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WASHINGTON, D.C. 20460

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OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

JUN 27 1985

MEMORANDUM

SUBJECT: Trichlorfon RS - Evaluation of data submitted under Accession Nos. 256446, 256447, and 257599
Registration No. 3125-9 Caswell No. 385

FROM: Irving Mauer, Ph.D., Geneticist
Section VI, Toxicology Branch
Hazard Evaluation Division (TS-769)

6-25-85

TO: Gary Otakie, PM 16
Registration Division (TS-767)

THRU: Jane E. Harris, Ph.D., 6/26/85 6/27/85
Head, Section VI
TB/HED(TS-769)

Sponsor/Registrant: Mobay Chemical Corporation

TB Evaluation: The following studies were reviewed and evaluated (see attached TOXICOLOGY BRANCH: DATA REVIEWS for individual studies):

26 pages

| EPA Acc.# | Mobay Report No. | Title | TB Evaluation |
|---|---|---|---|
| 256446 | 37204 | The Acute Oral and Intraperitoneal Toxicity of Five Trichlorfon Technical Samples to Rats | MINIMUM (Tox. Cat. II) |
| 256446 | 45153 | L 13/59 Acute Inhalation Toxicity Study on Rats | MINIMUM (Tox. Cat. II) |
| 256446 | 45160 | L 13/59 Subacute Inhalation Toxicity Study on Rats | [MINIMUM]* |
| 256446 | 49442 | Trichlorfon - Mutagenicity Test on Bacterial Systems | UNACCEPTABLE |
| 256446 | 66689 | Acute Toxicity of Trichlorfon in Adult Rhode Island Red Hens | MINIMUM |
| 256446 | 80616 | L 13/59 (DIPTEREX Active Ingredient) Tests for Skin and Eye Irritation | SUPPLEMENTARY (Tox. Cat. IV — Skin III — Eye) |
| 256446 | 80700 | Chronic Toxicity of Metrifonate | [**] |
| 256446 | 80974 | Trichlorfon - Melting Process Japan Neurotoxicity Study on Chickens | MINIMUM |
| 256446 | 85918 | Mutagenicity Evaluation of L 13/59 (C.N. Trichlorfon) in the Reverse Mutation Induction Assay with Saccharomyces Cerevisiae Strains S138 and S211a | ACCEPTABLE |
| 256446 | 86396 | Evaluation of L 13/59 C.N. Trichlorfon in the Primary Rat Hepatocyte Unscheduled DNA Synthesis Assay | ACCEPTABLE |
| 256446 | 86723 | An Acute and Subacute Neurotoxicity Assessment of Trichlorfon | MINIMUM |

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|---|---|---|---|
| 256446 | 86724 | Mutagenicity Evaluation of L 13/59 C.N. Trichlorfon in the Mouse Lymphoma Forward Mutation Assay | ACCEPTABLE |
| 256446 | 86733 | Trichlorfon - Mutagenicity Test on Bacterial Systems | [Same as #49442] |
| 256477 | 84009 | A Two-Generation, Two-Year Feeding Study of Trichlorfon in the Rat. | INVALID [Summary Only] |
| 257599 | 88978 | L 13/59 (c.n. trichorfon 1S0). Study for Skin-Sensitizing Effect on Guinea Pigs. | GUIDELINES (Moderate skin sensitizer) |

* Provisional. Urinalysis data should be submitted, as well as histopathological data for controls--see attached DATA REVIEW.

** Published summary of studies previously submitted by Mobay--see Registration Standard.

TOXICOLOGY BRANCH: DATA REVIEW

Chemical: Trichlorfon

Caswell: 385
EPA Chem.#: 057901

Study Type: Acute oral/ip $LD_{50}$ - Rat.

Citation: The Acute Oral and Intraperitoneal Toxicity of Five Trichlorfon Technical Samples to Rats.

Accession No./MRID No.: 256446

Sponsor/Testing Lab.: Mobay/Chemagro

Study No./Date: 37204/June 13, 1973

Test Material: Five batches of trichlorfon technical, as follows: 33048 (98.7% ai); 35873 (98.4% ai); 38865 (90.6%); 38866 (90.8% ai); 43093 (90.8% ai).

Procedures: Groups of four male and four female Sprague-Dawley rats weighing 200-230 g each were either intubated (after a 20-hr fast) or injected ip with graded doses of each batch of technical, and observed daily for 7 days. $LD_{50}$'s and confidence limits were calculated according to the method of Weil (Biometrics 8, 1952).

Results: All animals manifested "symptoms" (presumably OP toxicity) within the 7-day period of observation. The following $LD_{50}$'s were calculated:

| Batch | ORAL (mg/kg) | | IP (mg/kg) | |
|---|---|---|---|---|
| | Males | Females | Males | Females |
| 33048 | 184 (162-209) | 136 (125-147) | 195 (172-222) | 156 (133-183) |
| 35873 | 195 (162-306) | 151 (138-166) | 185 (156-219) | 165 (156-219) |
| 38865 | 274 (205-365) | 144 (130-160) | 165 (148-185) | 165 (148-185) |
| 38866 | 238 (207-274) | 144 (130-160) | 175 ( * ) | 156 (137-178) |
| 43093 | 273 (262-286) | 173 (156-192) | 218 (186-255) | 185 (165-207) |

* Confidence limits not calculable; 0/4 deaths at doses of 125 and 156 mg/kg, 4/4 at 195 and 243 mg/kg.

