean MCHC values for the 1000 and 3000 ppm males ($p<0.05$ or 0.01) and females ($p<0.01$) were less than those of the controls at 5 and 13 weeks. The percentage of reticulocytes was increased for the 3000 ppm males and females ($p<0.01$) at 5 and 13 weeks and for the 1000 ppm females ($p<0.05$) at 13 weeks. The number of erythroblasts was increased for 3000 ppm males and females ($p<0.01$) at 5 and 13 weeks. The myoblast/erythroblast ratio at 13 weeks was reduced for the 3000 ppm males and the 1000 and 3000 ppm females ($p<0.01$). In the clinical chemistry evaluation, the mean plasma cholinesterase activity was reduced from that of the control for the 3000 ppm females at both 5 and 13 weeks ($p<0.01$). The percentage of albumin was increased for the 3000 ppm females at 13 weeks ($p<0.01$). The globulin-1 percentage was reduced for the 3000 ppm females from that of the control at 5 and 13 weeks ($p<0.01$). The albumin/globulin ratio was increased for the 3000 ppm females at 13 weeks ($p<0.01$). In the necropsy, the absolute and relative spleen weights of the 3000 ppm females were greater than that of the control ($p<0.01$) at both 5 and 13 weeks. The mean absolute heart weight was greater for the 3000 ppm females at 13 weeks ($p<0.05$). The mean relative heart weights for the 1000 and 3000 ppm males ($p<0.05$ or 0.01) and the 3000 ppm females ($p<0.01$) were greater than those of the control at 5 and 13 weeks. The mean absolute liver weight was increased for the 3000 ppm females at 5 weeks ($p<0.05$). The mean relative liver weights for the 3000 ppm males and females were greater than those of the controls at 5 and 13 weeks ($p<0.01$). The mean relative kidney weights were increased for the 3000 ppm males and females ($p< 0.01$) and for the 1000 ppm males ($p< 0.05$). In the histopathology examination, the incidence of increased extramedullary hematopoiesis in the spleen was increased for the 1000 and 3000 ppm females at both 5 and 13 weeks (5 wks, 0: 0/6, 1000: 2/6, 3000: 6/6; 13 wks, 0: 0/10, 1000: 8/10, 3000: 10/10) and for the 3000 ppm males at 5 and 13 weeks (5 wks, 0: 0/6, 3000: 2/6; 13 wks, 0:0/10, 3000: 6/10). Brown pigment related to the breakdown of heme was noted in the sinusoidal and bile canaliculus cells of the liver and the tubular cells of the kidney of the 3000 ppm females at 13 weeks. Hypercellularity in the bone marrow was evident for the 1000 and 3000 ppm females at 13 weeks (0: 0/10, 1000: 6/10, 3000: 7/10). Other noted lesions for the 3000 ppm females were focal or generalized necrosis in the liver (0:0/10 vs. 3000: 4/10), extramedullary hematopoiesis in the liver (0: 0/10 vs. 3000: 5/10), myocardial fibrosis in the heart (0: 0/10 vs. 3000: 2/10), myelofibrosis and osteosis in the bone marrow (0: 0/10 vs. 3000: 3/10), atrophy of the thymus and the presence of thymal foam cells (0:0/10 vs. 3000: 3/10), sinus histiocytosis in the mesenteric lymph node (0:0/10 vs. 3000: 3/10), and cytoplasmic vacuolation in the adrenal cortex (0:0/10 vs. 3000: 3/10). **Adverse effects indicated:** anemia and hepatic necrosis. **Subchronic NOEL (M/F):** 300 ppm ((M) 19.4

mg/kg/day, (F) 22.4 mg/kg/day) (based upon hematologic effects noted for the 1000 ppm treated animals); **Study acceptable.** (Moore, 5/20/02)

52894-047; 184615; "Revised 13-Week Subchronic Oral Toxicity Study of S-53482 Pure in Rats"; (Akihiro Hagiwara; Daiyu-kai Institute of Medical Science, Azai, Ichinomiya, Japan; Project ID 8818; 6/24/89); Twelve Crj:CD (SD) rats/sex/group were treated in the diet with 0, 30, 300, 1000 or 3000 ppm of S-53482 (lot no. PYG-88021-M, purity: 98.4%) for 13 weeks ((M) 0, 2.28, 20.7, 69.7, 243.5 mg/kg/day, (F) 0, 2.21, 21.7, 71.5, 229.6 mg/kg/day). No animals died during the study. The mean body weights of the 3000 ppm males (91.7% of control) and females (90.2% of control) were less than those of the controls at the conclusion of the study ($p<0.05$). The 3000 ppm males and females suffered from anemia with reduced hemoglobin ($p<0.01$), hematocrit ($p<0.01$), MCV ($p<0.01$) and MCH ($p<0.01$). The mean percentages of reticulocytes and numbers of erythroblasts were increased for both sexes in the 3000 ppm group ($p<0.01$). The granulocyte/erythroblast ratio in the bone marrow was reduced for both males ($p<0.05$) and females ($p<0.01$) of the 3000 ppm group. The mean absolute ($p<0.05$) and relative spleen weights ($p<0.01$) were increased for the 3000 ppm males and females. The mean relative heart weight for the 3000 ppm females was greater than that of the controls ($p<0.01$). The mean relative liver weights were increased for both males and females of the high dose group ($p<0.01$) and for the males of the 1000 ppm group ($p<0.05$). The mean relative kidney weights for the 1000 ($p<0.05$) and 3000 ppm males ($p<0.01$) were greater than that of the controls. The absolute and relative thyroid weights of the 3000 ppm females were greater than those of the controls ($p<0.01$). In the histological examination, extramedullary hematopoiesis was noted in the spleen of the 3000 ppm males and females ((M) 0: 0/12 vs. 3000: 8/12, (F) 0: 0/12 vs. 3000: 12/12). **Adverse effect indicated:** anemia. **Subchronic NOEL:** (M/F) 1000 ppm ((M) 69.7 mg/kg/day, (F) 71.5 mg/kg/day) (based upon anemia in the 3000 ppm animals). **Study acceptable.** (Moore, 5/21/02)

