CALIFORNIA ENVIRONMENTAL PROTECTION AGENCY
DEPARTMENT OF PESTICIDE REGULATION
MEDICAL TOXICOLOGY BRANCH

SUMMARY OF TOXICOLOGY DATA
DICHLOBENIL

Chemical Code # 000112, Tolerance # 00231
SB 950 # 209

November 18, 1986
Revised 7/06/89, 12/04/89, 11/29/90, 10/28/91, 3/25/92, 7/30/92, 12/24/92, 11/30/93, 2/11/94, 6/10/94
Updated: 5/15/96

I. DATA GAP STATUS

Chronic toxicity, rat:	No data gap, possible adverse effect
Chronic toxicity, dog:	No data gap, no adverse effect
Oncogenicity, rat:	No data gap, possible adverse effect
Oncogenicity, hamster	No data gap, possible adverse effect
Reproduction, rat:	No data gap, no adverse effect
Teratology, rat:	No data gap, no adverse effect
Teratology, rabbit:	No data gap, no adverse effect
Gene mutation:	No data gap, no adverse effect
Chromosome effects:	No data gap, no adverse effect
DNA damage:	No data gap, no adverse effect
Neurotoxicity:	Not required at this time

Toxicology one-liners are attached.

All record numbers through 143933; volume 087 have been examined.
** indicates an acceptable study.
Bold face indicates a possible adverse effect.
File name: T960515
Revised by Stanton Morris, 12/04/89; M. Silva, 11/29/90; J. Kishiyama & M. Silva, 9/5/91; M. Silva, 10/28/91; Kishiyama & Silva, 3/25/92; M. Silva, 7/30/92, 12/24/92, 11/30/93, 2/11/94, 6/10/94, 5/15/96.
NOTE: The guidance document for reregistration of dichlobenil was published by EPA on 03/23/87.

II. TOXICOLOGY ONE-LINERS AND CONCLUSIONS

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

COMBINED, RAT

**** 231-015, 045297-045305;** "Chronic feeding study on Casoron in the rat", report # 314, Biosafety Research Center, Japan, 11/30/83; Choy, 11/18/86; Dichlobenil (96.7%) was tested at 0, 50, 400 and 3200 ppm in the diet in a chronic study in Fischer F344 rats; 70 rats/sex/group with two interim killings of 10 rats/sex/group at 52 and 78 weeks; adverse effects observed at 400 ppm and above; decrease in body weights, changes in many hematology and urinalysis parameters; increase in liver and kidney weights; **possible adverse effect** (although no increase in total tumor nor malignant tumor incidences, raw data at 110 week suggested tumorigenicity in liver at 3200 ppm in females; Peto analysis indicated increases in testicular interstitial cells, renal cancer in the males and adenoma of liver cells and malignant lymphoma and leukemia in the females); general systemic NOEL = 50 ppm; minor deviations from the EPA guidelines but the study is considered to be complete and ACCEPTABLE.

231-012, 045274; "Synopsis of the Results of a Chronic Feeding Study with Dichlobenil in the Rat". This document contains a summary of the study at DPR doc. # 231-015, rec. #'s 045297 - 045305 (Choy, 11/10/86).

CHRONIC TOXICITY, RAT

(Also Refer to COMBINED, RAT)

231-011, 045259; "Investigation as to the toxicity of H133 including a chronic experiment with rats"; National Institute of Public Health, Holland, date unknown, Report # 54/64 Tox; Gee, 2/6/86; Choy, 11/6/86; Dichlobenil (referred to as H133 in this report) (93.5%) tested at 0, 20, 50 and 100 ppm in the diet in a chronic study in Wistar rats; 20 rats/sex/group; decrease in body weight in female rats in all treated groups; no adverse effect, NOEL < 20 ppm; UNACCEPTABLE; incomplete; not upgradeable (multiple major deviations from the EPA guidelines)

EPA one-liner: Systemic NOEL = 20 ppm, Systemic LEL = 50 ppm

231-010, 037605: This document contains an identical copy of 231-011, 045259 (Gee, 02/06/86).

231-004, 038447; "Long-Term Feeding Study in Rats"; This document contains a summary of the study at DPR doc. # 231-011, rec. # 045259. No worksheet was done (Morris, 05/18/89).

231-009, 028388: This document contains an exact duplicate of 231-004, 038447. No worksheet was done (Morris, 05/22/89).

231-011, 045267: This document contains a summary of the study at DPR doc. # 231-011, rec. # 045259 (Choy, 11/6/86).

231-006, 016996; "Investigation as to the Toxicity of H 133 Including a Chronic Experiment with Rats." This document contains a summary of the study at DPR doc. # 231-011, rec. # 045259 (Gee, 05/09/85).

<u>Metabolite:</u>

231-014, 045295; "Effects of BAM in dietary administration to rats for two years", report #3980/71/138, Huntingdon Research Centre, England, 10/4/71; Choy, 11/13/86; BAM (2,6-dichlorobenzamide), a metabolite of dichlobenil, (purity unknown) was tested at 0, 60, 100, 180, and 500 ppm in the diet in a chronic study in Charles River CD rats; 35 rats/sex/group; adverse effects at 500 ppm; decrease in body weights, nonprogressive depression of PCV, RBC counts and Hb in males and females; increase of relative liver weight in females; NOEL = 180 ppm; incomplete; UNACCEPTABLE; not upgradeable (Purity of test compound, incomplete hematology, serum chemistry, urine analysis and histopathology study).

