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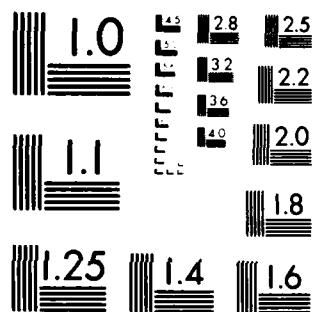
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INSTITUTE REPORT NO. 143

MUTAGENIC POTENTIAL OF:

4-nitrophenyl methy (phenyl) phosphinate
using the *Drosophila melanogaster* sex-linked recessive lethal test

NELSON R. POWERS, PhD, CPT MS

TOXICOLOGY GROUP,
DIVISION OF RESEARCH SUPPORT

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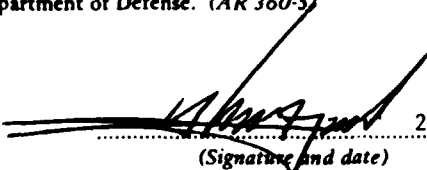
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REPORT DOCUMENTATION PAGE		READ INSTRUCTIONS BEFORE COMPLETING FORM
1. REPORT NUMBER Institute Report No. 143	2. GOVT ACCESSION NO. DD-A128183	3. RECIPIENT'S CATALOG NUMBER
4. TITLE (and Subtitle) Mutagenic Potential of 4-Nitrophenyl Methyl (Phenyl) Phosphinate Using the <u>Drosophila melanogaster</u> Sex-Linked Recessive Lethal Test	5. TYPE OF REPORT & PERIOD COVERED Final January - May 1982	
7. AUTHOR(s) Nelson R. Powers, PhD, CPT MS	6. PERFORMING ORG. REPORT NUMBER	
9. PERFORMING ORGANIZATION NAME AND ADDRESS Toxicology Group, Division of Research Support Letterman Army Institute of Research Presidio of San Francisco, CA 94129	8. CONTRACT OR GRANT NUMBER(s)	
11. CONTROLLING OFFICE NAME AND ADDRESS US Army Medical Research and Development Command Fort Detrick Frederick, MD 21701	10. PROGRAM ELEMENT, PROJECT, TASK AREA & WORK UNIT NUMBERS Project 35162772A875 WU 304	
14. MONITORING AGENCY NAME & ADDRESS (if different from Controlling Office)	12. REPORT DATE April 1983	
	13. NUMBER OF PAGES 31	
	15. SECURITY CLASS. (of this report) UNCLASSIFIED	
	15a. DECLASSIFICATION/DOWNGRADING SCHEDULE	
16. DISTRIBUTION STATEMENT (of this Report) THIS DOCUMENT HAS BEEN CLEARED FOR PUBLIC RELEASE AND SALE: ITS DISTRIBUTION IS UNLIMITED.		
17. DISTRIBUTION STATEMENT (of the abstract entered in Block 20, if different from Report)		
18. SUPPLEMENTARY NOTES		
19. KEY WORDS (Continue on reverse side if necessary and identify by block number) Mutagenicity, Toxicology, Sex-Linked Recessive Lethal Assay, <u>Drosophila melanogaster</u> , 4-nitrophenyl methyl (phenyl) phosphinate		
20. ABSTRACT (Continue on reverse side if necessary and identify by block number) 4-nitrophenyl methyl (phenyl) phosphinate (TW011) is being considered as a prophylactic agent in anticholinesterase poisoning. This compound was tested for mutagenic activity using <u>Drosophila melanogaster</u> Sex-Linked Recessive Lethal assay. It was found non-mutagenic after 72-hour feeding exposures to 0.002 and 0.0025 mM of TW011.		

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ABSTRACT

4-nitrophenyl methyl (phenyl) phosphinate (TW011) is being considered as a prophylactic agent in anticholinesterase poisoning. This compound was tested for mutagenic activity using Drosophila melanogaster Sex-Linked Recessive Lethal assay. It was found non-mutagenic after 72-hour feeding exposures to 0.002 and 0.0025 mM of TW011.

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PREFACE

TYPE REPORT: Drosophila melanogaster Sex Linked Recessive Lethal Assay

TESTING FACILITY: U.S. Army Medical Research and Development Command,
Letterman Army Institute of Research
Presidio of San Francisco, CA 94129.