52894-045; 184613; "Three-Month Oral Toxicity Study of S-53482 in Dogs"; (Minoru Nakano; Environmental Health Science Laboratory, Sumitomo Chemical Co., Ltd.,, Osaka, Japan; Project ID 1933; 4/26/91, amended, 1/11/93); Four beagle dogs/sex/group were dosed orally with 0, 10, 100 or 1000 mg/kg/day of S-53482 (lot no. PYG-89021-M, purity: 94.8%) in capsules for 13 weeks. There was no mortality or treatment-related effect upon mean body weights or food consumption. There were no treatment-related effects noted in the ophthalmology, electrocardiogaphy, urinalysis, fecal examination, liver and renal function tests or the bone marrow examination. The mean reticulocyte count was increased in a dose-related manner after 12 weeks of treatment for the males (p: NS). This apparent effect was not evident for the females. Mean total serum cholesterol concentration was increased for the 1000 mg/kg males at 4, 8 and 12 weeks ($p<0.05$ or $p<0.01$). The total cholesterol concentration was increased for the 1000 mg/kg females at the 3 time points, but was significant only at 4 weeks ($p<0.01$). Likewise, the mean serum phospholipids were increased for the 1000 mg/kg males at the 3 time points ($p<0.01$) and for the 1000 mg/kg females at 4 weeks ($p<0.01$). Mean serum alkaline phosphatase activity was increased for the 1000 mg/kg females at 8 and 12 weeks ($p<0.01$). In the necropsy examination, the mean absolute pituitary weight was decreased for the 1000 mg/kg males ($p<0.05$). However the relative pituitary weight was not significantly different from that of the control. Microscopic examination of the liver revealed increased bile ductule proliferation (0: 0/8 vs. 1000: 3/8) and increased fibrous tissue around centrilobular veins for the 1000 males (0:0/4 vs. 1000: 2/4). Further examination by electromicroscopy revealed proliferation and dilatation of the smooth endoplasmic reticulum in the hepatocyte (0:0/8 vs. 1000: 7/8). Target organ: liver. **No adverse effect indicated. Subchronic NOEL:** (M/F) 100 mg/kg/day (based upon the treatment-related effects in the liver of the 1000 mg/kg animals); **Study acceptable.** (Moore, 5/9/02)

52894-048; 184616; "21-Day Dermal Toxicity Study in Rats with S-53482"; (M.R. Osheroff; Hazleton Laboratories America, Inc., Rockville, MD; Study No. 343-230; 10/29/91); The skin of 5 Sprague-Dawley rats/sex/group was treated with 0, 100, 300, or 1000 mg/kg of S-53482 (lot no. PYG-89021M, purity: 94.8%) for 6 hours per day, 7 days per week for 3 weeks under an occlusive wrap. The test material, a powder, was made into a paste with corn oil. No deaths resulted from

the treatment. There were no treatment-related clinical signs and the treated animals exhibited body weight gain comparable to that of the controls. The mean hemoglobin and hematocrit values for the 1000 mg/kg females were lower than those for the controls ($p<0.05$). Otherwise, no treatment-related effects were noted in the clinical chemistry and necropsy evaluations. One of the females in the 1000 mg/kg group exhibited increased extramedullary hematopoiesis in the spleen. **No adverse effect indicated**. **NOEL: (M)** 1000 mg/kg/day (no treatment-related effects noted for the highest dose tested); **(F)** 300 mg/kg/day (based upon treatment-related effects upon the hematology of the 1000 mg/kg/day treatment group); **Study acceptable.** (Moore, 5/22/02)

