201-15799B

US EPA HPV Chemical Challenge Program

ROBUST SUMMARIES
FOR
CARBAMIC ACID, BUTYL-, 3-IODO-2-PROPYNYL ESTER
(CAS No. 55406-53-6)

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Robust Summaries, CAS No. 55406-53-6
Carbamic acid, butyl-, 3-iodo-2-propynyl ester

INTRODUCTION

Troy Chemical Corporation (www.troycorp.com) has prepared the following robust summaries for Carbamic acid, butyl, 3-iodo-2-propynyl ester, otherwise known as 3-iodo-2-propynl (IPBC) (CAS# 55406-53-6), as part of its contribution to the US Environmental Protection Agency's (EPA) High Production Volume (HPV) Challenge Program. The US HPV Challenge Program is directed at chemicals falling under the jurisdiction of the Toxic Substances Control Act (TSCA) and the initial list of HPV chemicals were identified from the 1990 TSCA Inventory Update. While IPBC was on the HPV candidate list, Troy is currently not aware of any uses of this compound governed by TSCA. Troy's production of IPBC is limited to pesticidal applications that are governed by the Federal Insecticide Fungicide and Rodenticde Act (FIFRA). Nonetheless, as the goal of the HPV Challenge Program is to ensure that the American public has access to basic information about the chemicals manufactured in the US, Troy has provided these robust summaries.

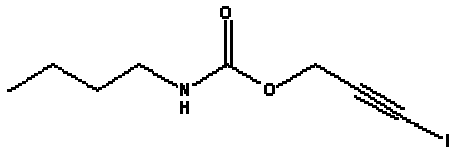
Background on IPBC and FIFRA Registration

IPBC is an off-white crystalline solid, non-metallic, non-phenol fungicide and mildewcide effective against a wide variety of fungal organisms.

Molecular Formula: $C_8H_{12}INO_2$

Molecular Weight: 281.0928

Chemical Structure:



The technical grade active pesticide ingredient is sold by Troy under the trade name Polyphase P100 (FIFRA registration #5383-50). Polyphase P100 is used to prevent the growth of fungi and mildew in press cakes and in fiber and yarn lubricants. Polyphase P100 also provides protection against mildew and rot of adhesives and fabrics such as awnings, canvas, footwear, jute, outdoor leisure upholstery, outdoor sports apparel and furnishings, sails, show curtains, tents, tarpaulins, vertical blinds and umbrellas.

Polyphase P100 may be used in both aqueous and solvent-based coatings and dyestuffs that are applied to the fabric.

IPBC was the subject of a review by EPA's Office of Pesticides as part of the FIFRA Reregistration Eligibility Decision (RED) program (Case 2725). As such, many of the studies contained in this robust summary submission were reviewed by EPA and are discussed in the RED.

1.0 Melting Point

Test Substance

Identity: Troysan Polyphase P-100
3-Iodo-2 Propynyl Butyl Carbamate

Method

Method/guideline followed:	OECD 105
GLP:	Yes
Year: (study performed):	1990

Test Conditions

OECD Method 102 (12 May 81) capillary tube method was used (also see ASTM E. 324-69). Three different batches were run in triplicate. In addition, an ultra-pure sample (99.9%) re-crystallized from CH_2CL_2 was also run in duplicate.

Results

Melting point value in °C:	64.72 – 66.34°C
Decomposition:	No
Sublimation:	No

Batch P8903-4839

1. 65.0 - 67.0°C
2. 64.5 - 67.0°C
3. 65.0 - 67.0°C

$\bar{x}$ = 64.83 - 67.0°C

Batch 9008-5789-43

1. 64.5 - 65.7°C
2. 64.5 - 66.4°C
3. 64.5 - 66.0°C

$\bar{x}$ = 64.5 - 66.03°C

Batch 8905 - 4757-1

1. 64.5 - 66.0°C
2. 65.0 - 66.0°C
3. 65.0 - 66.0°C

$\bar{x}$ = 64.83 - 66.0°C

Lot 3D-95-C 99.9% IPBC

1. 65.8 - 66.5°C
2. 65.8 - 66.5°C

$\bar{x}$ = 65.8 - 66.5°C

Conclusions

Melting range: 64.72 – 66.34°C

Reliability

Klimisch Code = 1

The study is reported as being conducted in compliance with EPA GLP 40 CFR 160 and was conducted according to EPA Guideline 63-5 and OECD Method 102. (Study Author)

References

Troy Chemical Corporation, "Melting Point of Troysan Polyphase P100 3-Iodo-1-Propynyl Butyl Carbamate," August 20, 1990.

2.0 Boiling Point

Test Substance

Identity: Troysan Polyphase P-100

Method

Method:	None cited
GLP:	No
Year:	1985

Test Conditions

Attached charts indicate the analysis was conducted by a thermogravimetric analyzer. (Reviewer)

Results

Boiling point value in °C:	NA
Pressure:	NA
Pressure Unit:	NA
Decomposition:	Yes

Conclusions

Thermogravimetric analysis shows the onset temperature of degradation is 192.2°C and the outset temperature is 246.2°C. The peak temperature of degradation was 221.1°C.

Reliability

Klimisch Code = 2

The study is reliable with restriction. The study was not conducted according to GLP; however, attached thermogravimetric results are provided with the report.

References

Netzsch Incorporated, "TG/DTA Analysis of Troysan Polyphase P-100," Correspondence from Neil Lander to Jane Yuster, September 5, 1985.

3.0 Vapor Pressure

Test Substance

Identity: Troysan Polyphase P-100
3-iodo-2propynyl butyl carbamate, CAS No. 55406-53-6, 97.25% pure

Method

Method:	OECD Method 104
GLP:	Yes
Year:	1992

This study utilized the gas saturation procedure for determining the vapor pressure of a compound. Assuming that the total pressure of a mixture of gases is equal to the sum of the pressures of component gases and that the ideal gas law is obeyed, the partial pressure of the vapor under investigation is computed from the total gas volume and the weight of material vaporized. A preliminary study was conducted to provide information regarding the desorption efficiency of extraction methods in recovering the vaporized compound from the sorbent used and to determine the maximum flow rate that will completely saturate the carrier gas with the test material.

The vapor pressure of Troysan Polyphase P-100 was investigated in triplicate at temperatures of 10, 20, 30, 44 and 49°C and at a nitrogen flow rate of approximately 30 mL/min. The solid test article was packed between glass wool in the front section of the saturator column. A trapping tube containing two sections of Tenax – a primary and a backup section – was connected to the effluent end of the saturator column.

Sample tubes were allowed to equilibrate at test temperature before the introduction of gas. High purity nitrogen entered through the sample tube and passed over each complete test system for approximately three hours. The nitrogen flow rate was measured in triplicate at the eluent end of the trapping tubes at three separate time intervals over the course of testing. The flow rate was measured using a soap bubble flow meter and was timed over a 10.0 mL volume. After three hours, the flow of nitrogen was discontinued and the trapping tubes were wrapped individually in aluminum foil until extraction. The procedure was repeated for each of the temperatures investigated.

The mass of vaporized Troysan Polyphase P-100 was determined by analysis of extracts from the trapping tubes. The primary and backup sections of Tenax were desorbed and analyzed separately. The Tenax from each section was placed in glass vials containing 3.0 mL of methanol. Each vial was vortexed for 5 minutes,

the solution filtered through a 0.45 um Acrodisc and collected into a second glass vial.

Quality Control (QC) samples and controls were prepared and analyzed concurrent with test samples. QC samples consisted of approximately 0.1 g of Tenax fortified with Troysan Polyphase P-100 at mass levels of 0.105, 0.158 and 0.263 ug. Control samples consisted of approximately 0.1 g of Tenax. Both the QC and control samples were desorbed with 3.0 mL of methanol, filtered through 0.45 um Acrodiscs and diluted to the appropriate volume. Before analysis all test samples were evaporated to dryness under a gentle stream of nitrogen. A volume of 1.0 mL of 45:55 acetonitrile:water was added to each vial.

Vapor pressure was calculated from the ideal gas equation:

$$p = (w/M)(RT/v)$$

where:

p = vapor pressure (Pa)
R = ideal gas constant (8.31 Pa $m^3mol^{-1}K^{-1}$)
M = molecular weight of the compound (g/mole)
T = temperature (K)
v = gas volume (m^3)
w = mass of sample vaporized (g)

The Henry's Law Constant (H) may be calculated as follows:

$$H = (16.04\ pM)/TS$$

where:

p = vapor pressure (mm of Hg)
M = molecular weight of the compound (g/mole)
S = solubility of chemical in water (mg/L)
T = temperature (K)
1 mm Hg = 133 Pascal

Note: The constant (16.04) has the units (K·mg·mole)/(L·mm Hg·g) and H is dimensionless

The concentration of Troysan Polyphase P-100 from preliminary and definitive samples equals the mass volatilized or fortified divided by the final volume after extraction. Once a concentration was detected, the mass detected equaled the reported concentration multiplied by the sample volume.

Test Conditions

An effective method detection limit (MDL) was established for the HPLC method, and reproducibility studies were conducted for the instrumental response and the retention time of Polyphase P-100. The desorption efficiency of Troysan Polyphase P-100 from Tenax™ was investigated using both methanol and acetonitrile as the extraction solvent.

Results

Vapour Pressure value:	4.19×10^{-4} Pa (30°C)
Decomposition:	Yes

Vapor pressure experiments were conducted in triplicate at each temperature while the flow rate was held constant at approximately 30 ml/minute. The vapor pressure of Troysan Polyphase P-100 was below the established detection limit at 10 and 20°C and was 4.19×10^{-4} Pa at 30°C. Since the HPLC system and conditions for the 30°C experiment were different from those at 10 and 20°C and detectable levels of Troysan Polyphase P100 were observed in the sorbent traps, further experiments were conducted at 30, 44 and 49°C. The chromatography at these higher temperatures were similar to those observed in the first series 30°C and, again, Troysan Polyphase P-100 was detected with reported vapor pressures of 8.1×10^{-4}Pa at 44°C and 4.3×10^{-2} Pa at 49°C. The logarithm of the vapor pressure was not a linear function of the inverse of the test temperature. Significant matrix interferences were observed to be present through testing. It is also suspected that Troysan Polyphase P-100 becomes unstable at temperatures above 30°C, which further complicated analytical detection. Troysan Polyphase P-100 was detected and had a corresponding vapor pressure of approximately 10^{-4} Pa (10-6 torr) at 30°C.

Conclusions

The vapor pressure of Troysan Polyphase P-100 near ambient temperature was below the detection limits of the analytical methods available. The reported vapor pressure at 20° for Troysan Polyphase P-100 is $< 2.3 \times 10^{-4}$ Pa ($<1.8 \times 10^{-6}$ torr). Troysan Polyphase P-100 does appear to have a detectable vapor pressure near 7×10^{-4} Pa (5×10^{-6} torr) at 30°C and vapor pressure of 8.1×10^{-4} and 4.3×10^{-2} Pa at temperatures of 44°C and 49°C, respectively. Fortifications of approximately 0.1 grams of Tenax™ with 105 ng Troysan Polyphase P-100 consistently indicated that this level was at or below the method detection limit. Preliminary testing (desorption efficiency) had less matrix interference from Tenax™ than was encountered in the definitive experiments.

Maintaining a consistent and non-interfering matrix from the Tenax™ proved to be the most difficult phase of the study. Extensive cleaning procedures were used to free the sorbent material from impurities. While this cleaning lowered the

matrix interferences, the problem persisted throughout the study. The impurities from Tenax™ had the same polarity and retention time as Troysan Polyphase P-100.

A noticeable change in the experiments occurred at temperatures at or above 30°C. Initial reservations about the observed vapor pressure at 30°C were based on back calculation of the vapor pressure to 30 from 20°C assuming a linear relationship (log v versus 1/T) with the inverse of test temperature. These considerations suggested that the method should have detected Troysan Polyphase P-100 at 20°C. Only significant changes in the chemical which subsequently could have a meaningful impact on the volatility of Troysan Polyphase P-100 could explain the apparent discontinuity in the data.

This consideration coupled with the observed changes in the chromatography at higher temperatures caused concern that the test chemical may be thermally unstable. If at temperatures equal to or greater than 30°C, the test chemical degrades to produce byproducts similar to Troysan Polyphase P-100 in structure and polarity while at the same time enhancing the volatility of the chemical, changes in both the matrix and the measured vapor pressure at 20 and 30°C would be expected. This is to say that the test chemical was not behaving as an ideal gas.

Upon discussions with the study sponsor it was revealed that available data suggests Troysan Polyphase P-100 thermal instability at elevated temperatures. The vapor pressure data generally supports the supposition that Troysan Polyphase P-100 is thermally unstable. The stability study focused on the change in purity of the active ingredient over time at elevated temperatures while the vapor pressure study attempted to detecte the vapor pressure of the pure test article at various temperatures.

The experimentally determined vapor pressure and knowledge of the water solubility of the test compound may be used to estimate the potential of that chemical to evaporate from water. The Henry's Law Constant (H) is an estimate of a chemical's volatility from water. The estimated Henry's Law Constant for Troysan Polyphase P-100 was calculated to be 1.9×10^{-7} using the following values: $p=1.8 \times 10^{-6}$ torr, m=281 g/mole, S= 156 mg/L at 20C and T (20°C) = 293K. (All conclusions by study author)

Reliability

Klimisch Code = 1

The data and the report were produced and compiled in accordance with all pertinent FIFRA/OECD GLP regulations (40 CFR Part 160).

The report shows that documentation of the test substance identity and purity was provided by the study sponsor. (Reviewer comment)

The study was conducted according to acceptable method (OECD 104). (Reviewer comment)

Reference

Springborn Laboratories, Inc., Wareham, MA, "Troysan Polyphase P-100 Determination of Vapor Pressure," June 25, 1992.

4.0 Partition Coefficient

Test Substance

Identity: Troysan Polyphase P-100Purity 99.9%, Lot number 3D-95-C

Method

Method:	OECD No. 107, "Partition Coefficient (n-octanol/water), Flask Shaking Method."
GLP:	Yes
Year:	1990

A 1 ml aliquot of the stock sample solution in Octanol was volumetrically pipetted into each of six 250 ml Nalgene centrifuge bottles containing 150 ml water, and an appropriate volume of octanol was added (1, 3 or 7 ml – duplicate analyses for each volume of Octanol). Note: each phase had previously been saturated with the other solvent. The centrifuge bottles were than capped and inverted manually 100 times in approximately 5 minutes followed by centrifugation for 20 minutes at 25°C to clarify the layers.

The octanol phase for each partition sample was withdrawn by dispopipette and placed in individual 10 ml centrifuge bottles, then centrifuged for approximately 10 minutes. A 1 ml portion of the octanol phase was removed by volumetric pipette and transferred to a 100 ml volumetric flask (for the samples containing 8 ml octanol). Each solution was diluted to volume with methanol and mixed thoroughly. An 8 ml portion of each solution was volumetrically pipetted into individual 10 ml volumetric flasks and 2 ml of internal standard stock solution was added by volumetric pipette. The solutions were mixed thoroughly.

After removal of the remainder of the octanol phase and the interfacial layer, a 100 ml portion of the water phase was transferred to a 125 ml separatory funnel. The aqueous phase was then extracted in three 5 ml portions of methylene chloride. The extracts were combined in an amber septum vial and evaporated to dryness under a stream of nitrogen. The residue was dissolved by adding 2 ml of internal standard stock solution, 1 ml of methanol, and 7 ml of a methanol: water (43:57) mixture by volumetric pipette. A portion of each sample for analysis was filtered through a 0.45 um syringe filter into individual autosampler vials, which were then sealed with Teflon® lined septa and screw caps. The samples were then analyzed by High Performance Liquid Chromatography (HPLC) to determine the response factors.

Calculations

1. A relative response factor (RRF) was calculated for each injection as follows:

$$RRF = \frac{\text{Peak Area Sample}}{\text{Peak Area Internal Standard}}$$

2. A linear regression equation was calculated relating the RRF of each standard solution to the theoretical Polyphase P100 concentration (ppm). The data obtained were evaluated for linearity (correlation coefficient > 0.999).

3. Using the regression equation established for the standards and the RRF for each sample solution, the concentration (ppm) of Polyphase P100 was calculated for each sample solution.

4. The partition coefficient was calculated for each set of test conditions as follows.

$$P = \frac{\text{concentration octanol}}{\text{concentration water}}$$

5. The mean value was determined for each set of test conditions, as well as for all determinations.

Test Conditions

No remarkable test conditions. (Reviewer)

Results

Log Kow: 2.81 (25 ± 1°C)

The partition coefficient was determined for Polyphase P100 (99.9%) at 25 ± 1°C. The preliminary estimate of the partition coefficient was 978 (log P = 2.99), determined from the solubilities of Polyphase P100 in octanol and water.

$$P_{estimate} = \frac{\text{Saturation } C_{octanol}}{\text{Saturation } C_{water}}$$

Weight of test substance added to each partition sample: 6.0248 mg

Measured Concentration (ppm)		Volume (mL)		Calculated Amount After Equilibration		Total Recovered	Calculated Partition Coefficient
Octanol	Water	Octanol	Water	Octanol	Water	(%)	(P)
2794.5	4.0852	2	150	5.589	0.6128	102.9	684
2827.1	4.7083	2	150	5.654	0.7062	105.6	600
							$\bar{x}$ = 642
1498.7	2.4488	4	150	5.995	0.3673	105.6	612
1456.5	2.2161	4	150	5.826	0.3324	102.2	657
							$\bar{x}$ = 635
667.69	1.0209	8	150	5.342	0.1531	91.2	654
762.86	1.1417	8	150	6.103	0.1713	104.1	668
							$\bar{x}$ = 661

$\bar{P}$ = 646 ± 33 (n = 6, RSD = 5%)

log $\bar{P}$ = 2.81

Analytical Accuracy and Precision:

The analytical procedure was evaluated for accuracy and precision using the data for the triplicate high and low standards.

Linearity was confirmed (correlation coefficient greater than 0.999) for the standard curve. Relative errors were less than or equal to 18.1%, and relative standard deviations (RSD) observed for triplicate determinations were less than or equal to 1.5%.

Conclusions

Log Kow: 2.81 (25 ± 1°C) (Study author)

Reliability

Klimisch Code = 1

The study is reliable without restriction. All work was conducted in accordance with the EPA Good Laboratory Practice standards (40 CFR 160). (Study author) The study was conducted according to OECD Method 107. (Study author)

References

Midwest Research Institute, Kansas City, MO, “Analysis of Polyphase P100 Octanol/Water Partition Coefficient (63-11),” MRI Project No. 9555-F (01), July 10, 1990.