Conclusions: The authors concluded that the toxicity of all batches was comparable.

TB Evaluation/Core: Minimum. Oral TOX. CAT. II
IP TOX. CAT. II

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TOXICOLOGY BRANCH: DATA REVIEW

Chemical: Trichlorfon

Caswell: 385
EPA Chem.#: 057901

Study Type: Acute inhalation $LC_{50}$ - Rat.

Citation: L 13/59 Acute Inhalation Toxicity Study on Rats. (G. Kimmerle)

Accession No./MRID No.: 256446

Sponsor/Testing Lab.: Mobay/Bayer AG Institut fur Toxikologie.

Study No./Date: Bayer Rpt. #5581 (Mobay #45153)/August 8, 1975.

Test Material: L 13/59 (technical trichlorfon), Batch 9609/73 (% ai not stated).

Procedures: Male and female Wistar II SPF rats weighing 160-190 g (10 per sex per experimental group) were exposed to aerosols of test compound (dissolved in a 1:1 mixture of ethanol and polyethylene glycol) in a dynamic flow inhalation chamber for either 1 hour at concentrations of 75, 229 and 419 $mg/m^3$ (determined quantitively by gc fitted with an electron capture detector), or for 4 hr at 6 concentrations ranging from 1.7 to 533 $mg/m^3$. All animals were observed for 14 days. Plasma and rbc cholinesterase activities were determined in one-half of each group exposed for 4 hr to the 3 lower concentrations of trichlorfon (1.7, 8.0 and 28 $mg/m^3$) immediately after exposure and 24 hr later.

Results: No deaths occurred after either exposure period (1 or 4-hr). No signs of toxicity were observed in animals exposed for 1 hr, nor in those exposed for 4 hr at concentrations of 1.7, 8.0, 28.0 and 73.7 $mg/m^3$. The two higher 4-hr exposures (296 and 533 $mg/m^3$) resulted in "...impairment of the general health condition, which persisted for 4-6 hours," but "...no indication of any damage to tissues." Exposure to 28 $mg/m^3$ trichlorfon for 4 hr (but not the two lower dosages) resulted in a 15 to 25 percent depression of circulating cholinesterase activity immediately after exposure in males (from 2.94 to 2.18 uM-AC in plasma; 3.74 to 3.26 uM in rbc), as well as in females (from 4.56 to 3.69 uM for plasma; 3.76 to 3.00 for rbc), all values recovering or exceeding pre-exposure activities by 24 hr.

Conclusions: $LC_{50}$ 1-hr exposure (males, females) > 419 $mg/m^3$. (0.4 mg/L)
$LC_{50}$ 4-hr exposure (males, females) > 533 $mg/m^3$. (0.5 mg/L)
TLC (threshold limit concentration) for cholinesterase depression = 28 $mg/m^3$.

TB Evaluation/Core: Minimum. TOX CAT.II

TOXICOLOGY BRANCH: DATA REVIEW

Chemical: Trichlorfon

Caswell: 385
EPA Chem.#: 057901

Study Type: Subchronic Inhalation - Rat

Citation: Subacute Inhalation Toxicity Study on Rats (G. Kimmerle)

Accession No./MRID No.: 256446

Sponsor/Testing Lab.: Mobay/Bayer AG Institute for Toxikologie

Study No./Date: Bayer #5582 (Mobay 45160)/August 8, 1975

Test Material: L13/59 (technical trichlorfon), Batch #9609/73 (percent ai not stated)

Procedures: Male and female SPF Wistar II rats (10/sex/group) were exposed 6 hr/day during a period of 3 weeks (total number of exposures = 15) to aerosols of the test material in a dynamic flow inhalation chamber at concentrations averaging 12.7, 35.4, and 103.5 mg/$m^3$ air (determined by gc fitted with an electron capture detector). As measured by a cascade impactor, 93 percent of the droplets were 1.0±0.5μ in diameter (the remainder, <5.0μ). A control group (10/sex) were exposed to the ethanol-polyethyleneglycol solvent at 20 ml/$m^3$. Clinical observations were recorded daily, body weights determined weekly, and clinical tests (hematology, chemistry, urinalysis) performed 24 hours after final exposure. Circulating cholinesterase was determined in one-half of each group after the fifth, tenth, and fifteenth exposure, and in brain at necropsy.