52894-044; 184612; "Four-Week Subacute Toxicity Study of S-53482 by Dietary Administration in Mice"; (Takaki Seki; Environmental Health Science Laboratory, Sumitomo Chemical Co., Ltd.,, Osaka, Japan; Project ID 1869; 10/16/90); Nine Crj:CD-1 (ICR) mice/sex/group were treated in the diet with 0, 1000, 3000 or 10000 ppm of S-53482 (lot no. PYG-89021-M, purity: 94.8%) for 4 weeks ((M): 0, 151.5, 419.9, 1366.5 mg/kg/day, (F) 0, 164.5, 481.6, 1698.3 mg/kg/day). No deaths resulted from the treatment. No treatment-related effect on body weight gain was evident. No treatment-related effects upon the hematology or clinical chemistry parameters. The mean absolute and relative liver weights for the 10000 ppm males were greater than those of the controls ($p<0.05$ and $p<0.01$). The mean absolute and relative liver weights for the 3000 ppm females ($p<0.05$ and $p<0.01$) and the mean relative liver weights for the 10000 ppm females ($p<0.01$) were greater than those of the controls. No treatment-related lesions were noted in the histopathological examination. **No adverse effects indicated. NOEL (M)** 3000 ppm (481.6 mg/kg/day (based upon increased absolute and relative liver weights for the 10000 ppm males), **(F)** 1000 ppm (164.5 mg/kg/day) (based upon increased absolute and relative liver weights for the 3000 ppm females); **Study supplemental**. (Moore, 5/8/02)

## METABOLISM STUDIES

### Metabolism, Rat

082; 184713; "Revised Metabolism of [phenyl-$^{14}$C]S-53482 in Rats"; (Haruyuki Matsunaga; Environmental Health Science Laboratory, Sumitomo Chemical Co. Ltd, Osaka, Japan; Project ID 2246; 1/29/93, revised, 1/10/96); Five Sprague-Dawley rats/sex were dosed by oral gavage with 1 or 100 mg/kg of [Phenyl-$^{14}$C]S-53482 (lot no. C-90-066 (c-90-042A), radiochemical purity: >99%, chemical purity: >99%, specific activity:195 mCi /mmol) and urine and feces samples were collected for 7 days. A third dosing group of 5 animals/sex was treated daily with 1 mg/kg of S53482 (lot no. LE-93003, purity: 99.5%) for 14 days, followed by a 1 mg/kg dose with the radiolabeled compound. Urine and feces samples were likewise collected for 7 days. Dosing via the oral route resulted in a greater percentage of the radiolabel being recovered in the feces. Males treated once or repeatedly with 1 mg/kg excreted approximately 70% in the feces and 30% in the urine. Females excreted 56 to 60% in the feces and 39 to 43% in the urine. Treatment with 100 mg/kg resulted in fecal excretion of 85% for the males and 78% for the females. The residual radiolabel in the animals 7 days after treatment was largely isolated in the blood cells and blood with lesser concentrations in the liver and kidneys. In the 100 mg/kg treatment group females, the radiolabel was also isolated in the thyroid at a notable level. Identification of metabolites met with limited success in the 1 mg/kg treatment groups with 51 to 63% of the radiolabel either not being identifiable or not extractable. For the 100 mg/kg group, these unidentifiable entities were reduced to approximately 27% due to the increased recovery of the unaltered test material. The identifiable metabolites revealed the following metabolic reactions: 1) hydroxylation of the cyclohexene ring, 2) cleavage of the imide linkage, 3) cleavage of the amide linkage in the benzoxazine ring, 4) acetylation of the amino group of the aniline derivative and 5) incorporation of a sulfonic acid group on the phthalimide moiety. No time-course blood collection was performed in an effort to characterize the pharmacokinetic parameters of uptake and elimination of the test material. No biliary excretion study was performed as well. However, without either of these analyses, the actual absorption from the gastrointestinal tract is not determinable. **Study unacceptable,** not upgradeable. (Moore, 7/26/02)