5.0 Water Solubility

Test Substance

Identity: Polyphase P-100
Technical Grade, Lot no. 89115175

Method

Method:	OECD 105
GLP:	Yes
Year:	1990

Test Conditions

Preparation of solutions

Internal standard solution. A solution of naphthalene was prepared by weighing 23.46 mg into a 50-mL volumetric flask and diluting to volume with methanol. The solution was mixed thoroughly. A 3-mL aliquot of this solution was volumetrically pipetted into a 250-mL volumetric flask, diluted to volume with methanol, and mixed thoroughly. This diluted naphthalene solution was treated as the internal standard stock solution.

Standard solutions. Duplicate stock solutions were prepared by accurately weighing 25.17 mg and 50.43 mg of the Polyphase P100 standard (Lot No. 30-95-C, 99.9%), transferring to individual 100-mL volumetric flasks, and diluting each flask to volume with methanol. The solutions were mixed thoroughly. Subsequent dilutions were made by volumetrically pipetting 1,3, and 10 mL of each stock solution into individual 25-mL volumetric flasks, pipetting 12.5 mL of the internal standard stock solution into each flask, and diluting to volume with methanol. The solutions were all thoroughly mixed. Triplicate dilutions were made for the highest and lowest standards. (Final concentrations: 10.068, 20.172, 30.204, 60.516, 100.68 and 201.72 ppm.) A portion of each solution was filtered through a 0.45-um syringe filter into an autosampler vial, then sealed with a Teflon-lined septum and screw-cap.

2.1.3.1.1.3 Sample solutions. The saturated solution was removed from each vial and placed in individual screw-capped centrifuge tubes. After centrifuging for 10 min, 5 mL of each aqueous solution was volumetrically pipetted into individual 10-mL volumetric flasks, and 5 mL of the internal standard stock solution was added to each flask by volumetric pipet. The solutions were thoroughly mixed. A portion of each solution was filtered through a 0.45-µm syringe filter into an autosampler vial, then sealed with a Teflon®-lined septum and screw-cap.

2.1.3.1.1.4 Internal standard blank solution. An internal standard blank solution was prepared by pipetting 5 mL of the internal standard stock solution into a 10-mL volumetric flask and adding 5 mL of water by volumetric pipet. The solution was thoroughly mixed. A portion of the solution was filtered through a 0.45-µm syringe filter into an autosampler vial, then sealed with a Teflon®-lined septum and screw-cap.

2.1.3.1.2 Instrument system. A single injection was made from each solution using the instrument and parameters described below.

Instrument System: Varian 5020
Detector: Waters 440
Injector: Waters WISP 7108 autosampler
Electronic Integration: Nelson 4400 Data System
Detection: Ultraviolet, 254 nm
Column: Waters µBondapak C_{18} (10 µm), 300 x 3.9 mm I.D.
Guard Column: Whatman Pellicular ODS, 20 x 2 mm I.D.
Solvent System:
- A: Water
- B: Methanol

Solvent Ratio: 40% A:60% B (premixed)
Flow Rate: 1.0 mL/min
Volume Injected: 50 µL
Retention Times:
- Polyphase P100: 8.2 min
- Naphthalene: 17.5 min

2.1.3.2 Calculations

1. A relative response factor (RRF) was calculated for each injection as follows:

$$RRF = \frac{\text{Peak Area Sample}}{\text{Peak Area Internal Standard}}$$

2. A linear regression equation was calculated relating the RRF of each standard solution to the theoretical Polyphase P100 concentration (ppm). The data obtained were evaluated for linearity (correlation coefficient > 0.999).

3. Using the regression equation established for the standards and the RRF for each sample solution, the concentration (ppm) of Polyphase P100 was calculated for each sample solution.

4. The average concentration (ppm) and standard deviation were calculated for the sample solutions.

The analytical procedure was evaluated for accuracy and precision using the data for the triplicate high and low standards. Linearity was confirmed (Correlation coefficient > 0.999) for the standard curve. Relative errors were less than or equal to 23.0% and relative standard deviations (RSD) observed for triplicate determinations were less than or equal to 18.4%.

Results

Value (mg/L) at temperature (25°C): 223 ± 7 mg/l (n=3)

Sample 1: 223.3 ppm
Sample 2: 230.0 ppm
Sample 3: 215.2 ppm
Average: 223 ± 7ppm (n=3)

Conclusions

Water solubility of Polyphase P100 is 223 ± 7ppm (n=3) at 25°C. (Study author)

Reliability

Klimisch Code = 1

Study was conducted in compliance with EPA Good Laboratory Practice standards (40 CFR 160). (Study author) The study was conducted according to OECD Guidelines No. 105 “Water Solubility” (May 12, 1981). Modifications were taken

from EPA Chemical Fate Test Guidelines CG-1500, "Water Solubility" (August 1982).

References

Midwest Research Institute, Kansas City, MO, "Analysis of Polyphase 100," MRI Project No. 9555-F (01), June 7, 1990.

7.0 Stability in Water

Test Substance

Identity: Troysan Polyphase P-100
3-iodo-2-propynyl-n [1-^{14}C]-butyl-[^{14}C]-carbamate (IPBC)

Method

Method/guideline followed:	Guideline 161-1
Type (test type):	Hydrolysis
GLP:	Yes
Year:	1990

Test Conditions

A P-100 stock solution of 21616 µg/mL was prepared in degassed acetone. Seven milliliters of this solution were transferred to a 1-liter volumetric flask and brought to volume with the appropriate buffer solution for a final concentration of 151.3 µg/mL. Forty milliliter aliquots of each solution were transferred to individual 50 mL Corex centrifuge bottles using Class A glassware and sealed with Teflon-lined screw caps (twenty-one samples were taken from each original solution). The samples were placed in a water bath and covered with a black cloth to prevent light penetration. Temperature of the water bath was maintained at 25 $\pm$ 0.1^{0}C.

Results

P-100 recovery from aqueous solutions was acceptable for all pH's. At time 0, average sample P-100 concentrations were 133.3 µg/mL at pH 5 (88.1% recovery), 119.4 µg/mL at pH 7 (78.9% recovery), and 131.6 µg/mL at pH 9 (87.0% recovery). Degradation of P-100 in pH 5 buffered solution was not detected using HPLC. Both the UV and ^{14}C data confirmed that P-100 remained intact at this pH. P-100 was slowly degraded in pH 7 buffered solution. Thirty days after treatment, greater than 10% of the applied P-100 had been converted to propargyl butyl carbamate, the only metabolite detected.

Conclusions

P-100 was very rapidly degraded in pH 9 buffered solution, slowly degraded in pH 7 buffered solution, and determined to be hydrolytically stable in pH 5 buffered solution. The only metabolite detected from pH 9 and 7 samples was propargyl butyl carbamate.

Reliability

Klimisch Code = 1

The study is reliable without restriction. The study was performed in accordance with the principles of Good Laboratory Practice with a few relatively minor exceptions.

References

EPL Bio-Analytical Services Inc., Harristown, IL, "Aqueous Hydrolysis of P-100," Laboratory Project ID 147-001, October 29, 1990.

The following preliminary study, providing similar information, was previously conducted:
Inveresek Research International, Scotland, "Troysan Polyphase (IPBC): Investigation of the Nature of the Hydrolysis Products of a Pesticide and their Analysis b GC/MS," Laboratory Project ID 131858, August 1986.

9.0 Biodegradation

Test Substance

Identity: Troysan Polyphase P-100
99% pure

Method

Method/guideline followed:	OECD 301F
Type (test type):	Aerobic
Year: (study performed):	2002
Contact time (units):	28 (days)

The percentage biodegradation of the test item was calculated based on the theoretical oxygen demand of 1.03 O_2/mg test item without nitrification ($ThOD_{NH4}$) and 1.25 O2/mg test item with nitrification ($ThOD_{NO3}$).

The percentage biodegradation was calculated according to the below given formula:

$$\% \text{ biodegradation} = \frac{\text{BOD (mg } O_2\text{/mg chemical)}}{\text{ThOD}_{\text{NH4 or NO3}} \text{ (mg } O_2\text{/mg chemical)}} \times 100\%$$

where:

BOD = Biochemical oxygen demand of the test or reference item

$$= \frac{\text{(mg } O_2 \text{ uptake/L by test and/or reference item) - (mg } O_2 \text{ uptake/L by inoculum control)}}{\text{mg test and/or reference item per liter}}$$

$ThOD_{NH4 \text{ or } NO3}$= Theoretical oxygen demand of the test or reference item without or with nitrification

The theoretical oxygen demand is the total amount of oxygen required to oxidize a chemical completely. It is calculated from the molecular formula assuming the turnover of H into H_2O, C into CO_2, P into P_2O_5, Na into Na_2O, halogens into their corresponding hydrogens and N into NH_4 and/or NO_3 according to EU Commission Directive 92/69 EEC C.4-D.

Test Conditions

Inoculum (concentration and source): The source was an aerobic activated sludge from a wastewater treatment plant (ARA Ergoiz II, Fullinsdorf, Switzerland) treating predominantly domestic wastewater. The sludge was washed once with tap water by centrifugation and the supernatant liquid phase was decanted. A

homogenized aliquot of the final sludge suspension was weighed, thereafter dried and the ration of wet to dry weight was calculated.

Based on this ratio, calculated amounts of wet sludge were suspended in test water to obtain a concentration equivalent to 4 g (± 10%) dry material per liter. During holding, the sludge was aerated at room temperature until use. Prior to use, the sludge was diluted with test water to a concentration of 1g/L (dry weight basis). This diluted activated sludge was used as inoculum to give final concentrations of 30 mg dry material per liter.

Concentration of test chemical, vehicle used, pre-acclimation conditions*:* Concentration = 50 mg/ L of test chemical. The amounts of test item were directly weighed into the test flasks. No emulsifiers of solvents were used. Ultrasound dispersion was employed for fifteen minutes to obtain a suspension of the test item as homogeneous as possible.

Reference substance:	Sodium Benzoate
Temperature of incubation (°C):	22°C – Temperature controlled with a built in thermostat and checked once per week
Sampling frequency:	Days 14 and 28
Appropriate controls and blank system used*:*	Sodium benzoate positive control and abiotic control were used.

The biochemical oxygen demand (BOD) of the test item IPBC in the test media was lower than in the inoculum controls. Consequently, IPBC was found to be not biodegradable under the test conditions within 28 days.

In the toxicity control, containing both IPBC at 51 mg/L and the reference item sodium benzoate at 100 mg/L, no inhibitory effect on the biodegradation of the reference item was determined.

In the procedure controls, the reference item sodium benzoate was biodegraded by an average of 97% on exposure Day 14, and reached an average biodegradation rate of 96% by the end of the test (Day 28), thus confirming suitability of the activated sludge.

Prior to test start the pH was 7.4 (measured in each test flask after the addition of the activated sludge inoculum). At the end of incubation, the pH was measured again in each test flask.

Results

Degradation % after time: $ThOD_{NH4}$ = 25% (28 days)

$ThOD_{NO3}$ = 21% (28 days)

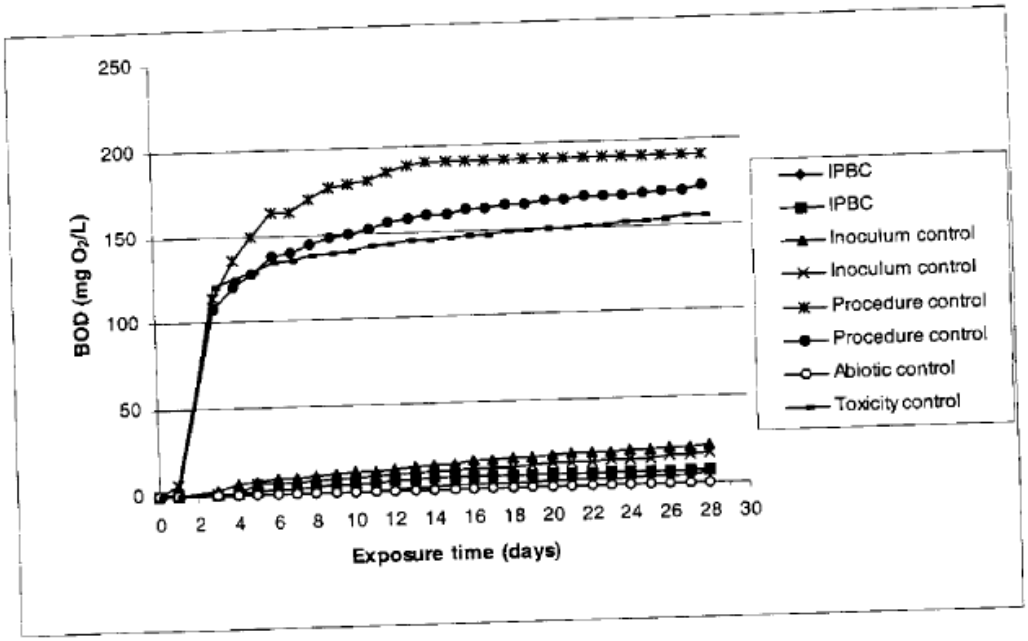
Breakdown products: No

The biochemical oxygen demand (BOD) of the test item IPBC in the test media was lower than in the inoculum controls. Consequently, IPBC was found to be not biodegradable under the test conditions within 28 days.

No degradation of the test item occurred in the abiotic control under the test conditions.

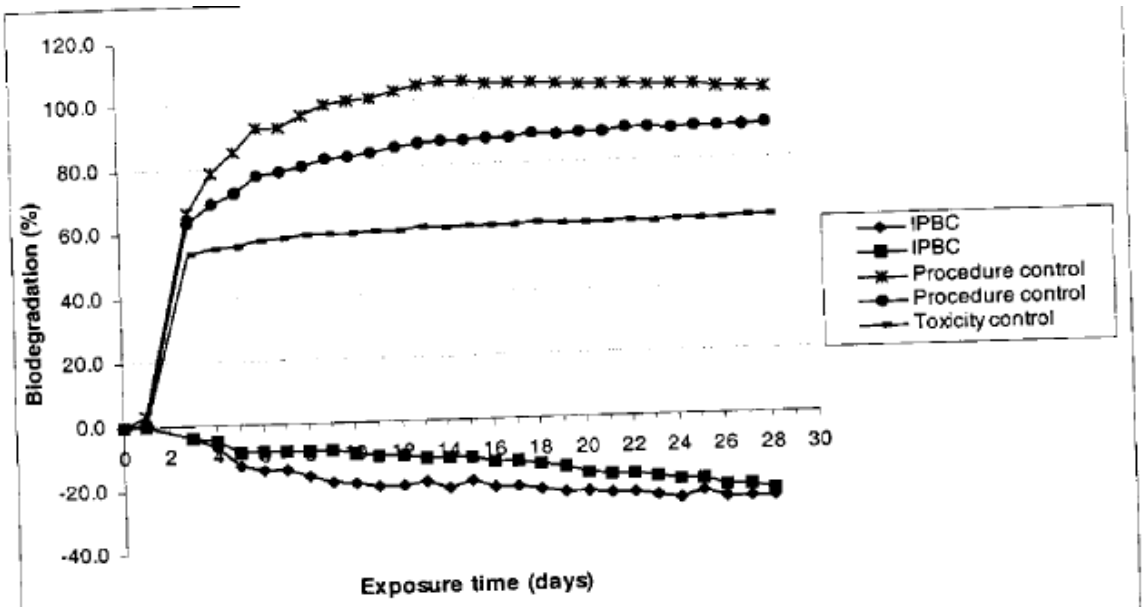
The percent biodegradation of the reference item sodium benzoate was calculated based on the theoretical oxygen demand of 1.67 mg O_2/mg (ThOD). The reference item was degraded by an average of 97% by Day 14, thus confirming suitability of the activated sludge. At the end of the test (Day 28) the reference item was degraded by an average of 96%.

Figure 1: Cumulative biochemical oxygen demand (BOD in mg 0_2/L) in the test flasks during the incubation period

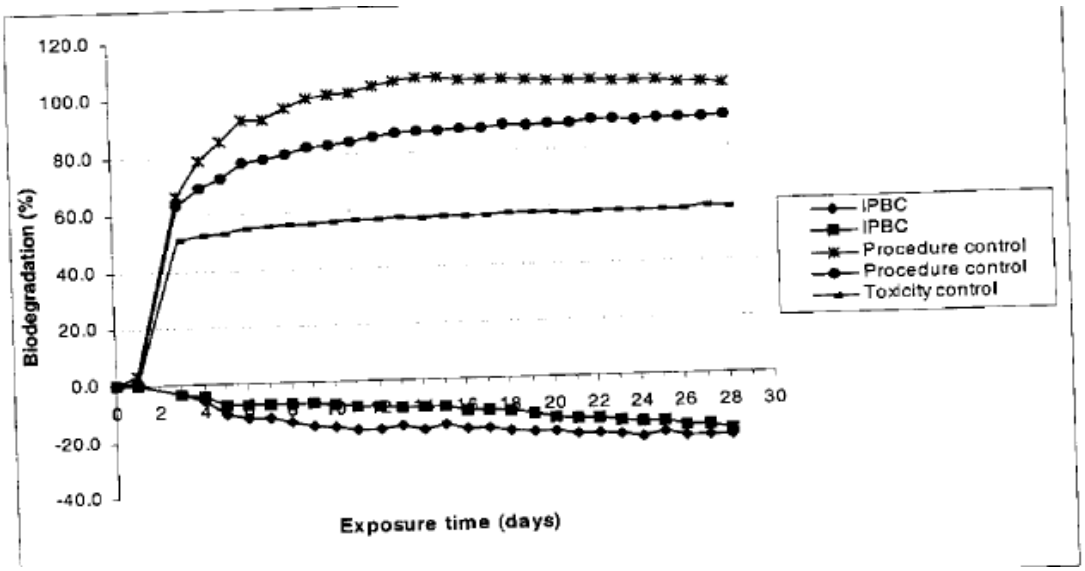


Biodegradation of IPBC with and without nitrification:

A. Without nitrification



B. With nitrification



Conclusions

IPBC was found to be not readily biodegradable under the test conditions within 28 days. (Reviewer)

Reliability

Klimisch Code = 1

The study is reliable without restriction as it was conducted according to OECD 301F and in compliance with the Swiss Ordinance on Good Laboratory Practice (GLP) (Feb 2, 2000)(RS 813.016.5), which is based on the OECD principles of GLP. (Study author)

References

RCC Ltd., Switzerland, "Ready Biodegradability of IPBC in a Manometric Respirometry Test," RCC Study No. 831172, March 11, 2002.

