

CALIFORNIA ENVIRONMENTAL PROTECTION AGENCY
DEPARTMENT OF PESTICIDE REGULATION
MEDICAL TOXICOLOGY BRANCH

SUMMARY OF TOXICOLOGY DATA
TRICHLORFON

CHEMICAL CODE: 88, TOLERANCE #198
SB 950 #: 014

March 9, 1987

Revised 7/8/88, 10/5/89, 9/15/92, 6/11/93, 9/28/95, 6/4/96, and 1/6/99

I. DATA GAP STATUS

Oncogenicity, rat: No data gap, possible adverse effect.
Chronic, rat: No data gap, possible adverse effect.
Chronic, non-rodent*: No data gap, no adverse effect.
Oncogenicity, mouse: No data gap, possible adverse effect.
Reproduction, rat: No data gap, possible adverse effect.
Teratogenicity, rat: No data gap, no adverse effect.
Teratogenicity, rabbit: No data gap, no adverse effect.
Gene mutation: No data gap, possible adverse effect.
Chromosome effects: No data gap, possible adverse effect.
DNA damage: No data gap, possible adverse effect.
Neurotoxicity: No data gap, no adverse effect.

* Data requirement was filled by a monkey study.

Toxicology one-liners are attached

In the one-liners below:

** indicates acceptable study

Bold face indicates possible adverse effect

Recent updates of this Summary were by T. Kellner, 6/11/93; P. Iyer, 9/28/95; P. Iyer and C. Aldous 6/4/96, and C. Aldous, January 6, 1999.

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All relevant records indexed by DPR as of 1/5/99 have been included in this Summary. This includes record numbers through 163507 (in Document No. 198-194). Also, all relevant record numbers of the series 900,000+ are included.

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

NOTE: Trichlorfon has some medical applications and is being evaluated for some new ones. It has synonyms such as Memaron and Metrifonate in pharmaceutical contexts (see DPR worksheet for Record No. 163507).

II. TOXICOLOGY ONE-LINERS AND CONCLUSIONS

A published article by Gibel *et al.*, 1973, (060:14170) notes that trichlorfon is rearranged in water to form DDVP (p. 6 of report). For this reason, risk assessment involving crop residues should consider residue chemistry data in evaluation of trichlorfon. Aldous, 5/1/86.

COMBINED, RAT

[Overall evaluation of 2 rat dietary "combined" studies: 198-144:073508 and 198-157:098125]. Title of the main study (Mobay Report No. 98569 = DPR Record No. 073508) is "Chronic Toxicity/Oncogenicity Study of Technical Grade Trichlorfon (Dylox®) with Rats", Mobay Chemical Corporation, 3/31/89. The other record (Mobay Report No. 98569-1 = DPR Record No. 098125) entailed only controls plus one dose level (2500 ppm: well above the highest dose level used in the main study, 1750 ppm). The primary purpose of this worksheet is to correlate the major findings of these two studies regarding cholinesterase enzyme inhibition, cholinesterase-inhibition related clinical signs, and major chronic effects. NOEL for cholinesterase enzyme inhibition is 100 ppm [based on a statistically significant (18%) inhibition in brain cholinesterase in 300 ppm males at 2-yr termination]. NOEL for clinical signs plausibly related to cholinesterase inhibition = 1750 ppm (based on urine staining in 2500 ppm females). NOEL for histopathology = 100 ppm (based on hyperplasia in small intestine, gastritis in the nonglandular stomach, and calcification of kidneys at 300 ppm). There was also a consistent increase in blood cholesterol at 300 ppm in males. Common findings at 1750 ppm in one or both sexes included histopathology in kidneys (especially chronic nephropathy, often with calcification), liver (cytoplasmic vacuolation, cystic degeneration, centrilobular degeneration, sinusoidal dilatation, and nodular regeneration), lung (type II pneumocyte hyperplasia, sometimes manifest as adenomatous hyperplasia; chronic inflammation), small intestine (hyperplasia, and occasionally erosion/ulcer or acute inflammation), non-glandular stomach (gastritis). In general, 2500 ppm caused similar lesions, often more severe. Two tumor types appeared to be increased due to treatment at 2500 ppm: the incidences were not statistically significantly elevated for either type. One of these was alveolar/bronchiolar tumors: with elevations of adenomas in males and of carcinomas in females. The other was renal tubular adenomas in 2500 ppm males. For reasons presented in this review, both tumor types appear to be associated with predisposing non-neoplastic lesions. These lesions were limited to high dose levels, and may not reflect increased risks of tumors at substantially lower dose levels. Studies have already been flagged for "possible adverse effects", considering especially the tumors, other histopathology at higher dose levels, and brain cholinesterase inhibition. Aldous, 5/24/96.

**** 198-144 073508**, "Chronic Toxicity/Oncogenicity Study of Technical Grade Trichlorfon (Dylox®) with Rats", (Mobay Corporation, Health, Environment, Safety and Plant Management, Corporate Toxicology Department, KS., report # 98569, 3/31/89). Technical grade trichlorfon, 98.8% purity, reference # 150-8-79, was fed in the diet for 2 years at 0, 92.2, 273 or 1518 ppm (mean analytical concentrations). Nominal concentrations were 0, 100, 300, with the highest level at 1000 ppm for 27 weeks, 1250 ppm for 5 weeks, 1500 ppm for 8 weeks, and 1750 ppm for the remaining 65 weeks, to give an average treatment level of 1518 ppm). There were 50 Fischer 344 rats/sex/dietary level. Acceptable (Green and Silva, 9/15/89). (See preceding 1-liner and associated worksheet by Aldous for findings of record Nos. 073508 and 098125).

198-157 098125 "A Combined Chronic Toxicity/Oncogenicity Study of Technical Grade Trichlorfon (Dylox®) with Rats", (W. R. Christenson, Mobay Corporation, Health, Environment, Safety and Plant Management Corporate Toxicology Department, Stilwell, KS, Report # 98569-1, 9/28/90). Trichlorfon technical, 98.5% purity, was fed to 50 Fischer 344 [CDF(F-344)/CrI/Br] rats/sex/group for 2 years and to satellite groups (sacrificed at 12 months) of 20/sex/group at 0 and 2500 ppm. Body weights averaged 4 to 11% less in the 2500 ppm group compared to control. Increases in non-neoplastic lesions (e.g. hepatocellular hyperplasia, hyperplasia and inflammatory foci of the lungs, renal calcification, nonglandular stomach gastritis) as well as increases in neoplastic lesions (renal tubular adenoma, alveolar/bronchiolar adenoma and carcinoma and mononuclear cell leukemia) were noted. Increased absolute and relative male and female kidney and liver weights were also reported. Possible adverse effects (i.e. small intestine hyperplasia) seen in study 198-144 073508 were confirmed by this study. **Supplemental** (Green, Kellner and Gee, 6/26/92). See overall evaluation of this study, and of the primary combined study above, immediately below the heading "Combined, Rat" (new 1-liner and associated worksheet by Aldous, 5/24/96).

198-166 118421 [Addendum to 198-157:098125 and -144:73508] Warren, D. "Combined Chronic Toxicity/Oncogenicity Study of Technical Grade Trichlorfon (Dylox®) with Rats" (Miles, Inc. No. 98569-2). This supplemental submission was in response to a request for additional data from the Health and Welfare Department, Health Protection Branch of Canada. These data included group mean body weight values for study (and satellite) males and females, details of control diet ingredients, individual clinical and behavioral observations from the 2500 ppm group that showed signs and a listing of the criteria utilized by the pathologist in determining the grading system for histopathological lesions. Supplemental information had no effect on study status. No worksheet. Kellner, 6/15/93.

CHRONIC TOXICITY, RAT

(NOTE: The accepted rat study (144 073548) and supplemental study (157 098125), supersede the following studies, which do not approach modern guideline standards).

198-018 908074 (Tab 19246) (5/31/62, Univ. of Chicago) Trichlorfon, presumed technical: 0, 50, 250, 500, and 1000 ppm in diet. Apparent NOEL = 500 ppm (reduced survival in M and F, reduced weight gain in M, slight cholinesterase inhibition in M RBC's). **Unacceptable**, incomplete, not upgradeable. No apparent adverse effects. Only 25 rats/sex/group, and histopathology on only 5/sex/group at term, Insufficient information to assess chronic or oncogenicity effects. J. Christopher 3/27/85.

EPA 1-LINER: Chemagro #19246 Oncogenic NOEL = 50 ppm Oncogenic LEL = 250 ppm (increased incidence of mammary tumors in females ChE NOEL = 250 ppm ChE LEL = 500

ppm (serum ChE) Syst. NOEL = 250 ppm (slight increase in testis weight to body ratio; and absence of primitive ova. No core grade.