231-006, 016994; "Effects of BAM in Dietary Administration to Rats for Two Years." This document contains a summary of the study at DPR doc. # 231-014, rec. # 045295 (Gee, 05/09/85).

231-009, 028393: This document contains a summary of the study at DPR doc. # 231-014, rec. # 045295. No worksheet was done (Morris, 05/22/89).

CHRONIC TOXICITY, DOG

Subchronic Study:

231-070 127948 "Dichlobenil - 4 Week Oral (capsule) Dose Range-Finding Study in the Beagle Dog, (Pickersgill, N., Pharmakon Europe, Study #: 65/509, 1/4/943). Dichlobenil was administered in capsules to Beagle dogs (2/sex/dose) at 0 (capsule), 1, 6, 36 and 72 mg/kg for 28 days. There were no unscheduled deaths. There was some vomiting in the dosed animals and a decreased body weight gain in females at 72 mg/kg. Food and water consumption were similar or slighly decreased in both sexes at 72 mg/kg. Males showed a slight decrease in activated partial thromboplastin times at ≥ 36 mg/kg, as well as an increase in platelets. Females showed an increase in hemoglobin, packed cell volume, RBC's, platelets, PT and APTT that appeared at ≥ 36 mg/kg. Some clinical chemistry effects were observed at ≥ 36 mg/kg in both sexes (**males:** decreased K, increased cholesterol, triglycerides, phospholipids, protein, alkaline phosphatase and ALAT; **females:** decreased glucose & ALAT and an increase in BUN, cholesterol, triglycerides, phospholipids, protein and alkaline phosphatase). There was an increase in absolute and relative liver weights at ≥ 36 mg/kg and males showed a slightly higher absolute and relative adrenal and thyroid weight at 72 mg/kg. Absolute thymus weights were decreased in males at ≥ 36 mg/kg. Females showed an absolute and relative increase in adrenal, thymus and spleen weights at ≥ 36 mg/kg and an absolute and relative decrease in ovary and uterine weights at ≥ 36 mg/kg. Histopathologically, 1 male at 72 mg/kg had mononuclear infiltration in the liver. There was also cytoplasmic aggregation in hepatocytes (males at ≥ 36 mg/kg & females at 72 mg/kg) and centrilobular hepatocytes with homogeneous cytoplasm at ≥ 36 mg/kg in both sexes. NOEL = 6 mg/kg. Possible adverse effects in liver and hematopoietic system at 72 mg/kg. These data are supplemental. M. Silva, 2/8/94.

231--074 132359 This volume is a duplicate of the study reviewed above (231-070 127948). M. Silva, 5/15/96.

Chronic Study:

** 231-087 143933 "Dichlobenil - 52 Week Oral (Capsule) Toxicity Study in The Beagle Dog," (Pickersgill, N., Pharmakon Europe; Study Number 65/510, Solvay Duphar Int., Doc. No.: 56352/41/95, 12/20/95). Dichlobenil (purity = 98.8%), was administered to Beagle dogs (5/sex/dose) by gavage (of gelatin capsules) at concentrations of 0, 1, 6, or 36 mg/kg/day for approximately 52 weeks. NOEL = 1 mg/kg/day (There was decreased body weight (both sexes) and food consumption (males only) at 36 mg/kg/day. Decreased blood parameters were observed in males at ≥ 6 mg/kg/day. Serum chemistry parameters were affected in both sexes at ≥ 6 mg/kg/day. Absolute and relative liver weights were increased in both sexes at ≥ 6 mg/kg/day. Uterine, ovarian and thymus weights were decreased at 36 mg/kg/day (females only). Thyroid weights were increased in males at 36 mg/kg/day. Females had increased thyroid weights at ≥ 36 mg/kg/day. Liver hepatic hypertrophy, adrenal cortical vacuolation and spleen hemosiderin were increased in both sexes at 36 mg/kg/day.) ACCEPTABLE. (Kishiyama & Silva, 5/13/96).

231-012, 051 045273, 088016 "Chronic (two-year) Toxicity Study With Dichlobenil (H133) in Beagle Dogs," (H.P. Til, V.J. Feron, J.W. Huismans & A.P. de Groot; Centraal Instituut Voor Voedingsonderzoek, TNO, Report #: R 2813, 1/69; WNC, 11/7/86). Dichlobenil (95.5% pure, batch #: 430) was administered in diet to pure bred beagle dogs (4/sex/group) at 0 (vehicle = feed), 20, 50 & 350 ppm. **No adverse effect indicated.** NOEL = 50 ppm (Treatment-related effects observed in both sexes at 350 ppm: increased alkaline phosphatase activity and glutamic-pyruvic transaminase--females only; increased liver glucose-6 phosphatase and glucose-6-phosphate dehydrogenase activity in liver; increased thyroid and liver weights; increased hepatic glycogen storage, accompanied by enlarged centrolobular hepatocytes and a slight hepatitis.) NOAEL ≥ 350 ppm. The study was previously reviewed as unacceptable (Gee, 2/6/88; Choy, 11/7/86 & Morris, 7/7/89), due to missing data. Some of the required data were submitted (volume 051, no record #) and reviewed (M. Silva, 5/18/90), however the study is now considered unacceptable and not upgradeable because dogs were treated with antibiotics and anesthetics while on the study. M. Silva, 7/28/92.
EPA one-liner: Core Minimum. Systemic NOEL = 20 ppm, Systemic LEL = 50 ppm.