10.0 Acute Toxicity to Fish

Test Substance

Identity: Troysan Polyphase P-100
Lot # P8907-4914-P100, Drum #F-41, 97.7% purity

Method

Method/guideline followed:	EPA Guidelines 72-1
Test type:	Flow-through
GLP:	Yes
Year (study performed):	1990
Species/Strain/Supplier:	Rainbow trout (*Oncorhynchus mykiss*) were obtained from Mount Lassen Trout Farms, a commercial supplier in California. (SLI Lot 90A)
Analytical Monitoring:	Yes

Both replicate solutions of the 0.019, 0.054 and 0.15 mg/L treatment levels and the control(s) were sampled and analyzed for Troysan Polyphase P-100 concentrations prior to the start of the definitive exposure. Results of these pretest analyses were used to judge whether sufficient quantities of test material were being delivered and maintained in the exposure aquaria to initiate the definitive test. During the in-life phase of the definitive study, water samples were removed from both replicate test solutions of each treatment level and the controls on test days 0 and 4 for analysis of Troysan Polyphase P-100. Each exposure solution sample was collected from the approximate midpoint of the aquarium with a volumetric pipet. In addition, three quality control (QC) samples were prepared at each sampling interval and remained with the set of exposure solution samples through the analytical process. The QC samples were prepared in dilution water at Troysan Polyphase P-100 concentrations which approximated the test concentration range. Results of these analyses were used to judge the precision and quality control maintained during the analysis of exposure solution samples. All samples were analyzed for Troysan Polyphase P-100 using high pressure liquid chromatography according to the methodology described in Appendix III. A method validation study conducted at SLI prior to the initiation of the definitive test established an average recovery of Troysan Polyphase P-100 of 89.0 ± 5.00% from freshwater.

Exposure period:	96 hours

Statistical methods:

The mean measured concentrations tested (day 0 and 4 analysis) and the corresponding mortality data derived from the toxicity test were used to estimate the median lethal concentrations (LC50) and 95% confidence intervals at each 24-hour interval of the exposure period. The LC50 is defined as the concentration of the test material in dilution water lethal to 50% of the test animal population at the stated exposure interval. If at least one test concentration caused mortality of greater than or equal to 50% of the test population, then a computer program (Stephan, 1977,1982) was used to calculate the LC50 values and 95% confidence intervals. Three statistical methods were available in the computer program: moving average angle analysis, probit analysis, and nonlinear interpolation with 95% confidence intervals calculated by binomial probability. Moving average angle and probit analyses yield statistically sound results only if at least two concentrations produce a mortality of between 0 and 100% of the test organism population. The selection of reported LC50 values and 95% confidence intervals was based upon an examination of the data base and the results of the computer analysis. Selection criteria included the establishment of a concentration-effect relationship (mortality), the number of concentrations causing partial responses, and the span of responses bracketing the LC50 value. If two or more statistical methods produced acceptable results, then the method which yielded the smallest 95% confidence interval was selected. The No Observed Effect Concentration (NOEC), defined as the highest concentration tested at and below which there were no significant toxicant-related mortalities or physical and behavioral abnormalities (e.g., lethargy, loss of equilibrium, darkened pigmentation) was also determined.

Remarks:

Biological observations of the exposed rainbow trout and observations of the physical characteristics of the test solutions (e.g., precipitate, film on solution's surface) were made at test initiation and at each subsequent 24-hour interval until termination of the test. During the exposure, the diluter system was visually inspected at least twice daily to insure proper function. Effects for this study were based on death, defined as the lack of movement by the exposed organisms (i.e., absence of gill movement and reaction to gentle prodding). Mortalities were recorded and removed from each aquarium every 24 hours during the exposure.

Dissolved oxygen concentration, temperature, and pH were measured daily in each replicate of each treatment level and the controls. Dissolved oxygen concentration was measured using a YSI Model # 57 dissolved oxygen meter, temperature was monitored with a Brooklyn alcohol thermometer, pH was measured using a LaMotte Model HA pH meter. Total hardness and alkalinity as $CaCO_3$ (APHA *et al.*, 1985) and specific conductivity were measured on day 0 in one replicate of all test concentrations and the controls. Specific conductance was measured with a YSI Model #33 S-C-T meter. Light intensity at the surface of the exposure solutions was measured with a General Electric Model #214 light meter. Continuous temperature monitoring in one replicate (the B replicate of the control solution) was performed with an Omega Data Aquisition Min/Max Temperature Apparatus.

Test Conditions

Prior to testing, fish were held in a 500 L fiberglass tank under a photoperiod of 16 hours light and 8 hours darkness for a minimum of 14 days prior to testing. The temperature in the holding tank was 12-13°C during this 14 day period. All fish were fed a commercial pellet food *ad libitum* daily during pretreatment, except during the 48 hours prior to initiation of the definitive study. In accordance with EPA GLP, routine analyses were also conducted on representative samples of the fish food for the presence of pesticides and PCBs and were found to be acceptable.

There was no mortality in the test fish population during the two days prior to testing. The test fish were not fed during the exposure period. The rainbow trout used during this study were all of the same year class as a representative sample (N=30) of fish from the test population had a mean standard length of 41 (38-47) millimeters and weight of 0.66 (0.54-0.86) grams.

Dilution water source: Well water
Dilution water chemistry:

The well water that flowed into the holding tank was characterized as having total hardness and alkalinity ranges as calcium carbonate of 29-36 mg/L and 20-23 mg/L, respectively, and a specific conductance range of 120-130 microohms per centimeter. Other parameters monitored in the holding tanks included the pH that ranged from 7.0 to 7.2, and the dissolved oxygen concentration with a range of 77-87% of saturation.

The dilution water used during this study was obtained from the same source as the water which flowed into the fish holding tank and was characterized as having a total hardness of 27 – 31 mg/L $CaCO_3$, a total alkalinity of 20 – 22 mg/L $CaCO_3$, a pH of 7.1 – 7.2 and a specific conductance of 120 – 140 umhos/cm. The ability

of several daphnid species to survive and reproduce in this water source over several generations of culture confirmed that the dilution water was of acceptable quality. In conformance with EPA-GLP, routine analyses were also conducted on representative samples from the dilution water source for the presence of pesticides and PCB's. None of the compounds have been detected in any of the water samples analyzed, in agreement with US EPA and ASTM guidelines. In addition, representative samples of the dilution water source were analyzed monthly for total organic carbon (TOC) concentration. Based on these analyses, the TOC concentration of the dilution water was determined to be 0.593 ppm in February 1990.

Stock and test solution and how they are prepared:

On exposure day 0, a clear colorless stock solution of 2.04 mg A.I./mL was prepared by diluting 0.522 grams (0.510 grams A.I.), weighed using an SP 182 balance, with acetone (CAS #67-64-1) to a total volume of 250 mL.

Concentrations dosing rate and conditions:

A 50 ml gas-tight Glenco syringe with a Sage pump was mechanically activated to deliver the Troysan Polyphase P-100 to the diluter system where it was diluted (60% dilution factor) to provide the desired concentration range (150, 90, 54, 32 and 19 µg A.I./L). The test material was delivered to the diluter system at a rate of 0.025 mL/minute. The delivered test material was mixed with dilution water which was delivered to the system at a rate of 0.341 L/min. This delivery rate (test material and dilution water) provided a solution containing a Troysan Polyphase P-100 concentration equal to the highest treatment level (150 µg/L nominal).

Exposure vessel system:

The test was conducted using an exposure system consisting of a constant flow serial diluter (Benoit, 1982), a temperature-controlled water bath, and a set of 14 exposure aquaria. The test system was designed to provide five concentrations of the test material, a solvent control and a dilution water control. The solvent control solution contained the maximum amount of acetone present in any test concentration (0.074 mL/L). All treatment levels and the controls were maintained in duplicate. Impartially positioned test aquaria were labelled to identify the test sample, nominal test material concentration and designated replicate. Test solutions were not aerated. A photoperiod of 16 hours of light and 8 hours of darkness with a light intensity of 70 - 110 footcandles at the test solution surface was maintained throughout the test period. Lighting was provided by Durotest Vita-Lite® fluorescent bulbs.

Each glass test aquarium measured 39 x 20 x 25 centimeters (cm) with a 19.5 cm high standpipe which maintained a constant test water volume of 15 L. The diluter was constructed to deliver 4.1 L/hour of solution to each replicate test aquarium, providing approximately 6.5 volume replacements per aquarium every 24 hours. Test aquaria were impartially positioned in a water bath containing circulating water designed to maintain the test solution temperatures at 12 ± 1 °C.

Number of replicates, fish per replicate: 2 replicates, 10 fish per replicate

Test temperature range: 10-13°C

Method of calculating mean measured concentrations: Arithmetic mean

Water chemistry in test (D.O., pH) in the control and one concentration where effects were observed.

In duplicate test aquaria, twenty organisms were exposed to five concentrations of Troysan Polyphase P-100, a solvent control and a dilution water control. Test concentrations (nominal) for the definitive study were based on toxicity information developed through preliminary testing.

During the test, nominal concentrations (based on active ingredient A.I.) of 150, 54, 32, and 19 ug active ingredient (A.I.) were maintained by introducing approximately 6.5 aquarium volumes per day of newly prepared test solution via a constant flow serial diluter apparatus. Each replicate solution was sampled and analyzed for Troysan Polyphase P-100 concentration prior to test initiation, and on days 0 and 4 of the exposure period. Based on these analyses the mean measured concentrations averaged 91%, or the nominal treatment levels.

Based on the results of these analyses, the mean measured test concentrations were 120, 79, 49, 24 and 22 ug A.I. /L Troysan Polyphase P-100. Throughout the exposure period test solutions were clear and contained no visible sign of undissolved material (e.g. precipitate, film on surface). Biological observations were made and recorded at test initiation and every 24 hours thereafter until the test was terminated.

Water quality parameters measured during the definitive study remained within acceptable ranges for the survival of rainbow trout and were unaffected by the concentrations of Troysan Polyphase P-100 tested.

The following protocol deviations occurred during the study. It is the opinion of the study author that these deviations did not affect the results of the study.

1. The protocol states that the total dissolved oxygen exceeds 90% of saturation at test initiation. During this study, total dissolved oxygen exceeded 90% of saturation at test initiation in all but two test vessels. These vessels measured 89% of saturation at test initiation.

2. The protocol states that light intensity at the water surface is within the range of 20 - 100 footcandles. During this study, light intensity at the water surface ranged from 70 - 110 footcandles.

Results

Nominal concentrations *(as mg/L):* 150, 90, 54, 32, 19 ug A.I./L (0.150, 0.090, 0.054, 0.032 and 0.019 mg A.I./L)

Measured concentrations *(as mg/L):* 120, 79, 49, 24, 22 ug A.I./L (0.120, 0.079, 0.049, 0.024 and 0.022 mg A.I./L)

Unit*:* ug A.I./L

Element value: Values based on mean measured concentrations
LC50(24 hr)> 120 ug A.I./L
LC50(48 hr)=97 ug A.I./L
LC50(72 hr)= 83 ug A.I./L
LC50(96) =67 ug A.I./L
NOEC (96hr) = 49 ug A.I./L

Statistical results:

TEST RESULTS

LC50 (µg A.I./L)[a]				
24-Hour[b]	48-Hour[c]	72-Hour[c]	96-Hour[c]	No Observed Effect Concentration Through 96 Hours (µg A.I./L)[a]
>120	97 (79 - 120)	83 (49 - 120)	67 (49 - 79)	49

[a] Based on mean measured concentrations of Troysan Polyphase P-100.
[b] LC50 value empirically estimated to be greater than the highest test concentration.
[c] LC50 value calculated by binomial probability; 95% confidence interval estimated by nonlinear interpolation.

Following 96 hours exposure, 100% mortality was observed in the highest treatment level (120 µg A.I./L). During the same period, mortality of 70% was observed in the 79 µg A.I./L treatment level. There was no mortality in the remaining concentrations (49, 24 and 22 µg A.I./L). One fish at the 49 and 24 µg A.I./L treatment levels exhibited lethargic behavior.

Conclusions

The No Observed Effect Concentration (NOEC) through 96 hours was 49 ug A.I./L Troysan Polyphase P-100. Based on criteria established by US EPA (1985), Troysan Polyphase P-100 would be classified as very highly toxic to rainbow trout. (Study author)

Reliability

Klimisch Code = 1, Reliable without restriction

Study followed EPA Guidelines 72-1.

The data and report for this study were produced and compiled in accordance with all pertinent EPA Good Laboratory Practice regulations with the following exceptions: stability, characterization and verification of the test substance identity and maintenance of records on the test substance is the responsibility of the test Sponsor. (Study author)

Study report references detailed purity and traceable source information for the test substance. (Reviewer)

References

Springborn Laboratories, Inc., "Troysan Polyphase P-100- Acute Toxicity to Rainbow Trout *(Oncorhynchus mykiss*) Under Flow-Through Conditions," SLI Report Number 90-03-3261, June 27, 1990.

11.0 Toxicity to Aquatic Plants

Test Substance

Identity: Polyphase P-100
99.1 % purity, Batch No. 0011-1954

Method

Method/guideline followed:	OECD 201
Test type:	Static, non-renewal
GLP:	Yes
Year (study performed):	2001
Species/strain # and source:	

The test organism used for the study was *Scenedesmus subspicatus* CHODAT, Strain No. 86.81 SAG, supplied by the "Sammlung von Algenkulturen" (Experimentelle Phykologie und Sammlung von Algenkulturen, Albrecht-von-Haller-Institut für Pflanzenwissenschaften, Universität Göttingen, D-37073 Göttingen, Germany). The algae had been grown in the RCC laboratories under standardized conditions according to the test guidelines.

Element basis:	Cell density, growth, biomass and growth rate
Exposure period:	72 hours
Analytical monitoring:	

For the analysis of the actual test item concentrations, the following samples were taken:

Just before the start of the test:	- one sample from the freshly prepared stock solution - duplicate samples from each test medium (without algae) - duplicate samples from the control (without algae)
After 72 hours: (stability samples)	- duplicate samples from each test medium (without algae) - duplicate samples from the control (without algae)

For the 72-hour stability samples, additional flasks with adequate volumes of the freshly prepared test media of all test concentrations and the control were incubated under the same conditions as in the actual test but without algae.

All samples were deep-frozen (at about -20 °C) immediately after sampling. Based on pre-experiments (without GLP) for investigation of the storage stability the test item is sufficiently stable in the test water under these storage conditions.

The concentrations of the test item Polyphase P-100 were analyzed in the duplicate test media samples from the test concentrations of nominal 4.6 - 46 µg/l from both sampling times (0 and 72 hours). The samples from the test concentrations of nominal 1.0 and 2.2 µg/l were not analyzed, since these concentrations were below the determined 72-hour NOEC. From the control samples, only one of the duplicate samples was analyzed from the corresponding sampling times.

Remarks:

Small volumes of the test media and the control (1.0–2.0 ml) were taken out of all test flasks after 24, 48, and 72 hours exposure, and were not replaced. The algal cell densities in the samples were determined by counting with an electronic particle counter (Coulter Counter®, Model ZM), with at least two measurements per sample.

In addition, after 72 hours exposure, a sample was taken from the control and from the test concentration of nominal 4.6 µg/l. The shape of the algal cells was microscopically examined.

The arithmetic mean area and the arithmetic mean growth rate were calculated for all test flasks per treatment.

The E_bC50 and $E_\mu C50$ values (the concentrations of the test item corresponding to 50% inhibition of algal biomass (b) and growth rate (µ) compared to the control, respectively) and the corresponding EC10 value and their 95%-confidence limits were calculated by Probit Analysis (Ref. 1, 2).

For the determination of the LOEC and NOEC, the calculated mean biomass and the mean growth rate at the test concentrations were tested for significant differences compared to the control values by a Dunnett-test (Ref. 3, 4).

Test Conditions

Test temperature range:	Maintained 21° C throughout study
Growth/test medium chemistry:	Synthetic water prepared according to the OECD test guidelines. Analytical grade salts were dissolved in sterile purified water.
Exposure vessel type:	Volumes of 15 ml algal suspension for each replicate were continuously stirred by magnetic stirrers in 50 ml Erlenmeyer flasks. The flasks were covered with glass dishes. They were incubated in a temperature controlled water bath at 21°C.

Water chemistry in test (pH) in at least one replicate of each concentration (at start and end of the test):

Nominal test item concentration (µg/l)	pH values	
	Start	End
Control	7.9	8.2
1.0	7.9	8.2
2.2	7.9	8.2
4.6	7.9	8.2
10.0	7.9	8.1
22.0	7.9	8.0
46.0	7.9	8.0

Stock solutions preparation (vehicle, solvent, concentrations):

A stock solution of nominal 10 mg/l was prepared by dissolving 30 mg of the test item completely in 3000 ml test water by ultrasonic treatment (30 minutes) and intense stirring (30 minutes at room temperature in the dark). This stock solution was diluted with test water by a factor of 100 (20 ml of the stock solution was made up to 2000 ml with test water) to obtain a diluted stock solution of nominal 100 µg/l. Adequate volumes of this diluted stock solution were further diluted with test water to prepare the test media of the desired test concentrations. The test media were prepared just before addition of algae (=start of the test; see Section 2.5.1).

Light levels and quality during exposure:

The test and control flasks were continuously illuminated at a measured light intensity of about 8780 Lux (mean) ranging from 8500 to 9270 Lux. The illumination was achieved by fluorescent tubes (Philips TLD 86W/840), installed about the test and control flasks.

Concentrations tested:	1.0, 2.2, 4.6, 10, 22 and 46 ug/L Test concentrations were based on the results of a range-finding test and on the results of a pre-experiment to the solubility of the test item (without GLP).
Controls:	Yes; with test water and without test material
Test design:	3 replicates per test concentration, 6 replicates for the control

Method of calculating mean measured concentrations: Arithmetic mean

The influence of the test item Polyphase P-100 on the growth of the green algal species *Scenedesmus subspicatus* CHODAT was investigated in a 72-hour static test according to the EU Commission Directive 92/69/EEC, C.3, 1992, and the OECD Guideline No. 201, 1984.

The nominal test concentrations were 1.0, 2.2, 4.6, 10, 22, and 46 µg/l. Additionally, a control was tested in parallel.

The algae were cultivated and tested in synthetic test water, prepared according to the test guidelines. Analytical grade salts were dissolved in sterile purified water to obtain the following final nominal concentrations:

Calculated water hardness of the test water: 0.24 mmol/l (= 24 mg/l) as $CaCO_3$

The test was started (0 hours) by inoculation of 10,000 algal cells per ml test medium. These cells were taken from an exponentially growing pre-culture, which was set up three days prior to the test at the same conditions as in the test.

The test design included three replicates per test concentration and six replicates of the control. Volumes of 15 ml algal suspension for each replicate were continuously stirred by magnetic stirrers in 50 ml Erlenmeyer flasks.