Results: One middose female died during the study, reportedly "...as the result of an accident." Only the high-dose group showed evidence of clinical toxicity, but in no group were body weight gains significantly different from controls (Report Tables 1a and 1b). Hematological values (hct, hb, cbc--Table 2 to 17), clinical chemistries (BUN, glu, GPT, GOT, AP, creat, chol, bili, total-P--Tables 18 to 25), and urinalysis (pH, glu, alb, blood, bili, urob, casts--no data provided) were comparable in all groups. Significant reductions in plasma (25 to 47%), rbc (25 to 30%) and brain (22 to 47%) cholinesterase activities were noted in both sexes exposed to 103.5 mg/$m^3$ (with larger depressions registered in females), as well as in middose females (35.4 mg/$m^3$); 25 to 30 percent reductions were also recorded in males after 15 exposures to 12.7 and 35.4 mg/$m^3$ trichlorfon (report Table 26a). Except for significant increases in absolute (20%) and relative (20 to 25%) spleen weights of mid- and high-dose males, gross pathological examination of thyroid, thymus, lung, liver, kidneys, adrenals, testes and ovaries were comparable between test groups and controls (Tables 27 to 40). Histopathological examination did not reveal

consistent variations from normal in any test group (5/sex from each group), even in animals exposed to the highest dosage showing evidence of clinical toxicity and cholinesterase depression.

Respiratory tract alterations (tracheal inflammatory cell infiltrations and mucus, pulmonary cell infiltrations and "emphysema") in all trichlorfon groups were stated to be "...commonly observed in animals maintained under conventional conditions."

[N.B.: However, a portion of the raw data for controls is missing from this review copy, covering histopathological examination of lungs, liver, spleen, kidneys, adrenals, stomach, ovary, testis, eyes, and bones.]

Reviewer Discussion: Neither the histopathological nor hematological data provide explanation for the splenomegaly observed in mid- and high-dose males, hence this may represent an incidental finding. Depression in plasma cholinesterase values of males at the LDT, as well as the mid-dose level, but only at the terminal sampling (ascertained from inspection of Table 26a of the report), also may not be biologically significant, since neither erythrocyte nor brain values conformed; on the other hand, consistent depressions in both circulating and tissue activities among mid-dose females represent an effect level. Based on the data submitted in this study, the NOEL for cholinesterase inhibition is set at 12.7 $mg/m^3$, with the LEL at 35.4 $mg/m^3$. For systemic effects, the NOEL is 35.4 $mg/m^3$, and the LEL 103.5 $mg/m^3$, based upon clinical toxicity (presumably OP effects, but stated as "...impairment of the general health condition").

TB Evaluation/Core: Provisionally MINIMUM, but urinalysis data should be submitted as well as the missing histopathological data for controls noted above.

TOXICOLOGY BRANCH: DATA REVIEW

Chemical: Trichlorfon

Caswell: 385
EPA Chem.#: 057901

Study Type: Mutagenicity - Gene Mutation (Ames Assay) and DNA Damage/Repair (rec-assay) in Bacteria.

Citation: Trichlorfon. Mutagenicity Test in Bacterial Systems.

Accession No./MRID No.: 256446

Sponsor/Testing Lab.: Mobay/Nitokuno Agricultural Chemicals Institute, Laboratory of Toxicology (Toyoda, Japan)

Study No./Date: Nitokundo Rpt. #28 (Mobay #49442)/June 19, 1976.

Test Material: Technical (DMN-200, 99 percent ai), dissolved in DMSO, and diluted with water.

Procedures: In the rec-assay, paper discs impregnated with 3, 30, or 300 mg test compound (dissolved in DMSO-water) were placed in contact with Bacillus subtilis cells of the sister strains NIG17 ($rec^+$) and NIG45 ($rec^-$), differing only in the ability to repair DNA damage. The cultures were incubated overnight at 37 °C, and growth inhibition (in mm) compared. Mitomycin-C (MC) served as a positive control. In a reversion (Ames) assay, Samonella typhimurium strains TA 1535, TA 1537, TA 98 and TA 100 were exposed (by plate incorporation) to test material at concentrations of 0.2, 2, 20 and 500 µg/plate, in the absence (non-activation) and presence (metabolic activation) of the S-9 fraction of liver homogenates from rats treated with phenobarbital (plus appropriate co-factors). Test plates were incubated for 2 days, and the number of revertent colonies compared to controls. The following reference mutagens served as positive controls: Furyl-furamide (AF-2) for non-activated TA 1535 and TA 100 cultures, and N-methyl-N'-nitro-Nitrosoguanidine (MNNG) also for TA 1535; Dexon (p-[dimethylamino] benzenediazo sodium sulfonate) for non-activated TA 1537 and TA 98; and acetylaminoflurorene (AAF) for activated TA 98 cultures.

Results: Trichlorfon did not inhibit growth of either B. subtilis strain (0 mm) at any concentration, whereas MC caused a differential inhibition of 8 mm (1 mm for the repair-proficient NIG 17 strain vs. 9 mm for the deficient NIG 45).