198-018 908076 (Tab 19247) (3/5/65, Univ. of Chicago, Doull *et al.*) Trichlorfon, presumed technical: 0, 100, 200, and 400 ppm in diet. NOEL cannot be estimated: too little information. No apparent adverse effects. Insufficient information to assess chronic or oncogenicity effects. An unacceptable long-term study terminated prematurely due to excess mortality. Too few animals evaluated microscopically to be meaningful for risk assessment. **Not acceptable**, not complete, not upgradeable. Reviewed by J. Christopher, 2/25/85 and 3/27/85.

EPA 1-LINER: Chemagro #19247 Syst. NOEL = 200 ppm Syst. LEL = 400 ppm
ChE NOEL = 200 ppm ChE LEL = 400 ppm (20% inhibition) Core grade: None

198-018 908078 Partial duplicate of 14163 from vol. 060 (Institut für Toxikologie, 7/66, Mobay AG Tab 19292) Trichlorfon, presumed technical: 0, 50, 250, 500, and 1000 ppm in diet. Rat feeding study in which no significant adverse effects were noted. The study was plagued with deficiencies. Apparent NOEL = 500 ppm (Minor cholinesterase inhibition at 1000 ppm). Substantial problems with respiratory disease (Tables 1-10) were noted. There were no histopathology data and no tissue accounting table. Test article was poorly defined, and numerous other deficiencies were noted. Rec. #14163 is a duplicate reviewed by Christopher, except certain raw data were included. Additional information includes hematology (5 rats/sex/group, term only), whole blood cholinesterase data from 10 rats/sex/group (week 89), and organ weight data. The additional data were not sufficient to warrant a separate formal review, as the major deficiencies remain. **Not acceptable**, not complete, not upgradeable. No apparent adverse effects. Insufficient information to assess chronic or oncogenicity effects. J. Christopher 3/27/85.

198-060, -088, -093 014163 More complete report of 908078. See above.

018, 060, 088, 093 014164 Histology data for report 908078. See above.

088 027018 Summary of 908074, 908076, and 908078.

088 027016 Journal article (Down to Earth, Vol. 35, No. 2, Spring 1979), contains summary information.

CHRONIC TOXICITY, DOG

Available dog chronic studies, taken together, do **not** indicate a significant adverse effect. Two chronic dog studies, 018/908072 and 060/14161, were re-examined by C. Aldous. Neither study adequately fills the non-rodent chronic study data gap. Most apparent effects were seen at comparatively high doses (800 ppm or higher). Dr. Christopher flagged both studies as having potential adverse health effects. This reviewer (C. Aldous) does not find evidence of specific, dose-related toxicity which warrants re-evaluation or flagging as a significant human health risk. In most cases, apparent effects were restricted to high dosage levels. In the case of possible male adrenal effects in the earlier study, 018/908072, group sizes were too small to attach much meaning to the presence of cortical hyperplastic nodules in all groups except controls. Comparable findings were not seen in the 1970 study, 060/14161.

198-060, -093 014161 (Tab and Registrant's study I.D.#29372). (11/24/70, Institute für Toxikologie) Trichlorfon, presumed technical: 0, 50, 200, 800, and 3200 ppm in diet for 4 years 4/sex/group). Apparent NOEL = 200 ppm (mortality, decreased body weights, unkempt appearance at 800 and 3200 ppm). Elevated SGOT and SGPT and some evidence of fatty livers noted at 3200 ppm. Cholinergic signs at 3200 ppm. **Not acceptable**, not complete, not upgradeable. Toxic effects limited to high doses. Numerous deficiencies, including inadequate histology, apparent confounding effects of lung infections, and lack of adequate individual data. J. Christopher 3/27/85, C. Aldous 4/18/86.

198-018 908072 (also vols. 060, 088, 093. Tab, Registrant study I.D.#8644). (2/3/62, Univ. of Chicago) Trichlorfon, presumed technical: 0, 50, 250, 500, and 1000 ppm in diet (2/sex/group). No NOEL indicated: hypertrophy or nodules in adrenal cortex: all dosages in males and 1000 ppm in females. Lymphoid atrophy and congestion in spleen [possible artifact due to technical error during autopsy], also decreased cholinesterase in serum and RBC at 500 ppm. **Not acceptable**, not upgradeable: too few animals, inadequate clinical chemistry, inadequate pathology/histology, insufficient individual data. J. Christopher 3/27/85.

CHRONIC, MONKEY

198-137 069086, Griffin, T.B., "Safety Evaluation and Tumorigenesis of Trichlorfon in Rhesus Monkeys: A Ten Year Study", (Coulston International Corporation, White Sands Research Center, Report #90237, 4/6/88). Trichlorfon, lot # PT9068/74, 99.1% purity, and lot # PT809731038, 98.6% purity, was administered 6 days/week for 10 years by imbibition from a syringe at 0 (Tang® orange-flavored drink), 0.2, 1.0, or 5.0 mg/kg/day with 5/sex/group. **No adverse chronic effect. Cholinesterase NOEL = 0.2 mg/kg/day (dose-related cholinesterase inhibition, over 20% in RBC and brain in both sexes, and in plasma of males). Brain cholinesterase inhibition at 10-yr termination was noteworthy: at dose levels of 1 and 5 mg/kg/day, respectively, brain cholinesterase inhibition was 53 and 83% of controls in males, and 24 and 78% of controls in females. Cholinesterase-related clinical signs NOEL = 1.0 mg/kg/day, based on pupillary constriction (2 high dose females only, both limited to the first month of study), muscle fasciculations (limited to 1 high dose female on one occasion: day 15 of study), and a noticeable increase in incidence of soft stools or diarrhea in high dose monkeys throughout the study, in comparison with other groups. There were no gross or microscopic findings attributed to treatment, despite periodic biopsies of liver tissue during the first 3 years of the study, and complete gross and histopathology examinations at termination. Thus the NOEL for chronic effects (other than those plausibly related to cholinesterase inhibition) was ≥ 5 mg/kg/day. **Acceptable**. (Green and Silva, 9/20/89). 1-liner update by Aldous, 5/16/96.

198-100 033961 Interim report to Record No. 069086, above.

ONCOGENICITY, RAT

NOTE: the acceptable rat "combined" study above supersedes any of the following studies. In particular, note that the dose level used in Record No. 014170 is an order of magnitude lower than higher doses used in the "Combined" study (Record No. 073508) and its associated study

(Record No. 098125). The latter studies define the oncogenicity concerns for the rat. Aldous, 5/30/96.

198-060 014170 (1/13/73, Mobay #39648) Published in Archive of Tumor Research 41(4):311-328 Trichlorfon, "The current commercial product". 0 and 15 mg/kg, gavage or im 2/wk. Reported increases in frequency of benign and malignant tumors, particularly in orally-dosed rats. Malignant tumors in orally dosed rats included two reticulosarcomas, vs. no malignant tumors in controls. Most common benign tumors were papillomas in the esophagus, with apparent statistically significant increase in the 15 mg/kg gavage-treated group. **Not acceptable**, not complete, not upgradeable. The report is sketchy and poorly organized. Histology was inadequate. Insufficient information for proper risk evaluation, however possible adverse effects noted. J. Christopher 2/25/85, C. Aldous 5/14/86.

198-070, -090, -091 038810, 027062 (also partial duplicate in vol. 113) (7/81, Albany Medical College) Combined 2-generation and 2-year oncogenicity study. Trichlorfon, presumed technical: 0, 100, 300, and 1000 ppm in diet. Apparent NOEL = 300 ppm (Cholinesterase inhibition at 1000 ppm in plasma, RBC's and brain). **Not acceptable**, not complete, not upgradeable. Study apparently suffered from severe management problems: no gross or histopathology data are presented, or hematology, blood chemistry data, etc. Tissues may have been lost (see p. 9 of report). No useful data. No apparent adverse effects. Insufficient information to assess chronic or oncogenicity effects. C. Aldous 9/18/85. [Title page to this report also found in 070:10797].

EPA 1-LINER: Carcinogenic potential negative up to 1000 ppm. No core grade.

198-092 027067 "Toxicology of Insecticidal Organophosphates," (Kimmerle, G. and Lorke, D., Pflanzenschutz-Nachrichten 21:111-142, 1968; Institute of Toxicology Farbenfabriken Bayer AG). Trichlorfon, (grade and purity not stated, but presumed technical) was administered at 30 mg/kg by gavage or injection 3/wk until death. Liver necrosis, fatty liver, cirrhosis, and nodular hyperplasia in liver at 30 mg/kg (only dose tested). **Not acceptable**, not complete, not upgradeable. Insufficient information to meaningfully assess chronic or oncogenicity effects. C. Aldous, reviewed prior to 3/9/87. No worksheet was done for this study.

198-113 055436 Partial duplicate of 038810, 027062.

198-088, -093 027009, 027010 Summary information.