52894-0137, -0138, -0139; 202104, 202105, 202106; "The Pharmacokinetics of [$^{14}$C]S-53482 in the Rat[1], Biliary Excretion of [$^{14}$C]S-53482 in the Rat[2], Tissue Distribution of [$^{14}$C]S-53482 in the Rat[3]"; (Gibson, N.A., G.R. Krautter, K. Jalali, and L.O. Ruzo; PTRL East, Inc., Richmond, CA and PTRL West, Inc., Richmond, CA; Project IDs: 1034E[1], 1035E/588W[2], 1036E/589W[3]; 2/19/97[1], 3/6/97[2], 3/17/97[3]); In the pharmacokinetic study, 7 Sprague-Dawley (Crl:CD:BR) rats/sex were dosed by oral gavage with 1 or 100 mg/kg of [Tetrahydrophthaloyl-1,2-$^{14}$C]S-53482 (Flumioxazin Technical) (lot no. RIS96014, specific activity: 121 mCi/mmole, radiochemical purity: 98.9%, chemical purity: 99.0%) mixed with; unlabeled S-53482 (lot no. 60208AG, purity: 99.9%). Blood samples were collected at 0, 1, 2, 4, 8, 24, 48 and 72 hours post-dose for the 1 mg/kg group and at 0, 2, 4, 8, 16, 24, 48 and 72 hours post-dose for the 100 mg/kg group. In the biliary excretion study, 3 rats/sex were dosed by oral gavage with 1 mg/kg of the test material and urine, feces and bile were collected at the following intervals: 0-6 hours, 6-24 hours, 24-48 hours, and 48-72 hours. In the tissue distribution study, 12 rats/sex/group were dosed by oral gavage with 1 or 100 mg/kg of the test material. Three animals/sex/group/time point were euthanized at Tmax, 1/2 Tmax, 1/4 Tmax and 168 hours post-dose. In the pharmacokinetic study, one female rat in the 1 mg/kg group died as a consequence of surgical trauma. Two females in the biliary excretion study died prior to the 72 hour sampling period due to possible dehydration as a result of sample collection. The following pharmacokinetic parameters were ascertained: 1 mg/kg treatment, Cmax (M) 0.255 ppm, (F) 0.213 ppm, Tmax (M/F) 4 hours, T1/2 (M/F) 12 hours, 100 mg/kg treatment, Cmax (M) 5.534 ppm, (F) 4.714 ppm, Tmax (M) 16 hours, (F) 8 hours, T1/2 (M) 28 hours, (F) 46 hours. At least 85 and 80% of the administered dose was absorbed by the 1 mg/kg males and females, respectively, over the 72 hours post-dose sampling period (% of dose recovered in the urine and bile). Thirty nine to 43% of the administered dose was recovered from the bile. Seventy eight to 80% of the administered dose was recovered in the first 24 hours post-dose. In the tissue distribution study, the radiolabel was primarily recovered from the gastrointestinal tract. Among the organs in the systemic circulation, the kidney and liver had the highest level of radiolabel over the course of the sampling period. Only a minimal amount of the radiolabel appeared to penetrate the blood-brain barrier. No sequestration of the label was noted in the fat. In the metabolic profile, approximately 1% of the test material was recovered unmetabolized. The tetrahydropthalimide moiety was the primary site of metabolism with hydrolysis, hydroxylation, and reduction of the double bond being noted. Sulfonation of the moiety as a secondary conjugation reaction also occurred. **Study acceptable.** (Moore, 1/23/03)

## SUPPLEMENTAL STUDIES

051; 184619; "Placental Transfer of S-53482 in Rats and Rabbits, Amended Report #1"; (N. Isobe; Environmental Health Science Laboratory, Sumitomo Chemical Co. Ltd, Osaka, Japan; Project ID 2461; 3/23/92, amended, 7/9/93); In the first study, 16 pregnant SD female rats and 8 pregnant JW/NIBS female rabbits were dosed by oral gavage on day 12 of gestation with 30 mg/kg of Phenyl-$^{14}$C]S-53482 (lot no. C-91-028A; radiochemical purity: >99%, chemical purity: >99%, specific activity:129 mCi /mmol) and unlabeled S-53482 (lot no. LE-93003, purity: 99.5%). Four rats and 2 rabbits/time point were euthanized at 1, 2, 4, and 24 hours post-dose and selected tissues were analyzed for total radiolabel and identifiable metabolites. In the second study, 30 pregnant rats and 14 pregnant rabbits were dosed with 30 mg/kg of the radiolabeled test material on day 12 of gestation. Fifteen rats/time point were euthanized at 1 and 24 hours post-dose and 7 rabbits/time point were euthanized at 2 and 24 hours post-dose. Selected tissues were analyzed for total radiolabel and metabolites. The studies specifically compared the relative concentration of the radiolabel in the fetal tissue as compared to the maternal plasma levels, denoted as the placental transfer factor. Recovery of the radiolabel in the urine and feces up to 24 hours post-dose was greater in the rat than the rabbit (76.6% vs. 30.2%) with 72 and 60% of the total recovered in the feces of the rat and rabbit respectively. Among the metabolites recovered in the feces, the unmetabolized compound constituted the predominant moiety (70% in the rat, 67% in the rabbit). Overwise, the profile of metabolites indicated the following metabolic reactions: 1) hydroxylation of the cyclohexene ring of the 3,4,5,6,-tetrahydrophthalimide, 2) cleavage of the imide linkage, 3) cleavage of the amide linkage of the benzoxazinone ring, 4) acetylation of the amino group of the aniline derivative, and 5) incorporation of the sulfonic acid group to the 3,4,5,6,-tetrahydrophthalimide which were common to both species. The peak

concentrations of the radiolabel in the various tissues was at 2 to 4 hours post-dose. The maximal levels of radiolabel in the fetus were 0.78 ppm in the rat and 0.2 ppm in the rabbit at 4 hours post-dose. The maximal placental transfer factor was 0.26 at 4 hours for the rat and 0.14 at 24 hours for the rabbit. These data indicate that transfer of the radiolabel from the plasma to the fetus was limited across the placental barrier. **Study supplemental** (non-guideline study). (Moore, 7/12/02)