In the Ames Assay, increases 2 to 3x control in absolute colony counts are shown (report Table 4) for non-activated test cultures of TA 1537 and activated cultures of TA 98 (both of which detect frame-shift mutagens), but less than twice control for TA

100 (detects mainly base-pair substitutions), none of which was dose-related. The (limited) positive control cultures treated with reference mutagens responded appropriately, with increases over respective controls of 5-fold (TA 100) to >20-fold (TA 1535, TA 1537, TA 98).

Reviewer's Discussion: (Ames Assay only)

Since (i) there is a range of control values for each of these strains used (even in the same lab over time, as ascertained since these studies were conducted) which would overlap the absolute increases reported, and (ii) no apparent dose-responsiveness was found, we would partially concur with the authors' conclusions that trichlorfon was non-mutagenic, but only up to the HDT (500µg/plate). Additionally, there are a number of deficiencies rendering this study inadequate, which should have been recognized had the investigators followed the referenced articles, e.g.:

(1) No toxicity data are provided.
(2) Ames Assay conducted with trichlorfon at the same time and since, indicate the HDT is insufficient.
(3) Lack of positive controls for activated tests with TA 1535, TA 1537 and TA 100.

TB Evaluation: Rec-assay, ACCEPTABLE.
Ames Assay, UNACCEPTABLE.

TOXICOLOGY BRANCH: DATA REVIEW

Chemical: Trichlorfon

Caswell: 385
EPA Chem.#: 057901

Study Type: Acute Delayed Neurotoxicity - Hen

Citation: Acute Toxicity of Trichlorfon in Adult Rhode Island Red Hens.

Accession No./MRID No.: 256446

Sponsor/Testing Lab.: Mobay/Albany Medical College, Department of Toxicology (E. Olajos and I. Rosenblum)

Study No./Date: Mobay #66689/(undated)

Test Material: (Not stated)

Procedures: Atropinized RIR hens (1.5 to 2.3 kg) were divided into two groups: Group A (3 hens) receiving a single dose of 200 mg/kg test material sc; and Group B (19 hens), a divided dose of 200 and 100 mg/kg sc 3 days apart. Group A birds and three from Group B were killed 24 hours after treatment; the remaining Group B birds were observed over a 3 to 4 week period, then sacrificed for determination of brain neurotoxic esterase activity (NTE) and histopathological examination of the central (CNS) and peripheral (PNS) nervous systems.

Results: Although no clinical observations were explicitly stated in the report text for Group A (200 mg/kg), Table I of the report indicated three Group B birds given a total dose of 300 mg/kg "...died from the acute toxic effects of trichlorfon one day after dosing," while 11 of the remaining 13 exhibited signs of delayed neurotoxicity (ataxia or paralysis) of Grade 2 severity (on the Cavanagh, et al., 1961, scale of 0-8). This response was considered "marginal" as compared to those from neurotoxic doses of the reference standards tri-o-cresylphosphate (TOCP) or diisopropyl fluorophosphate (DFP) (Grade 6), or that for leptophos, as previously reported in the literature (Cavanagh et al., 1961; AbouDonia, 1976).

Dose-related acute 24-hour post-treatment inhibition of NTE compared to an untreated control value of 13.4 (Table II) was evident, as indicated by the 37 to 41 percent depression reported for Group A birds (single dose of 200 mg/kg) compared to the 53 to 64 percent in Group B (200 + 100 mg/kg), but considerably less than that resulting from the authors' previous experience (unpublished data not reported here) with TOCP (81%) and DFP (89%). Compared to the return to normal 4 weeks after DFP (as reported by Johnson, 1975), however, brain NTE in Group B trichlorfon-treated birds (300 mg/kg total dose) was still inhibited 18 to 25 percent as measured 29 to 32 days after the last of the divided dosage.

Histopathological lesions were observed one month after dosing in both the CNS (cerebellar demyelinization, pyknotic Purkinje cells, inter alia) and PNS (sciatic nerve axonal swelling, and vacuolization of myelin), but not detected in CNS preparations of hens treated at either dosage sacrificed 24 hours after trichlorfon administration; 24-hour post-dose PNS samples were considered "equivocal in this regard."

Conclusion: Subcutaneously administered, marginally neurotoxic doses of 300 mg/kg trichlorfon (producing a Grade 2 delayed response in 9 to 15 days) induced a prolonged inhibitory effect on brain NTE (75 to 80% of normal) a month after dosing, as well as pathologic lesions in both CNS and PNS.

TB Evaluation/Core: MINIMUM. Although acute clinical effects at 200 mg/kg were not described, nor delayed sequelae investigated, the results of this study are consistent with other reports in the hen by Olajos (1979), Johnson (1970, 1975), Witter (1962)--see Registration Standard (1984). The acute clinical NOEL in birds pretreated with atropine would appear to be 200 mg/kg, but this level produced significant reduction in brain NTE.

TOXICOLOGY BRANCH: DATA REVIEW

Chemical: Trichlorfon

Caswell: 385
EPA Chem. #: 057901

Study Type: Eye Irritation - Rabbit

Citation: L13/59 (Dipterex Active Ingredient). Test for Skin and Eye Irritation.