198-088, -092 027011 Summary of 01470 and 027067.

198-088 027012 Journal article (Pesticides Abstracts, May 1974, Vol. 7, No. 5, Abstracts 74-1013-1266). Summary information.

198-060, -088, -092 014169, 027013 Journal article (Archive of Tumor Research, Vol. 37, No. 4 (1971), pp. 303-312). Summary information for 027067 and others.

198-088 027014 Summary information.

198-088, 092 027015 Summary of 014170.

198-092 027063, 027065, 027066, 027067 Summary information.

ONCOGENICITY, MOUSE

****198-138 069662** Hayes, R. H., "Oncogenicity study of technical grade trichlorfon (Dylox®) with mice", Mobay Corporation, Stilwell, KA, 7/28/88. Mobay Report No. 97471. Technical Grade Trichlorfon (Dylox), $\geq 97.3\%$ purity, was fed in the diet for 104 weeks at 0, 300, 900, and 2700 ppm (nominal) to 50 CD-1 mice/sex/group. There was no cholinesterase NOEL in this dose range (dose-related brain cholinesterase inhibition was significant at all dose levels). Plasma and RBC cholinesterase inhibition occurred at 900 to 2700 ppm. NOEL for effects other than cholinesterase inhibition was 300 ppm (liver relative weight increases in females at 900 to 2700 ppm). Common findings at 2700 ppm included increased urine staining in males, possibly caused by cholinesterase inhibition. Mammary tumors (adenomas, adenocarcinomas, or adenoacanthomas) were elevated in high dose females: incidences of 0, 300, 900, and 2700 ppm females were 1, 2, 0, and 8, respectively. These are considered treatment-related and thus a "**possible adverse effect**". A consideration of the high dose stressors and the lack of mammary tumors at 900 ppm suggest against a linear dose-response. Original review, including that of supplementary data in 198-141 070825 (a peer review of the histopathology of 10% of the mice, plus all neoplasms and possible test-article related lesions) was by Green and Silva, 9/18/89. Supplementary worksheet by Aldous and Iyer on June 3, 1996 made a change in study status to indicate a "possible adverse effect".

198-148 091348 supplies corrected, replacements for pages 20 (corrected purity of 98.4% vs originally reported 98.2%), 258 (illegible data are replaced with clearly printed figures), 895 and 1885 (death and necropsy dates are corrected from March 11, 1986, originally reported, to March 11, 1987) of record # 069662 in 198-138. The report remains **acceptable**. Green and Gee, 9/1/92.

198-194 163507 Stuart, B. P. (author of supplement), "Statistical analysis of survival and tumor data from an oncogenicity study of technical grade trichlorfon (Dylox®) with mice" (title of supplement). The supplementary data were prepared for FDA requirements in support of pharmaceutical products of trichlorfon (also called metrifonate in pharmaceutical literature). The analysis was also submitted to DPR, where it was reviewed as an addendum to Document # 198-138, Record # 069662. Supplement Report date: 7/24/98. Supplement Report # 97471-3. Analysis acknowledges that combined epithelial mammary tumor incidence was statistically significantly elevated in high dose females. Analysts noted several observations consistent with the high dose increased incidence being an incidental finding. Observations included (1) only combined epithelial mammary tumor incidences were statistically significant (but not any one morphological type), (2) there was no linear dose-response, since no such tumors were found at the medium dose level, (3) there was no reduction in latency, (4) there were no predisposing non-neoplastic lesions in mammary tissues, and (5) the combined epithelial mammary tumor incidence was only slightly higher than historical literature upper limits for individual studies. The 1996 DPR review considered similar factors, but concluded that the high dose tumor increase was plausibly treatment-related, and consistent with altered physiology at that dose level. New analyses do not alter study status, but provide a valid alternative interpretation of the data. Aldous, 1/5/99.

Pilot Study

198-138 057113, "Pilot Study on Trichlorfon with Mice", (Mobay Chemical Corporation, Environmental Health Research, Corporate Toxicology Department, report # 90278, 8/30/85),

Technical Trichlorfon (Dylox), 98.2% purity, fed in the diet for 8 weeks at 0 (corn oil), 100, 300, 900, and 2700 ppm (nominal) with 15 CD-1 mice/sex/group. **No adverse effect** indicated. Systemic NOEL \geq 2700 ppm (nominal). ChE NOEL = 100 ppm (nominal). **These data are supplemental to study #069662.** (Green and Silva, 9/15/89).

198-060 014160 (Tab #68788) (Albany Medical College, 6/6/80) Insufficient data: one-page summary only. No adverse effects were reported. J. Christopher 3/27/85.

The full 5-volume report is located at Stanley Research Center, EHR Data Center, and should be obtained by DPR.

198-060 014170 "Hepatotoxic and Cancerogenic Effect of Trichlorfon," (Archive of Tumor Research 41:311-328, 1973.) Trichlorfon (grade and purity not stated but presumed technical) was administered at (presumed) 15 mg/kg/day. Apparent adverse effect: "Myeloid leukoses" reported in mice, but no individual data. **Not acceptable**, not complete, not upgradeable. Too few animals, only one dose, insufficient information for assessment. J. Christopher, 3/27/85.

REPRODUCTION, RAT

****198-159 112029** Eigenberg, D. "A Two-Generation Dietary Reproduction Study in Rats Using Technical Grade Trichlorfon (Dylox®)" (Mobay Corp., Health, Environment, Safety and Plant Management, Corporate Toxicology Dept., Stilwell, KS, Report #101937, 12/20/91). Trichlorfon technical, 98.5% purity, was administered in the diet to 30 Sprague-Dawley rats/sex/group at nominal doses of 0, 150, 500 and 1750 ppm beginning at seven weeks of age for the F₀ parents and at weaning for the F₁ parents. Treatment-related reductions in birth weight in F_{1a} pups continued into adulthood with F₁ adults showing lower pre-mating and gestation body weight; food consumption was reduced in F₀ and F₁ dams during lactation. Dose-related kidney effects - dilated renal pelvis were seen in F_{1a} (F_{1a} + F₁ = 5, 7, 10 and 23 at 0, 150, 500 and 1750 ppm) and F_{2a} pups (F_{2a} = 2, 8, 6 and 31 at 0, 150, 500 and 1750 ppm). Gross and microscopic lesions were noted in the lungs (e.g. pneumonia) and kidneys (e.g. chronic nephropathy and hydronephrosis) of high-dose F₀ and F₁ adults. Cholinesterase (ChE) was inhibited in adult brain, plasma and RBC (NOEL = 150 ppm) and in pup brain and plasma at the high-dose. **Possible Adverse Effect:** Reduction in the birth and viability indices together with reductions in weight and kidney lesions in pups; **Reproductive NOEL = 500 ppm.** Initially reviewed as unacceptable (discrepancies in some of the data tables and inadequate justification for the dose levels used) Kellner and Gee, 8/19/92.

Upon submission of supplementary information 145 75193, 160 113393, 169 120990, 166 118420, 159 112029 and 173 127144 (data from pilot study for high dose determination), **change in study status to acceptable.** (P. Iyer, 9/12/95).

198-173 127144 159 112029 Supplementary submission (Miles No. 101937-3) provided justification for dose levels used in the reproductive study. Submission of data from pilot study (dated 11/16/90, memo to the study file) (P. Iyer, 9/12/95).

-166 118420 [Addendum to -159:112029] Eigenberg, D. "A Two-Generation Dietary Reproduction Study in Rats Using Technical Grade Trichlorfon (Dylox®)" (Miles, Inc. No. 101937-1). This supplemental submission was in response to request by the Health and Welfare, Health Protection Branch, Canada for the group mean mortality data for the adult

generations, individual body weights of pups at termination and definitions of the grading system for the histopathological lesions. No worksheet. Kellner, 6/15/93.

198-169 120990 [Addendum to -159:112029] Supplementary submission (Miles No. 101937-2) provided clarification and corrections to various tables related to reproductive parameters. Also included was justification for dose levels used in the reproductive study. Kellner and Gee, 6/2/93.

198-160 113393 is an **Adverse Effects Disclosure** dated 3/3/92 relating to the study entitled "A Two-Generation Dietary Reproduction Study in Rats Using Technical Grade Trichlorfon (Dylox)" (-159:112029). In addition to previously reported findings in Fischer rats (nephropathy, lung inflammation and cholinergic effects), this study reported new findings in Sprague-Dawley rats including dilated renal pelves in the pups, and mineralization and hydronephrosis in adults. Kellner, 8/27/92.