052; 184620; "Critical Period for Developmental Toxicity Induced by S-53482 in Rats"; (S. Kawamura; Environmental Health Science Laboratory, Sumitomo Chemical Co. Ltd, Osaka, Japan; Project ID DSB01; 6/24/93); Five mated female Crj:CD rats/group were dosed by oral gavage with 400 mg/kg of S-53482 (lot no. PYG-89021-M, purity: 94.8%) in 0.5% methylcellulose on days 11, 12, 13, 14, or 15 of gestation. All of the dams were euthanized on day 20 of gestation. The highest incidence of fetal mortality was noted for the dams treated on day 12. The surviving fetuses of the dams treated on day 12 had the lowest mean body weights and the highest incidence of ventricular septal defects. The critical time point of exposure was gestation day 12. **Supplemental Study** (Non-guideline study) (Moore, 7/12/02)

054; 184622; "Effects of S-53482, an N-phenylimide Herbicide, on Protoporphyrin IX Accumulation in Embryos, I. Species Difference in Protoporphyrin IX Accumulation between Rat and Rabbit Embryos"; (S. Kawamura; Environmental Health Science Laboratory, Sumitomo Chemical Co. Ltd, Osaka, Japan; Project ID DSB03; 10/21/96); Mated female Crj:CD rats and artificially inseminated female JW-NIBS rabbits were dosed with 1000 mg/kg of S-53482 (lot no. PYG89021-M, purity: 94.8%) on gestation day 12. The rats were euthanized at 0, 2, 6, 12, 18 or 24 hours post-dose. The rabbits were euthanized at 0, 2, 6, 12, 24, or 48 hours post-dose. Three embryos/female were pooled (except in the case of one rabbit when only 2 embryos were available) and wet weight and protoporphyrin IX (PPIX) content was determined for each embryo pool and for the liver of each female. The PPIX content in the rat embryos achieved a peak level at 12 hours post-dose which was 130 times that of the 0 time point. In contrast, the peak PPIX content in the rabbit embryos was achieved at 12 hours post-dose and was less than the baseline 0 time level for the rat. In the maternal rat livers, the peak PPIX content was achieved at 6 hours post-dose and was 12.5 times that of the 0 time level. For the rabbit livers, the PPIX content peaked at 12 hours and was 4.1 times that of the 0 time baseline level. The mean wet weight of the embryos increased over the time course of the study. No treatment-related effects were noted on the liver mean wet weights. **Supplemental Study** (non-guideline study). (Moore, 7/15/02)

055; 184623; "Effects of S-53482, an N-phenylimide Herbicide, on Protoporphyrin IX Accumulation in Embryos, II. Compound Difference in Protoporphyrin IX Accumulation in Rat Embryos"; (S. Kawamura; Environmental Health Science Laboratory, Sumitomo Chemical Co. Ltd, Osaka, Japan; Project ID DSB04; 10/21/96); Mated female Crj:CD rats were dosed with 0 (0.5% methylcellulose) or 1000 mg/kg of S-53482, S-23131 or S-23031 on gestation day 12. The rats were euthanized at 14 hours post-dose. Three embryos/female were pooled and wet weight and protoporphyrin IX (PPIX) content were determined for each embryo pool and for the liver of each female. The PPIX content in the embryos increased 272 and 291 times above that of the control for the groups which were treated with S-23121 and S-53482, respectively. In contrast, the mean PPIX content in the embryos of those treated with S-23031 increased only 1.24 times. For the maternal livers, the PPIX content increased 1.61, 3.63 and 2.88 times above that of the control for S-23031, S-23121 and S-53482, respectively. Treatment with both S-23121 and S-53482 resulted in a significant increase in the accumulation of PPIX in the embryonic tissues. **Supplemental Study** (non-guideline study) (Moore, 7/16/02)

056; 184624; "Effects of S-53482, an N-phenylimide Herbicide, on Protoporphyrin IX Accumulation in Embryos, III. Critical Period for Protoporphyrin IX Accumulation in Embryos"; (S. Kawamura; Environmental Health Science Laboratory, Sumitomo Chemical Co. Ltd, Osaka, Japan; Project ID DSB05; 1/6/97); Mated female Crj:CD rats were dosed with 0 or 400 mg/kg of S-53482 (lot no. PYG89021-M, purity: 94.8%). Artificially inseminated female JW-NIBS rabbits were dosed with 1000 mg/kg of the test material. Groups of both species were dosed once on

gestation days 10, 11, 12, 13, 14, or 15 and euthanized 14 hours post-dose. Three embryos/female were pooled and the wet weight and protoporphyrin IX (PPIX) content was determined for each embryo pool and for the liver of each female. The PPIX content in the rat embryos achieved a peak level after treatment on gestation days 11 and 12. The level was 69 to 84 times that of the control. The PPIX content in the rabbit embryos was not affected by the treatment at any time point during the treatment. There were no treatment-related effects on the wet weights of the embryos or maternal livers of either the rat or the rabbit. Exposure to the test material on gestation day 11 or 12 is the critical time point for accumulation of PPIX in the rat embryonic tissue. **Supplemental Study** (non-guideline study)**.** (Moore, 7/16/02)