Accesion No./MRID No.: 256446

Sponsor/Testing Lab.: Mobay/Bayer AG, Institute of Toxicology.

Study No./Date: Bayer #T9010306 (Mobay #80616)/1981.

Test Material: L13/59 (technical trichlorfon), Batch #809931188 (98.7% ai).

Procedures: Test material (unstated dose) was instilled in the eyes of albino NZ rabbits, and washed out after 5 minutes (5 rabbits) and 24 hr (3 rabbits). Assessment of irritation was according to the US-HEW criteria (37 FR 83, pp. 8534-5, April 24, 1972). Post dose observations were made at 1 hr, and at 1, 2, 3, 7, 14, and 21 days.

Results: Five-minute treatment with test material caused moderate conjunctival redness in all animals at 1 hr persisting for 72 hr, (but disappeared by 7 days), a low degree of chemosis which resolved by 72 hr in all but one rabbit, but no ulceration or corneal/iritic reactions. Twenty-four treatment was also positive for conjunctival redness and chemosis (but again, no ulceration or other reactions), disappearing by 7 days.

Conclusions: Trichlorfon is a moderate irritant to the ocular mucosae of rabbits.

TB Evaluation/Core: SUPPLEMENTARY. Dose instilled was unstated. TOX. CAT. III

TOXICOLOGY BRANCH: DATA REVIEW

Chemical: Trichlorfon

Caswell: 385
EPA Chem. #: 057901

Study Type: Skin Irritation - Rabbit

Citation: L13/59 (Dipterex Active Ingredient). Tests for Skin and Eye Irritation.

Accession No./MRID No.: 256446

Sponsor/Testing Lab.: Mobay/Bayer AG, Institute of Toxicology.

Study No./Date: Bayer #T9010305 (Mobay #80616)/September/October, 1981

Test Material: L13/59 (technical trichlorfon), Batch #809931188 (98.7% ai)

Procedures: Test material (dose unstated) was applied to the intact and abraded skin of six albino NZ rabbits (3 male, 3 female), and kept in place for 24 hr. Dermal reactions were assessed according to the Draize criteria (1959). Animals were observed for 7 days.

Results: No erythema or edema was observed in either abraded skin at 24 or 74 hr postdose. Hence PIS = 0.

Conclusions: L13/59 was considered "...not a skin irritant."

TB Evaluation/Core: SUPPLEMENTARY. Amount of test material applied was not specified. (TOX CAT. IV).

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TOXICOLOGY BRANCH: DATA REVIEW

Chemical: Trichlorfon

Caswell: 385
EPA Chem. #: 057901

Study Type: Chronic Toxicity - (various spp)

Citation: "Chronic Toxicity of Metrifonate" (published article, by L. Machemer, Acta Pharmacol. Toxicol. 49, Supplement V: pp. 15 to 28, 1981).

Accession No./MRID No.: 256446

Sponsor/Testing Lab.: (Published article from Bayer AG)

Study No./Date: Mobay #80700

[NOTE: This article is a summary of previous studies submitted by Mobay, which have already been evaluated for the Registration Standard].

TOXICOLOGY BRANCH: DATA REVIEW

Chemical: Trichlorfon

Caswell: 385
EPA Chem. #: 057901

Study Type: Neurotoxicity in Hens

Citation: Trichlorfon---Melting Process Japan. Neurotoxic Study on Chickens

Accession No./MRID No.: 256446

Sponsor/Testing Lab.: Mobay/Bayer AG Institute of Toxicology.

Study No./Date: Bayer Rpt #10811 (Mobay #80974)/April 20, 1982.

Test Material: L13/59, technical preparation DNR-136 (94.6% ai), administered orally as a 90 percent premix with Aerosil.

Procedures:

1. Adult White Leghorn hens weighing between 1.5 and 2.0 kg (10 per group except where noted) were gavaged once with test material (emulsified with aqueous Cremophor) at doses of 100, 150, 175 (20 hens) and 200 mg/kg (to determine an $LD_{50}$), weighed weekly and observed for 28 days. Gross pathology was performed on the survivors.

2. A second group of 30 atropinized hens was intubated with 185 mg/kg test material, the survivors challenged 21 days later (again following atropine protection) with 167 mg/kg, and then observed for a further 3 wk. All surviving birds were then sacrificed for histological examination of brain, spinal cord, and sciatic nerve. Six birds treated with the Cremophor carrier served as "negative" controls and 10 hens were gavaged once with 375 mg/kg of the reference positive substance, tri-ortho-cresyl phosphate (TOCP).

Results:

1. In the acute toxicity study, death occurred (within 1 and 24 hr) in 2 birds treated at 150 mg/kg, in 13 at 175 mg/kg, and in all 10 at 200 mg/kg. All trichlorfon-treated survivors lost weight during the first week (but regained starting weight by the third week), and exhibited varying degrees of typical OP toxicity lasting 4 days.

No indications of delayed neurotoxicity were reported in survivors observed up to 28 days. Gross pathology of all dead birds revealed ulcerated crops and stomachs, and the lungs in some had serosal bleeding.