213-051 This document contains a rebuttal letter for study 012 045273 as well as additional data for that study, including summary tables (with statistical analysis of data in the summary tables), an analysis of dichlobenil for stability, a QA statement and the purity of dichlobenil. M. Silva, 5/17/90.

231-010, 037612: This document contains an identical copy of 231-012, 045273 (Gee, 02/6/86).

231-006, 016997; "Chronic (Two-Year) Toxicity Study with Dichlobenil (H 133) in Beagle Dogs." This document contains a summary of the study at DPR doc. # 231-012, rec. # 045273 (Gee, 05/09/85).

231-070 (no record #) A letter was received from Solvay Duphar B.V. (1/4/94) which requested that DPR judge the acceptability of the proposed dose levels to be used in the definitive chronic dog study. The "adopted proposal of dose levels" is 0, 1, 6 and 36 mg/body weight/day). DPR considers this to be an acceptable range of doses, based upon the 28-day range-finding study: 070/127948). M. Silva, 2/11/94

No volume, 045273. A letter from Duphar Crop Protection Division (August 1, 1991) proposed to fill the ophthalmology requirement by performing detailed occular pathology in a hamster chronic feeding study. M. Silva, 9/4/91.

A letter (pages only) was submitted by Solvay Duphar B.V. (9/29/92), regarding the current status of the dog study. M. Silva, 12/17/92.

Metabolite:

231-014, 045292; "Toxicity studies on the 'PREFIX' residue 2,6- dichlorobenzamide: Two year oral experiment with dogs", report number TLGR.0028.71, Tunstall Laboratory, 9/71; Choy 11/12/86; 2,6-dichlorobenzamide (97%) was tested in a two year chronic study at 0, 60, 100, 180 and 500 ppm in the diet in beagle dogs; 4 animals/sex/group; decrease in body weight in females at 500 ppm; increase in liver wt./body weight ratio in males at 500 ppm; decrease in liver wt./body weight ratio in females at and above 60 ppm; sporadic increase of gamma globulin levels in females; NOEL = 60 ppm; no adverse effect; incomplete; UNACCEPTABLE; not upgradeable (no food consumption data, incomplete serum chemistry study, and no ophthalomological examination)

231-014, 045296: This document contains the individual animal data for 231-014, 045292 (Choy, 11/12/86).

231-006, 016995; "Toxicity Studies on the 'Prefix' Residue 2,6-Dichlorobenzamide: Two Year Oral Experiment with Dogs." This document contains a summary of the study at DPR doc. # 231-014, rec. # 045292 (Gee, 05/09/85).

231-009, 028392: This document contains a summary of the study at DPR doc. # 231-014, rec. # 045292. No worksheet was done (Morris, 05/22/89).

ONCOGENICITY, RAT

Refer to COMBINED, RAT.

ONCOGENICITY, MOUSE

Subchronic, Mouse:

231-051 088018 "A 13-Week Toxicity Study With Dichlobenil in Male and Female Mice," (A. Kemp, M. de Haan & R.L.F. Dawes, Duphar Crop Protection Division, Weesp, The Netherlands, 4/10/87). Dichlobenil (Batch #: FUN 86L22A, FUN 86L22B, FUN 86L22C & FUN 86L22D; 99.4% pure) was fed in diet to CD1 mice (20/sex control & 10/sex treatment) for 13 weeks at 0 (vehicle = feed), 25, 125, 625 & 3125 mg/kg diet. No adverse effect indicated. NOEL = 25 mg/kg diet (decreased food consumption in females at 3125 mg/kg diet; reduced glucose level in males at $\geq$ 625 mg/kg diet; increased level of cholesterol and phospholipids in both sexes at 3125 mg/kg diet; increased absolute and relative thymus weight in males at 3125 mg/kg diet and an increased absolute and relative testes weight at $\geq$ 125 mg/kg diet; decreased absolute and relative liver weight in both sexes at 3125 mg/kg diet; at 3125 mg/kg diet in males and $\geq$ 625 mg/kg diet in females there was increased glycogen storage--diffusely or in isolated single parenchyma cells; both sexes had increased severity of centrilobular swelling of parenchyma cells at $\geq$ 625 mg/kg diet; males showed inflammatory changes--pyelitis and interstitial nephritis--at $\geq$ 625 mg/kg diet and an increased incidence of basophilic cortical tubules--indicative of regeneration--at 3125 mg/kg diet.) NOAEL = 125 mg/kg diet. These data are supplementary. M. Silva, 5/14/90.

EPA 1-liner: Duphar Crop Protection has committed to performing a new mouse oncogenicity study. 1/24/89.