057; 184625; "Effects of SB Series Herbicides on Protoporphyrinogen Oxidase Activity in Rat and Rabbit Liver Mitochondria"; (S. Noda; Environmental Health Science Laboratory, Sumitomo Chemical Co. Ltd, Osaka, Japan; Project ID S0167; 6/26/95); The activity of protoporphyrinogen Oxidase (PPO) was assayed in rat and rabbit liver mitochondria and the IC50 values for S-53482 (lot no. PYG-89021-M, purity: 94.8%), S-23121 (lot no. PYG-88061, purity: 94.7%) and S-23031 (lot no. PYG-88092, purity: 94.7%) were determined. The hepatic mitochondrial fractions were procured from 5 week old Crj:CD (SD) female rats and a 5 month old JW-NIBS female rabbit. The enzymatically-mediated formation of protoporphyrin IX (PPIX) from protoporphyrinogen IX was measured by fluorescence spectroscopy. Any autooxidation of the product from its precursor was subtracted out of the total PPIX formed by incubating the substrate in the presence of the heat-inactivated enzyme. The PPO IC50 values for rat liver were 23, 36, and 2230 nM for S-53482, S-23121 and S23031, respectively. The IC50 values for rabbit liver were 300, 690, and 12500 nM for S-53482, S-23121 and S23031, respectively. These values correspond to the *in vivo* toxic effects noted for the respective compounds. **Supplemental Study** (non-guideline study) (Moore, 7/16/02)

058; 184626; "Histopathological Study of S-53482 Developmental Toxicity in Rat and Rabbit Embryos following Oral Administration to Dams at 1000 mg/kg on Day 12 of Gestation**";** (S. Kawamura and T. Yoshioka; Environmental Health Science Laboratory, Sumitomo Chemical Co. Ltd, Osaka, Japan; Project ID DSB02; 1/31/97); Mated female Crj:CD rats and artificially inseminated female JW-NIBS rabbits were dosed by oral gavage with 0 or 1000 mg/kg of S-53482 (lot no. PYG89021-M, purity: 94.8%) suspended in 0.5% methylcellulose on gestation day 12. Groups of both species (unless specified otherwise) were euthanized at 6, 12 (rats only), 24, 36 (rats only) or 48 hours post-dose. Embryos with their placentas attached were examined for external abnormalities. Embryonic blood was analyzed for the presence of cellular iron (Berlin blue staining). After fixation, serial sagittal sections through the whole embryo or serial transverse sections through the thoraco-abdominal region of the embryo were examined microscopically. Embryonic hearts and livers were further processed for examination by electron microscopy. In the rat, intrauterine death was first observed at 36 hours post-dose with 41 of the 44 embyros dying at 48 hours post-dose. No external abnormalities were noted at any time point post-dose. Examination of the embryonic blood revealed massive deposits of iron in the treated embryos by 24 hours post-dose. In the histological evaluation, degenerative erythroblasts were observed at 12 hours post-dose and persisted through 48 hours post-dose. Erythrophagocytosis was evident by 24 hours post-dose. In the heart, thin periventricular wall, thin muscular septum, and endocardial cushion hypoplasia were noted by 36 hours post-dose. Peripheral vascular dilatation was evident by 24 hours post-dose. In the liver, dilatation of the sinusoidal vessels was first evident at 24 hours post-dose. Hepatic necrosis and a thin anterior thoracic wall were only noted at 48 hours post-dose. In the ultrastructural evaluation, iron deposits were first noted in the erythroblast mitochondria at 6 hours post-dose in conjunction with dilatation of the mitochondria. At 12 hours post-dose, these lesions increased in severity and were accompanied with pyknosis or dilation of the nuclear membrane, hydropic change and erythrophagocytosis and persisted through 48 hours post-dose. Mitochondrial swelling was noted in the liver at 24 hours post-dose and persisted through 48 hours post-dose. The earliest observed lesions of deposition of iron and mitochondrial swelling were associated with the erythroblasts. The lesions which were observed subsequently were postulated to be a consequence of the disrupted heme synthesis. No effects were noted for the rabbit embryos. **Supplemental study** (non-guideline study). (Moore, 7/18/02)

059; 184627; “Inhibition of Protoporphyrinogen Oxidase Activity by S-53482 in Rat, Rabbit and Human Liver”; (C. Green and J. Dabbs; SRI International, Toxicology Laboratory, Menlo Park, CA; Project ID 4956-H01-93; 7/22/96); The inhibition of protoporphyrinogen oxidase (PPO) by S-53482 (lot no. PYG-89021-M; purity: 94.8%) was determined *in vitro* in the hepatic mitochondrial preparations of female Crl:CD rats, female New Zealand White rabbits and female humans. The kinetic constants were determined for PPO activity in human liver mitochondria. The Michaelis constant, Km, for PPO was 0.328 uM and the Vmax was 111 pmol PPIX/min/mg of protein. The Vmax value is in agreement with that reported for the rat. The Km is one tenth that of the rat. The IC50 values of PPO for the test material are 0.0173 uM for the human, 0.00715 uM for the rat and 0.138 uM for the rabbit. These results indicate that the *in vitro* inhibition of PPO activity by the test material in the human hepatic mitochondria more closely agrees with that observed in the rat than in the rabbit. **Study supplemental.** (Moore, 7/23/02)