The flasks were covered with glass dishes. They were incubated in a temperature controlled water bath at a temperature of 21 °C (Table 8), and continuously illuminated at a measured light intensity of about 8780 Lux (mean value), range: 8500 to 9270 Lux (minimum and maximum value of measurements at 9 places distributed over the experimental area at the surface of the test media). This illumination was achieved by fluorescent tubes (Philips TLD 36W/840), installed above the test flasks. The test vessels were labeled with the RCC study number and all necessary additional information to assure unmistakable identification.

Results

Nominal concentrations (mg/L): 0.001, 0.0022, 0.0046, 0.010, and 0.046 mg/L
Measured concentrations (mg/L): 0.0038, 0.0093, 0.0204, 0.0414 mg/L

Concurrent with the sample analysis, recoveries of spiked test water samples in the relevant concentrations (0.00439 and 0.0439 mg/l of the test item) were performed in duplicate. The average concentrations were found to be 106% and 95% of the spiked values, with an overall mean of 100% (n = 4). Therefore, no correction for possible losses during the analytical procedure is necessary.

With the exception of the sample pair of the lowest-level aged treatment samples (0.0046 mg/l), the average concentrations found in the treatment samples ranged from 87% to 97% of the nominal concentrations. For the sample pair of sampling day 3 (nominal 0.0046 mg/l) an average recovery of 76% was obtained.

NOEC, LOEC, or NOEL, LOEL:

The EC values were calculated for both parameters, the algal biomass (b) and the growth rate (μ), after 72 hours test duration:

Parameter (0-72 h)	Biomass b (μg/l)	Growth rate μ (μg/l)
EC50 95%-confidence limits	22 17 - 33	53 * 32 - n.d.
EC10 95%-confidence limits	5.8 3.1 - 8.4	13 3.7 - 21
EC90	> 46	> 46
NOEC	4.6	4.6
LOEC	10	10

* Value calculated by Probit analysis. The inhibition of the growth rate was 51% at the test concentration of nominal 46 μg/l.

Was control response satisfactory: Yes

No remarkable observations were made concerning the appearance of the test media. All test media were clear solutions throughout the test period.

The analytically determined test item concentrations in the analyzed test media varied in the range of 76 to 97% of the nominal values. The mean measured concentrations (calculated as the average over all measurements per test concentration) ranged from 82 to 93% of nominal. The test item was sufficiently stable during the test period of 72 hours under the test conditions, and consequently the reported biological results are based on the nominal concentration of the test item.

The test item Polyphase P-100 had a statistically significant inhibition effect on the growth (biomass and growth rate) of *Scenedesmus subspicatus* after the exposure period of 72 hours at concentrations of 10 μg/l (= 72-hour LOEC: lowest concentration tested with toxic effects) and above. The 72-hour NOEC (highest concentration tested without toxic effects) was determined to be 4.6 μg/l, since up to this test concentration both the mean biomass and the mean growth rate μ of the algae were not statistically significantly lower than in the control.

The microscopic examination of the algal cells after 72 hours test period showed no difference between the algae growing in the test concentration of nominal 4.6 μg/l and the algal cells in the control. The shape and size of the algal cells growing in test media containing the test item at up to this test concentration were obviously not affected.

In the control the cell density increased from nominal $N = 1 \times 10^4$ cells/ml at the start of the test (0 hours) to $N = 50 \times 10^4$ cells/ml (mean value) after 72 hours (Table 1). Thus, the algal growth in the control was sufficiently high under the test conditions and the validity criterion of increase of cell density by at least a factor of 16 over the duration of the study was fulfilled.

Conclusions

Polyphase P-100 had a statistically significant inhibition effect on the growth (biomass and growth rate) of *Scenedesmus subspicatus* after the exposure period 72 hours at concentrations of 10 ug/L. The 72-hour No Observed Effect Concentration (NOEC) was determined to be 4.6 ug/l. (Study author)

Reliability

Klimisch Code = 1. The study is reliable without restriction.

The study was conducted in accordance with Good Laboratory Practice requirements under Swiss legislation. The study was conducted according to OECD Method 201. (Study Author)

Study sponsor provided certificate of analysis for test substance. (Reviewer)

References

RCC Ltd, Switzerland, "Toxicity of Polyphase P-100 to *Scenedesmus Subspicatus* in a 72 Algal Growth Inhibition Test," RCC Study Number 790413, June 27, 2001.

12.0 Acute Toxicity to Aquatic Invertebrates

Test Substance

Identity: Troysan Polyphase P-100
Lot number 9102-6111, 97.25% Purity

Method

Method/guideline followed:	FIFRA Guidelines 72-3
Test type:	Flow-through
GLP:	Yes
Year: (study performed):	1991
Analytical Monitoring:	Yes

Both replicates of the high, middle and low treatment levels and the dilution water control were sampled and analyzed for Troysan Polyphase P-100 concentration prior to the start of the definitive exposure. Results of these pretest analyses were used to judge whether sufficient quantities of test material were being delivered and maintained in the exposure aquaria to initiate the definitive test. During the in-life phase of the definitive study, water samples were removed from both replicate test solutions of each treatment level and the controls on test days 0 and 4 for analysis of Troysan Polyphase P-100. Each exposure solution sample was collected from the approximate midpoint of the aquarium with a volumetric pipet. In addition, three Quality Control (QC) samples, formulated in test dilution water at concentrations of Troysan Polyphase P-100 similar to the nominal test concentration range were prepared at each sampling interval and remained with the exposure solution samples throughout the analytical process. Results of the analyses of the QC samples were used to judge the precision and quality control maintained during the analysis of exposure solution samples. All samples were analyzed for Troysan Polyphase P-100 using a High Performance Liquid Chromatographic (HPLC) procedure according to the methodology described in Appendix V. A method validation study conducted at SLI prior to the initiation of the definitive study established an average recovery of 83.1 ± 9.88% of Troysan Polyphase P-100 from seawater.

Species/Strain:	*Mysidopsis bahia*
Exposure period:	96 hours
Statistical methods:	Mean measured concentrations tested (based on day 0 and 4 analyses and the corresponding mortality data derived from the definitive toxicity test were used to estimate the median lethal concentration (LC50) and 95%

confidence intervals at each 24 hour interval of the exposure period.

The diluter was visually inspected at least twice daily during the definitive exposure period. Biological observations of the exposed mysid shrimp and observations of the physical characteristics of the test solutions (e.g., precipitate, film on solutions surface) were made at test initiation and at each subsequent 24-hour interval until test termination (96-hour). Effects for this study were based on death, defined as the lack of movement by the exposed organisms (i.e., absence of movement of respiratory and feeding appendages and reaction to gentle prodding). Mortalities were recorded and removed from each aquarium every 24 hours during the exposure.

Test Conditions

Test organisms:

Source, supplier: Springborn Laboratory brood stock, SLI Lot # 90A106
Original source was Aquatic Biosystems, a commercial supplier in Fort Collins , CO.

Pretreatment: Prior to tests the mysid were held in a 500 L fiberglass tank with a closed loop recirculating filtration system providing natural seawater to the holding tank. The natural seawater was characterized as having a salinity range of 31-32 and a temperature range of 24-27°C.

At an intensity of 20-100 foot-candles at the culture solution surface, light was provided by Durotest Vitalite fluorescent bulbs. The area in which the mysid was cultured received a regulated photoperiod of 16 hours light and 8 hours darkness.

Commercial aquarium heaters were used to maintain the culture solutions at 24-27°C.

The culture water was from the same source as the dilution water used during the preliminary and definitive tests.

Mysids were fed live brine shrimp (*Artemia salina*) nauplii twice daily. In accordance with EPA-GLP, routine analyses were conducted on representative samples of the food sources to detect the presence of pesticides and PCBs. Food sources were considered to be of acceptable quality since the total concentration of pesticide measured was less than 0.3 mg/kg (ASTM, 1985).

Age at study initiation: Juvenile, less than or equal to 24 hours.

Feeding: During the exposure period the mysid were fed live brine shrimp nauplli (*Artemia salina*) twice daily.

Stock solutions preparation (vehicle, solvent, concentrations)*:*

A 2.1 mg A.I./mL stock solution was prepared by dissolving 0.5365 g (0.5217 g as active ingredient) with acetone (CAS# 67-64-1) to volume in a 250 mL volumetric flask. The resultant stock solution was observed to contain small amounts of undissolved test material. The stock solution was then sonicated until total solubility had been achieved.

A 50-mL Glenco gas-tight syringe in conjunction with a Sage Syringe pump was calibrated to deliver 0.024 mL/min of the stock solution (2.1 mg A.I./mL) directly into the chemical mixing chamber which also received 0.250 L/min of seawater. The mixing chamber was positioned over a magnetic stirrer which continuously stirred the solution. The concentration of the solution contained in the mixing chamber was equivalent to the highest nominal test concentration (200 µg A.I./L). This solution was proportionally (60%) diluted to produce the remaining nominal test concentrations (120, 72, 43 and 26 µg A.I./L). A mechanical injector similar to that used to deliver the Troysan Polyphase P-100 stock solution was used to deliver acetone to the solvent control aquaria. The solvent control solutions contained a concentration of acetone which equaled the solvent in the highest test treatment level (96 µL/L).

Test temperature range: 25 ± 1°C

Exposure vessel type:

Test vessels consisted of 19.5-L glass aquaria which measured 39 x 20 x 25 centimeters (cm) with a constant level overflow located at 14.5 cm from the bottom of the test aquaria. This system allowed the aquarium volume to be maintained at 11-L. The diluter was calibrated to deliver 50 mL/min of exposure solution to each replicate test aquarium which provided approximately 6.5 volume replacements per aquarium every 24 hours. Exposure aquaria were labelled to identify the nominal test material concentration and designated replicate.

Dilution water source*:*

Dilution waster was collected from the Cap Cod Canal, Bourne Massachusetts with a pump (fiberglass reinforced thermoplastic housing) and a PVC pipe, and was then transported to the laboratory in 3400 L fiberglass tank. This was the same source as the water which flowed into the mysid pre-study holding tank.

The results of chemical analysis and the ability of several species of mysid to survive and reproduce over several culture generations confirmed that the dilution water used during this study was of acceptable quality.

Dilution water chemistry (hardness, alkalinity, pH, TOC, TSS, salinity, Ca/Mg ratio, Na/K ratio):
TOC concentration of the dilution water was 1.4 mg/L.

Lighting (quality, intensity and periodicity):
Test area was illuminated by Durotest Vita-Lite fluorescent bulbs at an intensity of 28-65 foot-candles. Photoperiodicity was 16 hours light and 8 hours darkness.

Water chemistry in test (D.O., pH) in the control and at least one concentration where effects were observed:

Table 1. The water quality parameters measured during the 96-hour flow-through toxicity test exposing mysid shrimp (*Mysidopsis bahia*) to Troysan Polyphase P-100.

Nominal Concentration (µg A.I./L)	0-Hour		24-Hour		48-Hour		72-Hour		96-Hour	
	A	B	A	B	A	B	A	B	A	B
					pH					
200	7.9	7.9	7.7	7.8	7.8	7.8	7.9	7.9	7.9	7.9
120	7.9	7.9	7.8	7.7	7.8	7.8	7.9	7.9	7.9	7.9
72	7.9	7.9	7.7	7.8	7.8	7.8	7.9	7.9	7.9	7.9
43	7.9	7.9	7.7	7.8	7.8	7.8	7.9	7.9	7.9	8.0
26	7.9	7.9	7.8	7.8	7.8	7.8	7.9	7.9	8.0	8.0
Solvent Control	7.9	7.9	7.7	7.7	7.7	7.7	7.9	7.9	8.0	8.0
Control	7.9	7.9	7.8	7.8	7.8	7.8	7.9	7.9	8.0	7.9
					Dissolved Oxygen, mg/L (% Saturation)					
200	6.2 (90)	6.2 (90)	6.3 (91)	6.2 (90)	6.1 (88)	5.9 (86)	6.0 (87)	6.0 (87)	5.7 (84)	5.6 (82)
120	6.2 (90)	6.3 (91)	6.1 (88)	6.0 (87)	5.6 (81)	5.7 (83)	5.6 (81)	6.1 (88)	5.4 (79)	5.6 (82)
72	6.3 (91)	6.3 (91)	6.0 (87)	6.1 (88)	5.9 (86)	5.8 (84)	5.7 (83)	5.8 (84)	5.4 (79)	5.4 (79)
43	6.3 (91)	6.3 (91)	6.0 (87)	6.0 (87)	5.9 (86)	6.0 (87)	5.9 (86)	5.9 (86)	5.5 (81)	5.4 (79)
26	6.3 (91)	6.2 (90)	6.2 (90)	6.2 (90)	6.0 (87)	6.1 (88)	6.1 (88)	5.8 (84)	5.5 (81)	5.4 (79)
Solvent Control	6.2 (90)	6.2 (90)	5.8 (84)	5.8 (84)	5.3 (77)	5.5 (80)	5.3 (77)	5.4 (78)	5.0 (74)	5.1 (75)
Control	6.8 (99)	6.7 (97)	6.9 (100)	6.9 (100)	6.4 (93)	6.6 (96)	6.5 (94)	6.6 (96)	6.5 (96)	6.6 (97)
					Temperature (°C)[a]					
	25		25		25		25		26	
					Salinity (°/oo)[a]					
	31		31		32		31		31-32	

[a] Values presented represent the daily temperature (Brooklyn Alcohol Thermometer) and salinity measured in all test concentrations and the controls at the stated observation interval.

Element (unit) basis):	Mortality; immobilization, lethargy
Test design:	2 replicates for each of 5 test concentration, ten organisms per replicate
Method of calculating mean measured concentration:	Arithmetic mean
Analytical monitoring:	Through out the exposure period, analytical measurements between replicate solutions and sampling intervals were observed to be generally consistent.

Twenty organisms (ten per replicate) were exposed in duplicate test vessel to five concentrations of Troysan Polyphase P-100, a solvent control (acetone) and a

dilution water control. During the test nominal concentrations of 200, 120, 72, 43 and 26 ug A.I. /L were maintained by introducing approximately 6.5 aquarium volumes per day of newly prepared test solution via a constant flow serial diluter. Each replicate solution was sampled and analyzed for the test substance on day 0 (initiation) and day 4 (termination) of the exposure period. Based on the results of these analyses, the mean measured exposure concentrations were 116, 56, 40, 19 and 17 ug A.I./L. Throughout the exposure period, undissolved test material (e.g. precipitate) was observed in the toxicant syringe tubing of the diluter system. Exposure solutions were observed to be clear and colorless and contained no visible sings of undissolved test material. Biological observations were made and recorded at test initiation and every 24 hours thereafter until the test was terminated.

Results

Nominal concentrations (mg/L): 0.200, 0.120, 0.072, 0.043 and 0.026 mg A.I./L
Measured concentrations (mg/L): 0.116, 0.056, 0.040, 0.019 and 0.017 mg A.I./L

LC50 (µg A.I./L)[ab]				No Observed Effect Concentration Through 96 Hours (µg A.I./L)[a]
24-Hour[c]	48-Hour[c]	72-Hour[d]	96-Hour[d]	
> 120	> 120	190 (100 - 2600)	88 (62 - 170)	19

[a] Based on mean measured concentrations of Troysan Polyphase P-100 (as active ingredient).
[b] Corresponding 95% confidence interval is presented in parentheses.
[c] LC50 value empirically estimated as greater then the highest concentration tested.
[d] LC50 value and corresponding 95% confidence interval calculated by probit analysis.

Following 96 hours of exposure, 55% mortality was observed among mysid exposed to the highest mean measured concentration tested (116 ug A.I./L). Mortality of 40 and 30% was observed among mysid exposed to the 56 and 40 ug A.I./L test concentrations, respectively. Sublethal effects (e.g. lethargy) were observed among all surviving mysid exposed to the 116, 56 and 40 ug A.I./L test concentrations. No significant toxicant related mortality (<10%) or sublethal effects were observed among mysid in the two lowest concentrations tested (19 and 17 ug A.I./L). The EC50 values and corresponding confidence intervals for Troysan Polyphase P-100 are reported. The No Observed Effect Concentration (NOEC) through 96 hours of exposure was established to be 19 ug A.I./L.

Lower than expected analytical recoveries on measured concentration values were attributed to adsorption of the test material to the high-density of organics present

in the dilution water. Based on the mean measured concentrations of Troysan Polyphase P-100, the treatment levels were defined as 116, 56, 40, 19 and 17 ug A.I./L and averaged 54% of the nominal concentrations. Coefficients of variation averaged 10% for all mean measured concentrations. Quality control samples were consistent with the predetermined recovery range and averaged 88% of the nominal fortified levels (200 – 30 ug/L). Based on the results of these analyses, it was established that the appropriate quality control was maintained during the analyses of the exposure solutions.

The mean measured concentrations, the corresponding mortalities and the observations made during the 96-hour test are presented in Table 3. Following 96 hours of exposure, 55% mortality was observed among mysids exposed to the highest mean measured concentration tested (116 µg A.I./L). Mortality of 40 and 30% was observed among mysids exposed to the 56 and 40 µg A.I./L test concentrations, respectively, while 5% mortality was observed among mysids exposed to the remaining two test concentrations (19 and 17 µg A.I./L). Sublethal effects (e.g., lethargy) were observed among all surviving mysids exposed to the 116, 56 and 40 µg A.I./L test concentrations and among one surviving mysid exposed to the 19 µg A.I./L test concentration. Since the mortality (5%) and sublethal effects observed in the 19 and 17 µg A.I./L treatment levels were equivalent to the solvent control group performance, the organisms at these treatment levels were considered unaffected by the exposure of Troysan Polyphase P-100. Further, ASTM (1989), EPA TSCA and FIFRA procedures recognize the limitations of acute toxicity testing, i.e., response less than or equal 10% is allowable in a control population and is considered within the expected range of naturally occurring variability. Therefore, the mortality (5%) observed in the lower treatment levels (19 and 17 µg A.I./L) is not considered an adverse response from exposure to Troysan Polyphase P-100. Based on these data, it was established that the effects observed during this study were concentration-dependent. The 96-hour concentration-response (mortality) curve is presented in Figure 2. The slope of this curve was calculated to be 2.2991. Table 4 summarizes the 24-, 48-, 72- and 96-hour LC50 values, the corresponding 95% confidence intervals and the No Observed Effect Concentration (NOEC). The 96-hour LC50 value (95% confidence interval) was calculated by probit analyses to be 88 (62 - 170) µg A.I./L. The NOEC established for this study was determined to be 19 µg A.I./L. Based on US EPA (1985) criteria, Troysan Polyphase P-100 would be classified as very highly toxic to mysid shrimp.