2. In the neurotoxicity study, 18 birds died within 2 days of the first dose, and a further 6 (of the 12 survivors) after the second (challenge) dose. Toxic signs subsided in survivors by the sixth day thereafter, and no delayed neurotoxic signs ensued. TOCP-treated hens were symptom-free for 6 days postdose, but thereafter developed ataxia and paralysis.

   No pathological neural lesions were found in surviving trichlorfon-treated hens, contrasted with severely degenerative alterations in ischemic nerve fibers of TOCP treatment.

   Neurotoxic esterase activity measured 24 hr (4 birds), and 48 hr (3 birds) after the first trichlorfon dose (185 mg/kg) was stated to reveal "No indication of a toxicologically relevant activity inhibition of NTE in brain, spinal cord and nn.ischiadici..." [Table 2 of the report recorded an average of 14 percent inhibition in 24-hr brain samples (range, 6 to 21%), but only a singular 11 percent depression in a cord sample from the same birds manifesting the highest brain reduction.]

Conclusions:

1. The $LD_{50}$ approximates 167 mg/kg, acute cholinergic signs were of short duration (4 days), and no indication of delayed neurotoxicity was evident.

2. The challenge study conducted at $LD_{50}$ doses also revealed no clinical, histological, or chemical (NTE activity) evidence of delayed neurotoxicity.

TB Evaluation/Core: MINIMUM

TOXICOLOGY BRANCH: DATA REVIEW

Chemical: Trichlorfon

Caswell: 385
EPA Chem. #: 057901

Study Type: Mutagenicity - Gene Mutation in Yeast

Citation: Mutagenicity Evaluation of L/3/59 (c.n. Trichlorfon) in the Reverse Mutation Induction Assay with Saccharomyces cerevisiae Strains S138 and S211α. Final Report.

Accession No./MRID No.: 256446

Sponsor/Testing Lab.: Mobay-Bayer AG/Litton Bionetics, Europe (Netherlands)

Study No./Date: Bayer Study #T1007987 (Mobay #85918)/June, 1983

Test Material: L13/59 (technical trichlorfon), Batch #809231110 (98.3% ai), a white powder, dissolved in DMSO for testing.

Procedures: Yeast cells of strains S138 (a frameshift methionine auxotroph) and S211α (base-pair substituted meth$^-$) were exposed for 3 hr to six concentrations of test substance from 33.3 to 10,000 µg/ml, both in the absence and presence of a mammalian metabolic activation system derived from Aroclor-induced rat liver S-9. Test plates were incubated for 3 days for population counts, and duplicates for 5 to 7 days for revertent counts. Solvent-treated plates served as negative controls; the reference mutagens, quinacrine mustard (for nonactivated S138 tests), ethylmethanesulfonate (for nonactivated S211α), and sterigmatocystin (both activation tests) served as positive controls.

Results: Test material was nontoxic to both strains up to 10,000 µg/ml, and no increase in methionine revertent frequencies (per $10^6$ survivors) recorded up to the HDT. Plates treated with the reference mutagens QM and EMS responded appropriately (200 to 500X solvent controls), but sterigmatocystin-treated plates only marginally (2 to 5X controls).

Conclusions: Trichlorfon was not mutagenic to S. cerevisiae S138 and S211a cells under the conditions and evaluation criteria employed in this assay.

TB Evaluation/Core: ACCEPTABLE

TOXICOLOGY BRANCH: DATA REVIEW

Chemical: Trichlorfon

Caswell: 385
EPA Chem. #: 057901

Study Type: Mutagenicity - Unscheduled DNA synthesis in rat hepatocytes (HPC/UDS).

Citation: Evaluation of L13/59 (c.n. Trichlorfon) in the Primary Rat Hepatocyte Unscheduled DNA Synthesis Assay.

Accession No./MRID No.: 256446

Sponsor/Testing Lab.: Bayer AG/Litton Bionetics, Kensington, MD

Study No./Date: LBI Project #20991, Assay #6908 (Mobay #86396)/ November, 1983.

Test Material: L13/59 (technical trichlorfon), Batch #80923110 (98.3% ai), a white powder, dissolved in WME tissue culture medium for testing.

Procedures: Hepatocytes collected by referenced procedures from an adult male Fischer-344 rat were exposed in vitro to eight concentrations of test material ranging from 0.5 to 100.0 ug/ml, and prepared for nuclear labeling (by $^{3}H$-thymidine). Mean net nuclear grain counts (MNGC) from triplicate preparations per test concentrations (150 total cells) were compared to the negative control (WME medium) and the reference mutagen (positive control), 2-acetylaminofluorene (2-AAF), using acceptable evaluation criteria.