ONCOGENICITY, HAMSTER

Subchronic, Hamster:

231-051 088017 "A 13-Week Toxicity Study With Dichlobenil in Male and Female Hamster Followed by a 4-Week Recovery Period," (Kemp, A., de Haan, M. & Jager, F.C., Duphar Crop Protection Division, Weesp, The Netherlands, 6/10/87). Dichlobenil (Batch #: FUX 0100000; 98.5% pure) was fed in diet to hamsters (20/sex control & 10/sex treatment) for 13 weeks at 0 (vehicle = feed), 60, 300, 1500 & 7500 mg/kg (diet). Actual doses, due to evaporation, were reported as 41, 209, 1289 and 4648 mg/kg (diet). The high dose was lowered to 5000 mg/kg, since 7500 mg/kg was not tolerated. A separate recovery group was maintained at 5000 mg/kg (10/sex/group for 5000 mg/kg & control). No adverse effect indicated. Nominal NOEL = 60 mg/kg (At 5000 mg/kg both sexes showed reduced body weight and food consumption. At ≥ 300 mg/kg males showed a dose related reduction in absolute and relative prostate weight, accompanied by mineralization. Absolute and relative liver weights in females were increased at ≥ 300 mg/kg--accompanied by an increase in swollen hepatocytes at ≥ 1500 mg/kg and male liver weights were increased at ≥ 1500 mg/kg. Males had decreased absolute and relative seminal vesicle weight, accompanied by a decreased number of spermatocytes in the epididymus and tubular degeneration in testis at 5000 mg/kg. At 5000 mg/kg both sexes displayed gall bladder calculi). These data are supplementary (pilot for a hamster oncogenicity study). M. Silva, 5/9/90.

Oncogenicity Study:

**** 231-064 112960**, "Dichlobenil Potential Tumorigenic Effects in Prolonged Dietary Administration to Hamsters," (P.R. Chambers, S.B. Hornby, W.A. Gibson, C. Gopinath, S. Harling and I. S. Dawe, Huntingdon Research Centre, Laboratory Project I.D. No.: PDR/511, 2/10/92). Dichlobenil (purity = 99.4%) in diet was fed to Bio FID Alexander Syrian hamsters (50/sex/group) at 675, 1500, or 3375 ppm. One hundred hamsters/sex fed normal untreated diet served as controls. Treatment period was 78 and 91 weeks for females and males, respectively. NOEL = 675 ppm (Decreased food consumption and mean body weight change were reported in both sexes primarily at 3375 ppm. Increased liver (both sexes) and decreased testes + epididymides adjusted weights were observed at ≥ 1500 ppm. Macroscopic pathology showed an increased incidence in liver enlargement (both sexes) and pale areas and masses in males at 3375 ppm. Microscopically, males showed an increased incidence in benign and malignant liver tumors at 3375 ppm. Non-neoplastic liver histopathology showed an increase in eosinophilic hepatocytes (males only), centrilobular hepatocyte enlargement, hepatitis, pigmented giant cells, pigmented sinusoidal cells and brown pigment in hepatocytes at 3375 ppm in both sexes but primarily in males.) **Possible adverse effect: Benign liver tumors occurred in 12% of males and 1 malignant liver tumor was also observed at 3375** ppm**.** ACCEPTABLE. (Kishiyama & Silva, 3/23/92).

231-058 097477, "Dichlobenil Potential Tumorigenic Effects in Prolonged Dietary Administration to Hamsters", (W. N. Hooks, R.J. Cary, K. Colman, W.A. Gibson, C. Gopinath, and I. S. Dawe, Huntingdon Research Centre Ltd. I.D. Number: PDR/478, 5/3/91). Dichlobenil Technical (2,6-dichlorobenzonitrile, purity = 99.4%, Batch #: FUN87E08A/FUX011000) was administered by admixture with the diet at concentrations of 5, 26, 132, or 675 ppm to 50 Bio FID Alexander Syrian hamsters/sex/group for 88 weeks (males) or 80 weeks (females). One-hundred hamsters/sex received untreated feed and served as controls. There was no evidence of oncogenicity. Treatment related changes included slightly lower body weight gain, increased incidence of smaller testes, and pale kidneys for males at 675 ppm. Reduction of adipose tissue was reported in males that died on study at 675 ppm. Systemic NOEL = 132 ppm (Decreased body weight gain (both sexes), increased liver weight and increased incidence of centrilobular enlargement of the liver in females was reported.) UNACCEPTABLE. No justification for dose selection (a proposed second study at higher doses may alleviate this concern). (Kishiyama & Silva, 8/30/91).

231-063 093239 "Dichlobenil: Potential Tumorigenic Effects in Prolonged Dietary Administration

to Hamsters (Supplemental Data)," (Hooks, W.N., Cary, R.J., Colman, K. and Gopinath, C., Huntingdon Research Centre Ltd., 6/27/91). This volume contains data supplementary to 058/097477 (chronological list of tumors and microscopic pathology--individual animal summary). M. Silva, 10/25/91.

REPRODUCTION, RAT

** 231-043, 065, 068 076143, 113477, 121307 "Dichlobenil: 2 Generation Oral (Dietary Administration) Reproduction Study in the Rat," (Barker, L., Hazleton UK, Project ID: 5798-65/18, Addendum 1, 1/92, Addendum 2, 1/92 & Addendum 3, 1/93--Pathology Reports). Dichlobenil (Batches FUN 87EO8A and FUX 011000; 99.4% pure), was fed to Crl:CD(SD)BR rats in the diet at 0 (vehicle = diet), 60, 350 or 2000 ppm continuously to both sexes for 2 generations. There were 30 rats/sex in the P and 25/sex in F1 adult generations. Parental & Pup NOEL = 60 ppm (Body weight gain and food consumption were reduced at 2000 ppm for parental animals of both sexes and generations. Body weight gain was reduced at 350 ppm for F1 adult males. Mean pup weights were reduced for both generations at 2000 and 350 ppm.) Reproductive NOEL $\geq$ 2000 ppm (No reproductive effects were observed at any dose). **No adverse effects.** The study was previously reviewed as unacceptable (Silva, 7/7/90, 3/24/92 & 11/24/93), but upgradeable. All requested histopathology was done for the P generation (presented in DPR volume/record #: 065/113477). The study is upgraded to acceptable. M. Silva, 6/2/94.