060; 184628; “Mechanism of Hematotoxicity of S-53482 by Dietary Administration in Rats”; (Y. Yoshida; Environmental Health Science Laboratory, Sumitomo Chemical Co., Ltd., Osaka, Japan; Project IDs S0065 and S0098; 6/26/95); Two studies were performed. In the 1st study, 30 female Crj:CD rats/group were treated in the diet with 0 or 3000 ppm of S-53482 (lot no. PYG-89021-M; purity: 94.8%) for up to 37 days. Six animals/group/time point were euthanized on study days 2, 5, 8, 15 and 37. An additional 24 females were treated with 10000 ppm of the test material up to 15 days. Six animals/time point were euthanized on days 2, 5, 8 and 15. In the 2nd study, 24 female rats/group were treated in the diet with 0 or 3000 ppm of the test material for up to 15 days and 6 animals/group/time point were euthanized on days 2, 5, 8 and 15. No animals died and there were minimal treatment-related effects upon the mean body weight and mean food consumption. In both studies, the mean number of red blood cells, hemoglobin concentration, hematocrit, MCV, and MCH of the 3000 ppm treatment group were less than that of the control from day 5 through day 15 ($p<0.05$ or $p<0.01$). In the 1st study, the hemoglobin concentration, hematocrit, MCV, and MCH values for the 3000 ppm group were less than those of the control on day 37 ($p<0.01$). For the animals treated with 10000 ppm in the 1st study, the mean number of red blood cells, hemoglobin concentration, hematocrit, MCV, and MCH were less than that of the control from day 5 through day 15 ($p<0.05$ or $p<0.01$). In the 1st study, the MCHC values for the 3000 and 10000 ppm groups were less than those of the controls on study days 8 and 15 and for the 3000 ppm group on day 37 ($p<0.01$). In the 2nd study, the MCHC value for the 3000 ppm group on day 15 was less than that of the control ($p<0.01$). The differential white blood cell analysis revealed first a decrease in the neutrophil count on day 2 in the 1st study for both treatment groups and then an increase from day 5 through day 15 for the 10000 ppm group ($p<0.01$) and for days 8 and 15 for the 3000 ppm treatment group ($p<0.01$). In the 2nd study, the neutrophil count for the 3000 ppm group was increased from day 5 through day 15 ($p<0.01$). Similarly the percentage of neutrophils decreased or increased based upon these results. The percentage of lymphocytes was inversely altered by the treatment-related effect on the neutrophil count. Reticulocytes decreased in number and percentage of the erythrocyte population on days 2 and 5 in both studies and increased on days 15 and 37 in the 1st study and on day 15 in the 2nd study ($p<0.01$). The number of erythroblasts per 100 white blood cells was increased from day 2 through to the termination of both studies ($p<0.05$ or $p<0.01$). In the 1st study, the myeloid/erthyroid ratio in the bone marrow reduced for both treatment groups from day 5 through termination of the respective groups ($p<0.01$). The percentage of siderocytes in the blood for both treatment groups increased from day 2 through the end of the study ($p<0.05$ or $p<0.01$). In the 2nd study, urinary coproporphyrin and free erythrocyte protoporphyrin in the blood increased from day 5 for the former parameter and from day 2 for the latter measurement and persisted through to the termination of the study ($p<0.01$). In the 1st study, the absolute and relative spleen weights for the treated animals were greater than that of the control from day 8 through day 37 ($p<0.1$). The mean relative liver weights for the treated animals were greater than that of the control animals at several time points during the study ($p<0.05$ or $p<0.01$). These results indicate that inititally the red blood cell parameters were universally reduced with subsequent recovery in the rbc count. However, the hemoglobin content, hematocrit, MCV, MCH and MCHC were depressed through to the termination of the studies. This condition describes a hypochromic, microcytic anemia. The increased percentage of siderocytes and concentrations of urinary coproporphyrin and blood protoporphyrin in the treated animals provides further evidence of an

interference in the synthesis of hemoglobin. **Study supplemental** (non-guideline study). (Moore, 7/25/02)