Results: Concentrations of 100 ug/ml (and higher) were cytotoxic and could not be analyzed for MNGC (7.4% cell survival at 100 ug/ml by trypan blue), but those of 25 ug/ml and below resulted in cultures indistinguishable from negative controls. At no concentration of test material did the MNGC exceed the evaluation criteria (MNGC > 6.56, or at least 10% of cells with 6 or more net grains, or 2% of cells containing 20 or more grains), the labeling remaining comparable to control values up to 50 ug/ml (cytotoxic range), in contrast to the positive control (2-AAF) which exhibited a MNGC of 7.11, with 47.3 percent of nuclei averaging greater than 6 grains and 6 percent with greater than 20 grains.

Conclusions: The test material was considered inactive in inducing UDS in rat hepatocytes up to levels of severe cytotoxicity.

TB Evaluation: ACCEPTABLE

TOXICOLOGY BRANCH: DATA REVIEW

Chemical: Trichlorfon

Caswell: 385
EPA Chem.#: 057901

Study Type: Neurotoxicity in Hens

Citation: An acute and Subacute Neurotoxicity Assessment of Trichlorfon, by V. Slott and D.J. Ecobichon, Canad J. Physiol. Pharmacol. 62:513-518, 1984.

Accession No./MRID No.: 256446

Sponsor/Testing Lab.: Published article (from McGill University Montreal, Canada).

Study No./Date: Mobay # 86723

Test Material: Dylox (technical trichlorfon, 97% ai) from Mobay

Procedures: Atropinized adult (12-15 mo) White Leghorn hens (4/group) were injected sc once with 100 or 300 mg/kg test material (acute assay), or (not treated with atropine) every 3 days with 100 mg/kg for a total of six doses (subchronic assay). Signs of neurotoxicity as well as circulating (plasma) and tissue (brain, spinal cord) cholinesterase and neurotoxic esterase (NTE) activities were compared to diisopropyl phosphorofluoridate (DFP) treatment (reference neurotoxin). Additionally, the sciatic nerve was examined enzymatically and histologically in the subchronic assay.

Results: Acute treatment. Both doses of trichlorfon caused a marked (equivalent) decrease in plasma (50% normal), brain (73%) and cord (60%) cholinesterase activity 24 hr after treatment (the higher dose being only marginally more inhibitory), but only slight depression in brain or neuronal NTE (70-80% of control). In contrast, DFP also depressed both tissue NTE levels.

Subchronic Treatment. Compared to the progressive clinical toxicity (ataxia, weakness, paralysis) and weight loss (>35%) in DFP-treated birds beginning 48 hr after initiation of treatment, only minor problems of equilibrium, coordination, and gait were observed for trichlorfon treatment, and mean body weight was only slightly depressed after the final dose (<15%, not significant), attributable to anorexia and decreased activity. Circulating and neural cholinesterase activities were equally depressed in both trichlorfon and DFP groups (40% and 60%, respectively), but no inhibition of neural NTE was found for trichlorfon treatment, in contrast to 55 to 70% depression for DFP. Except for minor cerebral edema and "slight widening of pericapillary and pericellular spaces" (without vacuolization or neuronal degeneration), and minor "thinning" of sciatic myelin sheaths in the trichlorofon-treated group, no distinct histopathological changes were observed, compared to the abnormal central architectural pattern, neuronal degeneration, and demyelinization seen for DFP treatment.

Conclusion: Although single doses of 100 or 300 mg/kg or a repeat schedule of 100 mg/kg/day trichlorfon caused marked inhibition of circulating and tissue cholinesterase activity and minor clinical effects, no evidence of delayed neurotoxicity was observed, as supported by lack of alterations in NTE levels and minimal morphological changes.

TB Evaluation/Core: Minimum.

TOXICOLOGY BRANCH: DATA REVIEW 004509

Chemical: Trichlorfon

Caswell: 385
EPA Chem.#: 057901

Study Type: Mutagenicity - Gene Mutation in Mammalian Cells in vitro (L5178Y/TK).

Citation: Mutagenic Evaluation of L13/59 C.N. Trichlorfon in the Mouse Lymphoma Forward Mutation Assay.

Accession No./MRID No.: 256446

Sponsor/Testing Lab.: Bayer AG/Litton Bionetics, The Netherlands.

Study No./ Date: LBI Genetics Assay No. E-9107 (Bayer R-2824, Study #T7008982; Mobay #86724)/April, 1984

Test Material: L13/59 (technical trichlorfon; Batch #809231110. (99.2% ai), a white powder dissolved in culture medium for testing.

Procedures: Following preliminary cytotoxicity testing, cells of the mouse lymphoma cell line, L5178Y $TK^{+/-}$ -3.7.2C, were exposed to test material at 7 concentrations ranging from 5 to 125 ug/ml without mammalian metabolic activation, or to a range of 7 concentrations of 1 through 145 ug/ml in the presence of an activation system consisting of the 9,000X supernatant from adult male rat liver induced by Aroclor 1254 (S-9) plus appropriate cofactors. Each portion of this assay was repeated once. Suspension growths, percent cloning efficiencies, percent relative growth, and mutant frequencies per $10^{-6}$ viable colonies (mf) were compared to solvent control (medium) and to the following reference mutagens (positive controls): ethyl methanesulfonate (nonactivation tests), and methylcholanthrene (activation tests).