Table 2. Concentrations of Troysan Polyphase P-100 measured in replicate (A,B) test solutions during the 96-hour flow-through exposure of mysid shrimp (*Mysidopsis bahia*).

Nominal Concentration (µg A.I./L)	Measured Concentrations (µg A.I./L) Day 0 A	Day 0 B	Day 4 A	Day 4 B	Mean Measured Concentration[a] (µg A.I./L)
200	110	130	110	110	116 (6.9)
120	59	57	57	51	56 (3.3)
72	41	48	35	35	40 (6.2)
43	19	17	17	23	19 (2.6)
26	18	17	16	19	17 (1.7)
Solvent Control	ND[b]	ND[b]	ND[b]	ND[b]	
Control	ND[b]	ND[b]	ND[b]	ND[b]	
QC #1[c]	29.7 (30.0)[d]		22.9 (30.0)		
QC #2	90.4 (100)		89.9 (100)		
QC #3	182 (200)		165 (200)		

[a] Mean measured concentrations are presented with the standard deviation in parentheses and were calculated using the actual analytical results (four significant figures) and not the rounded numbers (two significant figures) presented in this table.
[b] ND = not detectable (i.e., below the limit of quantitation as defined in Appendix V).
[c] QC = Quality Control sample.
[d] Value in parentheses represents the corresponding nominal fortified concentration for each QC sample.

Table 3. **Mean measured concentrations tested, corresponding mortalities and observations made during the 96-hour flow-through exposure of mysid shrimp (*Mysidopsis bahia*) to Troysan Polyphase P-100.**

Mean Measured Concentration (µg A.I./L)	Cumulative Mortality (%)											
	24-Hour			48-Hour			72-Hour			96-Hour		
	A	B	Mean	A	B	Mean	A	B	Mean	A	B	Mean
116	0	0	0	10	10	10[efg]	20	40	30[e]	60	50	55[e]
56	0	10	5[ab]	10	20	15[cgh]	30	20	25[e]	40	40	40[e]
40	0	0	0[bcd]	10	40	25[ch]	10	40	25[ef]	20	40	30[e]
19	0	0	0	10	0	5	10	0	5	10	0	5[i]
17	0	0	0	0	0	0	0	0	0	0	10	5
Solvent Control	0	0	0	0	0	0	10	0	5[i]	10	0	5[i]
Control	0	0	0	0	0	0	0	0	0	0	0	0

[a] Two of the surviving mysids were observed to be lethargic.
[b] One of the surviving mysids exhibited erratic swimming behavior.
[c] Several of the surviving mysids were observed to be lethargic.
[d] One of the surviving mysids exhibited darkened pigmentation and was observed to be lethargic.
[e] All of the surviving mysids were observed to be lethargic.
[f] All of the surviving mysids exhibited erratic swimming behavior.
[g] Several of the surviving mysids exhibited a darkened pigmentation.
[h] Several of the surviving mysids exhibited erratic swimming behavior.
[i] One of the surviving mysids were observed to be lethargic.

Figure 1. **Graphical illustration of the relationship between the mean measured concentrations (analysis on test days 0 and 4) and the nominal treatment levels of Troysan Polyphase P-100 established during the 96-hour flow-through exposure of mysid shrimp (*Mysidopsis bahia*).**

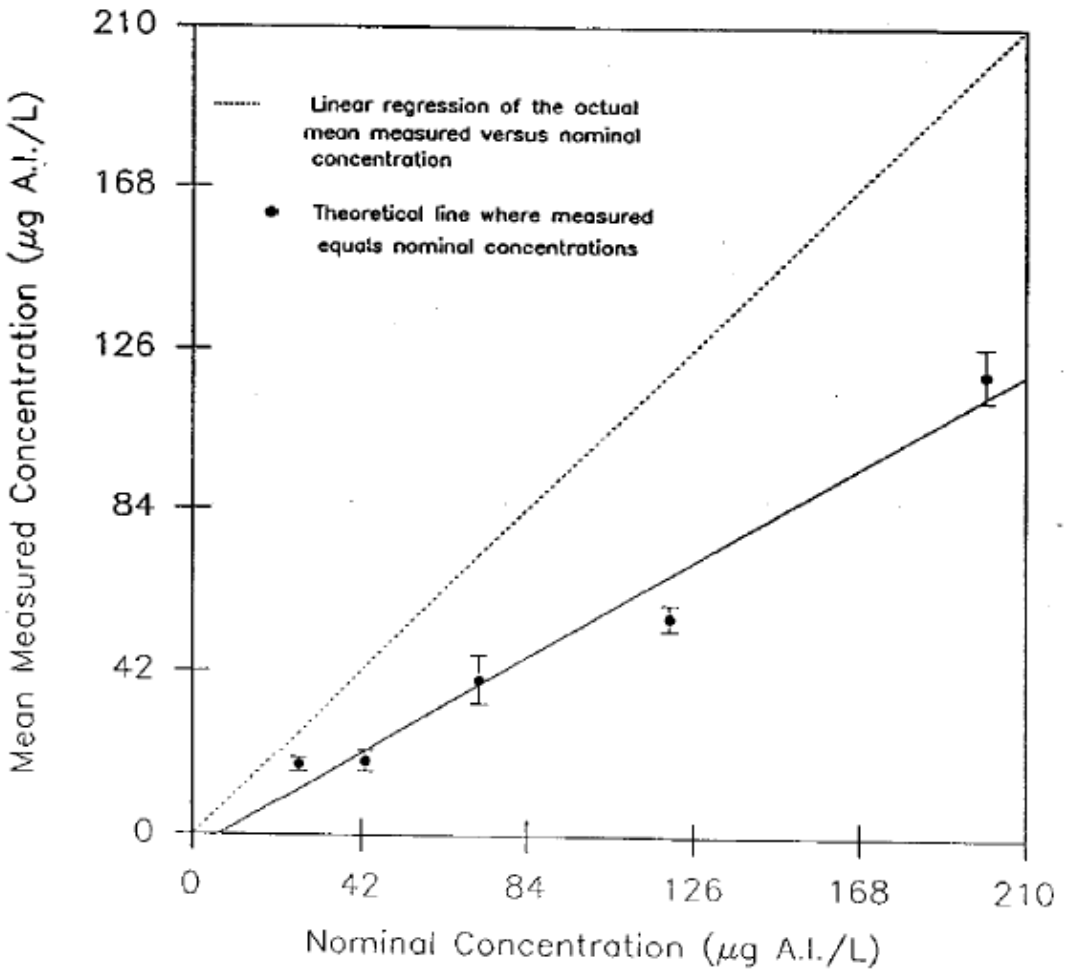
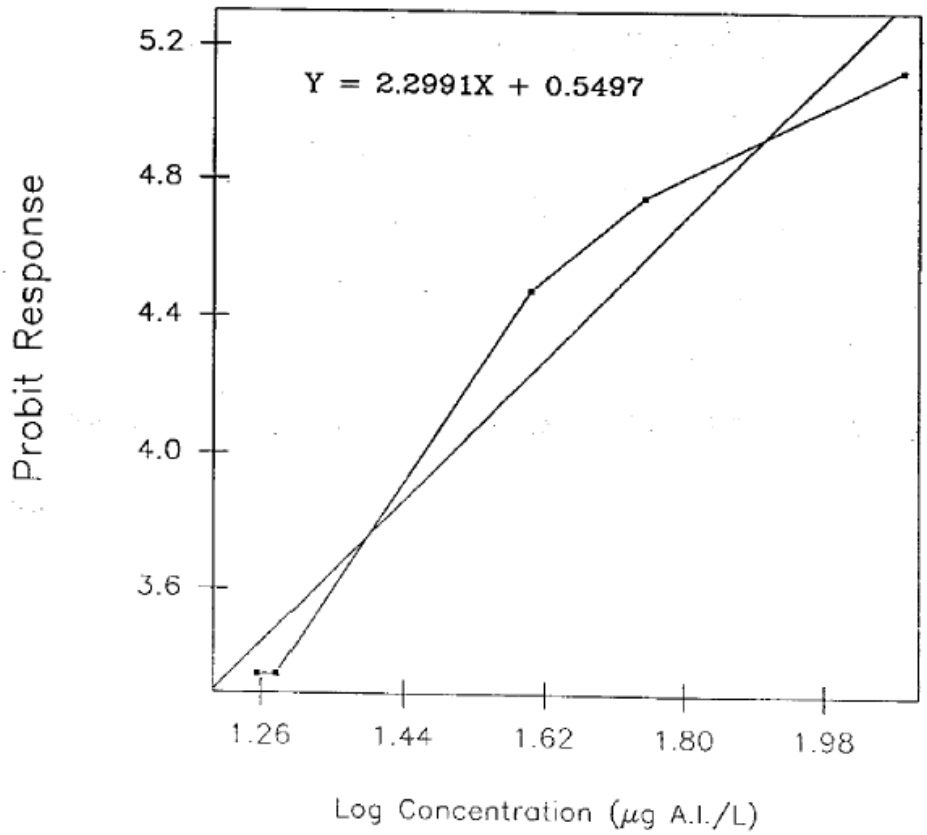


Figure 2. **The 96-hour concentration-response (mortality) curve for mysid shrimp (*Mysidopsis bahia*) exposed to Troysan Polyphase P-100.**



Conclusions

The No Observed Effect Concentration (NOEC) through 96 hours of exposure was established to be 19 ug A.I./L. Based on criteria established by US EPA (1985), Troysan Polyphase P-100 would be classified as very highly toxic to mysid shrimp. (Study Author)

Reliability

Klimisch Code = 1

The data and report were conducted in accordance with all pertinent EPA Good Laboratory Practice regulations (40 CFR 160) with the following exceptions:

stability, characterization and verification of the test substance identity are the responsibility of the test sponsor and routine water and food contaminant screening analysis for pesticides, PCBs and metals were conducted by Lancaster Laboratories, Lancaster PA. These data were not collected in accordance with Good Laboratory Practice procedures. (Study author)

Study sponsors provided traceable identification information and purity analysis on the test substance. (Reviewer)

Study was conducted according to protocol that generally meets FIFRA Guidelines 72-3. Based on the results of analyses (analytical validation) it was established that the appropriate quality control was maintained during the analyses of the exposure solutions. (Study author)

References

Springborn Laboratories Inc., Wareham MA, "Troysan Polyphase P-100 Acute Toxicity to Mysid Shrimp (*Mysidopsis bahia*) Under Flow Through Conditions," SLI Report Number 91-10-3954, December 24, 1991.

13.0 Acute Toxicity

Test Substance

Identity (purity): Troysan Polyphase P-100
3-iodo-2-propynyl butyl carbamate, Purity 99%

Method

Method/guideline followed: OECD 401
Type: Acute oral gavage
GLP: Yes
Year (study performed): 1984
Species/Strain: Rat/Sprague Dawley

Source of test animals was Charles River Breeding Laboratories Inc., Kingston, New York. Initial body weights of the rats did not exceed 20% of the mean weight.

Sex: Male and Female
No. of animals per sex per dose: 5 male, 5 female

Group	No. of Animals Males	No. of Animals Females	Dose Level mg/kg
1	5	5	250.0
2	5	5	500.0
3	5	5	1000.0
4	5	5	1500.0
5	5	5	2000.0

Vehicle: Corn Oil
Dukes Pure Corn Oil (Lot 52500)
Route of administration: Oral gavage

Test Conditions

Doses: 500, 1000, 1500, 2000 and 1500 mg/kg bw

The test rats were identified uniquely by ear tags and housed individually in suspended wire-mesh cages. Commercial rodent ration (Purina Rodent Laboratory Chow) and tap water were available *ad libitum* except where noted otherwise. The rats were acclimated to laboratory conditions for approximately one week prior to initiation of treatment.

The appropriate amount of test material was weighed on an electronic Arbor 126 balance and transferred to precalibrated beakers. A small amount was added to make a paste and the appropriate amount of vehicle was then added to the beakers. The suspension was mixed with a Tekmar Tissuemiser on a magnetic stirrer for approximately 5-10 minutes. The suspension of the test material was administered following an overnight fast from food but not water. Each rat was dosed at a factor of 10 ml/kg to achieve an appropriate dosage level.

All rats were observed for signs of toxic and pharmacologic effects at one, two and four hours post dose and once daily thereafter for 14 consecutive days in the definitive LD50 study. Mortality/moribundity was recorded twice daily. Individual body weights were recorded prior to treatment, on day 7, and at death or termination. At termination (day 14) all surviving rats were sacrificed by carbon dioxide asphyxiation. Necropsies were performed on animals that either died or were sacrificed. Observations were recorded.

The mortality data were analyzed separately for males by a modified Behrens-Reed-Muench Cumulant Method (Thakur and Fezio, 1981). For the females and for the combined sexes, LD50s and their 95% confidence intervals were computed using a maximum likelihood Logit Method (Bliss, 1952).

Results

LD50 Value:

Sex	LD50 mg/kg	95% Confidence Limits
Male	1795	(1437, 2243)
Female	1056	(783, 1329)
Combined	1470	(1226, 1713)

Number of deaths at each dose level:

500 mg/kg	0 mortalities
1000 mg/kg	0 mortalities
1500 mg/kg	3 Female (Day 2)
2000 mg/kg	3 Female (Day 3), 1 Female (Day 6)
2500 mg/kg	1 Female (Day 2), 2 Female (Day 3)
2500 mg/kg	1 Male (Day 4), 3 Males (Day 5)

Based upon the findings of this study, the acute oral LD50 in males was calculated to be 1795 mg/kg of body weight with 95% confidence limits of 1437 and 2243 mg/kg; the oral LD50 in females was calculated to be 1056 mg/kg of body weight with 95% confidence limits of 783 and 1329 mg/kg; and the combined sex LD50

was calculated to be 1470 mg/kg of body weight with 95% confidence limits of 1226 and 1713 mg/kg.

Cumulative mortality data are presented in tables. In the 1500 mg/kg group, three females were found dead on Day 2 post dose. In the 2000 mg/kg group, three females were found dead on Day 3, and one female was found dead on Day 6. In the 2500 mg/kg group, one female was found dead on Day 2, two females were found dead on Day 3; one male was found dead on Day 4 and three males and the remaining females were found dead on Day 5. LD50s and their 95% confidence limits were calculated based on these findings.

Summaries of clinical observations and individual body weights are presented in tables in the report. Clinical observations included soft feces, rough haircoat, urine stains, and slight depression and/or depression in all groups; ataxia, hunched appearance and prostration in the 1500, 2000 and 2500 mg/kg groups; labored respiration in the 1500 and 2000 mg/kg groups; and tremors in the 2500 mg/kg group. In general, animals surviving to termination gained weight and the body weights of animals found dead decreased after initiation.

All surviving animals had no observable gross pathology findings at necropsy. In the animals that were found dead, gross pathology findings were noted for the stomach, intestines, kidneys and liver.

Conclusions (Study author)

LD50 Value:

Sex	LD50 mg/kg	95% Confidence Limits
Male	1795	(1437, 2243)
Female	1056	(783, 1329)
Combined	1470	(1226, 1713)

Reliability

Klimisch Code = 1

Study authors certify that the work in this study was preformed according to the EPA Good Laboratory Practice regulations. Study conducted according to OECD 401. (Study author)

References

Hazleton Laboratories America Inc., Vienna, VA, "Acute Oral Toxicity Study in Rats 3-iodo-2-propynyl Butyl Carbamate-Final Report," July 13, 1984.

15.0 Genetic Toxicity In Vitro

(A) Unscheduled DNA Synthesis

Test Substance

Identity : Troysan Polyphase P-100
IPBC

Method

Method/guideline followed:	Not reported
Type:	Unscheduled DNA Synthesis
System of testing:	Nonbacterial, rat hepatocytes
GLP:	Yes
Year (study performed):	1988
Species/Strain or cell type And/or cell line:	Rat, Fischer 344 (young adult males) supplied by Charles River (U.K.). Primary cultures of adult rat hepatocytes (HPCs) were initiated according to modified procedures of Williams et al (1982).

For HPC/DNA repair studies, cell suspensions containing at least 1 x 10^5 cells in 1 ml WMES were immediately seeded on to 13 mm diameter coverslips in multiwell dishes and placed in a 95% air, 5% CO_2 humidified 37°C incubator. Two hours after seeding, coverslips were washed with 1 ml of WME leaving only attached viable cells.

HPC/DNA Repair Assay

Immediately after washing, IPBC and 10 uCi.ml^{-1} tritiated thymidine ([6^{3}H]-TdR), were added to the culture in 1 ml serum-free WME. IPBC was tested in quadruplicate wells at the following concentrations: 3, 4.5, 6, 7.5, 9, 10.5, 12, 13.5 ug.ml^{-1}. Additionally, the positive

After 7 days, autoradiographs were developed in D19 developer for 4 min, placed in a stop bath of acidified tap water for 30 s, immersed in fixer for 5-10 min, then washed in running tap water for 5 min.

Slides were stained in methyl-green/pyronin Y solution, washed, air dried and coverslipped with D.P.X. control, Michler's ketone at concentrations of 4, 8 and 16 µg.ml^{-1} and DMSO at 10 µl.ml^{-1} were tested in parallel with the test sets.

HPCs were incubated at 37°C for a total of 18-20 h in the presence of the IPBC/tritiated thymidine mixture. Each culture was rinsed in 3 successive 1 ml washes of WME containing 1 mM thymidine after which 3 of each set of 4 cultures were treated with 1% sodium citrate for 10 min to allow the nuclei to swell, which permits better quantification of nuclear grains. The fourth culture was used to monitor the toxicity of each treatment; viable cell count (trypan blue exclusion) was determined to estimate cell survival relative to the vehicle control. The cell cultures for autoradiography were fixed in three 30 min changes of methanol: glacial acetic acid (3:1), air dried, and coverslips mounted cell surface upwards upon glass slides with D.P.X. Using a Kodak Beehive safelight fitted with a red filter, slides were dipped into K2 emulsion, prewarmed for 1 h at 48°C. Slides were dried and then places in a plastic slide box which was wrapped in foil and stored at -20°C.

Quantification of Repair Synthesis:

The cells were examined microscopically at approximately 1000 x magnification under oil immersion using a Leitz Dialux 20L microscope. Unscheduled DNA synthesis (UDS) was measured by counting nuclear grains and subtracting the average number of cytoplasmic grains in three nuclear-sized areas adjacent to each nucleus (background count). This value was referred to as the net nuclear grain count. The coverslips were coded to prevent bias while grain counting. The data were recorded as the average net grain counts for 3 cultures.