SPONSOR: U.S. Army Medical Research and Development Command
U.S. Army Medical Institute of Chemical Defense
Aberdeen Proving Grounds, Aberdeen, MD 21005

PROJECT/WORK UNIT/APC: 35162772A875 Toxicity Testing of Phosphinate Compounds, APC, TLO4

GLP STUDY NUMBER: 82001

STUDY DIRECTOR: COL John T. Fruin, DVM, PhD, VC,
Diplomate, American College of Veterinary Preventive Medicine

PRINCIPAL INVESTIGATOR: CPT Nelson R. Powers, PhD, MS

REPORT AND DATA MANAGEMENT: A copy of the final report, test compound, study protocol, raw data and SOP's will be retained in the LAIR Archives.

TEST SUBSTANCE: 4-nitrophenyl methyl (phenyl) phosphinate (LAIR Code: TW011)

INCLUSIVE STUDY DATES: 25 January - 26 May 1982

OBJECTIVE: The purpose of this study was to determine the mutagenic potential of 4-nitrophenyl methyl (phenyl) phosphinate in an invertebrate model.

ACKNOWLEDGMENTS


The author wishes to thank Paul Waring, BS, for providing technical assistance and background information concerning formulation of the test substance. The author also wishes to thank SP5 Kincannon, BS; SP4 Mullen, BS; and SP4 Rodriguez, BS, for their assistance in performing the research.

The present generation is only a caretaker of the human genome of future generations.

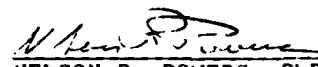
Malling and Vakovic 1978

Signatures of Principal Scientists involved
in the Study

We the undersigned, believe the GLP Study numbered 82001, described in this report to be scientifically sound and the results and interpretation to be valid. The study was conducted to comply, to the best of our ability, with the Good Laboratory Practice Regulations for Nonclinical Laboratory Studies as outlined by the Food and Drug Administration.

 5 Jan 83

JOHN T. FRUIN DVM, PhD / DATE
COL, VC
Study Director

 5 Jan 83

NELSON R. POWERS, PhD / DATE
CPT, MSC
Principal Investigator



DEPARTMENT OF THE ARMY
LETTERMAN ARMY INSTITUTE OF RESEARCH
PRESIDIO OF SAN FRANCISCO, CALIFORNIA 94129

REPLY TO
ATTENTION OF:

SGRD-ULZ-QA

7 December 1982

MEMORANDUM FOR RECORD

SUBJECT: Report of GLP Compliance

I hereby certify that in relation to LAIR GLP study 82001, the following inspections were made:

12 Jan 82/0930 hours
12 Jan 82/1600
27 Jan 82
9 Feb 82
14 Apr 82

The report and raw data for this study were audited on 18 Oct 82.

Routine inspections with no adverse findings are reported quarterly, thus these inspections are also included in the 21 Apr 82 and 7 Jul 82 report to management and the Study Director.

JOHN C. JOHNSON
CPT (P), MSC
Quality Assurance Officer

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Mutagenic Potential of: 4-nitrophenyl methyl (phenyl) phosphinate using the Drosophila melanogaster sex-linked recessive lethal test--Powers (Toxicology Series 39)

Currently the use of organophosphinates as prophylactic agents for anti-cholinesterase poisoning is being investigated. Due to the potential success of these investigations and consequent widespread use of these compounds, their mutagenicity is being studied. Among the various screening programs, the Drosophila melanogaster sex-linked recessive lethal assay (SLRL) has been performed. This report contains our findings from the SLRL studies.

Rationale for SLRL Testing

In addition to the tests for acute and chronic toxicity, evaluation of genetic damage from exposure to chemicals must be considered. A variety of tests using Drosophila are available for the detection of specific types of genetic changes. The most sensitive assay which detects the broadest range of mutations is the SLRL test (1-3). This test uses insects of a known genotype and detects lethal mutagenic changes in 800 to 1000 loci on the X-chromosome, representing 80% of the X-chromosome or 20% of the entire genome (4,5). To date, the SLRL test has been used in most of the research on the mutagenic response of Drosophila to test substances (1,3,4).