231-011, 045258, 045260; "Investigation as to the toxicity of H 133 including a chronic experiment with rats", National Institute of Public Health, Holland, date unknown; Gee, 2/6/86; Choy, 10/29/86; Dichlobenil (referred to as H133 in this report) (93.5%) tested at 0, 20, 50 and 100 ppm in the diet in a two-generation study in Wistar rats; 10 rats/sex/group (except 5 rats/sex/group in 100 ppm treated F_1s); decrease in body weight gain in both F_0 and F_1 at high dose; **possible adverse effect**; increased infertility in F_0 and F_1 females in 100 ppm treated groups; NOEL = 50 ppm; unacceptable; incomplete; not upgradeable (multiple major deviations from the EPA guidelines)

EPA one-liner: Reproductive NOEL = 50 ppm, Reproductive LEL = 100 ppm (HDT; decreased viability of F1A pups; decreased fertility for F2A and F2B parents)

231-010, 037606: This document contains an exact duplicate of DPR doc. #231-011; rec. #'s 045258, 045260 (Gee, 02/06/86).

231-011, 045266; "Reproduction Studies in Rats". This document contains a summary of the study at DPR doc. # 231-011; rec. #'s 045258, 045260 (Choy, 11/06/86).

231-004, 005646; "Reproduction Studies in Rats". This document contains a summary of the study at DPR doc. # 231-011; rec. #'s 045258, 045260 (Morris, 05/18/89).

231-009, 028390: This document contains an exact duplicate of 231-004, 005646. No worksheet was done (Morris, 05/22/89).

No volume number, 045273 A letter dated March 27, 1991 from Duphar Crop Protection Company, stated that detailed histopathology of the preserved reproductive organs from the control and high dose parental animals (from study 043/076143) would be performed. M. Silva, 9/4/91.

231-066 115685 "Dichlobenil: 2 Generation Oral (Dietary Administration) Reproduction Study in the Rat. Addendum 2 - Revision to the Pathology Report." This document includes group incidence in histopathological findings in ovaries, vagina and seminal vesicles for the P generation (summary table only, no individual findings). M. Silva, 7/30/92.

A letter from Solvay Duphar B.V. dated 9/29/92 stated that the requested histopathology would be performed to complete the rat reproduction study (DPR Document/Record #: 231-043, 065/076143, 113477). The expected completion date was not indicated. M. Silva, 12/18/92.

Metabolite:

231-013, 045286; "Results of Reproduction Study of Rats Fed Diets Containing 2,6-Dichlorobenzamide (BAM) Over Three Generations"; Hine Laboratories, 10/70; Choy, 10/30/86; 2,6-Dichlorobenzamide (BAM) (99.5%) tested at 0, 60, 100 and 180 ppm in the diet in a three-generation study in Long-Evans rats; 10 males and 20 females/dose group; decrease in body weight of F_2 females and weanlings of F_1 and F_3 at 180 ppm; increase in kidney weight of F_3 females at 180 ppm; increase in liver weight of F_3 males at 180 ppm and females at both 100 and 180 ppm; no adverse effect; NOEL = 60 ppm; UNACCEPTABLE; incomplete; not upgradeable (diet analysis, food consumption data, justification of the highest dose)

231-006, 016992: This document contains a summary of the study at DPR doc. # 231-006; rec. # 045286. No adverse effect was indicated (Gee, 05/09/85).

231-009, 028394: This document contains a summary of the study at DPR doc. # 231-006; rec. # 045286. No worksheet was done (Morris, 05/22/89).

TERATOLOGY, RAT

** 231-012, 032, 051 045275, 066112 "Oral Embryotoxicity/Teratogenicity Study with Dichlobenil in rats," (H.B.W.M. Koeter, Civo Institute TNO, Netherlands, project number B83-1045,1/84). Dichlobenil (98.8% stated purity; batch #: FUX0030000, sample FUN82B07A) was administered by gavage to 21-23 mated (detection of vaginal sperm = day 0 of gestation) female Wistar rats / dose on gestation days 6 - 16 at 0 (vehicle = 1% gum tragacanth), 20, 60, or 180 mg/kg/day. Maternal NOEL = 20 mg/kg/day; NOAEL $\geq$ 180 mg/kg/day (decreased food consumption, food efficiency, and weight gain at 60 and 180 mg/kg/day during gestation days 6 - 16). Fetal NOEL = 20 mg/kg/day (increased total skeletal variants and absent or incomplete ossification of phalanges and sternebrae at 180 mg/kg/day and increased visceral variants at 60 mg/kg and 180 mg/kg/day). The study status of unacceptable with a possible adverse effect (Choy, 11/5/86) was changed, after data re-evaluation, to no adverse effect (volume/record #: 032 066112--Morris, 06/01/89) and to ACCEPTABLE after dose justification was provided (volume 051). M. Silva, 5/16/90.