Results: Relative growth (compared to medium control) in repeat assays was affected by all concentrations of test material, ranging from 90 percent at the LDT decreasing to about 1 percent at the HDT in nonactivated assays, and from 70 percent at 10 ug/ml to 5 percent at 145 ug/ml in the first activation test and 75 percent at the LDT decreasing to about 8 percent at 100 ug/ml in the second (145 ug/ml was too toxic for mutant analysis). Dose dependent increases in mf were recorded at nonactivated concentrations of 50 ug/ml and above in the first test and 75 ug/ml and above in the repeat, and with activation at 70 ug/ml and above initially and 50 ug/ml and above in the replicate.

Conclusion: In independent repeat assays, trichlorfon (Batch #809231110, 99.2%) induced significant increases in mf, both in the absence and presence of metabolic activation.

TB Evaluation: ACCEPTABLE.

TOXICOLOGY BRANCH: DATA REVIEW 004509

Chemical: Trichlorfon

Study Type: Mutagenicity - Gene Mutation and DNA Repair in Bacteria (AMES and rec-assays)

Citation: Trichlorfon. Mutagenicity Test on Bacterial Systems.

Accession No./MRID No.: 256446

Sponsor/Testing Lab.: Bayer AG/NITOKUNO Agricultural Chemicals Institute, Laboratory of Toxicology, Japan.

Study No./Date: NITOKUNO Rpt #87 (Mobay #86733)/December 2, 1977.

Test Material: Technical Trichlorfon, (DMN-200, 99% ai)

[NOTE: This appears to be the same study as NITOKUNO Report #28 (Mobay #49442), dated June 19, 1976.]

TOXICOLOGY BRANCH: DATA REVIEW

Chemical: Trichlorfon

Caswell: 385
EPA Chem.#: 057901

Study Type: Reproduction - Rat

Citation: A Two-Generation, Two-Year Feeding Study of Trichlorfon in the Rat (Inst. Exptl. Path. Tox., Albany Medical College).

Accession No./MRID No.: 256447

Sponsor/Testing Lab.: Mobay/Albany Medical College

Study No./Date: Mobay #84009/February 28, 1983.

Test Material: Technical (98.6% ai)

[Note: This submission entitled, "Dylox Supplement No. 1, February 28, 1983", under cover letter from Mobay, January 31, 1985, is a summary and synopsis only of a study completed in 1981, and contains no data sheets].

004509

TOXICOLOGY BRANCH: DATA REVIEW

Chemical: Trichlorfon

Caswell: 385
EPA Chem.#: 057901

Study Type: Skin Sensitization - Guinea Pig

Citation: L 13/59 (c.n. trichlorfon 150). "Study for Skin Sensitizing Effect on Guinea Pigs".

Accession No./MRID No.: 257599

Sponsor/Testing Lab.: Mobay/Bayer AG Institute of Toxicology

Study No./Date: Bayer Study #T6018557 (Mobay #88978)/February 2, 1985.

Test Material: L 13/59 (technical trichlorfon), Batch #809-331-149 (98.7% ai), dissolved neat in Freund's Adjuvant diluted 1:1 in DW (first injection), and formulated as a 1 percent preparation with 0.9 percent NaCl-2 percent Cremaphor EL (second injection and topical inunction) and with additional Freund's mixed in equal parts (third injection).

Procedures: The Magnusson-Kligman (1969) maximization procedure was employed. Briefly, three groups (20, 10, and 10) of young adult male guinea pigs (strain BOR: DHPW, 344-422 gm) were primed by intradermal injections of 0.1 ml test compound in Freund's adjuvant in parallel on each flank, the first group ("test" animals) only receiving two successive injections of the 1 percent Cremaphor formulation medially and caudally. One week later, the test group of 20 was inuncted with 25 percent trichlorfon in 2 percent Cremaphor EL-saline, and test sites covered for 48 hr; the two "control" groups received only the vehicle. Two weeks after topical induction, hypoallergenic dressings (10% sodium lauryl sulphate in paraffin oil) soaked in 25 percent test compound formulations were placed on the left flank sites of the test and first control groups, and fastened with Saniplast (occlusive) for 24 hr; control dressings (soaked only with vehicle) were placed on the right flank sites. Two weeks later, a second challenge of 12.5 percent formulation was applied to the left sides of the test and second control groups, in order to eliminate concentration-related effects. Reactions were appraised 24 and 48 hr after removal of dressings, and assessed according to Kligman's (1966) classification scale.

Results: Adjusted values for the test group of 20 animals (i.e., the number of animals reacting on compound side less number reacting on control side) for the first challenge was 11 (55%) compared to 0 for first control group, and for the second challenge was 9 (45%) versus 1 (10%) for the second control group. In addition to irritation, responding test compound animals exhibited slight hardening and scabbing of the treated area, observed after both challenges.

Conclusions: Trichlorfon technical is considered to be a sensitizer, classified (according to Kligman) as a "moderate contact allergen."

TB Evaluation/Core: Guideline.