061; 184629; "Pathogenesis of Developmental Effects Produced by S-53482, an N-phenylimide Herbicide, in Rats"; (S. Kawamura; Environmental Health Science Laboratory, Sumitomo Chemical Co. Ltd, Osaka, Japan; Project ID DSB06; 2/20/97); Pregnant female Crj:CD rats were dosed by oral gavage with 0 (0.5% methylcellulose) or 400 mg/kg of S-53482 (lot no. PYG-89021-M, purity: 94.8%) on day 12 of gestation. The rats were euthanized on days 13, 14, 15, 16, 17 or 20 days of gestation. Uteri were removed and the number of implantations were counted. Live fetuses were examined for enlargement of the heart and edema. The fetuses in half of the litters on days 14, 15, 16, 17 and 20 days of gestation were examined for closure of the interventricular foramen. In the remaining litters fetal blood was collected and pooled for the evaluation of treatment-related effects upon hematology and blood chemistry. In addition, bone and cartilage of approximately half of the fetuses were examined for treatment-related effects. The incidence of fetal mortality increased by day 15 of gestation (0: 12.3% vs. 400: 43.6%). Although the percentage of mortality increased to 64.1% on day 16, this was likely an excessively high incidence as the percent mortality was 35.9 and 38.7% for litters euthanized on days 17 and 20. The onset of a heart enlargement was evident on day 14 (0: 0 vs. 400: 63.2%) and progressively diminished on days 16, 17 and 20. Likewise, the incidence of edema peaked on day 15 and had largely declined to 0 by day 20. The closure of the interventricular foramen in the heart was delayed with only 57.7% of the treated fetuses demonstrating closure at 20 days of gestation compared to 95.2% in the control group. Treatment resulted in lower red blood cell counts and reduced hemoglobin and protein concentrations in the fetal blood on days 13 through 16 with incipient recovery to control levels evident on day 17. In the skeletal examination, delayed ossification of the ribs was noted for the treated fetuses on day 17 (0: 0 vs. 400: 5.6%). On day 20, the incidence of wavy ribs (23.9%) and bent scapula (8.5%) was noted for the treated group in comparison with 0 incidence for the control. Physiologically, the anemia suffered by the treated fetuses preceded the effects of enlarged heart and edema and likely contributed to these effects. Likewise, the author of the report surmised that the skeletal abnormalities may have been due to reduced serum protein levels with delayed development of osteoblast progenitors and low fetal serum alkaline phosphatase levels. **Study supplemental** (non-guideline study). (Moore, 7/3/02)

065; 184633; "Protoporphyrinogen Oxidase Activity in Rat and Rabbit Tissues: Inhibition by Three Test Chemicals"; (C. Green and J. Dabbs; SRI International, Toxicology Laboratory, Menlo Park, CA; Project ID 3013-H01-91; 7/9/93); Hepatic mitochondria were procured both from adult female Crl:CD Sprague-Dawley rats and adult female New Zealand White rabbits. Fetal tissue was derived from embryos of pregnant rats and rabbits on gestation day 12 or 15 and pooled. This tissue was used to isolate fetal mitochondria. Using these samples, the kinetic parameters were determined for protoporphyrinogen oxidase (PPO) activity in the liver and in the embryonic tissue of 12 and 15 day old fetuses of both species treated with S-53482, S-23121, or S-23031 *in vitro*. The Km and Vmax values of PPO for both species increased between gestation day 12 and 15 and then decreased to an adult level. For the rat, the Km and Vmax values ranged from 3.1 to 8.2 uM and 93.2 to 270 pmol/mg protein/min, respectively. For the rabbit, the Km and Vmax values ranged from 1.8 to 12.3 uM and 26.7 to 161 pmol/mg protein/min. The IC50 values for the inhibition of PPO was determined for S-53482 (lot no. PYG-89021-M, purity: 94.8%), S-23121 (PYG-88061, purity: 94.7%) and S-23031 (lot no. PYG-88092-M) in the liver and in the embryonic tissue of 12 and 15 day old fetuses of both species. The IC50 values for the different tissues were from least to greatest, S-53482, S23121 and S-23031. For S-53284, the values ranged from 5.9 to 12.1 nM in the rat and from 51.9 to 308 nM in the rabbit. For S-23121, the values ranged from 10.8 to 46.7 nM in the rat and from 1270 to 6490 nM in the rabbit. For S23031, the values ranged from 204 to 793 nM in the rat and from 4750 to 5920 nM in the rabbit. **Study supplemental.** (Moore, 7/26/02)

Eleven supplemental studies with record nos. ranging between 184619 to 184633 were performed in an effort to elucidate the apparent sensitivity of the rat to treatment-related hematotoxic and teratogenic effects. The test material is an herbicide which inhibits protoporphyrinogen oxidase (PPO) thereby interfering with both chlorophyll and heme synthesis.

Inhibition of the enzyme results in an accumulation of protoporphyrin IX (PPIX). The results of these studies indicate that PPO is more readily inhibited in the rat liver than in the liver of the rabbit with a corresponding accumulation of PPIX. Although the transplacental movement of the test material was somewhat limited in the rat, its concentration in the developing rat embryo was greater than that observed in the rabbit embyro. The PPIX concentration in the treated rat embryo was quite elevated above that of the baseline level in contrast to that observed for the rabbit embyro. The hypothesized mechanism of action for teratogenic effects entails the inhibition of PPO, resulting in fetal anemia, followed by hypoxia, enlargement of the fetal heart, decreased protein synthesis, edema, and enlargement of the pericardial sac. An *in vitro* characterization of human, rat and rabbit hepatic PPO kinetic parameters demonstrated that the human enzyme was more similar to that of the rat than the rabbit. The Vmax value was similar to the Vmax of the rat with a Km value one tenth that of the rat. The PPO IC50 values for the test material were as follows: rat, 0.00715 uM, human, 0.0173 uM, and rabbit, 0.138 uM. These results indicate that humans may be more similar to the rat in sensitivity for hematotoxic and teratogenic effects than to the rabbit.