EPA 1-liner: Core Minimum, 1/24/89.

231-032, 066112; "Oral Embryotoxicity/Teratogenicity Study with Dichlobenil in Rats Addendum to CIVO Report No. V84.026/231045"; This document contained statements and additional data about test material purity (batch FUX0030000, sample no. UN82B07A), doses used, and embryo effects seen in doc. # 231-012, rec. # 045275. Evaluation of this submission resulted in the possible adverse effect being changed to no adverse effect indicated (Morris, 06/01/89).

TERATOLOGY, MOUSE

231-010, 037611; "Investigation to an eventual teratogenic effect of H133 in mice"; National Institute of Public Health, The Netherlands, report # 54/64 Tox; Gee, 02/06/86; Dichlobenil, no purity; 33 females; 0 or 60 mg/kg by oral gavage, days 3-14, NOEL cannot be evaluated, no adverse effect reported, almost no information, incomplete, inadequate

231-011, 045263: This document contains an exact duplicate of DPR doc. # 231-010, rec. # 037611 (Choy, 11/5/86).

231-011, 045264: This document contains a summary of the study at DPR doc. # 231-010, rec. # 037611 (Choy, 11/6/86).

231-004, 005647: This document contains a summary of the study at DPR doc. # 231-010, rec. # 037611 (Gee, 05/10/85).

TERATOLOGY, RABBIT

** 231-042 076142 "Dichlobenil: Oral (Gavage) Teratology Study in the Rabbit (Experiment II) Repeat Study," (L. Barker, author and Study Director; Hazleton UK, Project ID: 5942-65/19, 8-89). Dichlobenil (batch FUX 010000; 98.5% pure) was administered to mated New Zealand White rabbits (day of mating = day 0 of gestation) by gavage at 0 (1% gum tragacanth), 15, 45 or 135 mg/kg/day (18/group). Maternal NOEL = 45 mg/kg/day (decreased bodyweights on gestation days 7-13--3 animals continued to lose weight until the end of treatment; decreased food consumption; increased incidence of intrauterine deaths). Developmental NOEL = 45 mg/kg/day (lower bodyweights and increased incidence of major defects, primarily associated with 3 high dose litters). No adverse effects. ACCEPTABLE. D. Shimer & M. Silva, 5/17/90.

231-042 076141 "Dichlobenil: Oral (Gavage) Teratology Study in the Rabbit (Experiment II) Duphar Report No. 56645/85/87," (L. Barker, author; Hazleton UK, 8-89). Dichlobenil (Batch No. FUX 010000; purity = 98.5%) was administered by gavage to mated (day of mating = day 0 of gestation) New Zealand White rabbits at levels of 0 (vehicle = 1% gum tragacanth), 15, 45 or 135 mg/kg/day on days 7-19 of gestation (16 or 17/group). NOEL (maternal and fetal) = 135 mg/kg/day (HDT). No adverse effects. UNACCEPTABLE (3, 4, 4 and 8 does died or were killed after aborting their fetuses (from groups 1 to 4 respectively), so at termination, the number of rabbits/group was greatly decreased. Animals which were killed had a Pasteurella respiratory infection. The validity of the experiment is therefore in question). D. Shimer & M. Silva, 5/2/90.

GENE MUTATION

Microbial Systems:

** 231-011, 045249; "Ames Test to Assess the Potential Mutagenic Effect of Dichlobenil"; Duphar B.V., Weesp, The Netherlands, 10/83; dichlobenil, 99.44%; The Ames assay was carried out in *Salmonella typhimurium* strains TA1535, TA1537, TA1538, TA98, and TA100 at 0, 40, 200, 1000, or 5000 ug/plate with or without S9 activation system from Aroclor 1254-induced rat liver. The test material precipitated at 5000 ug/plate. No adverse effect was indicated. The study was changed from unacceptable (Choy, 10/24/86) to ACCEPTABLE (Morris, 07/06/89).

EPA 1-liner: Core Acceptable.

231-032, 066113: This document contained a statement of purity for the test material (batch FUX003000, 99.44%) used in doc. # 231-011, rec. # 045249. Evaluation of this

submission resulted in a change of status from unacceptable to acceptable (Morris, 06/06/89).

** 231-011, 045247; "Report on the results of mutagenicity test on DBN with bacteria"; Institute of Environmental Toxicology, Japan; 01/22/81; dichlobenil, 99.5%; The Ames assay was carried out in *Salmonella typhimurium* strains TA100, TA1537, TA1535, WP2 hcr, TA98, TA1537, and TA1538 at 0, 5, 10, 50, 100, 500, 1000, or 5000 ug/plate with or without S9 activation system from Aroclor 1254-induced rat liver. No adverse effect was indicated. The study was changed from unacceptable (Choy, 10/23486) to ACCEPTABLE (Morris, 07/06/89).

EPA 1-liner: Core Acceptable.