Genetic Basis of SLRL Test

The basic mechanism of the SLRL test is that the X-chromosome of the father is passed on to the daughter; the sons receive the X-chromosomes from the mother. The recessive lethal mutations located on the X-chromosomes are expressed in males in a hemizygous condition, and since the Y-chromosome does not contain the dominant, wild-type alleles to suppress their manifestation, this results in death.

The SLRL test relies on the fact that among the progeny of females carrying a recessive lethal mutation on one of her X-chromosomes (heterozygous for a recessive lethal mutation), half of the sons will die. By the use of suitable genetic markers, the class of males carrying the X-chromosomes of treated grandfathers can easily be determined. If a lethal mutation was induced, this class will be missing and is easily scored. This test is described as the Basc or Muller-5 test (5,6). The assay system uses strains which prevent the crossing-over in females, heterozygous for the lethal-bearing chromosome; transfer of the lethal from the paternal to the maternal X-chromosome by genetic recombination restores viability of the chromosomes under test and leads to erroneous results, consequently, males receiving the X-chromosome survive. Since combinations of suitable inversions effectively inhibit the occurrence of crossing over, females used for the test carry two scute inversions; the left-hand part of SC⁵ and the right-hand part SC⁸ covering the whole X-chromosome, and a smaller inversion In-S in the Basc and dl-49 in the msy chromosome (5).

Description of Test

The test (7) was developed in 1948 for determining genetic changes which in hemizygous but not homozygous or heterozygous conditions kill the developing individual (egg to pre-adult stage). Such genetic factors, recessive lethal mutations, can be induced on all chromosomes. Only two test generations are needed to detect if sex-linked recessive lethal mutations have been induced on the X-chromosome.

In the SLRL test, wild-type males, normal round red eyes, are exposed to the test materials (treated). (We use Canton-S (CS)). Such an exposure will be regarded as a recessive lethal mutation if it affects the X-chromosome. These males are mated to homozygous females (we use First Multiple Number 6 (FM6)) carrying the Basc chromosome. This chromosome is expressed as bar (narrow-shaped) eyes, white-apricot in color. The bar serves as a genetic marker in homozygous or hemizygous conditions. It is kidney-shaped in heterozygous females. The progeny of this cross now consists of females heterozygous for the treated X-chromosome, characterized by kidney-shaped red eyes and males of the Basc phenotype that have received their X-chromosome from their Basc mother. Each F₁ female represents one paternal X-chromosome, treated in the male gametes. These siblings are mated to produce the F₂ generation. This generation now consists of males of two phenotypic expressions, those with round red eyes (hemizygous carrying the treated X-chromosome from the F₁ female) and those with bar-shaped white-apricot eyes (hemizygous for the Basc chromosome); and females of two phenotypic expressions, kidney-shaped red eyes (heterozygous, carrying the treated X-chromosome from the F₁ females and the Basc chromosome) and those with bar-shaped white-apricot eyes (homozygous for the Basc chromosome). This generation is inspected for the presence of males

with round red eyes. If this class is missing, it can be concluded that the treated male gamete contained a recessive lethal mutation. Thus, this test relies upon the disappearance of a whole Mendelian class (males with round red eyes).

Brooding

As part of the SLRL testing a brooding scheme was used to sample sperm cells exposed to the test substance. This is done as chemicals often exhibit stage-specificity on different stages of germ cell development. The brooding scheme was done at intervals of 3, 2, 2 and 3 days. This insures that sperm exposed to the test material are in different stages of development: Brood 1 = mature and near-mature sperm; Brood 2 = primarily spermatids; Brood 3 = primarily meiotic stages; and Brood 4 = primarily spermatogonia. This procedure safeguards against the possibility that chemicals with more pronounced effects in earlier stages of spermatogenesis are not erroneously dismissed as false negatives.

Objective of Study

The objective is to assess the mutagenic potential of the organophosphinate compound 4-nitrophenyl methyl (phenyl) phosphinate (TW011) by using Drosophila melanogaster in the Sex-Linked Recessive Lethal Assay.

