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DATA EVALUATION RECORD

TRICHLORFON

Mutagenicity: 1. Gene Mutations in Salmonella typhimurium and Escherichia coli
2. Chromosome aberrations (mitotic recombination) in Saccharomyces cerevisiae
3. DNA Repair Damage in E. coli and Bacillus subtilis
4. Unscheduled DNA Synthesis in Human Fibroblasts

CITATION: Simmon VF. 1979. In vitro microbial mutagenicity and unscheduled DNA synthesis studies of eighteen pesticides. Unpublished report. EPA-600/1-79-041 (Contract No. 68-01-2458), October 1979. U.S. EPA, Research Triangle Park, NC.

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DATA EVALUATION RECORD

STUDY TYPE: 1. Gene Mutations in Salmonella typhimurium LT-2 strains and Escherichia coli WP2.
2. Chromosome aberrations in Saccharomyces cerevisiae D3 (mitotic recombination).
3. DNA repair damage in E. coli p3478 and Bacillus subtilis M45.
4. Unscheduled DNA synthesis in human fibroblasts (WI38).

CITATION: Simmon VF. 1979. In vitro microbial mutagenicity and unscheduled DNA synthesis studies of eighteen pesticides. Unpublished report. EPA-600/1-79-041 (Contract No. 68-01-2458), October 1979. U.S. EPA, Research Triangle Park, NC.

ACCESSION NUMBER: Not available.

MRID NUMBER: 00028625.

LABORATORY: SRI International, Menlo Park, CA.

TEST MATERIAL: Trichlorfon technical manufactured by Chemagro (Batch 5-00-7003) and supplied by Battelle [purity not stated].

PROTOCOL:

1. Trichlorfon was dissolved in DMSO for all assays.

2. The compounds were assayed for mutagenicity using the following in vitro procedures:

   a. Reverse mutation (with and without S9; see "3e") in Salmonella typhimurium strains TA1535, TA1537, TA1538, TA98, and TA100, and in Escherichia coli WP2 uvrA.

   b. Mitotic recombination in Saccharomyces cerevisiae D3 (with and without S9; see "3e").

   c. DNA repair assays in E. coli strains W3110 (repair proficient) and p3478 (repair deficient), and in Bacillus subtilis strains H17 (repair proficient) and M45 (repair deficient).

   d. Unscheduled DNA synthesis (UDS) - Human Fibroblast, WI-38 cells [number of passages not stated] with and without S9 mix (see "3e").

3. a. Plate incorporation assays, performed according to the standard Ames protocol were used for the S. typhimurium assay. A procedure similar to the Ames assay was used for the E. coli WP2 uvrA assay except Oxoid nutrient broth was added to supply a trace amount of tryptophan required for mutant colony development. Trichlorfon was added to plates at several concentrations ranging from 1 to 10,000 µg/plate. Negative controls were run with DMSO. Positive controls utilized 2-anthramine, N-methyl-N'-nitro-N-nitroso-guanidine and AF2 [2-aminofluorene].

b. Overnight cultures of S. cerevisiae D3 (diploid heterozygous for adenine deficiency) were suspended at a concentration of 10^8 cells/ml in a 67 mM phosphate buffer solution (pH 7.4). To sterile test tubes, the following were added: 1.3 ml of the culture, 0.50 ml of either S9 or buffer, and 0.2 ml of a pesticide solution in DMSO or 0.2 ml of DMSO alone. The trichlorfon concentrations tested ranged from 1 to 5 percent. The supension was incubated for 4 hours at 30°C after which the suspension was plated onto tryptone-yeast agar plates. After 2 days of incubation at 30°C, followed by 2 days of incubation at 4°C to enhance the red pigment development (indicative of adenine-deficient homozygosity), the plates were scored for survival (10^{-5} dilution) and recombination (10^{-3} dilution). Positive controls were not stated. However tables for results of the assay were missing from the copy available for review, and these tables may have contained further information.

c. DNA repair was measured by the relative toxicity (zone of inhibition) of the test material to two E. coli strains (W3110, polA$^+$ and p3478, polA$^-$) according to the standard Rosenkranz procedure and by the relative toxicity of the test material to two B. subtilis strains (H17, rec$^+$ and M45, rec$^-$). The bacteria were added to top agar, which was allowed to solidify, after which a sterile disc impregnated with the trichlorfon in DMSO was placed in the center of the plate. The concentrations tested were not stated. However, tables for results of these assays were missing from the copy available for review, and these tables may have described the concentrations used. The diameter of the inhibition zones of the test bacteria were measured and compared. Negative controls utilized chloramphenicol because of its equal toxicity to each strain. Positive controls used 1-phenyl-3,3-dimethyltriazine.