Test Conditions

Criteria for evaluating results:

Evaluation Criteria

The net nuclear grain count was determined for 50 randomly selected cells on each coverslip. Only normally-appearing nuclei were scored and any occasional nuclei blackened by grains too numerous to count were excluded as cells in which replicative DNA synthesis occurred rather than repair synthesis. If the actual count for any nucleus was less than zero (i.e. cytoplasmic count is greater than nuclear count), then the negative value was used in the calculation of the mean value. The mean net nuclear grain count was determined from the triplicate coverslips (150 total nuclei) for each treatment condition.

Several criteria have been established which, if met, provide a basis for classifying a test material as positive in the UDS assay. These criteria are formulated on the basis of published results and laboratory experience and are used in lieu of a statistical treatment to indicate a positive response. While the criteria are arbitrary guidelines that may not be applicable to all assays and may need revision as the data base alters, they represent a reasonable approach to the evaluation of the test material.

The test material was considered to be positive in the UDS assay at concentrations that caused:

1. An increase in the mean net nuclear grain count to at least 6 grains per nucleus in excess of the concurrent vehicle control value, and/or

2. The percentage nuclei with 6 or more net grains to increase above 10% of the examined population, in excess of the concurrent vehicle control value and/or

3. The percentage nuclei with 20 or more net grains to reach or exceed 2.0% of the examined population.

If the vehicle control showed an average of 6 net grains per nucleus or 1% of the cells had 20 net grains per nucleus, the assay would have been considered invalid.

The test material was considered inactive in this assay if none of the above conditions was met and if the assay included the maximum applied dose or other doses that are shown to be toxic by the survival measurements. If little or no toxicity was demonstrated for any of the applied doses and the test material remained soluble in the culture medium, the assay would have been considered inconclusive and would have been repeated with higher doses after consultation with the sponsor. However, a concentration of 5 $mg.ml^{-1}$ would not have been exceeded.

The positive control nuclear labelling was not used as a reference point to estimate mutagenic or carcinogenic risks associated with the UDS activity of the test material, but only to demonstrate that the cell population employed was responsive and the method was adequate for the detection of unscheduled DNA synthesis. Even if the positive control results were inconclusive, a significant dose related response with the test substance would be sufficient evidence to conclude that the test substance caused UDS.

Results

Cytotoxic concentration:	13.5 ug/ml
Genotoxic effects:	Negative

Cell Viability at the Completion of the Dosing Period (Table 1 and 2)

In the first experiment, cell viability values for Solvent Controls 1 and 2 were 85.3 and 88.6% respectively. Viability of cultures treated with Michler's ketone at 4, 8 and 16 $\mu g.ml^{-1}$ were 80, 78.5 and 64.3% respectively. IPBC was toxic at all but the bottom dose, (7.8 $\mu g.ml^{-1}$), which had a viability of 76.1%. Due to the excessive toxicity, this experiment was abandoned (see Table 1).

In the second experiment, cell viability values for Solvent Controls 1 and 2 were 72 and 72.7% respectively. In cultures treated with Michler's ketone, cell viability decreased as the concentration increased. Viability was 91.3, 85.7 and 81.0% at the 3 concentrations used; 4, 8 and 16 $\mu g.ml^{-1}$ respectively.

A dose-related trend was observed in the cultures treated with IPBC. Viability decreased from 84.4% for the culture treated with 3 $\mu g.ml^{-1}$, down to 25% for the culture treated with 13.5 $\mu g.ml^{-1}$.

In the third experiment, the solvent controls had a viability of 81.8% for Solvent 1 and 80.8% for Solvent 2. Viability of cultures treated with Michler's ketone at 4, 8 and 16 $\mu g.ml^{-1}$ were 79.8, 88.5 and 80.8% respectively.

Cultures treated with IPBC showed decreasing viability with increasing concentrations, with the exception of the second and third lowest dose in comparison to the doses immediately above and below. Viability decreased from 80.7% for 3 $\mu g.ml^{-1}$ down to 14.6% for 13.5 $\mu g.ml^{-1}$ (see Table 2).

IPBC

In Experiments 2 and 3, primary cultures of adult rat hepatocytes were treated with IPBC at concentrations of 3, 4.5, 6, 7.5, 9, 10.5, 12 and 13.5 $\mu g.ml^{-1}$. Evidence of toxicity was noted in cells treated with 13.5 $\mu g.ml^{-1}$ in Experiments 2 and 3. Although there were few cells on the slide at this concentration in Experiment 2, scoring was possible.

In Experiments 2 and 3, none of the cultures satisfied any of the evaluation criteria defining a positive response in the UDS Assay.

Conclusions

It is concluded that IPBC did not induce unscheduled DNA synthesis in adult rat hepatocytes primary cultures when tested in dimethyl sulphoxide at concentrations extending into the toxic range.

Reliability

Klimisch Code = 2

Study author certifies that study was conducted according Good Laboratory Practices (GLP); however GLP standard is not cited. Study design is well described. (Reviewer)

Purity of test compound not reported. The study is reliable with restriction. (Reviewer)

References

Inveresk Research International, Scotland, “IPBC: Assessment of Gentoxicity in an Unscheduled DNA synthesis Assay Using Adult Rat Hepatocyte Primary Cultures,” IRI Project Number 737447, January 21, 1988.

(B) Mutation

Test Substance

Identity: Troysan Polyphase P-100 (IPBC)
Purity 97%; Batch number p-2848-8603-P100

Method

Method/guideline followed:	Ames (1975)
Type:	Bacterial reverse mutation
System of testing:	Bacterial
GLP:	Yes
Year (study performed):	1989
Species/Strain:	*Salmonella typhimurium*, Strains TA 1535, TA1537, TA1538, TA 98, TA 100
Metabolic activation:	Yes

Mutation Tests

Two independent mutation tests were conducted using 5 bacterial strains (TA 1535, TA 1537, TA 1538, TA 98 and TA 100). The dose levels used in the first of these experiments and selected on the basis of the results of the toxicity test, were 1, 3, 10, 33, 100 and 333 µg per plate. Dose levels used in the second experiment, and based on the results of the first experiment, in the presence of S9 mix were 3, 10, 33, 100, 333 and 1000 µg per plate. Dose levels used in the absence of S9 mix were 1, 3, 10, 33, 100 and 333 µg per plate, excepting TA 1535 which ranged between 3 and 1000 µg per plate.

Triplicate plates were prepared for each bacterial strain and dose level in both the presence and absence of S9 mix.

Test Conditions

Positive and negative control groups and treatment:

Positive control substances used were 2-aminoanthracene (2-AAN) (Lot No. 33F-0816) from the Sigma Chemical Company Limited, Fancy Road, Poole, Dorset, England and sodium azide (NaN_3) (Lot No. 6529052) from BDH Limited, Poole, Dorset, England and 9-aminoacridine (9-AA) (Lot No. N333JE) and 2-nitrofluorene (2-NF) (Lot No. A3A) from IIT Research Institute, Chicago, Illinois 60616, USA.

Solvent: Dimethylsuphoxide was the solvent for the test substance and positive controls, except sodium azide; this latter substance was dissolved in sterile, ultra-pure water.

Criteria for evaluating results (e.g. cell evaluated per dose group):

Evaluation of Results

A test was considered acceptable if for each strain:

i) the bacteria demonstrated their typical responses to crystal violet, ampicillin and u.v. light.

ii) at least 2 of the vehicle control plates were within the following ranges: TA 1535, 4-30; TA 1537, 1-20; TA 98, 10-60; TA 100, 60-200 and TA 1538, 5-35.

iii) on at least 2 of the positive control plates there were x 2 the mean vehicle control mutant numbers per plate, or in the case of TA 100, x 1.5 the mean vehicle control mutant numbers per plate.

If the mean colony count on the vehicle control plates was less than 10 then a value of 10 was assumed for assessment purposes. In such cases a minimum count of 20 was required on at least 2 of the positive control plates.

iv) no toxicity or contamination was observed in at least 4 dose levels.

v) in cases where a mutagenic response was observed, that no more than one dose level was discarded before the dose which gave the highest significant mean colony number.

Where these criteria were met, a significant mutagenic response was recorded if there was:

i) for S. typhimurium strains TA 1535, TA 1537, TA 1538 and TA 98, at least a doubling of the mean concurrent vehicle control values at some concentration of the test substances and, for S. typhimurium strain TA 100, a 1.5-fold increase over the control value. If the mean colony count on the vehicle control plates was less than 10 then a value of 10 was assumed for assessment purposes. In such cases a minimum count of 20 was required before a significant mutagenic response was identified.

ii) a dose related response, although at high dose levels this relationship could be inverted because of, for example, (1) toxicity to the bacteria generally, (2) specific toxicity to the mutants and (3) inhibition of foreign compound metabolising enzymes where mutagens require metabolic activation by the liver.

iii) a reproducible effect in independent tests.

Results

Cytotoxic concentration:

- With metabolic activation: 1000 ug per plate
- Without metabolic activation: 1000 ug per plate

Genotoxic effects:

- With metabolic activation: Negative
- Without metabolic activation: Negative

Toxicity Test Results:

Precipitation and toxicity to the bacteria occurred at the 3 highest concentrations of 1000, 3333 and 10000 µg per plate, in both the presence and absence of S9 mix, inducing no growth of the background lawn. At 333 µg per plate there was a reduction in colony number and a thinning of the background lawn in the presence of S9, while in the absence of S9 no growth occurred.

P100 induced no mutagenic activity in Salmonella typhimurium, in either the presence or absence of S9 mix, in either of 2 independent experiments. Toxicity to the bacteria was observed at the higher concentrations of P100.

Vehicle Control Results:

The vehicle control values were within the normal ranges experienced in this laboratory and reported in the literature with these strains of S. typhimurium.

Positive Control Results:

The results obtained in the positive control groups were within the normal ranges expected for each bacterial strain and activation condition.

Results:

All tests were acceptable according to the study criteria except the second test with TA 1537 where the vehicle control values were unacceptably high. This part of the test was repeated.

Toxicity to the bacteria with a corresponding reduction in colony number occurred at the top dose of 333 µg per plate in the absence of S9 mix.

Toxicity to the bacteria occurred at concentrations ranging from 333 to 1000 µg per plate with a corresponding reduction in colony number.

Precipitation occurred at 1000 µg per plate in Test 2 in the presence of S9 mix and in TA 1535 in the absence of S9 mix.

No mutagenic activity was observed in any of the 5 strains used, in either the presence or absence of S9 mix, in either test.

Conclusions

It is concluded that P100 is not mutagenic in S. typhimurium when tested in dimethylsulphoxide at concentrations extending into the toxic range.

Reliability

Klimisch Code = 1

The study was conducted in compliance with EPA Good Laboratory Practices 40CFR 150. (Study author)

The study was conducted according to recognized protocol by Ames et al (1975) (Reviewer)

References

Inveresek Research International Limited, Scotland, “Troysan Polyphase P-100 IPBC-97%, Batch No. P-2848-8603-P100: Testing for Mutagenic Activity With *Salmonella typhimurium* TA 1535, TA 1537, TA 1538, TA 98 and TA 100,” IRI Report Project number 740140, June 1989.

16.0 Repeated Dose Toxicity

Test Substance

Identity (purity): Troysan Polyphase P100
98.7%

Method

Method/guideline followed:	OECD 409
Test type:	Subchronic dietary exposure
GLP:	Yes
Year (study performed):	1997
Species:	Albino Rabbits
Strain:	New Zealand White
Route of administration:	Oral (dietary)
Duration of test:	90 days
Doses/concentration levels:	500, 2000, 4000 ppm diet
Sex:	21 Male, 19 Female
Exposure period:	90 days
Frequency of treatment:	daily, dietary
Control group and treatment:	5 Male, 5 Female
Post exposure observation period:	Daily for 90 days
Statistical methods:	Mean values of all dose groups were compared to controls at each time interval. Statistically significant differences from the controls were determined.

Test Conditions

Supplier:	HRP Denver, PA
Number of test animals:	40 total (21 males, 19 females)

Note: Group II animal No. 2503 was incorrectly sexed and identified as female by the supplier and was place in Group II as a female. This mis-sexing was not revealed until the terminal sacrifice. Data for this animal were reported under the Group II male data. The deviation from protocol was considered not to have affected the results of the study. (Study author)

Females were nulliparous and non-pregnant.

Animals considered suitable for study based on pretest physical examination and body weight were distributed into four groups of five animals per sex using a computerized random sorting program.

Weight at test initiation:
Mean body weights for each group were comparable.

Weight at Initiation of Treatment (kilograms):		Mean	Range
	Males:	2.3	2.2 to 2.5
	Females:	2.2	2.1 to 2.4

Age at receipt: Approximately 3 months
Age at initiation of treatment: Approximately 4 months
Experimental Design:

Group	Dietary Level[a]	Number of Animals							
		Initial		Clinical Laboratory Studies: Pretest, Days 45 and 90		Terminal Sacrifice: 3 Months		Microscopic Pathology[c]	
	ppm	M	F	M	F	M	F	M	F
I	0[b]	5	5	5	5	5	4	5	5
II	500	6[d]	4	6	4	6	4	6	4
III	2000	5	5	5	5	5	5	5	5
IV	4000	5	5	5[e]	5	4	5	5	5

[a]Diets were prepared with test material as supplied, i.e., no correction for percent active ingredient was made.
[b]Control animals received untreated (re-pelleted) diets.
[c]Selected tissues were evaluated histopathologically for all animals in Groups I and IV. In addition, based on findings seen in the high-dose animals, the livers of all Group II and III animals were evaluated histopathologically.
[d]Group II Animal No. 2503 was identified as a female but discovered to be a male at postmortem examination. Data for this animal are reported with the Group II male data.
[e]Clinical laboratory studies were performed on four animals at Day 90 due to the death of one animal on Test Day 45.

Feed: Rabbit Diet No. 5325 High Fiber (PMI Feeds Inc. St. Louis, MO) Analysis of feed included in study report. Stability of test material in pelleted dietary mixture was established and documented in study report.

Feeding: Animals were on a restricted feeding regimen for the first several days after receipt. The rabbits were not fed on the day of arrival. The following three days they received approximately 25,50 and 100 grams of feed per day. For the remainder of the acclimation period and during the study they were fed

approximately 125 grams of feed daily. During the study fresh diets were presented daily. Any feed remaining in the feeder from the previous day was discarded.

Water: *ad libitum* by automated watering systems. Analysis of water provided in study report.

Environmental Conditions:

Light:	12 hour light/dark cycle
Temperature:	12 – 24°C
Humidity:	28 – 74%

Observations (frequency):

- Mortality and gross signs of toxicological or pharmacologic effects (Twice daily)
- Detailed physical examination for signs of local of systemic toxicity, pharmacologic effects and palpation for tissue masses (Twice pretest and once weekly during study)
- Ophthalmoscopic examination (Pretest and Day 90)
- Body weight (Twice pretest, Day 0 and at sacrifice)
- Food Consumption (Two daily intervals pretest, daily for first five weeks of study and twice per week for the remainder of the study)
- Test Material Intake: Calculated from food consumption data and based on actual analytical concentrations in feed
- Hematology (Days 45 and 90)

  Hemoglobin concentration; Hematocrit; Erythrocyte count; Reticulocyte count; Platelet count; Mean corpuscular volume Mean corpuscular hemoglobin concentration; Prothrombin time Activated partial thromboplastin time; Total and differential leukocyte counts; Erythrocyte morphology

- Clinical chemistry (Days 45 and 90)

  Aspartate aminotransferase; Alanine aminotransferase; Blood urea nitrogen; Fasting glucose; Total protein; Albumin; Globulin (Calculated); A/G Ration (Calculated) ; Creatinine ; Total bilirubin Sodium; Potassium; Chloride; Calcium; Inorganic phosphorus Gamma-glutamyltransferase

- Postmortem examinations (Performed on all animals at sacrifice or death)

External surface and all orifices; evaluation of the external surfaces of the brain and spinal cord; evaluation of the organs and tissues of the cranial, thoracic, abdominal and pelvic cavities and neck; and evaluation of the remainder of the carcass

- Postmortem organ weights (at sacrifice or death)

  Adrenal glands, brain, kidneys, liver, testes with epididymides, thyroid glands (with parathyroids)

- Postmortem-tissues were preserved and examined microscopically.

Results

NOAEL (NOEL): 500 ppm (diet) (13 mg/kg bw/day)
LOAEL (LOEL): Not established

Actual dose received by dose level by sex:

Group (ppm)	Overall Mean Test Material Intake (mg Troysan Polyphase P100/Kg body weight/day) [range][a]	
	Males	Females
II (500)	13 [9-17]	13 [9-18]
III (2000)	75 [59-94]	78 [61-102]
IV (4000)	151 [87-179]	146 [61-170]

[a]Represents a mean of 13 mean weekly values and a range of values over this period.

Concentration of test material in feed:

Group (ppm)	Homogeneity Analytical Data (% of nominal) Level in Mixer[a]		
	Top	Middle	Bottom
II (500)	70.0/67.0/67.4 Mean: 68.1	63.4/60.8/53.4 Mean: 59.2	54.6/70.8/81.0 Mean: 68.8
IV (4000)	96.9/97.4/95.6 Mean: 96.6	97.8/94.6/95.0 Mean: 95.8	91.7/90.5/88.9 Mean: 90.4

[a]Presents results for the three diet samples analyzed per level and a mean of these data.

Mortality:

No treatment-related mortality occurred during the study. One high-dose male was sacrificed moribund on Day 45 shortly after being bled for the scheduled hematology/clinical chemistry assessment. Morbidity was attributed to handling-related stress during the blood collection procedure and not treatment-related. No other mortality occurred among the treated groups.

One control female died on Day 90 after being bled for the terminal hematology/clinical chemistry assessment. Death was attributed to handling-related stress during the blood collection procedure. No other mortality was seen in the control group.

Summary of results:

In this study, Troysan Polyphase P100 was incorporated into the pelleted ration and administered to male and female New Zealand white rabbits at nominal dietary concentrations of 0, 500, 2000 and 4000 ppm over a 13-Week period. No adverse effect of treatment at the 500 or 2000 ppm dietary levels was evident from body weight or food consumption data. At the 4000 ppm level, animals adjusted more gradually to the test rations, ate less during the early weeks of study and gained less weight. Treatment produced hepatic changes at the two higher concentration levels. These effects consisted of slightly elevated GGT enzyme levels in the high-dose group only, increased liver weights in the females at the two higher dietary levels and microscopic changes consisting of centrilobular hepatocellular enlargement (cell swelling) and brown pigment in the cytoplasm of hepatocytes and sinusoidal macrophages. The no-observed-effect level (NOEL) for this study was the 500 ppm dietary level. This was equivalent to a dose level of approximately 13 mg/kg/day.