MATERIALS AND CONDITIONS

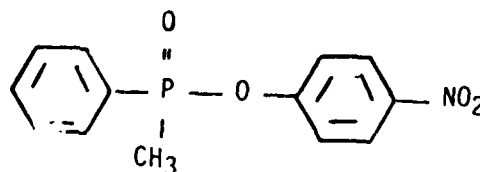
Test Substance

Chemical name: 4-nitrophenyl methyl (phenyl) phosphinate

LAIR Code : TW011

CAS: none

Molecular Structure: $C_{13}H_{12}NO_4P$



Molecular Weight: 277.2

Vehicle

Due to the instability of (TWQ11) when prepared in an aqueous system, a mixture of Tween 80TM, ethanol, citrate buffer and water were prepared to stabilize the test compound (LAIR SOP OP-STX-45 - Preparation of Compounds Unstable in Water for SLRL Assay). This mixture of Tween 80TM, ethanol, citrate buffer and water was itself appropriate for consumption by the test insects.

CAS: N/A

Molecular Structure: N/A - mixture

Molecular weight: N/A - mixture

See Appendix A for Analytical Data.

Test Model

Insect Genus and Species: Drosophila melanogaster

Strains: Canton-S (CS), a wild-type stock, characterized by round-red eyes. This stock was selected for mutagenicity studies because it has shown a relatively low constant spontaneous mutation frequency (8).

First Multiple Number 6 (FM6) a laboratory stock containing the homozygous Basc chromosome for females and the hemizygous Basc X-chromosome for males. This strain carries the phenotypic markers for yellow body (Y), bar shaped eye (B) and white-apricot colored eye (W) and several superimposed inversions which prevent "crossing over" (exchange of chromosome segments) with homologous non-inverted X-chromosomes.

Both strains are presently being reared in the insectary at Letterman Army Institute of Research. The original stock colonies were obtained from the University of Wisconsin, Madison, Wisconsin.

Diet

The diet is the standard medium used for colony rearing of D. melanogaster. Materials and instructions for its preparation are contained in LAIR SOP-OP-STX-5 Drosophila Media Preparation.

Restraint

Ether for anesthesia was used only when conducting matings of F₂ and F₃ generations and for general colony maintenance.

Identification System

Each CS male from the LC_{50} 72-hour exposure (test, negative, positive control), had a unique number assigned and placed on the vial in which its progeny was produced (LAIR SOP-OP-STX-8 Sex-linked Recessive Lethal (SLRL) Drosophila melanogaster Mutagenicity Test). In this manner progeny were traced back to the parental male which had been subjected to the test compound or controls.

Environmental Conditions

All studies were conducted within the insectary at a temperature of $21 \pm 4^{\circ}C$, relative humidity of $50 \pm 5\%$ and a photoperiod of 12 hours light and 12 hours dark. All insect colonies were reared in polypropylene bottles and SLRL testing was done in glass vials (LAIR SOP-OP-STX-6 Drosophila Stock Colony Maintenance).

Dosing

Dosing was done in compliance with LAIR SOP-OP-STX-7 Drosophila melanogaster Exposure Procedures and LAIR SOP-OP-45 Preparation of Compounds Unstable in Water for SLRL Assay, by allowing the CS strain (wild-type) male to feed upon 250 μ l of various concentrations of the test chemical formulated with Tween 80TM, ethanol, citrate buffer, water and 1% fructose. These males formed the test groups. Concurrent exposure of the Tween 80TM, ethanol, citrate buffer, water and 1% fructose (250 μ l) for CS males to feed upon were designated as negative control (spontaneous mutation frequency) and 1 mM ethylmethane sulfonate with Tween 80TM, ethanol, citrate buffer, water and 1% fructose (250 μ l) for CS males to feed upon were designated as positive control. Ethylmethane sulfonate is a known mutagen and was used to confirm the ability of the test organism to produce SLRL mutations (9). Dosing was done continuously for 72 hours with test compound replenishment every 24 hours for a total of 3 exposures. For test chemical exposure, a pilot toxicity study was done to establish the upper and lower limits of mortality. Based on the results of this study a second study was conducted from which an exposure level was selected, based on the LC_{50} level for treated males after 72 hours of exposure (4). It was these males that were used in the SLRL testing. For TW011, groups of 100 CS males were exposed to each dose level in this second study of 0.0005, 0.001, 0.0015, 0.002, 0.0025 and 0.003 mM, this was replicated four times. The male insects surviving the LC_{50} (or as close to the LC_{50} as possible) in each of the four replicates were selected for the four replicates of the SLRL Assay.