231-011, 045241; "Genetic Effects of Herbicides: Induction of Mitotic Gene Conversion in *Saccharomyces Cerevisiae*", Mutation Research, vol. 22, 1974, pp. 111-120; dichlobenil, commercial preparation, purity not stated. Three trials were conducted in which 5 x 10^7 cells of *Saccharomyces cerevisiae*, strain D_4 (auxotrophic for adenine and tryptophan) were exposed to 2.0 mls of 1.0 mg/ml for 16 hours. The cells were then seeded at 10^6 cells / plate on 5 plates with media deficient in adenine and 5 plates deficient in tryptophan. The plates were scored 6 days later. Parallel platings of 250 cells / plate on complete media produced 110% viability. No increased gene conversion to heterotrophic mutants was seen. No adverse effect was indicated. The study was UNACCEPTABLE because cytotoxicity was not seen at the highest dose (Choy, 11/06/86; Morris, 05/22/89).

Mammalian Systems:

231-011, 045244; "The Mutagenic Potential of Dichlobenil Technical in the In Vitro Mouse Lymphoma L5178Y Cell Mutation Test", HRC Report No. PDR 381/831001; Huntingdon Research Centre, Cambridgeshire, England; 4/9/84; dichlobenil, stated purity 99.44%; The forward mutation rate at the thymidine kinase locus was measured in mouse lymphoma cells L5178Y $TK^{+/-}$ by selecting for growth of $TK^{-/-}$ mutants in the presence of trifluorothymidine 48 hours after a 3 hour exposure to the test material. Two tests were conducted in the presence of S9 activating system from Aroclor 1254-induced rat liver, one with exposures of 0, 20, 30, 40, 50, 60, or 70 ug/ml, and the other with 0, 5, 10, 20, 25, or 30 ug/ml. Two tests were conducted without S9 at 0, 130, 160, 190, 220, 250, or 280 ug/ml. A **possible adverse effect** was indicated by increased mutation frequencies in all test groups. The study status was UNACCEPTABLE because of inadequate rationale for dose (Choy, 10/15/86; Morris, 05/10/89).

EPA 1-liner: Core Acceptable.

231-032, 066115: This document contained a statement of purity for the test material (batch FUX003000, 99.44%), cytotoxicity measurements, statistical analysis, and criteria for a positive test used in doc. # 231-011, rec. # 045244. Evaluation of this submission resulted in no change of the study status (Morris, 05/10/89).

231-006, 016993; "Evaluation of Herbicides for Possible Mutagenic Properties", J. Agr. Food Chem., 20:649-656 (1972). This document contains a summary of a mutagenicity study on the test material. No adverse effect was indicated (Gee, 05/10/85).

231-011, 045243: This document contains an exact duplicate of 231-006, 016993 (Choy, 10/10/86).

Summary Statement: The two bacterial mutation studies fill the data requirement for the gene mutation test type with no adverse effect indicated. A possible adverse effect was indicated in the mouse lymphoma study. However, the study was unacceptable and the adverse effect findings were equivocal. Collectively, the data from all the evaluated gene mutation studies do not support the

finding of a possible adverse effect for dichlobenil (Morris, 06/01/89).

CHROMOSOME EFFECTS

** 231-011, 045245; "Test on Mutagenic Potential of Dichlobenil In Vitro Study of Metaphase Chromosomes of Human Lymphocytes" (HCR Report No. PDR380/83977); Huntingdon Research Centre, Cambridgeshire, England, 4/3/84; dichlobenil, 99.44%; Cultured human lymphocytes were exposed to 0.1, 0.5, or 1.0 ug/ml for 2 hours with or 24 hours without S9 activation system from Aroclor-1254-induced rat liver. Cytotoxicity was demonstrated at 1 ug/ml by Z 50% decrease in the mitotic index. Scoring 100 cells / dose revealed no dose-related increases in chromatid gaps, breaks, interchanges, or acentric fragments. No adverse effect was indicated. The study status was changed from unacceptable (Choy, 10/23/86) to ACCEPTABLE (Morris, 05/09/89).

231-032, 066114: This document contained a statement of purity for the test material (batch FUX003000, 99.44%) and a rationale for the doses used in doc. # 231-011, rec. # 045245. Evaluation of this submission resulted in a status change from unacceptable to acceptable (Morris, 05/09/89).

231-011, 045248; "Micronucleus test on dichlobenil"; Duphar B.V., Weesp, The Netherlands, 10/83; dichlobenil; 99.44%; Two doses, 24 hours apart, of 150, 300, or 600 mg/kg were given by oral gavage to 5 mice / sex / group. Six hours after the second dose, bone marrow sample were taken and examined by light microscopy for micronucleated polychromatic erythrocytes. No adverse effect was indicated. The study was UNACCEPTABLE and not upgradeable because the compound was not tested to cytotoxic levels and only one sacrifice time was used (Choy, 10/24/86; Morris, 07/06/89).
EPA 1-liner: Core Acceptable.

231-032, 066118: This document contained a statement of purity for the test material (batch FUX003000, 99.44%) and a rationale for the doses used in doc. # 231-011, rec. # 045248. Evaluation of this submission resulted in no status change and no worksheet was done (Morris, 05/09/89).