198-145 75193 Eigenberg, D. "Proposed Doses For a Two-Generation Dietary Reproduction Study in Rats Using Trichlorfon" Submitted to MT DPR as justification for the dose range used in study -159:112029. A dose of 3000 ppm was reported to cause maternal death during the pre-mating period in a multi-generation reproduction study. Hypercholesterolemia, small intestine hyperplasia, gastritis of the nonglandular stomach and significant reduction in ChE activity was seen at 1750 ppm in a chronic study in rats. A high dose of 1750 ppm was established by the sponsor for the two-generation reproduction study based primarily on brain cholinesterase (ChE) depression (approximately 26% inhibition for males and 55% for females after 12 months). Kellner, 8/26/92.

198-060, -087, -093, 102 014162 (1/23/69, Institute für Toxikologie, Bayer AG #24855) Trichlorfon, presumed technical 0, 100, 300, 1000, and 3000 ppm in diet; Apparent NOEL = 300 ppm for parental and reproductive effects: Parental body weights decreased at 1000 and 3000 ppm, decreased live young/litter at 1000 ppm and upwards. Decreased fertility of F₀ parents, fewer pups/litter in the 3000 ppm F_{1b} offspring, additional high incidence of pup losses in same group by day 5, with no survivors in that group of pups to mating age; **Unacceptable** study, incomplete, not upgradeable. No histological examinations of parental animals. Other deficiencies noted in original review. Rev. by J. Christopher 3/27/85 and C. Aldous 4/18/86.

EPA 1-LINER: Bayer AG #1195 (1/1/69) NOEL = 300 ppm LEL = 1000 ppm (lower body weight gain in females; smaller litter size; reduced fertility. 3000 ppm produced lower body weight gain in females; smaller litter size; lower lactation index; decrease in F_{1a} body weight; reduced fertility.

070, 090, 091 038810, 035780 (Part of an aborted multi-faceted study mentioned elsewhere in this Summary of Toxicology Data), (7/81, Albany Medical College, New Mexico facility) Trichlorfon, presumed technical, 0, 100, 300, and 1000 ppm in diet. No apparent parental or reproductive toxicity, however information scant. Study stopped shortly after the F_{1a} generation was born. **Not acceptable**, not complete, not upgradeable. This study appears to have had management problems: it is the reproduction phase of the rat chronic study, reported in the same volume under Rec. #27062, an unacceptable study. No histology data were reported for either phase of that study, and tissues were apparently lost (p. 9 of report). C. Aldous 9/18/85.

198-070 010797 (Company number = 84009) 1-page summary of 091/35780.

198-073 014810 Summary dog data.

TERATOLOGY, RAT

198-114 059215 "A Teratology Study with Dylox Technical (Trichlorfon) in the Rat," (Miles Laboratories, Mobay No. 94638, 6-22-87). Trichlorfon (Batch EHR 150-8-79; purity = 99.0%) was given to CrI CD BR rats in the diet at 0, 500, 1125 or 2500 ppm on Days 6-15 of gestation (day 0 = day sperm detected). There were 33 mated rats per group; 5/group were sacrificed on day 16 for cholinesterase testing and 28 were sacrificed on day 20. **No adverse effect. Maternal NOEL < 500 ppm (Plasma, erythrocyte and brain cholinesterase inhibition at 500, 1125 and 2500 ppm; reduced body weight gain and food consumption at 2500 ppm). Developmental NOEL = 1125 ppm (delayed ossification of the skull, ribs, vertebrae and pelvis and wavy, curved and or bulbous ribs). **ACCEPTABLE.** Shimer, 4-26-88. M. Silva, 6/17/88.

198-060, -087 014159 "Studies of Embryotoxic and Teratogenic Effects on Rats Following Oral Administration," (5/29/79, Bayer AG). Trichlorfon technical (purity = 98.4%) was administered at 0 (vehicle = 0.5% Cremophor EL), 10, 30, and 100 mg/kg/day by gavage to inseminated FB 30 (Long Evans) rats (25/group) from day 6-15 of gestation (detection of sperm = day 0 gestation). **No adverse effect indicated.** Maternal NOEL = 100 mg/kg/day (some diarrhea observed at 100 mg/kg/day). Developmental NOEL = 100 mg/kg/day (no effects observed at any dose). **Unacceptable** (no data from pilot study; absence of toxic signs indicate that an MTD was not reached). **Upgradeable** (dose justification and data from pilot study are requested). J. Christopher 2/27/85, 3/28/85. C. Aldous 5/1/86.
EPA 1-LINER: Bayer AG #8400 5/29/79 (Mobay Report #69298) Teratogenic, maternal, fetotoxic NOEL > 100 mg/kg/day (HDT) Core grade: Minimum

198-060, -087, -092 014171 "Trichlorfon: Studies of Product for Possible Embryotoxic and Teratogenic Effects on Rats," (Mobay #30242; 2/15/71, Institut für Toxikologie, Bayer AG). Trichlorfon technical (purity = 98.3%) administered at 0 (vehicle = 1% tragacanth), 10, 30, and 100 mg/kg/day by gavage to mated FB 30 (Long Evans) rats (20/group), during day 6-15 of gestation (presence of sperm = day 0 gestation). Maternal NOEL = 100 (diarrhea was observed in 25% of animals at 100 mg/kg/day). Developmental NOEL = 100 mg/kg/day (HDT: no effects were observed at any dose). **No adverse effect indicated. Unacceptable** (no evidence of an MTD; no individual data). **Possibly upgradeable** (dose justification and individual data are requested). J. Christopher 2/25/85 and 3/27/85.

198-087 026993 "Embryotoxicity Tests in Rats After Oral Application," (Inst. für Toxikol., 5/29/79). Trichlorfon technical (purity = 98.4%) was administered by gavage to "pregnant" rats (no strain given) at 0 (vehicle not stated), 10, 30 and 100 mg/kg (25 rats/group) during gestation days 6-15. **No adverse effect indicated.** Maternal NOEL = 100 mg/kg (some animals had diarrhea). Developmental NOEL > 100 mg/kg (no effects at any dose). **Not acceptable** (summary only: not a complete report). **These data are considered supplementary to 060/087/14159 and 060/087/092 14171.** M. Silva, 6/29/88.

198-087 026996 "Dipterex Teratogenicity in the Rat, Hamster and Mouse When Given by Gavage," (NIEHS, June, 1979). Trichlorfon (grade and purity not stated) were administered by gavage to mated CD rats at 0--vehicle only during days 6-15 of gestation (11 rats; vehicle = 0.5% methylcellulose in distilled water), 0 (two groups of rats gavaged with vehicle on day 8 or 10--10 rats/group) and 480 mg/kg/day-total dose, divided into 3 doses/day (10 rats day 8, 10 rats day 10). Maternal NOEL < 480 mg/kg/day under conditions of this study (clinical signs of

cholinesterase inhibition). Developmental NOEL < 480 mg/kg/day under conditions of this study (increased fetal malformations and decreased fetal weights at 480 mg/kg/day). **Possibility of an adverse effect cannot be determined from this study. NOEL not established. Not acceptable** (only summary data provided). **Not upgradeable** (only one dosage tested). **This study provides supplementary data only.** J. Parker, 9/17/85.

198-087 026998 "Experimental Study of the Effect of a Series of Phosphoroorganic Pesticides (Dipterex and midan) on Embryogenesis," (All-Union Scientific Research Institute of the Hygiene and Toxicology of Pesticides, Polymers, and Plastics, Kiev, USSR, publ. in *Env. Health Perspectives* 13: 121-125, 1976). Dipterex (trichlorfon: grade and purity not specified) were administered by gavage to "pregnant" Wistar rats (10-11 rats/group) at 0 (vehicle not stated) or 8 mg/kg daily through gestation (sperm detection = day 1 gestation), and 80 mg/kg as single dose either on day 9 or 13. Maternal NOEL unknown (no maternal data were provided). Developmental NOEL = 8 mg/kg/day (increased fetal deaths and abnormalities at single dosages of 80 mg/kg). **Existence of an adverse effect cannot be determined without maternal data. Not acceptable** (only summary data provided, equivalence to products of USA not established, not a standard study, and not enough dosages to establish dose-effect relationship). **Not upgradeable.** No more information needed from this report. **This report contains supplementary data.** J. Parker, 9/16/85.

198-087 026999 "Chlorophos Action on the Embryogenesis of Rats," (dated ca. 1970, Lab not stated in English). Chlorophos (trichlorfon, grade and purity not stated) was administered to rats at 0, 0.005, 0.02, 0.2, and 9.0 mg/m³, inhalation, (details of administration procedures not given). Investigators claimed "all these concentrations exerted embryotropic action which found its expression in external and internal abnormalities in the development of the embryos, biochemical shifts in the organs of the females and the fetus, such as histopathological and histochemical changes in the placenta." No further information provided. **Since the report contains no further information, the occurrence of adverse effects cannot be adequately evaluated. Not acceptable** (no data, no report; two sentence summary only). **Not upgradeable. Supplementary data only.** J. Parker, 9/16/85.