Test Format

The CS males surviving the LC_{50} of the test chemical after 72 hours of exposure and those males subjected to the concurrent negative controls were used in the SLRL assay. Survivors from the test

chemical and negative control compound were scored by mating 25 dosed CS males (wild-type) to FM6 virgin females (Base chromosome). This was done by placing 3 FM6 virgin females in a vial with one CS male, the vial was labeled with that male's unique number. At intervals of 3, 2, 2 and 3 days the CS male was transferred to successive groups of 3 FM6 virgin females in vials with that male's unique number. These intervals of days corresponded to broods 1,2,3 and 4. This procedure was replicated 4 times. Scoring of the mutants resulting from positive control exposure was based on mating of 10 CS males using the above mating scheme. This was replicated 4 times. After sufficient numbers of flies had emerged, a maximum of 25 (minimum of 5) kidney-shaped red eyed F_1 females were selected at random and mated with their sibling bar-shaped white-apricot eyed male). Each pair was placed in an individual vial, and these vials from the same unique numbered father were placed together and labeled with that unique number for reference. After 2 to 3 weeks the F_2 progeny were examined and scored for the absence of round red eyed males, which indicated that a lethal mutation had taken place in the treated male. To confirm this, an F_3 cross was conducted from each vial scored as a lethal mutation, 3 F_2 females (kidney-shaped red-eyed) were crossed with one bar-shaped white-apricot eyed male. Experimental conclusions were based on the spontaneous mutation frequency (negative control) compared to the mutation frequency induced by the test chemical.

Historical Listing of Significant Study Events

See Appendix B for the Historical Listing of Study Events.

Statistical Analysis

This testing was conducted to examine 2500 X-chromosomes in each of 4 replication yielding 8000 to 10,000 X-chromosomes. The mutation frequency of the test compound to the negative control (spontaneous mutation frequency) by means of the Fisher's exact test using a 2 x 2 table (10, 11); a more conservative test, the Kastenbaum-Bowman test (12, 13, and 14) was also considered. Both tests were based on the number of lethal and non-lethal culture vials of the total number examined (each culture vial contains F_2 progeny and is regarded as an X-chromosome (5)) from each unique numbered male (control and treated). Vials without F_2 progeny or less than 5 progeny (F_2) were scored as failure. In addition the mutation frequency of each of the four broods was also analyzed.

Change in Procedure During Study

The following deviations from the Standard Operating Procedures were made in the study: LAIR SOP OP-STX-4-Preparation of Compounds Unstable in Water for SLRL Assay, to prepare the various concentrations of TW011 with the vehicle various amounts of water and a constant volume of fructose and diluent were required. However, in place of the various amounts of water, a "blank" (Tween 80, ethanol

(100%), and citrate buffer (5.0 mM)) was substituted, this was done to aid in solubility and stability; LAIR SOP-OP-STX-4-Preparation of Compounds Unstable in Water for SLRL Assay, to prepare the stock, a specific weight of TW011, and amounts of Tween 80, ethanol (100%), citrate buffer (5.0 mM) and water were required. However, in preparation of the stock no water was used, again this aided in solubility and stability. In LAIR SOP-OP-STX-7 Drosophila melanogaster Exposure Procedures, the filter papers upon which the test and control solutions were placed for feeding were washed in 150 ml of deionized water with 2 to 3 drops of 1 N HCL, as explained in Laboratory Notebook 678.1, page 35. This also helped to stabilize the TW011 during exposure for feeding; LAIR SOP OP-STX-8 Sex-Linked Recessive Lethal (SLRL) Drosophila melanogaster Mutagenicity Test, required the testing of 8000 to 10,000 Drosophila melanogaster X-chromosomes. The resulting number of X-chromosomes from the negative control (9208) were within this range. The resulting number of X-chromosomes from TW011 was 7426; however, this proved to be an adequate sample size based on the mutation frequency resulting from the concurrent negative control according to the Kastenbaum-Bowman tables (13).