DNA DAMAGE

231-011, 045246; "Report on the Results of Mutagenicity Test on DBN with Bacteria"; Institute of Environmental Toxicology, Japan; 01/22/81; dichlobenil, 99.5%; The Rec-assay was carried with *B. subtilis* by measuring the zone of inhibition of growth around disks of filter paper impregnated with 0, 20, 50, 100, 200, 1000, 2000, or 5000 ug. A repair-deficient strain (M45) was compared to a wild-type reference strain (H17). No inhibition of growth was seen for either strain. Appropriate positive and negative controls were used. No adverse effect was indicated. The study was UNACCEPTABLE and not upgradeable because trials using metabolic activation were not used (Choy, 10/23/86; Morris, 05/17/89).

EPA 1-liner: Core Acceptable.

231-011, 045250; "Autoradiographic Assessment of DNA Repair in Mammalian Cells after Exposure to Dichlobenil Technical"; Huntingdon Research Centre, Cambridgeshire, England, 5/4/84; dichlobenil technical, 99.44%; In 2 independent trials, 2 dishes of confluent monolayers of HeLa S3 cells were arrested in G_0 and exposed for 3 hours in the presence of [^{3}H] thymidine to 0, 0.05, 0.1, 0.2, 0.4, 0.8, 1.6, 3.2, 6.4, 12.8, 25.6, 51.2, or 102.4 ug/ml with or without S9 activation system from Aroclor 1254-induced rat liver. One hundred nuclei / dish were analyzed by autoradiography for ^{3}H incorporation. No adverse effect was indicated. The study was UNACCEPTABLE and not upgradeable because nuclear grain counts were not corrected for background cytoplasmic counts,

cell viability was not assessed, and dose-effect relationships for the positive controls were not demonstrated (Choy, 10/27/86; Morris, 05/22/89).

EPA 1-liner: Changed from Core Acceptable to Unacceptable, 1/24/89.

231-032, 066116: This document contained statements about the purity of the test material (batch FUX003000, 99.44%), lack of cytoplasmic grain counts, and the significance of positive findings in doc. # 231-011, rec. # 045250. Evaluation of this submission resulted in no status change and no worksheet was done (Morris, 05/09/89).

231-011, 045251; "Evaluation of Dichlobenil Technical in the In Vitro Transformation of BALB/C-3T3 Cells with S9 Activation Assay"; Litton Bionetics, Inc.; 7/84; dichlobenil, 99.44% stated purity; Twenty dishes / dose of 3 X 10^4 BALB/C-3T3 mouse cells were exposed for 4 hours to 667, 2667, 4000, 6000, or 7500 ug/ml in the presence of S9 from Aroclor 1254-induced male Fisher 344 rat livers and scored for transformed foci 24 - 31 days later. Fifty % relative survival was seen at 4000 ug/ml in a preliminary cytotoxicity test. No adverse effect was indicated. The study was UNACCEPTABLE not upgradeable because a test was not performed without S9. (Choy, 10/28/86; Morris, 05/16/89, 12/04/89).

231-032, 066117: This document contained statements about the purity of the test material (batch FUX003000, 99.44%) in doc. # 231-011, rec. # 045251. Evaluation of this submission resulted in no status change and no worksheet was done (Morris, 05/16/89).

231-048, 085319: This document contained statements that answered DPR's concerns about the adequacy of the positive controls in doc. # 231-011, rec. # 045251. Evaluation of this submission resulted in no status change and no worksheet was done (Morris, 12/04/89).

231-048; 085320; "Evaluation of dichlobenil technical in the in vitro transformation of BALB/3T3 cells assay", LBI Project No.: 20992; Litton Bionetics, Inc., Kensington, MD; 7/84; DNA Damage (844); dichlobenil, 99.44% stated purity; Twenty dishes / dose of 3 X 10^4 BALB/C-3T3 mouse cells were exposed for 72 hours to 240, 480, 720, 900, or 1125 ug/ml and scored for transformed foci . 4 weeks later. Preliminary cytotoxicity test showed 16% relative survival at 1000 ug/ml. No adverse effect was indicated. The study was UNACCEPTABLE not upgradeable because a test was not performed with metabolic activation (Morris, 12/04/89).

Summary Statement: Although no individual study is acceptable, data in the studies above (DPR doc. #'s 231- 011, 032, 048; rec. #'s 045251, 066117, 085319, 085320) collectively fill the data gap for the DNA Damage test type with no adverse effect (Morris, 12/04/89).

SUPPLEMENTAL INFORMATION

231-006, 017140; "The Toxicity of WL Compounds Numbers 3379 and 5792 for Vertebrates." This document contains a summary of toxicity studies on the test material. No worksheet was done (Morris, 05/18/89).

231-006, 017151; "Toxicology of the Herbicide Dichlobenil (2,6-Dichlorobenzonitrile) and its Main Metabolites", Food and Cosmetic Toxicology, Vol. 6, pp. 261-269. This document contains a summary of toxicity studies on the test material and metabolites. No worksheet was done (Morris, 05/18/89).

231-004, 005645; "Cumulative Toxicity of Dichlobenil." This document contains a summary of toxicity studies on the test material. No worksheet was done (Morris, 05/18/89).

231-009, 028389: This document contains an exact duplicate of 231-004, 005645. No worksheet was done (Morris, 05/22/89).

231-011, 045262; "Investigation of the Possible Initiating Properties of H133 in Combination with Croton Oil in Mice." This document contains a study type that is not required. No adverse effect was indicated (Choy/Parker, 11/18/86).

231-010, 037610: This document contains an exact duplicate of 231-011, 045262 (Gee, 02/06/86).