198-087 027000 "Developmental Toxicity in the Rat After Ingestion or Gavage of Organophosphate Pesticides (Dipterex, Imidan) During Pregnancy," (NIEHS preprint for published report in *Environmental Health Perspectives* 13, pp. 121-125, 1976; see 087:26997). Trichlorfon technical (purity = 98.5%) was administered to mated CD rats (9-47 rats/group) in diet at 0, 76, 145, 375, 432, and 519 mg/kg/day or by gavage at 0 (vehicle for gavage = 0.5% methylcellulose in water), 50, 75, 150, 200, and 250 mg/kg/day during days 6-17 of gestation (detection of sperm = day 1 gestation). Maternal NOEL (diet) = 76 mg/kg/day (significant decrease in food intake at \geq 145 mg/kg/day; significant decrease in weight gain at \geq 432 mg/kg/day). Developmental NOEL (diet) = 76 mg/kg/day (a significant increase in fetal death and lower body weight at \geq 432 mg/kg/day; an increase in malformed fetuses at \geq 145 mg/kg/day). Maternal NOEL (gavage) < 50 mg/kg/day (a significant increase in deaths at \geq 150 mg/kg/day; a significant decrease in food intake at \geq 50 mg/kg/day). Developmental NOEL (gavage) = 50 mg/kg/day (a significant decrease in fetal weight at \geq 75 mg/kg/day). **No adverse effect indicated. Not acceptable** (not complete: summary data only). **Upgrade unlikely unless a complete report can be provided.** J. Parker, 9/16/85.

198-087 026997 Journal article (*Environmental Health Perspectives*, Vol. 13, pp. 133-140, 1976). Duplicate information for 027000.

TERATOLOGY, RABBIT

** 198-147 088680, "Teratology Study in the Rabbit with Dylox Technical (Trichlorfon)", (G. R. Clemens, *et al*, Toxicology Department, Miles Inc., Elkart, IN., Report # 100195, 6/19/90), Trichlorfon technical, 99% purity, was administered by gavage to 20 to 25 artificially inseminated American Dutch female rabbits per group on gestation days 6-18 at 0 (distilled water), 10, 35, and 110 mg/kg/day. By gestation day 18, 14 of 25 inseminated females at 110 mg/kg/day died. Maternal clinical symptoms at 35 and 110 mg/kg/day included tremors, rapid respiration, ataxia, salivation, and prostration. **No Adverse effects.** Maternal NOEL = 10 mg/kg/day (clinical symptoms of ChE inhibition in the mid and high dose groups). Developmental NOEL = 35 mg/kg/day (reduced fetal weights and increased resorptions at 110 mg/kg/day). **Acceptable.** (Green, Kellner, and Gee, 9/1/92).

198-156 089841 is a supplemental submission in response to a September 28, 1990 request for additional information from Canada's Bureau of Chemical Safety, Ottawa, Ontario. Tabulated group mean animal data on the clinical/behavioral observations and information relating to classification of early and late resorptions is provided. The report (-047:88680) remains **acceptable.** Kellner and Gee, 9/1/92.

198-063, -087 010665 (6/8/79, Bayer Report #69299) Trichlorfon, technical: 0, 5, 15, and 45 mg/kg/day by gavage. Maternal NOEL = 15 mg/kg/day (decreased weight gain, two abortions in 45 mg/kg/day group). No developmental toxicity. **Unacceptable,** not complete. Upgrade appears possible on receipt of additional information (see supplementary review worksheet, J. Christopher 3/27/85, C. Aldous 8/14/86).

EPA 1-LINER: Bayer AG 8400 (Mobay #69299) Teratogenic NOEL = > 45 mg/kg (HDT). Maternal NOEL = 15 mg/kg Maternal LEL = 45 mg/kg (decreased weight gain). Fetal toxic NOEL = 15 mg/kg Fetal toxic LEL = 45 mg/kg (2 litters aborted) Core grade: Minimum

TERATOLOGY, RODENT (NON-RAT)

Two studies discussed below are from the same published report. Although they provide useful data, they are not adequate for satisfying DPR data requirements. Malformations were not seen at dosages below 400 mg/kg/day in either study. There is no apparent need for further information about rodent teratogenicity studies other than in the rat. Aldous, 5/1/86.

198-087 035781 (Published article: "Dipterex teratogenicity in the rat, hamster, and mouse when given by gavage" (June 1979, *Environmental Health Perspectives* 30:105-113) Reviewed 9/17/85 Only summary data were provided in a golden hamster study by NIEHS. Animals considered pregnant (30) were dosed at 400 mg/kg/day during days 7-11 of gestation and subsequently, lower dose levels were added (20, 5, 25, 10 at 0-gavaged with distilled water, 100, 200, 300 mg/kg/day) and additional hamsters (23) were administered 400 mg/kg/day only on day 8 to determine fetal sensitivity to exposure on a single day of gestation. There was an increase in fetal malformations at the highest dosage (400 mg/kg/day, days 7-11), primarily edema, cleft palate, patagium (fold of skin connecting forelimbs and hindlimbs), and fused ribs. Increases in fetal death, also an increase in the numbers of runted fetuses and a decrease in mean fetal weight (all signif., $p < 0.05$) were noted at 400 mg/kg/day. The parental NOEL was apparently 100 mg/kg/day, as signs of cholinesterase inhibition were observed at 200 mg/kg/day. Food intake of dams was decreased in a dose-related fashion at 300 to 400

mg/kg/day. The high dosages administered were made possible by giving the daily dose in three portions, 3.5 hours apart. J. Parker, 9/17/85.

198-087 035782 The mouse data in the same NIEHS report; dosing was administered in three daily portions, as in the hamster segment of the study. Dosing by gavage was administered to 16, 5, 3, 4 and 25 presumed pregnant mice during days 6-10 of gestation. Also some animals were dosed on days 10-14 of gestation (12, 23, 7, 4, 6 and 20 at 0-no gavage, 0-gavage with distilled water, 300, 400, 500 and 600 mg/kg/day). Dosing at 600 mg/kg/day was also attempted at specific times of gestation (11, 13 and 28 at 0-no gavage, 0-gavage with distilled water and 600 mg/kg/day on day 8; 11, 14 and 18 at 0-no gavage, 0-gavage with distilled water, and 600 mg/kg/day on days 8-10; 2 and 4 at 0-gavage with distilled water and 600 mg/kg/day on days 10-12; and 14, 16 and 26 at 0-no gavage, 0-gavage with distilled water and 600 mg/kg/day on days 12-14). Food intake of dams decreased significantly at dose levels of 500 mg/kg/day and maternal weight gain was diminished significantly at doses of 400 mg/kg/day and above, with dosing on days 10-14 of gestation. Fetal weight was reduced at doses of 300 mg/kg/day and above. Stunting, here defined as fetuses weighing less than 0.5 g or weighing less than two thirds the weight of the largest littermate, occurred only in the 600 mg/kg/day group. Incidence of malformations was increased at 500 and 600 mg/kg/day, predominantly as cleft palate. No behavioral responses consistent with cholinesterase inhibition were observed, however the maximum dosage was limited by maternal food intake and weight gain. J. Parker, 9/17/85.

GENE MUTATION

Mammalian cells

****198-060, -089 014154** (86724) (4/84, Litton E-9107) Mouse lymphoma 0 - 125 (-S9) and 0 - 145 (+S9) : g/ml, cytotoxic at concentrations higher than these; 4 hours incubation, two trials. **Acceptable.** A significant increase in mutation frequency occurred with and without activation in both trials. J. Christopher 2/28/85.

Bacteria

****198-060, -089 014156** (1979, Institute of Environmental Toxicol., Japan) Salmonella, 5 strains, 0 - 20,000 : g/plate with and without activation, in duplicate, no repeat trial. Also included Escherichia coli WP2 hcr. Evaluated as **acceptable**. An increase of 2-3 fold in mutations in TA100 and E. coli with and without activation in a dose response. Positive for mutagenesis. J. Christopher 2/27/85.

****108-149 039417** [EPA-contracted studies on multiple compounds, volume assigned to acephate file], SRI International, Oct. 1979. Report title: "In vitro microbiological mutagenicity and unscheduled DNA synthesis studies of eighteen pesticides". Trichlorfon, technical, Batch 5-00-7003. Gene mutation (842), Salmonella (TA1535, TA1537, TA1538, TA98, and TA100) and E. coli [WP2 (uvrA)]. 0-10,000 : g/plate. Repeat trials with TA100 and with E. coli [WP2 (uvrA)]. Increases in revertants with TA100 and with E. coli [WP2 (uvrA)] at higher concentrations, but not with other Salmonella strains. **Acceptable.** J. Gee 2/25/87.