RESULTS

Measurements of TW011 formulated with the vehicle and its stability were made using a spectrophotometer. The various concentrations (mM) of TW011 used in the exposures were prepared from a stock of 0.05 mM (theory). The concentrations exposed to the test organisms; 0.0005, 0.001, 0.0015, 0.002, 0.0025 and 0.003 mM, in theory. The stock and resulting concentrations, were far below the detection limits of the spectrophotometer to measure accurately the actual amounts (mM) and rates of hydrolysis of TW011. However, based on the resulting increasing mortality rate during the exposure period at the LC₅₀ concentration it was assumed the test compound was toxic throughout the period of exposure and therefore stable.

The concentrations producing the mean percentage mortality and the standard deviation after 72 hours exposure from which the surviving males were selected for SLRL testing for each of the four replicates TW011 are shown in Appendix C Table 1. The 100 CS males exposed to each of the four replications of concurrent negative controls showed a total mean mortality of 2.5%. The results of the SLRL assay (mutation frequency) for each replicate and overall results for test and concurrent controls (negative and positive) for TW011 are presented in Appendix C, Table 2. In addition the analysis of mutation frequency for each brood for test chemical exposure and concurrent controls are presented in Appendix C Table 3. The progeny from CS males exposed to concurrent positive controls, 1 mM ethyl methane sulfonate, showed SLRL mutation frequency of 9.25%. Tabular data from this study (GLP 82001) for each male of the test substance, TW011 and concurrent negative and positive controls are in the archives of Letterman Army Institute of Research, Presidio of San Francisco, California.

Compliance was made with the following standard operating procedures: SOP-OP-STX-3, Positive Control Substances; LAIR SOP-OP-STX-5, Drosophila Media Preparation; LAIR SOP-OP-STX-6, Drosophila Stock Colony Maintenance; LAIR SOP-OP-STX-7, Drosophila melanogaster Exposure Procedures; LAIR SOP-OP-STX-8, Sex-linked Recessive Lethal (SLRL) Drosophila melanogaster Mutagenicity Test.

DISCUSSION

The spontaneous mutation frequency was 0.206% based on 9,208 X-chromosomes, while the mutation frequency resulting from TW011 was 0.242% based on 7426 X-chromosomes (Appendix C, Table 2). To detect a doubling of the spontaneous mutation frequency, the criteria used by many investigators, as an indicator of the test compound being at least a weak mutagen, are based on a particular sample size. To detect a doubling of the spontaneous mutation frequency (0.206%) required 7000 X-chromosomes based on the tables in Kastenbaum-Bowman (13). The resulting number of X-chromosomes from TW011 was 7426; therefore, this proved to be an adequate sample size for analysis by the Kastenbaum-Bowman test (12).

The results of the Fisher's exact test LAIR SOP OP-STX-10, Execution of Fortran V Program FEXP, showed non-significant differences between the mutation frequency of the negative control and TW011 (Appendix C, Table 2); the P-value (0.1194) was not significant at the 5% level ($P > 0.05$). This indicated non-significant differences between the mutation frequency (0.242%) due to TW011 and the negative control (spontaneous mutation frequency (0.206%)). The P-value for the extremes was 0.7887. The Kastenbaum-Bowman test revealed non-significant differences between TW011 and the negative control at the 5% level ($P > 0.05$) ($M = 37$, $K = 0.45$).

The results of the analysis of each brood Appendix C, Table 3, (corresponding stage of sperm), when exposed to treatment or control indicate that for broods 1, 2, 3 and 4 the P-values and their extremes from the Fisher's exact test (10, 11) were: 0.2000, 0.5240; 0.2944, 0.6304; 0.2509, 0.6026; and 0.1018, 0.1174, respectively. These P-values are non-significant at the 5% level ($P > 0.05$). This indicated non-significant differences between the mutation frequency for each brood due to TW011, and the spontaneous mutation frequency due to the negative control.

It should be noted that the total number of culture vials examined (F_1 progeny) (7426) resulting from TW011 was less than the total number of such vials resulting from the negative control (9208). This reduction in progeny (F_1) from parental (P_1) exposure to TW011 is apparent in broods 2, 3 and 4 in Appendix C, Table 3. This may indicate TW011 effects the fecundity of the test organisms exposed to it.