Conclusions

No observed effect level established at 500 ppm dietary level, which is equivalent to a dose of approximately 13 mg/kg bw/day. (Study author)

Reliability

Klimisch Code = 1

The study was conducted in compliance with OECD Good Laboratory Practices and in accordance with OECD Method 409. (Study author)

References

Huntington life Sciences, East Millstone, NJ, “A Subchronic (3-Month) Toxicity Study of Troysan Polyphase P100 in the Rabbit via Dietary Administration - Final Report,” February 13, 1997.

17.0 Reproductive Toxicity

Test Substance

Identity (Purity): Troysan Polyphase P-100
Batch No. P 2710-8511-R100, 3-Iodo-2-Propynyl butyl carbamate

Method

Method/guideline followed:	EPA Guideline 83-4
Type:	Two-generation reproductive toxicity
GLP:	Yes
Year (study performed):	1987
Species:	Rat
Strain:	Sprague Dawley Crl: CD (SD)BR
Route of administration:	Oral, dietary in feed
Doses/concentration levels:	120, 300, 750 ppm in diet
Sex:	25 Male, 25 Female per dose
Control group and treatment:	25 Male, 25 Female with untreated diet
Frequency of treatment:	Daily in diet
Duration of test:	2 generations (290 days)
Premating exposure period for (P and F1) as appropriate:	Animals were treated for fourteen weeks and then allowed to mate for maximally 21 days to produce one litter.

Statistical methods:

For body weight, body weight gain, litter weight, and mean pup weight, the analysis of variance was performed with one factor TREATMENT. In case of suspected significance, the four groups were compared at each time by the Newman-Keuls test[2,3,4] for multiple group comparisons ($p < 0.05$).

All significances found (at least $p < 0.05$) are presented in the respective tables.

The statistical evaluation was performed with the standard software package SAS® (Statistical Analysis System).

Calculations:

The mating performance (in days) was calculated for each group as:

$$\frac{\text{Sum of days until successful insemination}}{\text{Number of inseminated animals}}$$

The fecundity index (in per cent) was calculated for each group as:

$$\frac{\text{Number of pregnant animals}}{\text{Number of inseminated animals}} \times 100$$

The insemination index (in per cent) was calculated for each group as:

$$\frac{\text{Number of inseminated animals}}{\text{Number of paired animals}} \times 100$$

The fertility index (in per cent) for male and female animals was calculated for each group as:

$$\frac{\text{Number of males shown to be fertile}}{\text{Number of males mated}} \times 100 = \frac{\text{Number of pregnant females}}{\text{Number of mated females}} \times 100$$

The gestation index (in per cent) was calculated for each group as:

$$\frac{\text{Number of females with live pups}}{\text{Number of pregnant animals}} \times 100$$

The live birth index (in per cent) was calculated for each litter as:

$$\frac{\text{Number of pups alive day 1}}{\text{Total number of pups born}} \times 100$$

The viability indices (in per cent) were calculated for each litter as:

$$\frac{\text{Number of pups alive day 4/7/14/21}}{\text{Number of pups alive day 1/4*/7/14}} \times 100$$

The weaning index (in per cent) was calculated for each litter as:

$$\frac{\text{Number of pups alive day 21}}{\text{Number of pups alive day 4*}} \times 100$$

Percentage sex ratio was calculated as:

$$\frac{\text{Number of males}}{\text{Number of pups}} \times 100 \qquad \frac{\text{Number of females}}{\text{Number of pups}} \times 100$$

*) number of pups alive on day 4 post-partum after adjustment of litter size

Test Conditions

Test animals:

Male and female rats of Sprague Dawley Crl:CD (SD)BR strain were obtained from a “specific pathogen free” colony at Charles River Wiga GmbH, 8741 Sulzfeld, West Germany. All animals were examined upon arrival for external signs of ill health. Prior to the start of dosing. All animals were acclimatized to the laboratory for at least two weeks prior to the start of treatment.

F1 Animals:

Selection of weaned F1a pups to form F1 generation was made as follows: after all the F1a pups were weaned, one to three male and one to three female pups were selected from each litter - a minimum of 25 males and females per group - using a system of randomly drawn cards.

Selected F1 animals were identified by a combination of the litter number and Arabic numerals, if necessary. The animals were earmarked to ensure individual identification. Each cage was identified by a group-related coloured card.

Environmental Conditions:

The room was maintained at a temperature of 20 to 25 °C (except on six occasions when 19, 19, 26, 27, 26, and 18 °C were measured) and a relative humidity of 40 to 70 per cent (except on two occasions when 30.5 and 35 per cent were measured).

The minimum and maximum temperature were recorded once daily. The humidity was checked twice daily (except on 46 days at the beginning of the study due to a technical problem with the measuring instrument).

The animals were exposed to a constant artificial (fluorescent) light cycle of twelve hours light and twelve hours dark.

P and F1 animals were individually housed, except during mating when one male was housed with one female and during lactation when each female was housed with its litter, in solid floor macrolone cages of type III with stainless steel lids (dimensions: 420 mm x 260 mm x 150 mm; E. Becker & Co GmbH, 4620 Castrop-Rauxel, West Germany) during the pre-mating and mating period.

Diet and Water:

Throughout the study the rats were allowed free access to powdered diet (Ssniff R 10, Ssniff Spezialdiäten GmbH, 4770 Soest, West Germany; see Appendix XVI).

Tap water was available ad libitum from plastic water bottles attached to each cage.

Number, age, sex per dose for P, F1 and F2, if appropriate:

P Generation:

The P animals were treated for fourteen weeks and then allowed to mate for maximally 21 days and to produce one litter (F1a).

Group number	Colour code	Dose level ppm	Number of males	Number of females
1	buff	0	25	25
2	blue	120	25	25
3	green	300	25	25
4	red	750	25	25

F1 Generation:

At the end of lactation, one to three male and one to three female F1a off-spring were selected from each litter (25 males and females per group) for further study. This gave group sizes of:

Group number	Colour code	Dose level ppm	Number of males	Number of females
1	buff	0	25	25
2	blue	120	25	25
3	green	300	25	25
4	red	750	25	25

Mating:

Each P generation male was housed with one P generation female from the same dose group for a maximum of 21 days.

The F1 generation animals were mated after 14 weeks of maturation when one male was mated with one female from the same dose group in such a way that sibling mating was avoided.

For both generations, mating success was checked daily by vaginal smearing. The day on which sperm was observed was designated day 0 of gestation. Smearing of individual females was discontinued when mating was confirmed or at the end of the 21 day mating period.

Evaluation of Effects:

Appearance, behavior and general observation:

All P animals and selected F1 animals were observed at least once daily for signs of ill-health or overt signs of toxicity.

Each litter was examined daily during the pre-weaning period and any abnormalities of the progeny were recorded.

Body weight:

The body weight of the parental animals was recorded at weekly intervals during the pre-mating period.

Inseminated parental P and F1 females were weighed on days 0, 6, 12, 15, and 20 post-coitum and parental lactating females weighed on days 1, 4, 7, 14, and 21 of lactation.

Food consumption:

The amount of food consumed by the parental animals was recorded weekly during the pre-mating period, for days 0 to 6, 6 to 12, 12 to 15, and 15 to 20 post-coitum, and for days 1 to 4, 4 to 7, 7 to 14, and 14 to 21 during lactation.

The food consumption was then evaluated as mean daily food consumption per measuring period.

Necropsy:

All animals were examined externally and a macroscopic examination was carried out of all tissues and organs in situ. Major organs were incised and the cut surfaces examined.

In addition, the following organs were removed: epididymides, testes, seminal vesicles, prostate, ovaries, uterus, cervix, and vagina.

Testes were fixed in Bouin's fluid and all other tissues in 10 per cent neutral buffered formalin.

Following fixation, sections of all tissues from the control and high dose group were prepared in paraffin wax, stained and subsequently evaluated by the study pathologist.

Littering Groups – P Generation

All P females in each dose group were allowed to litter and to rear their young to weaning.

The duration of gestation was recorded and the numbers of live and dead pups were counted. The pups were sexed and weighed on days 1, 4, 7, 14, and 21 post-partum.

On day 4 post-partum, appropriate litters were culled to a maximum of eight pups (four males and four females where possible).

Pups dying during lactation were examined for external and visceral abnormalities.

During lactation the pups in each litter were examined daily and the physical development of the progeny was assessed by monitoring the following events:

Pinna unfolding:	the day on which the pinnae became detached
Generalised hair growth:	the day on which generalised growth of body hair was observed
Tooth eruption:	the day on which the upper incisors were observed to penetrate the gum
Eye opening:	the day on which the upper and lower eye lids separated.

For each developmental event the number of pups in each litter showing the observation was daily recorded until all the pups in the litter showed the observation.

After weaning two males and two females per litter, where possible, were randomly selected for examination of pupillary reflex and startle response.

One to three males and one to three females per litter (25 males and females per group) were selected after weaning to produce the F2a generation. The remaining pups were subjected to gross necropsy.

After the lactation period of 21 days all P females were sacrificed and examined according to section 7.6. The number of implantations was recorded.

The uteri of apparently non-pregnant females were immersed in a 10 per cent solution of ammonium sulphide to reveal evidence of implantation (Salewski technique[1]).

Examination of selected F1 Females Used for Mating:

All F1 females in each group were allowed to litter and to rear their young to weaning. The duration of gestation was recorded and the numbers of live and dead pups were counted. The pups were sexed and weighed on day 1, 4, 7, 14, and 21 post partum.

On day 4 post-partum, appropriate litters were culled to a maximum of eight pups (four males and four females where possible).

Pups dying during lactation were examined for external and visceral abnormalities.

During lactation the pups in each litter were examined daily and the physical development of the progeny was assessed by monitoring the following events:

Pinna unfolding:	the day on which the pinnae became detached
Generalised hair growth:	the day on which generalised growth of body hair was observed
Tooth eruption:	the day on which the upper incisors were observed to penetrate the gum
Eye opening:	the day on which the upper and lower eye lids separated.

For each developmental event the number of pups in each litter showing the observation was daily recorded until all the pups in the litter showed the observation.

All pups were subjected to gross necropsy after weaning.

After the lactation period of 21 days, all F1 females were sacrificed and examined according to section 7.6. The number of implantations was recorded.

The uteri of apparently non-pregnant females were immersed in a 10 per cent solution of ammonium sulphide to reveal evidence of implantation (Salewski technique[1]).

Results

After conclusion of the present study with Troysan Polyphase including the evaluation of the data, the results can be summarized as follows:

1. There were no abnormalities of clinical condition in the parental animals in either generation considered to be associated with treatment. Necropsy revealed no treatment-related findings.

2. Treatment with Troysan Polyphase at 300 and 750 ppm was associated with reduced body weight gain in P male animals during the pre-mating treatment period.

   At 750 ppm the body weight gain of the F1 male animals was also slightly reduced during the pre-mating treatment period.

3. At 750 ppm the mean food consumption of the P and F1 male animals was slightly reduced during the pre-mating treatment period.

4. There was no effect of treatment in either generation on mating performance or fertility.

5. Live birth index was slightly reduced in either generation at 750 ppm. Post-natal viability was however not affected.

   Post-natal growth of the offspring of the treated P and F1 animals was comparable to the controls.

6. Physical and functional development of the offspring of the treated animals was similar to the controls in both generations.

Mortality:

There were no treatment-related mortalities of the parental animals.

One female of the P generation (animal no. 186) of group 4 (750 ppm) died on day 9 post-partum. Necropsy showed findings in the spleen, kidneys, urinary bladder, and lungs.

The occurrence of one death is considered to be incidental.

Clinical Observations and necropsy findings:

P generation - males

No treatment-related clinical signs could be observed.

Necropsy of males revealed a few isolated findings as dilatation of the renal pelvis or tumour caudal left of the liver or tumour in the right abdominal cavity or severe emaciation due to missing upper incisors and abnormally long lower incisors.

In one male of group 2 (120 ppm) the testes were reduced in size.

These findings were considered to be not treatment-related.

P generation - females

No treatment-related clinical signs could be observed.

Necropsy findings as dilatation of the renal pelvis or grossly granulated cut surface of the liver were occasionally observed in all groups.

One animal in group 2 (120 ppm) showed 100 per cent intra-uterine deaths at necropsy.

These findings were considered to be incidental.

Body weight:

P generation - males

Body weight gain during the pre-mating period was slightly reduced in group 3 (300 ppm) and clearly reduced in group 4 (750 ppm) during the treatment period.

P generation - females

Body weight gain of females during the pre-mating treatment, the gestation, and the lactation period was comparable in all groups.

Food Consumption – P Generation:

Mean food consumption of group 4 (750 ppm) males was slightly reduced during the pre-mating treatment period.

Mean food consumption of group 2 (120 ppm) and group 3 (300 ppm) males throughout the pre-mating treatment period was comparable with the control.

Mean food consumption of P females was generally similar in all groups throughout pre-mating treatment period, gestation, and lactation.

Mating Performance and Fertility – P Generation:

There was no effect of treatment with Troysan Polyphase on mating performance or fertility.

Litter Data – P Generation:

Duration of gestation (Table 18, Appendix V)
All pregnant animals littered on day 21 or 22 of gestation. The mean duration of gestation was normal in all groups.

Number of pups (Table 18, Appendix V)
The live birth index was slightly reduced in group 4. Between days 1 and 4 p.p. pup losses were observed in all groups. After adjustment of litter size (day 4 p.p.) pup loss was similar in all groups.

The sex ratio was similar in all groups on days 1 and 21 of lactation.

Pup weight (Table 18, Appendix V and VI)
There was no effect of treatment on post-natal growth. Mean pup weight in the treated groups was comparable with the control group.

Physical development of the F1a pups (Figures 9 to 12, Appendix VII)
Concerning all developmental landmarks examined (pinna unfolding, hair growth, incisor eruption, eye opening) its mean onset in the dose groups was comparable to the control group.

Functional tests (Table 19)
The employed functional tests (pupillary reflex, startle response) revealed no effect of treatment.

Malformations (Table 20)
One F1a pup of group 4 (750 ppm) showed externally an aplasia of the tail. The incidence of one malformation was considered to be incidental.

Necropsy findings (Table 20)
Macroscopic examination of F1a pups died or sacrificed revealed no treatment-related changes.

Parental Animals – F1 Generation:

Mortality (Table 21, Appendix VIII)
One F1 male animal of group 4 (750 ppm) died on day 36 after start of mating; necropsy showed the right kidney developed as a tumour. This finding was considered to be incidental.

Clinical observations and necropsy findings (Appendix VIII)
No treatment-related changes in appearance, behaviour, and general condition were observed.

Necropsy of F1 males and females revealed a few isolated findings in all groups (e.g. hydronephrosis and/or dilatation of the renal pelvis, or testis enlarged or reduced, or small cyst on the urinary bladder, or cyst on the right ovary or right uterine horn developed rudimentarily). These findings were considered to be not related to treatment.

Body weight - males (Tables 22 to 23, Figure 13, Appendix IX)
Body weight gain of F1 males during the pre-mating treatment period was slightly lower in group 4 (750 ppm) than in the concurrent control group.

In group 2 (120 ppm) and group 3 (300 ppm) mean body weight gain during the pre-mating treatment period was comparable with the control group.

Body weight - females (Tables 24 to 29, Figures 14 to 16, Appendix IX)
Mean body weight gain of females during the pre-mating treatment, the gestation, and the lactation period was comparable in all groups.

Food consumption (Table 30 to 33, Figures 17 to 20, Appendix X)
Mean food consumption was slightly reduced throughout most of the pre-mating treatment period in F1 males of group 4 (750 ppm).

In groups 2 and 3 of F1 males and in all dose groups of F1 females, mean food consumption was comparable with the control group.

Mating performance and fertility (Table 21)
Mating performance and reproductive indices were comparable in all groups.

Litter Data – F1 Generation:

Duration of gestation (Table 38, Appendix XII)
The mean duration of gestation was normal in all groups.

Number of pups (Table 38, Appendix XII)
The live birth index was slightly lower in group 3 (300 ppm) and especially in group 4 (750 ppm).

Between days 1 and 4 p.p. pup losses were observed in all groups. After adjustment of litter size (day 4 p.p.) pup loss was similar in all groups.

The sex ratio was similar in all groups on days 1 and 21 of lactation.

Pup weight (Table 38, Appendix XII)
From day 1 to 14 p.p. mean pup weight was similar in all groups. On day 21 p.p. mean pup weight of group 3 (300 ppm) and group 4 (750 ppm) was slightly lower than in the control group. This finding was considered to be incidental.

Physical development of the F2a pups (Figures 21 to 24, Appendix XIV)
The onset of all physical parameters examined (pinna unfolding, hair growth, incisor eruption, eye opening) was comparable in all groups.

Functional tests (Table 39)
The employed functional tests (pupillary reflex, startle response) revealed no effect of treatment.

One male pup in group 2 (120 ppm) showed blindness on the right eye. This finding was considered to be incidental.

Malformations (Table 40)
One F2a pup of group 2 (120 ppm) showed an exophthalmia and one of group 3 (300 ppm) showed a hydronephrosis at necropsy. The occurrence of these malformations was considered to be incidental.

Necropsy findings (Table 40)
Macroscopic examination of F2a pups died or sacrificed revealed no treatment-related changes.

Histopathological Examination:

P Generation

Two males in group 1 (Control) and one male in group 4 (750 ppm) had atrophy of the seminiferous tubules of the testes. The group 4 male also had an absence of sperm in one epididymis. A small focus of chronic inflammation was observed in the prostate of one group 1 male.

Lesions observed in the females were: an ovarian cyst in one group 1 female, dilated lumens of the uterus in three females of group 1 and four females of group 4, an implantation site in the uterus of one female of group 4, acute inflammation in the uterus of one female of group 4, chronic inflammation in the uterus of one female in group 1, one endometrial stromal polyp of the uterus in each of the groups 1 and 4, and minimal accumulations of acute inflammatory cells in the cervix of four females in group 1 and ten females in group 4.

F1 Generation

Atrophy of seminiferous tubules was observed in one male each in groups 1 (Control) and 4 (750 ppm). One male in group 4 had an absence of sperm in one epididymis.

Dilatation of the uterus was observed in three females in group 1 and nine females in group 4.

Discussion

The lesions observed in the P and F1 generations microscopically were lesions that are frequently observed in rats and were not considered compound-related.

Conclusions

Study author concluded:

In conclusion, administration of Troysan Polyphase by admixture to the basic powdered diet at concentrations of 300 and 750 ppm elicited slight toxicity in the P male animals (reduced body weight gain and reduced food consumption - group 4 only - during the pre-mating treatment period). Body weight gain and food consumption were also slightly reduced in F1 males of the high dose group (750 ppm) during the pre-mating treatment period.