198-060, -089 014158 (49442) (6/19/76, Agricultural Chemicals Inst.# 28) Salmonella, 4 strains, with and without rat liver S9, phenobarbital-induced, 0 - 500 : g/plate, one plate, one

trial. **Unacceptable** (2/27/85): no repeat trial, high concentration not justified: should use higher, if possible.

No significant increase in reversion rate. NOTE: Data are almost identical to 17602. Reason why results differ from 14156 could be concentrations used. J. Christopher 3/4/85.

198-060, -089 017602 (54353) (1977, Agricultural Chemicals Inst.#87) Salmonella, 4 strains, with and without rat liver activation (phenobarbital induced), 0 - 500 : g/plate, one plate, one trial. **Unacceptable**: no justification of high dose, single trial without duplicates. No increase in mutations reported. J. Christopher 2/27/85.

198-089 027046 (1980, Litton, publ. in Mutation Res. 76:169) C. Aldous 9/12/86 Escherichia coli WP2 and WP2 uvrA mutants. Review article referencing Simmons et. al. at SRI. No data.

198-060, -089 027044 (85918) (1983, Litton Bionetics E-9107) Saccharomyces cerevisiae S138 and S211a (both haploid strains), reverse mutation assay, 0 -10,000 : g/ml in DMSO as vehicle, 3 hours preincubation before plating, with and without S9, single trial with each strain. **Unacceptable** (2/27/85): no repeat trial. Also, DMSO is not the best choice of a solvent. **No adverse effect** reported. J. Christopher 2/27/85.

198-060 014153 Supplemental data.

198-089 027021, 027049, 027050 Summary information.

198-089 027035 Journal article (Mutation Research, 40 (1976) 19-30). Summary information.

198-089 027045 Journal article (Tox. and Applied Pharm. 41 (1) 196, 1977). Summary information.

Conclusion: Adverse effects are evident in all the acceptable tests including: mouse lymphoma, Salmonella and E. Coli mutagenicity. They are also noted in study 089 27046. Two of the studies (record #s: 060/089 14158/49442 and 060/089 17602/54353), negative with Salmonella apparently did not use a high enough dose of trichlorfon compared to the studies where a positive response was observed. Study 060/089 270(85918) was negative with Saccharomyces cerevisiae and perhaps trichlorfon was not able to penetrate the cell wall of this haploid yeast strain to induce mutation. Therefore, since the more sensitive mutagenicity tests (such as Salmonella mutagenicity) were positive and the majority of all tests showed positive adverse effects, DPR will consider the positive studies in the evaluation of possible toxic effects. M. Silva, 7/6/88. A recent in vivo cytogenetic test (-170:121312) was negative. Kellner and Gee, 6/21/93.

CHROMOSOME EFFECTS

**198-140, -143 070348, 072394, "L 13/59 (trichlorfon), Sister Chromatid Exchanges (SCEs) in Chinese Hamster Bone Marrow Cells", (Laboratory for Mutagenicity Testing, Darmstadt, Germany, Report # 94788, 2/25/87), L 13/59 (trichlorfon), batch # 809431103, 98.9% purity, single dose administered by gavage at 0 (distilled water), 30, 100, and 300 mg/kg with 6

Chinese hamsters/sex/group. Bone marrow of 5/sex/group was sampled at 24 hours (colcemide 2.5 hours prior to sacrifice). **No increase in sister chromatid exchange frequency. Acceptable.** (Green and Silva, 9/15/89).

198-170 121312 Herbold, B. "In Vivo Cytogenetic Study of the Spermatogonia in Chinese Hamster to Evaluate for Induced Clastogenic Effects" (Institute of Toxicology for Industrial Chemicals, Bayer AG, Fachbereich Toxicology, Germany. Miles Report # 103298, 9/22/92). Trichlorfon (98.5% purity, Batch # 238000109) was administered to 5 male Chinese hamsters/dose/sampling period through single intraperitoneal injections of 0 (0.5% aqueous Cremophor) or 100 mg/kg; sampling of gonadal cells was performed 6, 24, and 48 hours after treatment (24 h only for controls). **No Adverse effects: no increases in chromosomal aberrations were reported. ACCEPTABLE. (Green, Kellner and Gee, 6/15/93).

198-140 070347, "Cytogenetic Study of Human Lymphocyte Cultures In Vitro to Test for Chromosome Damage", (Bayer AG, Institut für Toxikologie, Wuppertal, FRG, report # 91236, 7/23/86), cytogenetic assay with human lymphocytes from 1 male and 1 female donor, 4 replicates/group (2/donor), with activation at 0 (DMSO), 300, 1000, 3000 : g/ml and without activation at 0 (DMSO), 3, 10, 30 : g/ml. Colchicine added 21 hours after test compound. Cultures harvested at 24 hours. Mitotic activity reportedly reduced at 10 and 30 : g/ml without activation (65.2% and 56.5% of negative control, respectively) and at 1000 and 3000 : g/ml with activation (72.7% and 47.7% of negative control, respectively). **Possible adverse effect** (chromosomal damage) indicated. **Unacceptable**, possibly upgradeable with submission of individual mitotic activity and separate replicate data. (Green and Silva, 9/15/89)

****198-115 059211** "Sister Chromatid Exchange Assay in Chinese Hamster Ovary Cells," (Microbiological Associates, Mobay No. 94410, 7-15-86). Dylox technical (purity = 97.3%) was tested in a sister chromatid exchange assay using Chinese hamster ovary cells at 0 (vehicle = distilled water), 10, 25, 50 and 100 : g/ml (no S-9) or 0, 30, 75, 150 and 300 : g/ml (with S-9). **Possible adverse effect.** Under conditions of this study, Dylox technical produced a statistically significant increase in sister chromatid exchange both with S-9 (≥ 75 : g/ml) and without S-9 (≥ 50 : g/ml). Positive controls functioned as expected. **Acceptable.** Shimer, 4-26-88. M. Silva, 6-17-88.

198-060, -089, -108 017603 (68925) (1979, Bayer) Mouse dominant lethal test. Fifty male mice were given 250 mg/kg orally in a single dose and mated for 4 day periods 12 times. Dose selection was based on a preliminary test, the selected dose being the estimated m.t.d. Individual data included. **Inadequate** based on lack of concurrent positive control. No adverse effect reported. J. Christopher 2/27/85.

198-060, -089 017604 (69164) (1980, Martin Luther Inst.) Mouse dominant lethal test. 29 males were given 405 mg/kg (i. p.?) in one dose (short term) or 54 mg/kg/day over 5 weeks, then mated for 8 weeks. **Unacceptable** due to insufficient information, inadequate negative controls (one week only), no concurrent positive control. J. Christopher 2/27/85.

198-089 027019 (1971, IBT: not on EPA list) Mouse dominant lethal. 12 males per group were given 50 or 100 mg/kg i.p. and mated 1:3 per week over 6 weeks. **Unacceptable** (9/12/85): no concurrent positive control, no individual data. No adverse effect reported.

198-060, -089 014157 (68783) (1979, Bayer #8505) Mouse micronucleus test. 5/sex/dose were given 125 or 250 mg/kg twice by oral gavage and sacrificed after 6 hours following the protocol of Schmid, which is **unacceptable**. Also, no evidence m.t.d. was approached despite preliminary study (no data presented). No evidence of an adverse effect of increased micronuclei formation is reported. J. Christopher 2/27/85.

198-089 027047 (1981, Roswell Park, publ in Mutation Res. 88: 307 Chen, Hsueh, Sirianni and Huang) Chinese hamster V79 and sister chromatid exchanges without activation, at 0 - 80 : g/ml. **Unacceptable** (9/12/85) dose selection not justified, no activation included, inadequate data presentation. A concentration-dependent increase in SCE's to greater than 2-fold and cell cycle delay at 80 : g/ml. [Note: Consider 27054 as part of the same study the +S9 portion, also showing increase in SCE's.] Gee, 9/12/85.

198-089 027054 (1982, Roswell Park, publ. in Environmental Mutagenesis 4:624. Chen, Sirianni and Huang. Chinese hamster V79 cells and sister chromatid exchanges, 5-60 : g/ml with S9 only. **Unacceptable** (9/12/85): no description of test article, activation only, inadequate data. Concentration-dependent increase in SCE's is reported to greater than 2-fold per cell. Consider with 27047 above. Gee, 9/12/85.

198-089 027042 (1979, publ. in Arch. Environm. Contam. Toxicol. 8: 309 (1979). Chromosome aberrations in workers in a pesticide manufacturing plant. Trichlorfon was one of several pesticides to which workers had been exposed (including phosmet). An increase in stable chromosome-type aberrations is reported over normals but "factory employee controls" were elevated even more. Also, missing information on other exposures/disease, etc., so cannot evaluate reliably any toxic effect. 9/12/85.