The results of the analysis of each brood Appendix C, Table 3, (corresponding stage of sperm), when exposed to treatment or control indicate that for broods 1, 2, 3 and 4 the P-values and their extremes from the Fisher's exact test (10, 11) were: 0.2000, 0.5240; 0.2944, 0.6304; 0.2509, 0.6026; and 0.1018, 0.1174, respectively. These P-values are non-significant at the 5% level ($P > 0.05$). This indicated non-significant differences between the mutation frequency for each brood due to TW011, and the spontaneous mutation frequency due to the negative control.

It should be noted that the total number of culture vials examined (F_1 progeny) (7,426) resulting from TW011 was less than the total number of such vials resulting from the negative control (9,208). This reduction in progeny (F_1) from parental (P_1) exposure to TW011 is apparent in broods 2, 3 and 4 in Appendix C, Table 3. This may indicate TW011 effects the fecundity of the test organisms exposed to it.

CONCLUSION

The mutation frequency of TW011 at the concentrations tested and method of exposure was not significantly different from the concurrent spontaneous mutation frequency. This suggests non-mutagenic activity associated with this compound, under these test conditions.

RECOMMENDATION

Due to the decrease in F_1 progeny resulting from TW011 an additional dominant lethal test (5) might indicate whether genetic or non-genetic damage is responsible for this reduction of X-chromosomes resulting from TW011 compared to the negative control.

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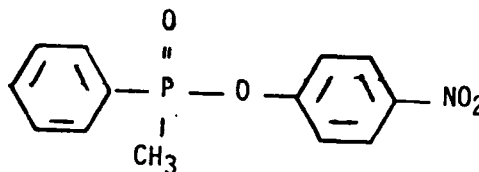
APPENDICES

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Analytical Data, Test Substances

Chemical name: 4-nitrophenyl methyl (phenyl) phosphinate

Molecular structure: $C_{13}H_{12}NO_4P$



pH: N/A non aqueous

Physical state: solid crystals

Boiling point: N/A

Melting point: 85-86 C

Compound refractory: N/A

Stability: Under refrigerated conditions, Dr. Lieske (Biomedical) Laboratory, Aberdeen Proving Grounds, Aberdeen MD, 21005) believed the compound would remain stable for two years

Names of contaminants and percentages: unknown

Manufacturer: Ash Stevens
Detroit Research Park
5861 John C. Lodge Freeway
Detroit, Michigan 48202

Manufacturers Lot No.: XLIX-40

This sample was kept from exposure to light and frozen, as required.

Analytical data: 4-nitrophenyl methyl (phenyl) phosphinate
formulated with Tween 80TM, ethanol, citrate
buffer and water.

APPENDIX A

The various concentrations of TW011 used in this study were formulated in the following manner:

A stock solution containing TW011 [10 mg, 1 ml Tween 80, 0.5 ml EtOH (100%) and 1 ml citrate buffer (5.0 mM)].

A blank containing [1 ml of Tween 80, 0.5 ml EtOH (100%) and 1 ml citrate buffer (5.0 mM)].

A diluent containing [24 ml Tween 80, 16 ml EtOH (100%), 100 ml citrate buffer (5.0 mM) and 20 ml H₂O (dist'l) at a pH 4.0].

Various proportions of TW011 stock and blank were combined to give a final yield of 1 ml; resulting in various concentrations of TW011. This was then formulated with 8 ml of diluent and 1 ml of 10% fructose.

A control containing [1 ml of blank, 8 ml of diluent and 1 ml of 10% fructose].

pH: 4.0

Physical state: liquid/clear yellow

Boiling point: N/A

Melting point: N/A

Compound refractory: N/A

Stability: Hydrolysis measurements were conducted immediately after preparation and 24 hours later on the various concentrations of TW011. However, such concentrations were below the ability to have accurate spectrophotometer readings.

Names of contaminants and percentages: unknown

Manufacturer: Tween 80 is a preparation of polyoxyethylene 20 and sorbiton monooleate, and is approved for use in humans. It is manufactured by Fisher Scientific Company - Chemical Manufacturing Division, Fair Lawn, NJ 07410.