There was no evidence of toxicity in either of the two generations of animals given diet containing Troysan Polyphase at a concentration of 120 ppm.

Life birth index was slightly reduced in either generation at 750 ppm. The post-natal development of the offspring during the lactation period was however not affected.

There were no effects on fertility or general reproductive performance over two successive generations in animals given Troysan Polyphase in the diet at concentrations of 120, 300, and 750 ppm.

Reliability

Klimisch Code = 1

The study was certified as being conducted in accordance with US EPA Good Laboratory Practice standards as set forth in US Federal Register Vol. 48, No. 230 (November 29, 1983) with the following deviations: the stability of the test article/carrier mixture had not been determined at the time of the study; the final report does not contain analytical data relating to the test article or test article/carrier. Despite these deviations from particular requirements of the regulations, there is no reason to believe that the scientific integrity of the study was compromised in any way. (Study author)

The study was conducted according to US EPA Guideline No. 83-4 (Pesticide Assessment Guidelines). (Study author)

References

Hazleton Laboratories, West Germany, “Two Generation Oral (Dietary Administration) Reproduction Toxicity Study in the Rat (One Litter per Generation),” Report No. 548-511/3, October 16, 1987.

18.0 Developmental Toxicity/Teratogenicity

Test Substance

Identity (purity): Troysan Polyphase P-100
97%, 3-Iodo-2-propynyl butyl carbamate, Lot 910662.69

Method

Method/guideline followed:	Guideline 83-3
GLP:	Yes
Year (study performed):	1992
Species:	Rabbit
Strain:	New Zealand White
Route of administration:	Oral gavage
Doses/concentration levels:	2, 20, 50 mg/kg/day
Sex:	20 Female per dose
Exposure period:	Days 6-18 of gestation
Frequency of treatment:	Daily
Control group of treatment:	20 female
Duration of test:	29 days

The in-life phase of the study was initiated with the artificial insemination of female rabbits (gestation Day 0) and concluded with terminal sacrifice on gestation day 29.

Statistical methods:

Continuous maternal and fetal data, including body weights, body weight gain, food consumption, number of fetuses, implantation sites and corpora lutea, were analyzed using one way analysis of variance (ANOVA) followed by Dunnett's test [5]. The Mann-Whitney U test was used to compare post-implantation loss and resorptions [6]. Fetal sex ratios were analyzed using the Chi-square test [7]. Fisher's Exact test was used to analyze the incidence and number of fetal malformations and variations utilizing the dam (litter) as the experimental unit [8]. All analyses were two-tailed with a minimum significance level of 5%.

The study group design and dosage levels tested were as follows:

Group	Number of Females	Dosage Material	Dosage Level (mg/kg/day)*	Dosage Conc. (mg/ml)	Dosage Volume (mg/kg/day)
1	20	Vehicle	0	0	0.5
2	20	Troysan Polyphase P-100	2	4	0.5
3	20	Troysan Polyphase P-100	20	40	0.5
4	20	Troysan Polyphase P-100	50	100	0.5

*Dosage levels for this study were selected based on results of a preliminary dose range-finding study (SLS Study No. 3228.15)[1].

Test Conditions

Test Animals:

The females were examined upon receipt and daily thereafter during acclimation for signs of physical or behavioral abnormalities. Only healthy animals were maintained for possible assignment to the study. Mortality checks were performed twice daily, in the morning and afternoon. Individual body weights were measured on the day following receipt and just prior to randomization for assignment to study groups. The females were acclimated to the laboratory environment for a period of 22 days prior to randomization.

Weight range at start of study: 3 to 4 kg
Age at study initiation: 6 months
Controls: Control females were administered the vehicle (Corn oil) under the same experimental conditions.
Number of animals per dose per sex: 20 females per dose
Vehicle: Mazola Corn Oil, Lot JUL1592A
Mating procedure: Artificial insemination

Semen was collected from male New Zealand White rabbits obtained from the same source and supplier as the females, and evaluated for volume, motility, and concentration. The semen was diluted with 0.9% physiological saline and maintained in a water bath at 35-37°C during the insemination procedure. Approximately 0.5 ml of the diluted semen was introduced into each female's vagina. Semen from one male was used to inseminate an equal number of females in each study group. Immediately following insemination, the females were administered human chorionic gonadotropin, via the marginal ear vein. The day of insemination was considered day 0 of gestation.

Dose Preparation:

The test article was ground using a mortar and pestle and passed through a No. 40 mesh sieve prior to weighing. A specified amount of Troysan Polyphase P-100 for each dose group was weighed into a calibrated beaker. An appropriate amount of the vehicle was added to each beaker and the mixtures were stirred. A sufficient quantity of vehicle was then added to each mixture to achieve the desired concentrations. Each mixture was stirred for an additional 10 minutes and dispensed into daily aliquots. The dosing mixtures were prepared fresh weekly and stored in amber bottles at room temperature. During each weekly preparation, a sufficient quantity of corn oil was also dispensed into daily aliquots for administration to control animals. Daily aliquots were stirred for 10 minutes prior to dispensation and continuously during dosing. The dosing mixtures were not adjusted for the stated test article purity of 97%.

Dosage preparations were administered orally, by gavage, as a single dose daily from gestation day 6 through gestation day 18. Individual doses were adjusted based on the most recent body weight data. Oral administration of the test article was selected since this is a potential route of human exposure.

Homogeneity, Stability and Concentration Analysis:

Prior to study initiation, the analytical method supplied by the Sponsor was validated and homogeneity and stability analyses were performed on concentrations of the test article which were expected to encompass those used in the study (5 and 500 mg/ml). Although a concentration of 4 mg/ml was ultimately utilized for the low-dose group, further stability and homogeneity testing was not considered necessary since the test article was stable and homogeneous in the vehicle at concentrations as low as 5 mg/ml. Each fresh dosing preparation was analyzed for test article concentration. Analytical methodology is described in detail in the Analytical Chemistry Report which is presented in Appendix B.

Environmental Conditions and Husbandry:

Animals were housed throughout the study in an environment-controlled room with a 12-hour light/12-hour dark cycle. The controls were set to maintain a room temperature of 61-70°F and a relative humidity of 40-60%. Room temperature and relative humidity were measured and recorded a minimum of once daily. On two days, the animal room temperature was outside the specified range by +2°F. On nine occasions, the relative humidity was outside the specified range by -9 to +17%. These occurrences had no apparent impact on the outcome of the study.

Animal housing and care were based on the standards established by the American Association for Accreditation of Laboratory Animal Care (AAALAC) and the guidelines set forth in the Guide for the Care and Use of Laboratory Animals, NIH Publication No. 86-23, 1985.

Diet and Drinking Water:

Purina Certified Rabbit Chow® #5322 and municipal tap water were provided to each animal ad libitum. The feed was analyzed by the supplier for nutritional components and environmental contaminants. The lot number and expiration date of each batch of food used during the study were recorded. The tap water was purified by reverse osmosis or deionization (backup purification system) and supplied to the animals by an automatic watering system. Water supplying the facility is analyzed on an annual basis for contaminants according to SLS Standard Operating Procedures. The results of the feed and water analyses are maintained at SLS.

On February 5, 1992, an apparent overnight malfunction of the watering system was detected and corrected. This occurrence had no impact on the outcome of the study.

Clinical observations performed and frequency:

1. Clinical Observations

During the experimental period, all animals were observed daily for clinical signs of toxicity including physical or behavioral abnormalities. Mortality checks were performed twice daily, in the morning and afternoon. In addition, during the treatment period, the rabbits were observed between one-half hour and two hours following dosing for overt signs of toxicity.

2. Body Weights

Individual body weights were measured on gestation days 0, 6, 9, 12, 15, 19, 24, and 29. Body weight changes were calculated for the following gestation intervals: 0-6, 6-9, 9-12, 12-15, 15-19, 19-24, 24-29, 6-19, 19-29 and 0-29.

3. Food Consumption

Individual food consumption was measured daily during gestation. Food consumption was calculated as grams/animal/day and grams/kg/day for the following gestation intervals: 0-6, 6-9, 9-12, 12-15, 15-19, 19-24, 24-29, 6-19, 19-29 and 0-29.

4. Unscheduled Death or Euthanasia

Females that were found dead, euthanized moribund or exhibited signs of abortion during the study were necropsied. Body cavities (cranial, thoracic, abdominal and pelvic) were opened and examined. Uterine contents were examined and the number of implantation sites was recorded. The number of corpora lutea on each ovary was also recorded. Abnormalities were recorded and representative tissue samples from internal lesions were preserved in 10% neutral buffered formalin for possible histological examination.

5. Scheduled Euthanasia and Cesarean Section

All surviving females were sacrificed on gestation day 29 with sodium pentobarbital administered intravenously (via the marginal ear vein) and subjected to a gross necropsy examination. The thoracic, abdominal and pelvic cavities were opened and the viscera examined. Abnormalities were recorded and representative tissue samples from internal lesions were preserved in 10% neutral buffered formalin for possible histological examination.

The uterus was removed from the body, examined externally, weighed and then opened for internal examination. The number of viable and nonviable fetuses and early and late resorptions was recorded beginning with the left distal uterine horn, noting the position of the cervix, and continuing up the right uterine horn. Corpora lutea were recorded for each ovary. Uteri with no macroscopic evidence of implantation were opened and placed in 10% aqueous ammonium sulfide solution for detection of early embryolethality as described by Salewski [2].

The gravid uterus weight of group 2 female #1816 was inadvertently not recorded at cesarean section. This deviation had no apparent impact on the outcome of the study or on the interpretation of study results.

6. Fetal Morphological Observations

Fetuses were examined for external, internal (visceral), and skeletal abnormalities. Findings were classified based upon the severity of the anatomical change(s) and the extent of their potential for interference with organ and/or body function.

a. External Examination

Each fetus was examined for the occurrence of external abnormalities. The fetuses were weighed and tagged individually. The tags displayed the study, dam and fetus numbers. The crown-rump length of late resorptions was measured and the tissue was then discarded.

b. Visceral Examination

Each fetus was dissected and examined using a technique similar to that described by Staples [3]. During the procedure, the sex of the fetus was determined. The examination was performed using a low power dissection scope.

c. Skeletal Examination

Each fetus was eviscerated, skinned and fixed in 95% isopropyl alcohol. Following fixation, the fetuses were macerated in a 1-2% aqueous potassium hydroxide solution, stained with Alizarin Red S, and cleared in glycerin [4]. Subsequent skeletal examinations were performed using substage lighting.

This study was performed to detect and evaluate the potential embryotoxic or teratogenic effects of Troysan Polyphase P-100 when administered orally to pregnant rabbits during the period of major organogenesis. The study design consisted of a vehicle control and three treatment groups. Each group contained twenty artificially inseminated female New Zealand White rabbits. The test article was suspended in Mazola® corn oil and administered at dosage levels of 2, 20 and 50 mg/kg/day from gestation day 6 through gestation day 18. All doses were given at a constant volume of 0.5 ml/kg. Control animals were administered corn oil under the same experimental conditions and at an equivalent dose volume. The animals were observed daily for clinical signs of toxicity. Body weights were measured on gestation days 0, 6, 9, 12, 15, 19, 24, and 29 and food consumption was measured daily. Surviving animals were sacrificed on gestation day 29 and subjected to cesarean section. Fetuses were weighed individually, sexed and examined for external, visceral and skeletal abnormalities.

Results

NOAEL (NOEL) maternal toxicity:	20 mg/kg/ day
NOAEL (NOEL) developmental toxicity:	50 mg/kg/day

Maternal data with dose level:

Substantial toxicity was produced by the test article at the 50 mg/kg/day level where two females died and one was sacrificed moribund between gestation days 21 and 23. Additional indications of maternal toxicity at the 50 mg/kg/day level consisted of adverse clinical signs, body weight loss and reduced food consumption. One female of the 50 mg/kg/day group aborted on gestation day 24. In contrast to these changes, no adverse maternal effects were observed at the 2 or 20 mg/kg/day levels. There were no statistical differences in cesarean section parameters among the groups. However, at the 50 mg/kg/day level there was a slight increase in mean post implantation loss and a corresponding decrease in the mean number of viable fetuses. It is noteworthy that these differences occurred in the presence of substantial maternal toxicity. All other cesarean section parameters were comparable among the groups.

Fetal data with dose level:

No treatment-related fetal malformations or developmental variation were observed in the study.

A. Analytical Chemistry Evaluations

Appendix B (Analytical Chemistry Report)

Prestudy analytical chemistry evaluations indicated that Troysan Polyphase P-100 was homogeneous and stable in corn oil for up to eight days when stored at room temperature. Analysis of dosing preparations resulted in average test article recoveries ranging from 97.8 to 103.8% indicating that the mixtures were accurately prepared.

B. Maternal Survival and Pregnancy Status

Table 1 (Summary Data)

Two 50 mg/kg/day females were found dead and one 50 mg/kg/day female was sacrificed moribund during the study. These deaths/sacrifices occurred between gestation days 21 and 23. In addition, one 50 mg/kg/day female aborted on gestation day 24 and one control female died due to an apparent gavage error on gestation day 13. All other females survived to scheduled sacrifice on gestation day 29.

The pregnancy rate was 85% in the 20 mg/kg/day group and 95% in the control, 2 and 50 mg/kg/day groups.

C. Clinical Observations

Table 2 (Summary Data)
Appendix C (Individual Data)

For the control female which died, clinical observations including salivation, fecal stain and decreased activity were noted following dosing on the day of death. No clinical signs were observed for this female prior to this time.

In animals which died or were sacrificed during the study at the 50 mg/kg/day level, clinical observations were noted for several days prior to death or sacrifice. These findings included few feces, no feces, fecal stain, soft stools, mucoid stools, fecal stain, and reddish colored material in the cage/tray. Additional findings for the female sacrificed moribund at the 50 mg/kg/day level included reddish colored material in cage/tray, yellow or white crusty material around the nose and/or mouth, emaciation, dehydration, unkempt appearance and prostration.

Clinical signs of toxicity were also observed for surviving females at the 50 mg/kg/day level. These findings included few feces, no feces and soft stools. No adverse clinical signs were observed in surviving females of the control, 2 or 20 mg/kg/day groups.

D. Body Weights and Weight Gain

Tables 3 and 4 (Summary Data)
Appendices D and E (Individual Data)

Mean maternal body weights and body weight gain were comparable among the control, 2 and 20 mg/kg/day groups throughout the study. At the 50 mg/kg/day level, statistically significant body weight loss occurred during gestation days 6-9, 9-12, 15-19 and 6-19, while slight weight loss occurred during gestation days 12-15. Mean body weights at the 50 mg/kg/day level were slightly lower on gestation days 12 and 15, and statistically lower on gestation day 19 as compared to control females. During the final days of gestation (days 24-29), body weight gain of the 50 mg/kg/day females was markedly higher than controls indicating that a partial recovery had occurred from the earlier reductions in body weight gain.

E. Food Consumption

Tables 5 and 6 (Summary Data)
Appendices F and G (Individual Data)

Food consumption calculated as grams/animal/day and grams/kg/day was comparable between the control, 2 and 20 mg/kg/day groups throughout the study. Food consumption at the 50 mg/kg/day level was statistically lower during gestation days 6-9, 9-12, 12-15, 15-19, and 6-19 as compared to controls. During the final days of gestation (days 24-29), food consumption of the 50 mg/kg/day females was markedly higher than controls indicating that a partial recovery had occurred from the earlier reductions in food consumption.

F. Maternal Necropsy Observations

Table 7 (Summary Data)
Appendix H (Individual Data)

Necropsy findings for the two females found dead at the 50 mg/kg/day level (#1849 and #1850) included dry red or wet dark brown matting in the urogenital area and base of tail, wet light brown matting around nose, several tan areas on the external and cut surfaces of the liver, and left apical lobe of the lung light brown. For the female sacrificed moribund at the 50 mg/kg/day level (#1885), necropsy findings included yellow or white crusty material around the nose and/or mouth, small thymus, pale kidneys, eroded areas and multiple black foci on the mucosal surface of the stomach, blackish-red mucoid material mixed with food contents in the stomach, and multiple tan areas on the external and cut surfaces of the liver. Gross necropsy findings for the 50 mg/kg/day female which aborted (#1866) were limited to green fluid contents in the cecum and hairloss on the hindlimbs and abdominal area.

For the control female found dead during the study (#1825), gross necropsy findings consisted of wet dark brown matting in the anogenital area, clear wet matting around the mouth, dark red areas on the caudal lobes of the lungs, and dark red foci on the glandular mucosa of the stomach. In surviving animals, gross necropsy observations were generally unremarkable.

G. Cesarean Section Observations

Table 8 (Summary Data)
Appendices I, J and K (Individual Data)

There were no statistical differences in cesarean section parameters among the groups. However, in the 50 mg/kg/day group, a slight increase in mean post-implantation loss and a corresponding decrease in the mean number of viable fetuses was observed. All other cesarean section parameters were comparable between the control and treatment groups.

H. Fetal Morphological Observations

Tables 9 and 10 (Summary Data)
Appendices L, M and N (Individual Data)
Appendix O (Historical Control Data)

No statistically significant differences in fetal malformation or developmental variations were noted among the groups. A low incidence of dissimilar malformations known to occur spontaneously in this strain of rabbit were observed in each study group, including the control. The incidence of developmental variations was generally similar among the groups.

Conclusion

The study author concluded:

Substantial toxicity was produced by the test article at the 50 mg/kg/day level where two females died and one was sacrificed moribund between gestation days 21 and 23. Additional indications of maternal toxicity at the 50 mg/kg/day level consisted of adverse clinical signs, body weight loss and reduced food consumption. One female of the 50 mg/kg/day group aborted on gestation day 24. In contrast to these changes, no adverse maternal effects were observed at the 2 or 20 mg/kg/day levels. There were no statistical differences in cesarean section parameters among the groups. However, at the 50 mg/kg/day level, there was a slight increase in mean post-implantation loss and a corresponding decrease in the mean number of viable fetuses. It is noteworthy that these differences occurred in the presence of substantial maternal toxicity. All other cesarean section parameters were comparable among the groups. No treatment-related malformations or developmental variations were observed in the study.

Based on the results of this study, a dosage level of 20 mg/kg/day was considered a no-observed-effect level (NOEL) for maternal toxicity. A dosage level of 50 mg/kg/day was considered a NOEL for teratogenicity.

Reliability

Klimisch Code = 1 Reliable without restriction

Study was conducted in compliance with the EPA Good Laboratory Practice regulations (40 CFR 160) and according to Guideline 83-3. (Study author).

References

Springborn Laboratories Inc., Spencerville, OH, “Teratology Study in Rabbits With Troysan Polyphase P-100- Final Report,” SLS Study No. 3228.16, August 17, 1992.