d. WI-38 cells were grown in T-25 tissue culture flasks with Eagle's Basal Medium containing 10 percent fetal calf serum. The cells were grown to confluency to produce contact-inhibited cells in synchronous cultures in the G_0 phase of the mitotic cycle. ^{3}H-TdR was added to cells treated with the test material and to the positive and negative control cultures. The positive controls used were (without S9) 4-nitroquinoline-N-oxide (4NQO) and (with S9) dimethylnitrosamine (DMN). The negative control was DMSO. The test compounds were all assayed with and without metabolic activation by S9. After incubation for 3 hours in the presence of

the pesticide and ^{3}H-TdR (without S9) or after incubation of cells, pesticide, ^{3}H-TdR, and S9 for 1 hour, cells were then incubated an additional 3 hours in the absence of pesticide. DNA was then extracted from all treated and control cell cultures. The DNA was quantified by diphenylamine reaction, and ^{3}H-TdR incorporation was measured by scintillation counting.

Results were expressed as disintegrations per minute (dpm) of incorporated ^{3}H-TdR per unit of DNA, and the rates of incorporation of treated and control samples were compared. An acceptable assay was defined as one in which the positive control compound could be predicted "within 95 percent confidence limits by regressions of average dpm/µg DNA versus dpm/µg DNA for background." Regressions were done over a range of background dpm/µg DNA of 0-450.

Statistics to evaluate the results of these assays included parametic One-Way Classification of Variance or nonparametric Kruskal-Wallis One-Way Analysis of Variance. Bartlett's test of variance was used to decide which method was appropriate. If Bartlett's test was negative, the parametric analysis was considered appropriate, but, if the variances were not equal, the nonparametric analysis was employed. To be considered a positive assay, the results had to be statistically significant at the 99 percent confidence level and a dose-response relationship had to be shown.

e. Assays a and b were performed with and without metabolic activation by the S9 fraction from Sprague-Dawley rats injected ip with the polychlorinated biphenyl, Aroclor 1254. The WI-38 cells in assay d were activated by S9 from Swiss-Webster mice [treatment of mice not stated].

RESULTS:

Trichlorfon was found to be mutagenic in all microbiological assays except for the DNA repair (relative toxicity) tests with B. subtilis and E. coli. In the Ames assay, trichlorfon at 5, 7.5, and 10 mg/plate was mutagenic in TA100 only. Its mutagenic response was 3- to 5-fold above [range of three experiments] the number of spontaneous revertants. The 5-fold value was in the absence of S9 and the 3-fold increase was obtained when S9 was present. At 1 mg/plate or less, trichlorfon was not mutagenic to any strain of S. typhimurium. With E. coli WP2, trichlorfon was also mutagenic (at 1 mg/plate in one test and at 5 mg/plate in the other two tests) with and without S9 activation; approximately 2-fold above the number of spontaneous revertants. From the dose response curves [presumably obtained from mean values from all experiments] with S. typhimurium TA100 and E. coli WP2, it appeared that trichlorfon was mutagenic at concentrations between 5 and 10 mg per plate. Trichlorfon did not cause DNA-repair damage to E. coli and B. subtilis in their respective assays.

Trichlorfon increased mitotic recombination in S. cerevisiae D3 in the presence and absence of S9 activation (similar response with and without S9). The recombination rate was about 1×10^{-5} without trichlorfon treatment. At trichlorfon concentrations of 1 to 5 percent (10–50 mg/ml), the recombination rate ranged from about 5×10^{-4} to 5×10^{-3}.

The unscheduled DNA synthesis (UDS) assay with trichlorfon was considered positive, and showed a dose-related increase. In the first test, the increase was statistically significant (99 percent confidence limit) but failed to show a dose response relationship, while the second test was statistically positive and gave a dose response relationship. In the presence of metabolic activation, trichlorfon failed to give a positive UDS response; however, the constraints of solubility with S9 prevented the authors from testing at the same concentrations which gave a positive result without S9, i.e., 100–10,000 µg/ml (0.1 to 10 mg/ml).

CONCLUSIONS:

Based on positive findings cited from the results above, the authors correctly concluded that trichlorfon has a broad spectrum of mutagenic and potentially other genetic activity. The experiments were well designed and are considered to have produced reliable results. In conclusion, trichlorfon was moderately mutagenic in bacterial reverse mutation assays in a concentration range between 5 and 10 mg/plate, induced mitotic recombination in yeast cells at 10–50 mg/ml, and induced unscheduled DNA synthesis in human fibroblasts at 0.1–10 mg/ml.

CORE CLASSIFICATION: Acceptable.