198-089 027041 (10/23/81, Inst. Public Health, Moscow) [Translation of an article?] Male mice were given a single oral dose of 0.5 to 0.0002 of the LD50 (not given), 5/dose, and bone marrow was examined after 24 hours for Chromosomal aberrations. A dose-dependent increase of greater than 5-fold is reported. **Unacceptable:** inadequate protocol, missing information. J. Remsen (Gee) 9/12/85.

198-089 027033 (1975, Abstract translated from Russian.) Rats (or mice?) were fed mixtures of pesticides at 1/15 and 1/200 of LD50 (not given) of nitrophen and zineb, each administered alone or as mixtures with trichlorfon or other pesticides. Apparently trichlorfon was determined to increase the mutagenic effect of zineb (especially chromatid gaps and breaks). **No data.** No way to evaluate. J. Remsen (Gee) 9/12/85.

198-089 027020 Journal article (Toxicology and Applied Pharmacology, 23. 283-325 (1972)). Summary information.

198-089 027032 Journal article (Mutation Research, 28 (1975) 405-420). Summary information.

198-089 027037 Journal article (in Italian). Summary information.

198-089 027022, 027026 Supplemental information.

Conclusion: No adverse effects were observed in the mouse dominant lethal studies, which test for embryo and fetal deaths induced by chromosome damage in germ tissue. A micronucleus test showed no adverse effect (060/089 14157/68783) while another test where bone marrow was examined after treating mice with trichlorfon at 1/2 LD50 showed an increase in chromosomal aberrations (089 27041). In 14157/68783 it was stated that no MTD was reached and therefore 27041 may have been positive due to use of a higher dose of trichlorfon (value for LD50 was not given). Of the highly sensitive *in vitro* sister chromatid exchange tests, which measure chromosome mutations, two were positive for adverse effects (including the acceptable studies 115 59211) while another acceptable study (140, 143 070348, 072394) was negative. One of the most recent studies, where human lymphocytes were used in a cytogenetic assay, showed increased chromosomal damage induced by trichlorfon. Therefore, since increases in chromosomal damage was observed using human cells and the majority of all tests showed positive adverse effects, DPR will consider the positive studies in the evaluation of toxic effects due to trichlorfon. M. Silva, 7/5/88. A recent *in vivo* cytogenetic test (170:121312) was negative. Kellner and Gee, 6/21/93.

DNA DAMAGE

198-060, -089 014155 (86396) (11/1983, Litton Bionetics) Rat hepatocytes were tested for unscheduled DNA synthesis at 0 - 50 : g/ml (100 : g/ml was toxic), 20 hours, counted grains in 3x50 cells per concentration. **Acceptable
No evidence of UDS. J. Christopher 2/28/85.

198-089 027051 (2/1/84, Bayer). E. coli pol mutants tested at 0 - 10,000 : g/plate with and without activation on disk. **Unacceptable** but upgradeable (9/12/85): missing information on protocol and discussion of findings (inverse of what would be anticipated for a genotoxic effect). Questionable effect on DNA. A possible adverse effect was noted in the initial review (J. Remsen (Gee), 9/12/85). Upon reevaluation, J. Remsen & J. Parker, 3/25/86, evaluated as negative for adverse effect: with other similar studies its biological meaning is in doubt.

198-060, -089 017602, 038813, 038815 (54353) (1977, Agricultural Chemicals Inst.#87) B. subtilis rec assay, 3, 30 and 300 : g/disk, no activation. **Unacceptable** (2/27/85)-- no activation, insufficient range of concentrations. No difference in growth between rec+ and rec- strains is reported. J. Christopher, 2/27/85.

198-060, -089 014158, 038812 (49442) (1976, Agricultural Chemicals Inst., report No. 28) B. subtilis rec assay at 3, 30 and 300 : g/disk, no activation = **unacceptable** (2/27/85). No difference in growth is reported. J. Christopher, 3/4/85.

198-060, -089 014156, 038814 (69367) (1979, Inst. of Environ. Toxicol.) Bacillus subtilis rec assay, 0 to 2000 : g/disk. **Unacceptable** (2/27/85): no activation was used, single values only. Marginal increase in growth inhibition at 1000 (1mm) and 2000 (3mm) between the two strains. J. Christopher, 2/27/85.

198-089 027034 11/75 B. subtilis at 300 : g/disk showed no differential growth inhibition. J. (Remsen) Gee, 9/12/85.

198-089 027048 Abstract in Environmental Mutagenesis 3:327 (1981) on Saccharomyces D3 and D4, positive for mitotic crossing over, gene conversion, and reversion. S.R.I. J. (Remsen) Gee, 9/12/85.

108-149 039419 [EPA-contracted studies on multiple compounds, volume assigned to acephate file], SRI International, Oct. 1979. Report title: "In vitro microbiological mutagenicity and unscheduled DNA synthesis studies of eighteen pesticides". Trichlorfon, technical, Batch 5-00-7003; Muta-DNA/Other (844). E. coli, W3110/p3478 and B. Subtilis H17/M45. Filter disk at 0, 0.01, 0.1, 1.0 and 5.0 mg on 6 mm disk minus activation only. No cytotoxicity in any strain indicates a "no-test". **No evidence for DNA damage. Unacceptable**, not upgradeable [no activation] J. Gee 2/25/87.

108-149 039420. [EPA-contracted studies on multiple compounds, volume assigned to acephate file], SRI International, Oct. 1979. Report title: "In vitro microbiological mutagenicity and unscheduled DNA synthesis studies of eighteen pesticides". Trichlorfon, Trichlorfon, technical, Batch 5-00-7003; Muta-DNA/Other, UDS (844). Human cell line, WI-38 (no passage number) with and without mouse liver activation; two trials, 6 replicates each, at 0 to 2000 : g/ml, -S9; at 0 to 4000 : g/ml, +S9; increase in dpm/: g DNA without activation; **Unacceptable**, but upgradeable [see review] J. Gee 2/26/87.

****108-149 039418** [EPA-contracted studies on multiple compounds, volume assigned to acephate file], SRI International, Oct. 1979. Report title: "In vitro microbiological mutagenicity and unscheduled DNA synthesis studies of eighteen pesticides". Trichlorfon, technical, Batch 5-00-7003; Muta-DNA/Other (844). Saccharomyces cerevisiae D3 mitotic recombination test. Two trials with and without rat liver activation at 0.1, 0.5, 1.0, 2.0, 4.0 or 5.0 % concentration; clear and dose-related increase in mitotic recombinants with and without activation. **Acceptable** J. Gee 2/25/87.

198-089 027027, 027045, 027049, 027050 Summary information.

198-089 027024, 027028, 027029 Supplemental information.

Conclusion: The discrepancy between the negative results for unscheduled DNA synthesis in rat hepatocytes (Record #14155) and in WI-38 human diploid fibroblasts (Record #39420) may in part be explained by the apparently large difference in cytotoxicity of trichlorfon. With hepatocytes, cytotoxicity was evident at 100 : g/ml while no toxicity was reported at 2,000 : g/ml (-S9) or 4000 : g/ml (+S9) with WI-38. The induction of unscheduled DNA synthesis is consistent with the gene mutation effects seen in bacteria (Record #'s 14156 and 39417) and mammalian cells (Record #'s 14154), which demonstrate the ability of trichlorfon to form DNA lesions which could initiate a DNA repair response. In the two studies in Salmonella with negative findings (Record #'s 14158 and 17602), the maximum concentration was 500 : g/plate, far below that at which the increase in revertants was seen in the other studies (>1000 : g/plate). One of the reasons for rejecting Record #'s 14158 and #17602 was the inadequacy of the highest concentration. The positive effect on mitotic recombination in Saccharomyces (Record #39418) also required comparatively high concentrations ($\geq 0.5\%$). The induction of sister chromatid exchanges and chromosome aberrations (Record #'s 27047, 27054 and 27041) is also consistent with the other genotoxic effects. Taken all together, trichlorfon appears to be weakly genotoxic. J. Gee, 3/9/87.

NEUROTOXICITY , HEN

Acute Hen Neurotoxicity

198-063 010666 "An Acute and Subacute Neurotoxicity Assessment of Trichlorfon," (Slott, V. and Ecobichon, D.J., *Canadian Journal of Physiology and Pharmacology*, **62**: 513-518, 1984; McGill University, 10/5/83). The toxicity of trichlorfon (purity not stated), reported to elicit delayed neurotoxicity in humans and chickens, was studied by administering single subcutaneous doses of 100 or 300 mg/kg to adult White Leghorn hens. At 24 hours post-treatment, hens were observed for visible signs of neurotoxicity, were euthanized, and samples of blood plasma, brain and spinal cord (cervical and thoracic regions) were obtained for quantification of cholinesterase and neurotoxic esterase (NTE) activities. In subacute studies, hens were dosed with trichlorfon (100 mg/kg) every 72 hours for a total of 6 doses. 72 hours after the final dose, the hens were euthanized and the brains, spinal cords and distal sciatic nerves were removed for enzymatic and/or histological examination. Parallel acute and subacute studies were conducted using diisopropyl phosphorofluoridate (DFP as positive control) at subcutaneous dosages of 1.0 mg/kg. In the acute studies, both DFP and trichlorfon markedly inhibited tissue cholinesterase activities but only DFP elicited significant NTE inhibition. In the subacute studies, DFP produced a characteristic central-peripheral distal axonopathy in the 18-day period of study which was confirmed by clinical and morphological evidence and by marked inhibition of neuronal NTE. Trichlorfon caused little or no obvious neurotoxicity, an observation that was supported by minimal morphological changes and impairment of walking ability and no inhibition of brain or spinal cord NTE. **No adverse effect. This study is supplemental to 60378.** M. Silva, 6/28/88.