Historical Listing of Significant Study Events

23-27 Nov 81	The formulation of 4-nitropheny methyl (phenyl) phosphinate with Tween 80 and 1% fructose (LAIR SOP OP-STX-45 Preparation of Compounds Unstable in Water for SLRL Assay) for pilot toxicity testing to establish the dosing range after 72 hour exposure.
25 Jan-24 Mar 82*	Removal of all adult insects from CS colony and collecting of newly emerged adult CS males, 24 hours later for toxicity testing (LAIR SOP OP-STX 7 <u>Drosophila melanogaster</u> Exposure Procedures)
27 Jan-27 Mar 82 [†]	Selection of CS male survivors after toxicity testing and concurrent positive and negative control exposure at 72 hours from the concentration that resulted in LD ₅₀ (approximately).
29 Jan, 1,3,5 Feb 82 [‡]	Each exposed CS male was crossed with 4 groups of 3 virgin FM6 females in corresponding vials labeled Broods 1, 2, 3, and 4. Replicate 1. (Run 35).
19,22,24,26 Feb 82	Replicate 2, (Run 36).
5,8,10,12 Mar 82	Replicate 3, (Run 37).
27,30 Mar, 1,3 Apr 82	Replicate 4, (Run 38).
16 Feb-26 May 82	The F ₂ crosses of all broods for all 4 replicates were made and scored, the (F ₃) were made and scored for all 4 replicates.

*The events enclosed in parentheses required two days for each replicate, e.g., (1) 25-26 Jan 82, (2) 15-16 Feb 82, (3) 1-2 Mar 82, (4) 23-24 Mar 82.

[†]The events enclosed in parentheses required three days for each replicate, e.g., (1) 27-29 Jan 82, (2) 17-19 Feb 82, (3) 3-5 Mar 82, (4) 25-27 Mar 82.

[‡]Dates for each of the four broods.

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TABLE 1

Concentrations and Corresponding Mean Percentage Mortality and Standard Deviation for TW011* fed to CS Males for the SLRL Assay.

Replication Number	Conc. mM	%Mortality [†] $\bar{X} \pm \text{s.d.}$
1	0.002	42.0 \pm 9.80
2	0.0025	68.0 \pm 13.98
3	0.002	62.0 \pm 18.14
4	0.002	54.0 \pm 19.50

*TW011 = 4-nitrophenyl methyl (phenyl) phosphinate, formulated with Tween 80, ethanol (100%), citrate buffer (5.0mM), and 1% fructose

†Based on a sample size ranging from 95 to 100 CS males.

TABLE 2

Sex-Linked Recessive Lethal
Assay of TW011

Compound	Replication*				Total	%Mutation
	1	2	3	4		
TW011†	5/2159	9/1580	2/2094	2/1593	18/7426	0.242
Neg Cont‡	8/2411	4/2162	3/2300	4/2335	19/9208	0.206

*Data are recorded as number of SLRL events/number of X-Chromosomes tested. All single mutations, i.e. no "clusters" or "multiples" were detected.

†TW011 = 4-nitrophenyl methyl (phenyl) phosphinate, formulated with Tween 80, ethanol (100%), citrate buffer (5.0 mM), and 1% fructose.

‡Negative Control = Tween 80, ethanol (100%), citrate buffer (5.0 mM) and 1% fructose.

TABLE 3
Sex-Linked Recessive Lethal Assay
for Each Brood of TW011

Compound	Brood*			
	1	2	3	4
TW011 [†]	7/2184	3/1873	4/1668	4/1701
Neg. Cont. [‡]	8/2249	4/2382	6/2376	1/2201
p values				
Fisher's Exact	0.2000	0.2944	0.2509	0.1018

*Data are recorded as number of SLRL events/number of X-Chromosomes tested. All single mutations i.e., no "clusters" or "multiples" were detected.

[†]TW011 = 4-nitrophenyl methyl (phenyl) phosphinate (formulated with Tween 80, ethanol (100%), citrate buffer (5.0 mM) and 1% fructose.

[‡]Negative Control = Tween 80, ethanol (100%), citrate buffer (5.0 mM) and 1% fructose.

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