198-063 010667 "Trichlorfon - Melting Process Japan Neurotoxic Study on Chicken," (Bayer AG Institute of Toxicology, 4/20/82). Trichlorfon technical (purity = 94.6%) was administered by gavage to White Leghorn chickens in 2 tests. Test 1: a single oral dose of 100 (10 hens), 150 (10 hens), 175 (20 hens) or 200 mg/kg (10 hens) followed by a 28 day observation period (no atropine). Test 2: two doses at 167 (1st dose = 30 hens) and 185 (2nd dose = hens remaining alive) mg/kg were administered along with atropine (50 mg atropine sulphate/kg i.m. at each dosing) and a vehicle control (2% Cremophor EL in distilled water: 6 hens) in a 21 day interval. Dosing for test 2 was followed by a 21 day observation period. At 24 and 48 hours after treatment (185 mg/kg) a neurotoxic esterase (NTE) assay was performed using 4 and 3 mice for the respective time points. **No adverse effect.** LD50 = 167 mg/kg (Test 1). In test 2, no signs of delayed neurotoxicity were observed and NTE was not inhibited by 185 mg/kg trichlorfon. **Unacceptable** (No analysis of dosing material; randomization procedure was not described; no body weights measured in test 2; no individual clinical signs were reported in test 1 and no clinical signs at all were reported for test 2; no statistics were performed; no GLP statement included). The study is possibly **upgradeable** if the above information can be provided for evaluation by DPR. M. Silva, 6/23/88.

198-060, -090 014152 (No testing lab nor test date given). Test article = trichlorfon (not further characterized). Divided dose: 200 mg/kg sc, followed by 100 mg/kg, sc, in atropinized hens. Sacrifice apparently 23 days after last dose. The enzyme "neurotoxic esterase" was inhibited, and mild clinical signs were observed after a delay of 9 days or more. Central and peripheral nervous system pathology was observed microscopically in the only hen observed histologically after repeated exposure. This hen had axonal swelling in peripheral nerve, and vacuolization of

myelin. In the brainstem, "diffuse areas of circumscribed erosion of the myelinated fibers" were seen (p. 8 of report). Cerebellar Purkinje cells appeared to be damaged as well.

Unacceptable: insufficient data. No adverse effects were found. J. Christopher 2/28/85, and C. Aldous 5/1/86.

198-090 027060 Neurotoxicity, hen. (9/82, Univ. of Illinois, Coll. of Vet. Sci.) Trichlorfon, technical: repeated dermal dosing of 50 mg/kg/day for 3 days followed by a 58 day observation period. Other segments of the study involved lower daily dosages, either orally or dermally, over 90 days. Although no delayed neurotoxicity effects were observed, the numbers of animals were far too few, the dosages not justified, and other deficiencies were noted.

Unacceptable C. Aldous 9/16/85.

198-090 027055 A desmethyl metabolite of trichlorfon was tested (3-12-74). The most relevant portion of the study involved a dose of 2500 mg/kg, repeated after 28 days, and survivors were sacrificed 21 days later. The 4 survivors did not indicate TOCP-type neuropathy. The purpose for the study was not given, and the study is **not acceptable**. See worksheet. C. Aldous 9/16/85.

198-090 027056 Journal article (Biochem. J. (1970) 120, 523-531) containing duplicate information to 014152.

Subchronic Hen Neurotoxicity

198-127 060378 "Subchronic Delayed Neurotoxicity Study of Trichlorfon Technical (Dylox®) with Hens", Hayes, R. H. and Ramm, W. W., Mobay Corporation, Stilwell, KS, 8/28/87. Mobay Report No. 94821. Technical trichlorfon (purity = 98.8%) was given to White Leghorn hens by gavage for 90 consecutive days at dose levels of 0 (vehicle = corn oil:acetone 9:1), 3, 9 or 18 mg/kg/day (12/group). TOCP was used as a positive control. **No adverse effect. NOEL for delayed neurotoxicity parameters = 18 mg/kg/day (no clinical signs of delayed neurotoxic damage, no related histopathology findings, no alterations seen in ladder and turf test). There was no "NOEL" for whole blood cholinesterase inhibition (consistently reduced by about 20-30% at 9-18 mg/kg/day: slight but statistically significant reduction at 3 mg/kg/day in latter weeks of the study). Clinical signs typical of cholinesterase inhibition (ataxia and decreased activity) were common at 18 mg/kg/day. Hypertrophy of the esophagus (and often in one or more structures of the upper alimentary tract such as the crop, gizzard or proventriculus) was the only histopathology change, occurring at 18 mg/kg/day. Originally reviewed as unacceptable (attainment of an MTD was unclear in review by Shimer and Silva, 6/21/88). [NOTE: The CDFA worksheet by Green and Silva (9/15/89) summarized the range-finding study (DPR Document No. 198-142, Record No. 071215: Mobay Report No. 94281-1). Excessive mortality at 27 to 36 mg/kg/day justified the dose range selected for the main study and upgraded status of that study to **acceptable**]. Updated with additional worksheet (Iyer and Aldous, 5/30/96).

Conclusion: The majority of hen neurotoxicity studies, including the most recent and acceptable study (subchronic study 127 060378), showed a total absence of delayed neurotoxicity. One study however, 060/090/14152 stated a possible adverse effect due to NTE inhibition and nerve pathology in the only hen examined. This study had no test article characterization nor were there sufficient data to adequately assess the presence of an adverse effect. DPR maintains that trichlorfon does not cause a delayed neurotoxic effect in hens. Silva, 10/5/89.

NEUROTOXICITY, RAT

****198-184 143222** "A Subchronic Dietary Neurotoxicity Screening Study with Technical Grade Trichlorfon (Dylox®, Dipterex®) in Fischer 344 Rats (825(b); L. P. Sheets, Bayer Corporation, Agriculture Division, Stilwell, Kansas; Study# 92-472-ND, 12/1/95). Trichlorfon technical (Dylox®, Batch# 2030021/542102252, purity of 98.4%, dissolved in corn oil) was administered in the feed to 18 Fischer 344 rats/sex/dose at 0, 100, 500 and 2500 ppm for 13 weeks. Clinical observations (at 2500 ppm) consisted of perianal stain (males) and increased incidence of urine stain (females). High-dose males showed lower body weights (4-5%) compared to controls lower during most of the study. Functional Observational Battery (FOB)- high-dose males had perianal staining (week 4) and decreased righting response (week 8); females at this level showed increased urine stain for weeks 8 and 13. Reduced motor and locomotor activity (18 to 28%) was noted during weeks 4 and 8 for 2500 ppm males and during week 4 for 2500 ppm females. ChE Inhibition High-dose rats showed >20% reductions in plasma, erythrocyte and brain ChE activity and reductions in plasma and erythrocyte ChE at 4 weeks were similar in magnitude to those at 13 weeks. At 500 ppm, inhibitions of plasma and erythrocyte ChE ranged from 22-31% during week 4. **NOEL(M/F)=100 ppm**,((M)=6 mg/kg, (F)=7 mg/kg, based on ChE inhibition). Microscopic lesions (segmental, ballooning separation of myelin lamellae from the axon, i.e., primary myelinopathy) were reported in the cervical and lumbar spinal nerve roots of high-dose rats. **[NOAEL(M/F)=500 ppm; (M)=35 mg/kg/day, (F)=31 mg/kg/day]** based on degeneration of myelin in cervical and lumbar spinal nerve roots. **ACCEPTABLE**. Kellner, 5/23/96.

SUPPLEMENTAL STUDIES, DOG

198-087 027003 "90-day testicular maturation study" (6/25/71, IBT (not listed on IBT tracking system as to whether audited)) Test article named THFA, which is not a current synonym for trichlorfon. 0, 200, 400, and 800 ppm in diet. No apparent effects on male gonads. Not complete, **not acceptable**, study design is of supplementary nature: hence study cannot be upgraded to fill a data requirement. Test article not defined. The reason for undertaking this study was not explained, and this explanation should be requested of the registrant. C. Aldous 9/12/85.